USDA/APHIS Environmental Assessment

Monsanto Company and Forage Genetics International Petition 04-110-01p for Determination of Non-regulated Status for Roundup Ready® Alfalfa Events J101 and J163

October 2004

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture, has prepared an environmental assessment in response to a petition (APHIS Number 04-110-01p) received from Monsanto Company and Forage Genetics International seeking a determination of non-regulated status for their genetically engineered alfalfa designated as events J101 (OECD unique identifier MON-ØØ1Ø1-8) and J163 (OECD unique identifier MON-ØØ163-7) under APHIS regulations at 7 CFR Part 340. The plants have been engineered with a gene that confers tolerance to the herbicide glyphosate.

U.S. Department of Agriculture

Animal and Plant Health Inspection Service

Biotechnology Regulatory Services

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I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture (USDA), has prepared an Environmental Assessment (EA) in response to a petition (APHIS Number 04-110-01p) from Monsanto Company (St. Louis, MO) and Forage Genetics International (West Salem, WI) (hereafter FGI) seeking a determination of non-regulated status for their genetically engineered Roundup Ready® alfalfa designated as events J101 (OECD unique identifier MON-ØØ1Ø1-8) and J163 (OECD unique identifier MON-ØØ163-7). Monsanto Company and FGI seek a determination that events J101 and J163 and their progeny do not present a plant pest risk and, therefore, become no longer regulated articles under regulations at 7 CFR Part 340.

Events J101 and J163 were engineered to be glyphosate tolerant by inserting a gene that codes for the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) into the alfalfa genome. The gene is from the common soil bacterium *Agrobacterium* sp. strain CP4 and was introduced into alfalfa via an *Agrobacterium*- mediated transformation protocol.

This EA specifically addresses the potential for impacts to the human environment through the use in agriculture of events J101 and J163. It does not address the separate issue of the potential use of the herbicide glyphosate in conjunction with these plants. The United States Environmental Protection Agency (EPA) has authority over the use in the environment of all pesticidal substances, under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The Food and Drug Administration (FDA) has authority over food and feed issues of all plants used as food or feed.

Field trials of J101 and J163 alfalfa have been conducted under APHIS notification procedures (7 CFR 340.3). In accordance with APHIS procedures for implementing the National Environmental Policy Act (NEPA) (7 CFR 372), this EA has been prepared prior to issuing a determination of nonregulated status for J101 and J163 alfalfa in order to specifically address the potential for impact to the human environment through unconfined cultivation and use of the regulated articles in agriculture.

II. INTRODUCTION

A. Development of Events J101 and J163 Alfalfa

Monsanto and FGI have submitted a "Petition for Determination of Non-regulated Status" to the USDA, APHIS (APHIS number 04-110-01p) for genetically engineered alfalfa that is tolerant to the broad spectrum herbicide glyphosate. Glyphosate tolerant alfalfa would offer farmers a new option for weed control.

The management of weeds in alfalfa fields can be an expensive, labor intensive, and sometimes complicated operation. Often farmers use pre-emergent herbicides that will stop weed seeds from germinating. However, this assumes that weeds will always be a problem in all parts of the field. With J101 and J163 and progeny, farmers will have the option of applying herbicide after weeds have germinated and only in the areas of the field where there are weeds. Glyphosate is one of the most environmentally friendly herbicides commercially available.

These alfalfa plants were genetically engineered to be glyphosate tolerant by inserting a gene (from *Agrobacterium* sp. strain CP4) that code for the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) into the alfalfa genome. This gene, along with its regulatory sequences, was introduced into these alfalfa plants via an *Agrobacterium*-mediated transformation protocol. This is a well-characterized procedure, which has been widely used for over a decade for introducing various genes of interest directly into plant genomes.

APHIS authorized the first field testing of these alfalfa plants starting in 1998 and they have been field tested in the United States under the APHIS authorization numbers noted in Appendix B. Events J101 and J163 alfalfa have been evaluated extensively to confirm that they exhibit the desired agronomic characteristics and do not present a plant pest risk. The field tests have been conducted in agricultural settings under physical and reproductive confinement conditions.

B. APHIS Regulatory Authority

APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority granted by the Plant Protection Act (7 U.S.C. 7701-7772), regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. An organism is no longer subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. 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. These alfalfa plants have been considered regulated articles because they contain non-coding DNA regulatory sequences derived from plant pathogens and the vector agent used to deliver the transforming DNA is a plant pathogen.

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 therefore should no longer be regulated. If APHIS determines that the regulated article is unlikely to present a greater plant pest risk than the unmodified organism, the Agency can grant the petition in whole or in part. In such a case, APHIS authorizations (i.e., permits or notifications) would no longer be required for field testing, importation, or interstate movement of the non-regulated article or its progeny.

C. U.S. Environmental Protections Agency and Food and Drug Administration Regulatory Authorities

The genetically engineered alfalfa is also subject to regulation by other agencies. The EPA is responsible for the regulation of pesticides 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 prior to distribution or sale, unless exempt by EPA regulation. Under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended (21 U.S.C. 301 *et seq.*), pesticides added to (or contained in) raw agricultural commodities generally are considered to be unsafe unless a tolerance or exemption from tolerance has been established. Residue tolerances for pesticides are established by EPA under the FFDCA, and the FDA enforces the tolerances set by the EPA. A final EPA decision is pending.

The FDA 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. Under this policy, FDA uses what is termed a consultation process to ensure that human food and animal feed safety issues or other regulatory issues (e.g., labeling) are resolved prior to commercial distribution of bioengineered food. Monsanto/FGI submitted a food and feed safety and nutritional assessment summary for events J101 and J163 in October 2003. A final FDA decision is pending.

III. PURPOSE and NEED

APHIS has prepared this EA before making a determination on the status of J101 and J163 alfalfa as regulated articles under APHIS regulations. The developer of these alfalfa plants, Monsanto and FGI, submitted a petition to USDA-APHIS requesting that APHIS make a determination that these alfalfa plants shall no longer be considered regulated articles under 7 CFR Part 340.

This EA was prepared in compliance with the National Environmental Policy Act (NEPA) of 1969 as amended, (42 USC 4321 *et seq.*) and the pursuant implementing regulations (40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 372).

IV. ALTERNATIVES

A. No Action: Continuation as a Regulated Article

Under the Federal "no action" alternative, APHIS would not come to a determination that these alfalfa plants are not regulated articles under the regulations at 7 CFR Part 340. Permits issued or notifications acknowledged by APHIS would still be required for introductions of J101 and J163 lines of glyphosate tolerant alfalfa. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from the unconfined cultivation of glyphosate tolerant alfalfa.

B. Determination that J101 and J163 Alfalfa Plants are No Longer Regulated Articles, in Whole

Under this alternative, these glyphosate tolerant alfalfa plants would no longer be regulated articles under the regulations at 7 CFR Part 340. Permits issued or notifications acknowledged by APHIS would no longer be required for introductions of glyphosate tolerant alfalfa derived from these events. A basis for this determination would include a "Finding of No Significant Impact" under the National Environmental Policy Act of 1969, as amended (42 USC 4321 *et seq*.; 40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 342).

C. Determination that J101 and J163 Alfalfa Plants are No Longer Regulated Articles, 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 some but not all lines requested in the petition. In some petitions, applicants request deregulation 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. This request is for the two events J101 and J163 and their progeny.

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 line could only be grown without APHIS authorization in certain geographic areas.

V. POTENTIAL ENVIRONMENTAL IMPACTS

Potential impacts to be addressed in this EA are those that pertain to the use of events J101 and J163 and its progeny in the absence of confinement.

A. Alternative A: No Action

If APHIS takes no action, commercial scale production of events J101 and J163 and their progeny is effectively precluded. These plants could still be grown in field trials for variety development as they have been for the past several years under APHIS authorizations (notifications). APHIS has evaluated field trial data reports submitted on events J101 and J163 and their progeny, and has noted no significant adverse effects on non-target organisms, no increase in fitness or weediness characteristics, and no effect on the health of other plants. The Agency expects that future field tests would perform similarly.

With respect to commercial production, if APHIS were to take no action, alfalfa growers would still have the same options available to them for weed control in their fields as they currently have. Control measures can be complicated by type of weeds (over 90 weeds were reported as being significant in alfalfa), growth stage of specific weeds, growth stage of the alfalfa, carry-over effects on the following crops, and field environmental conditions. Statistics presented (Hower et al., 1999, Gianessi and Marcelli, 2000) on the usage of pesticides on the nation's alfalfa crop (seed and forage) document significant use of 19 herbicides, other than glyphosate, in the U.S. between 1988 and 1997. Planted area of alfalfa during this time period was reported to be 21,000,000 to 23,000,000 acres.

The data on the estimated use of the herbicides in alfalfa in 1997 in the following table are taken from Gianessi and Marcelli (2000) (http://www.ncfap.org/database/default.htm).

Herbicide	Application Rate (Lbs A.I./A)	Total Lbs. A.I. (1000s)
Benefin	1.2-1.35	119
Bromoxynil	0.26-0.45	37
Clethodim	0.1-0.2	4
Diruron	1.2-1.6	271
EPTC	2.6-3.5	695
Glyphosate	0.35-1.45	175
Hexazinone	0.25-1.0	316
Imazethapyr	0.03-0.11	28
Metribuzin	0.25-0.75	319
Norflurazon	1.0-1.5	43
Paraquat	0.25-0.69	355
Pronamide	1.0-1.3	24
Sethoxydim	0.11-0.5	132
Terbacil	0.50-0.64	47
Trifluralin	0.75-2.16	950
2,4-DB	0.3-1.5	389
Total		3,904

A range of application rates were reported, since recommended rates vary based on the type of weeds, environmental conditions and type of herbicide mixture.

In addition to chemical control measures, growers would also likely continue the use of mechanical and cultural practices such as mowing, tillage, burning, flash grazing and companion crops.

B. Alternative B: Approval of the Petition, in Whole

If APHIS were to grant the petition for non-regulated status in whole, alfalfa events J101 and J163 and their progeny would no longer be considered regulated articles. APHIS' assessments of the environmental impacts are discussed in the following sections.

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 alfalfa events J101 and J163 and their progeny or in other plants. We also considered whether data indicate that unanticipated unintended effects would arise from engineering of these plants. APHIS considered information from the scientific literature as well as data provided by the developer when conducting their field trials.

Recipient organism

The plant material used for development of events J101 and J163 was FGI proprietary alfalfa clone R2336 from a high yielding, fall dormant breeding population. The initial plants, selected for tolerance to glyphosate, were designated J101 and J163, and various populations were developed from these events to provide the data presented in the petition. The breeding history and progeny resulting from events J101 and J163 can be found in Figure VI-8, p. 113 of the petition. Alfalfa is not listed as a Federal noxious weed or on other weed lists such as:

Federal Noxious Weed List (http://www.aphis.usda.gov/ppq/weeds/noxwdsa.html),
Washington State Weed Lists (http://www.nwcb.wa.gov/weed_list/weed_listhome.html),
California Weed Species Lists (http://www.extendinc.com/weedfreefeed/list-b.htm),
Montana County Noxious Weed List (http://www.weedawareness.org/weed%20list.html),
North Dakota Noxious Weeds (http://www.ext.nodak.edu/extpubs/plantsci/weeds/w1103w.htm).

Transformation system

Events J101 and J163 were developed using a disarmed (i.e. pathogenicity genes removed) *Agrobacterium*-mediated transformation system of sterile alfalfa seedling cotyledons. Post-transformation, *Agrobacterium* were eliminated from tissues by a 7-week culture on antibiotic-containing medium. Glyphosate was used to select for transformed tissues containing the *epsps* gene construct. This technique using disarmed *Agrobacterium* followed by selection has a 20-year history of safe use and has been used for transformation of a variety of plant species and tissues (Howard *et al.*, 1990).

DNA sequences inserted into alfalfa events J101 and J163

Data supplied in the petition and reviewed by APHIS (Section V.A., pp. 38-68) support the conclusion that events J101 and J163 contain the following sequences: (1) a 35S promoter from a modified figwort mosaic virus (P-FMV), (2) coding sequence for a chloroplast transit peptide from *Arabidopsis thaliana*, (3) the 5-enolpyruvylshikamate-3-phosphate synthase gene (*epsps*) from *Agrobacterium* sp. strain CP4, and (4) DNA containing polyadenylation sequences from the 3' non-translated region of the *Pisum sativum* (pea) *rbc*S E9 gene. The non-coding 35S promoter is from the plant pathogen figwort mosaic virus. This sequence, however, cannot cause plant disease and serves a purely regulatory function for the *epsps* gene. The *epsps* gene is from the soil-inhabiting bacterial plant pathogen, *Agrobacterium* sp. strain CP4. It encodes the EPSPS protein which functions to impart tolerance to the broad spectrum herbicide glyphosate. It does not cause disease and has a history of safe use in a number of deregulated genetically engineered plants (e.g., corn, cotton and soybean varieties).

Evaluation of intended effects

As expected, as a result of introduction of the *epsps* gene into the alfalfa genome, the resulting plants are tolerant to glyphosate, the active ingredient in the herbicide Roundup®.

Analysis of inheritance: Data were provided and reviewed by APHIS that demonstrate stable integration and inheritance of the *epsps* gene and its associated regulatory sequences over several breeding generations. Statistical analyses show that glyphosate tolerance is inherited as a dominant trait in a typical Mendelian manner (petition Table V-1, p. 71).

Analysis of gene expression: Data on EPSPS (5-enolpyruvylshikamate-3-phosphate synthase) protein concentrations were collected from field trials conducted at multiple locations. Using standard laboratory ELISA techniques, protein concentrations from alfalfa forage were determined (petition Table V-8, p. 97). EPSPS protein concentrations on a fresh weight basis averaged 257 µg/gram in plants with event J101, 270 µg/gram in plants with event J163, and 252 µg/gram in plants from the population containing both events J101 and J163. EPSPS enzymes are ubiquitous in plants and microorganisms and have not been associated with hazards from consumption or to the environment. Genetically engineered crops that contain this recombinant protein and have been granted non-regulated status include corn, soybean, cotton, rapeseed and sugar beet (http://www.aphis.usda.gov/brs/not_reg.html). In 2004, significant acreages of corn (10.5 million acres or 13% of the total), upland cotton (4.2 million acres or 30% of the total) and soybean (63.6 million acres or 85% of the total) grown in the U.S. were planted with herbicide tolerant varieties (http://usda.mannlib.cornell.edu/). Although the data include all herbicide tolerant varieties, glyphosate tolerant ones (containing EPSPS) predominate. All the genetically engineered glyphosate tolerant varieties have also undergone review by the FDA (http://www.cfsan.fda.gov/~lrd/biocon.html) and are allowed for food and feed use.

Analysis of the intended trait: Numerous field trials were conducted (Appendix B of this EA) to evaluate events J101 and J163 in different environments. Standard field trials evaluated (1) agronomic performance, (2) disease and pest resistance performance, and (3) seed multiplication. Agronomic practices used to prepare and maintain each field trial were characteristic of each representative region. Where the glyphosate herbicide Roundup® was used in trials, no negative impacts from application of the Roundup® were noted.

Analysis of possible unintended effects: Expression of EPSPS in events J101 and J163 alfalfa is not expected to cause plant disease or influence susceptibility of J101 and J163 or their progeny to diseases or other pests. Data addressing disease susceptibility and overall agronomic performance were collected in order to assess possible effects from introduction of the *epsps* gene and its associated regulatory sequences. The petitioner has described these trials, conducted over several years in a variety of locations, and presented these data in Section VI of the petition (starting on p. 99). Approximately 760 observations were presented in the petition from 18 location-years on susceptibility to disease, insects and weeds. All of the observations noted no differences between the populations with and without events J101 and J163. An additional 152 observations from 15 location-years were presented on abiotic stresses with no differences noted between populations with events J101 and J163 and control populations. Other phenotypic characterizations comparing J101 and J163 populations with conventional and control populations were also completed. Data were provided and assessed by APHIS on numerous characteristics related to the morphology of flowers, pollen and seed, seed germination and dormancy, seed yield, and various plant growth characteristics such as forage yield, seedling vigor, regrowth after cutting, survival, and fall dormancy. No qualitative or quantitative observations indicated any biologically meaningful differences from control populations or differences outside the range of conventional alfalfa norms.

Al-Kaff et al. (1998) have noted gene silencing effects when transgenic plants have been infected by a virus with DNA sequence homology to a portion of the introduced genes. None of the viral diseases of alfalfa is related to figwort mosaic virus (http://image.fs.uidaho.edu/vide/refs.htm and http://www.apsnet.org) (a *caulimovirus,* from which the promoter for the *epsps* gene originates), so silencing of the *epsps* gene should not occur.

In addition to field studies on agronomic parameters, Monsanto/FGI analyzed alfalfa for compositional changes as part of their submission to FDA in the consultation process. While FDA uses these data as indicators of possible nutritional changes, APHIS views them as general indicators of possible unintended changes. Compositional analyses evaluating carbohydrates, protein, ash, minerals, fiber, lignin, fat, and 18 amino acids (a total of 35 different components) identified three statistically different values compared with the control population for J101, seven statistically different values for J163, and 11 statistically different values for the paired J101 X J163 population. However, all analyses fell within the 99% tolerance interval developed from the conventional varieties grown in the same locations, providing additional evidence that J101, J163 and the paired J101 x J163 populations do not exhibit unexpected or unintended effects.

Potential Impacts on Relative Weediness of Events J101 and J163 Compared to Conventionally Bred Alfalfa

APHIS assessed whether J101 or J163 alfalfa populations are any more likely to become a weed than the non-transgenic control populations or other currently cultivated alfalfa. This assessment considers the basic biology of alfalfa and an evaluation of unique characteristics of J101 and J163 alfalfa populations.

Almost all definitions of weediness stress as core attributes the undesirable nature of weeds from the point of view of humans; from this core, individual definitions differ in approach and emphasis (Baker, 1965; de Wet and Harlan, 1975; Muenscher, 1980; Booth et al., 2003). The parent plant in this petition, *Medicago sativa* L., is not listed as a serious weed in A *Geographical Atlas of World Weeds* (Holms et al., 1991) or as a weed in *World Weeds: Natural Histories and Distribution* (Holms et al., 1997), *Weeds of the North Central States* (http://www.ag.uiuc.edu/~vista/html_pubs/WEEDS/list.html), *Weeds of the Northeast* (Uva et al.,1997), or *Weeds of the West* (Whitson et al., 1992) nor is it listed as a noxious weed species by the U.S. Federal Government (7 CFR Part 360). Occasionally, alfalfa volunteers in fields the year after stand termination. These volunteer plants can be controlled by mechanical means or several other registered herbicides besides glyphosate. Alfalfa possesses few of the characteristics of plants that are notable of successful weeds (Baker, 1965; Keeler, 1989; Booth et al., 2003).

As part of a bilateral agreement between the United States and Canada, USDA/APHIS and the Canadian Food Inspection Agency (CFIA) have generated documents that outline basic data requirements for developers of genetically engineered plants (http://www.aphis.usda.gov/brs/international_coord.html). One of these documents, Appendix II, outlines the environmental characterization data requirements for unconfined releases. As a part of the entire package requesting a determination of non-regulated status, these data are designed to address characteristics that influence both reproductive biology and survival biology of the transgenic plant compared to its non-transgenic counterpart.

In trials conducted in the United States, no differences were observed between J101 or J163 populations and non-transgenic control populations with respect to the plants ability to persist or compete as a weed. APHIS considered data relating to plant vigor, seedling emergence, seed germination, seed dormancy and other characteristics that might relate to increased weediness. No unusual characteristics were noted that would suggest increased weediness of J101 and J163 plants. Additionally, no characteristics relating to disease or insect resistance that might affect weediness were noted. These characteristics were consistent over all field trial locations. J101 and J163 alfalfa plants are still susceptible to the typical insect and disease pests of alfalfa.

Potential Impacts from Outcrossing of Lines J101 and J163 to Wild Relatives

APHIS evaluated the potential for hybridization and gene introgression to occur from J101 and J163 to sexually compatible wild (free-living) relatives, and considered whether such introgression would result in increased weediness. Alfalfa is sexually compatible with several subspecies within the *M. sativa* complex (Small and Jomphe, 1989). The center of origin for the genus *Medicago* is generally believed to be in the Caucasus, northwestern Iran and northeastern Turkey; the genus is not native in North America (see this EA's Appendix A). An additional 18 *Medicago* species are known to be naturalized (free-living) or possibly so within the United States, of which only *M. lupulina* (black medic) is widely naturalized throughout the United States. None of these species are native to the United States, and none are sexually compatible with *M. sativa*.

Potential Impact on Threatened or Endangered Species or Non-target Organisms Including Beneficial Organisms

APHIS evaluated the potential for deleterious effects or significant impacts on non-target organisms, including those on the Federal Threatened and Endangered Species (TES) list of the U.S. Fish and Wildlife Service (FWS) (http://endangered.fws.gov/wildlife.html#Species), from cultivation of J101 and J163 alfalfa and its progeny. The gene that codes for the enzyme EPSPS that confers glyphosate tolerance is from the bacterium *Agrobacterium* sp. strain CP4. This gene is similar to the gene that is normally present in alfalfa and is not known to have any toxic property. Field observations of events J101 and J163 revealed no negative effects on non-target organisms. The lack of known toxicity for this enzyme suggests no potential for deleterious effects on beneficial organisms such as bees and earthworms. The high specificity of the enzyme for its substrates makes it unlikely that the introduced enzyme would metabolize endogenous substrates to produce compounds toxic to beneficial organisms.

A number of researchers have conducted laboratory investigations with different types of arthropods exposed to genetically engineered 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 genetically engineered 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 these crops (Nahas et al., 2001; Dunfield and Germida, 2003, Siciliano and Germida 1999).

EPSPS has received an exemption from tolerance requirement from the EPA on all raw agricultural commodities (http://www.epa.gov/fedrgstr/EPA-PEST/1996/August/Day-02/pr-840.html). APHIS has not identified any other potential mechanisms for deleterious effects on beneficial organisms.

From the above analysis of both qualitative and quantitative information from the petition and published data, APHIS concludes that the unconfined release of J101 and J163 and their progeny would not harm any non-target or Federally listed (or proposed) threatened or endangered species. Consistent with APHIS' U.S. Fish and Wildlife Service TES assessment requirements, this is a "no harm" decision.

Potential Impacts on Agricultural and Cultivation Practices

Current weed control practices in alfalfa can be somewhat complicated and can vary substantially among spring seedings, fall seedings, and established stands and between hay and seed production. Current practices include mechanical tillage, companion crops, mowing, flash grazing, early harvest, burning, and both pre-and post-planting application of broadleaf and grass herbicides (Hower et al. 1999). Each of these practices has its limitations and can be significantly impacted by growing conditions, soil pH, weed species, target weed size, crop size, cost, etc. Glyphosate, a non-selective herbicide (e.g., Roundup®), would provide post-planting control of most annual grass and broadleaf weeds in glyphosate tolerant alfalfa under a wide range of growing conditions. Glyphosate would control larger broadleaf weeds than currently available herbicides and allow more application flexibility when environmental conditions prevent the timely application required by some currently used herbicides. In addition, glyphosate would provide a different herbicide mode of action in the growers' crop rotation, which is important in preventing the development of herbicide resistant weeds. Glyphosate is applied like any other post-emergent herbicide used in any other crop. Glyphosate tolerant alfalfa may alter current alfalfa cultivation practices by allowing for reduced herbicide use in comparison to current practices in order to achieve the same crop yield.

Alfalfa forms a symbiotic relationship with the bacterium *Sinorhizobium meliloti* (formerly *Rhizobium melilto*), that transforms nitrogen in the atmosphere, which cannot be utilized by the plant, into a form that is useable by plants. This process of nitrogen fixation reduces the plants dependence on soil nitrogen. *Sinorhizobium meliloti* strains were able to grow on glyphosate as the sole source of phosphorus in the presence of aromatic amino acids (Liu et al., 1991), which indicates that the application of glyphosate on glyphosate tolerant alfalfa should have no effect on the nitrogen fixation process of this symbiotic relationship.

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 alfalfa , will be significantly impacted by the expected commercial use of this product since: (a) non-transgenic alfalfa will likely still be sold and will be 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 tolerant.

No transgenic varieties of alfalfa are currently in commercial production. Varieties derived from events J101 and J163 should not present new and different issues with respect to impacts on organic farmers. With the exception of seed production fields, alfalfa does not typically set viable seed in fields used to produce forage. Although these fields may produce flowers that may release pollen, the fields are typically harvested before the seed is set and allowed to mature, because high quality forage is the desired product.

Potential Impact on Sprout Production

Although alfalfa is mainly used for animal feed, humans do consume alfalfa sprouts. APHIS considered the likelihood of events J101 and J163 entering the sprouting industry. The following are the reasons why APHIS considered this use of glyphosate tolerant alfalfa to be a very low probability: (1) Only a small amount (estimated to be about 7%) of the alfalfa seed produced and marketed in the United States is used for sprouting purposes (Bass et al., 1988). (2) Glyphosate tolerant alfalfa seed will be labeled to indicate that it is glyphosate tolerant since this claim is a valuable agronomic characteristic. (3) Glyphosate tolerant alfalfa seed will use many of the conventional practices to maximize seed yield and these recommended practices include the use of pesticides during production (Hower et al., 1999). (4) The seed to be used for planting forage fields will most likely be coated with bacterial inoculant and /or fungicide and colored (petition Addendum 1). (5) Each container of glyphosate tolerant seed to be sold will have a designation (i.e. lot number) that can trace its origin to the field in which it was produced. (6) Applicants intend to produce all glyphosate tolerant alfalfa seed under production contracts that will preclude the legal sale of the seed for food use and forage producers who purchase Roundup Ready (i.e., glyphosate tolerant) alfalfa seed will be required to sign an agreement that expressly prohibits the production of any seed. (7) The sprouting industry endorses the use of certified sprouting seed, the criteria for which include seed production practices such as field history, pesticide use and origin of seed. Each of these criteria by itself would be adequate to disqualify glyphosate tolerant alfalfa for certified sprouting purposes (DeWaal, 1998; Oregon State University, 2004; International Specialty Supply, 2004).

Potential Damage to Raw or Processed Agricultural Commodities

APHIS review of the information provided by the applicant regarding the components and processing characteristics of these plants revealed no differences in any component that could have a direct or indirect plant pest effect on any raw or processed commodity. APHIS believes that the modifications for herbicide tolerance should not affect this commodity in any significant manner.

Other Environmental Statutes and Considerations

Executive Order (EO) 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations," requires Federal agencies to conduct their programs, policies, and activities that substantially affect human health or the environment in a manner so as not to exclude persons and populations from participation in or benefiting from such programs. It also enforces existing statutes to prevent minority and low-income communities from being subjected to disproportionately high and adverse human health or environmental effects. Each alternative was analyzed in its ability to affect minority and low-income populations. None of the alternatives were found to pose disproportionately high or adverse human health or environmental effects to any specific minority or low-income group.

EO 13045, "Protection of Children from Environmental Health Risks and Safety Risks," acknowledges that children may suffer disproportionately from environmental health and safety risks because of their developmental stage, greater metabolic activity levels, and behavior patterns, as compared to adults. The EO (to the extent permitted by law and consistent with the agency's mission) requires each Federal agency to identify, assess, and address environmental health risks and safety risks that may disproportionately affect children. None of the alternatives is expected to have disproportionately high or adverse human health or environmental effects to children.

EO 13112, "Invasive Species", states that Federal agencies take action to prevent the introduction of invasive species and provide for their control and to minimize the economic, ecological, and human health impacts that invasive species cause. The non-engineered plant is grown in the U.S. and based on the data submitted by the applicant and reviewed by APHIS, the engineered plants are not significantly different in any fitness characteristics from their parent that might increase their invasive potential.

Potential Impacts on Biodiversity

After careful evaluation, APHIS believes that events J101 and J163 alfalfa exhibit no traits that would cause increased weediness, that their cultivation should not lead to increased weediness of other cultivated or feral alfalfa or any sexually compatible relatives, and that they are unlikely to harm non-target organisms common to the agricultural ecosystem or Federal threatened or endangered species as recognized by the U.S. Fish and Wildlife Service. Based on this analysis, APHIS believes that it is unlikely that events J101 and J163 alfalfa or their progeny will pose a significant impact on biodiversity.

Consideration of Potential Environmental Impacts Associated With the Cultivation of Events J101 and J163 outside the United States

APHIS has also considered potential environmental impacts outside the United States and its territories associated with a determination of non-regulated status for J101 and J163 alfalfa. *Medicago sativa* is a prized forage used worldwide; the primary center of the genus is in the Caucasus, northwestern Iran and northeastern Turkey (see the EA's Appendix A). Many of the wild *Medicago* species are annuals, with which the perennial species do not hybridize or hybridize only with great difficulty under natural conditions. None of the *Medicago* species were noted to be serious weeds.

Any international trade in alfalfa 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. 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 (OECD). APHIS periodically holds bilateral or multilateral 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. All the existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new alfalfa cultivars internationally apply equally to those covered by an APHIS determination of non-regulated status under 7 CFR Part 340.

In 1992, world leaders agreed on a strategy for "sustainable development". One feature of this agreement was the Convention on Biological Diversity (CBD). The U.S. government has not ratified the CBD. One part of the CBD is the Cartegena Protocol on Biosafety, which entered into force on September 11, 2003. The Biosafety Protocol is designed to ensure an adequate level of safety in the transfer, handling and use of "living modified organisms" addressing the potential adverse effects on conservation, sustainable use of biological diversity, taking into account risks to human health. Signatory countries are required to implement a system to address these issues. The CEQ report's biodiversity considerations for incorporation into environmental impact analysis under the National Environmental Policy Act are similar to those addressed by the Biosafety Protocol.

C. Determination that J101 and J163 Alfalfa Plants are No Longer Regulated Articles, in Part

If APHIS were to grant the petition for non-regulated status in part, alfalfa events J101 and/or J163 and their progeny would no longer be considered regulated articles, with some restriction. If an in-part determination would be a geographic restriction, all of the environmental considerations under Part B would be applicable to such a determination.

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VII. CONSULTATIONS

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Appendix A: Alfalfa biology and the potential for introgression into related species

Medicago sativa L., alfalfa (sometimes called lucerne), is a perennial herbaceous legume (Lesins and Lesins, 1979; Hill, 1987) that is grown worldwide, and is a member of the family Fabaceae (Leguminosae), tribe Trifolieae (Mabberley, 1998; Small and Jomphe, 1989; Quiros and Bauchan, 1988). Alfalfa is insect pollinated, primarily by a few bee species; the flower is specialized and only pollinated once, after which it is tripped and no longer available for insect visitors to pollinate it again (Viands et al., 1988; Hill, 1987). The mature plant has a deep taproot, making it possible to use soil moisture from depths of about 20 feet (6 meters) or more (Teuber and Brick, 1988; Barnes and Sheaffer, 1995). In addition, alfalfa forms a symbiotic relationship with *Sinorhizobium meliloti* (formerly *Rhizobium meliloti*), thereby reducing its dependence on soil nitrogen (Vance et al., 1988; Ferguson et al., 2002). Flowers develop at the shoot apex when the plant transitions from vegetative to reproductive growth, which generally takes place between the 6^{th} and 14^{th} nodes depending on genetics and the environment. The stems are indeterminate, so vegetative and reproductive growth can occur simultaneously (Teuber and Brick, 1988; Viands et al., 1988).

The Old World genus *Medicago* L. (including some *Trigonella* species) consists of about 85 species (Small and Jomphe, 1989; Bena, 2001; Mabberley, 1998), with most being annuals and one quarter or fewer being perennial herbs (and a few shrubs). Domesticated alfalfa (*M. sativa*) originated in Asia Minor, Iran, Transcaucasia and Turkmenistan several millennia ago (Quiros and Bauchan, 1988; Muller et al., 2003). It can now be found growing wild (free-living) from Spain (Muller et al., 2001) to China and North Africa to Sweden. It has also become acclimatized as a crop in South Africa, Australia, New Zealand, South America and North America. The *M. sativa* complex, which was introduced into North America early by Europeans for forage and includes all the commercial alfalfa varieties, is a group of closely related subspecies, including the cultivated *M. sativa* ssp. *sativa* and *M. sativa* ssp. *falcata* (synonym *M. falcata*) (Small and Jomphe, 1989). Of the 11 other species in *Medicago* section *Medicago* (all of which are perennials), only *M. hybrida* is found free-living in North America (Small and Jomphe, 1989; Quiros and Bauchan, 1988; Michaud et al., 1988; Kartesz, 2004).

In addition to the *M. sativa* complex within which all of the subspecies are sexually compatible to some degree, an additional 17 and possibly 18 *Medicago* species have been recognized as being naturalized (free-living) or possibly so in the United States (USDA-NRCS, 2004; Kartesz, 2004). All of these 18 species are annual species, except for the species *M. hybrida* (in *Medicago* section *Medicago*) hybrids of which have only been produced experimentally by embryo culture. No annual species are known to hybridize with *M. sativa* (Quiros and Bauchan, 1988; McCoy and Bingham, 1988; and the petition's Appendix 4).

Medicago lupulina (black medic) is the species that might be of most concern within this list of 18 species. It is considered a weed in lawns and waste places and in forages since its seeds frequently contaminate forage legume seed crops. Black medic is an annual (possibly sometimes short-lived perennial) self-pollinating species and is known to occur throughout the United States. Successful hybridizations between *M. sativa* and *M. lupulina* have been reported (Southworth, 1928; Fryer, 1930; Shrock, 1943). However, because of many subsequent experiments, there is general agreement that these putative "hybrids" were most likely not hybrids but due to self-fertilization (Lesins and Gillies, 1972; Fridriksson and Bolton, 1963; Valizadeh et al., 1996). For more details on this topic, see Section E.1 (p. 284) and Appendix 4 of the petition. Based on all the recent data available on this subject, APHIS' opinion is that hybridization between *Medicago sativa* and *M. lupulina* has an extremely low to non-existent probability of occurring in a non-experimental or even in an experimental setting.

Alfalfa is not considered a serious weed, a noxious weed or an invasive species in the United States, even though feral (free-living) populations are fairly common and volunteers may occur in succeeding crops. Generally feral populations, many of which are along roadsides, are not a problem, and generally no attempts are made to control these populations. In some instances, these feral populations are considered advantageous and are encouraged (petition Appendix 3, p. 375, 12/31/02 Letter from South Dakota State University). More detailed information concerning feral populations of alfalfa and control of feral populations is in the petition's Section E.4 (p. 287), F.3 (p. 293) and Appendix 3 (p. 369). Alfalfa is frequently used in different crop rotations, varying with the region. The use of glyphosate tolerant alfalfa is not expected to change current crop rotation options or patterns. More detailed information on crop rotations is addressed in Section F.1 (p. 291) and Section F.4 (p. 302) of the petition. Less than 100% stand termination can result in volunteer alfalfa plants in the following crop. Therefore good stand termination procedures would still be a good method of eliminating volunteer glyphosate tolerant alfalfa plants. More detailed information on stand termination is addressed in the petition's Section B.6 (p. 259) and specifically for glyphosate tolerant alfalfa in Section E.2 (p. 292). If volunteers of glyphosate tolerant alfalfa are in a crop, management practices and recommendations to control these volunteers can be found in its Sections F.3 (p. 293) and F.5 (p. 303). Based on the available information on this subject, APHIS' opinion is that alfalfa is not an important weed in the United States, but care should be taken with other glyphosate tolerant crops that may be chosen to follow glyphosate tolerant alfalfa.

Possible movement of the transgene via pollen from events J101 and J163 to other members of the *Medicago* genus would be species and geographically specific. Movement of the transgene to plants within the *M. sativa* complex can be expected if the plants are located relatively near each other. Based on a search for *Medicago* populations in the United States (http://www.natureserve.org/explorer) 14 matches were found. All of the matches were considered to be non-native species.

APHIS believes that if the glyphosate tolerance trait moves from J101 and J163 to other sexually compatible *Medicago* species in the United States, this will not have a significant impact. Since all *Medicago* species are not native to the Western Hemisphere, there will be no impact on the natural genetic resources of these species from release in the United States. If glyphosate tolerant individuals did arise through interspecific hybridization, the tolerance would not confer any competitive advantage to these plants unless challenged by glyphosate. This would only occur in managed ecosystems where glyphosate is applied for broad spectrum weed control, or in plant varieties developed to exhibit glyphosate tolerance and in which glyphosate is used to control weeds. As with glyphosate tolerant alfalfa volunteers, these individuals, should they arise, could be controlled using other available chemical and/or mechanical means. Undesired crosses, if they developed, could potentially result in the loss of glyphosate as a tool to control them. However, this can be avoided by the use of sound crop management practices.

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Appendix B: APHIS authorizations for field tests of events J101 and J163 alfalfa

1998 Field Trials

98-093-08n

1999 Field Trials

99-047-03n

2000 Field Trials

00-040-10n
00-053-07n
00-053-14n
00-053-17n
00-055-03n
00-063-18n
00-069-04n
00-139-01n
00-139-02n
00-171-02n
00-182-04n
00-207-01n
00-243-06n
00-272-04n

2001 Field Trials

01-009-04n
01-009-05n
01-009-08n
01-010-09n
01-011-03n
01-016-33n
01-017-08n
01-017-09n
01-029-12n
01-053-08n
01-058-09n
01-058-10n
01-080-05n
01-092-07n
01-092-08n
01-107-01n
01-108-09n
01-156-01n
01-159-01n
01-163-01n
01-163-02n
01-164-01n
01-164-02n
01-164-03n
01-164-04n
01-164-05n
01-164-06n
01-164-07n
01-205-04n
01-205-05n
01-205-06n
01-211-06n
01-211-08n
01-211-09n
01-219-02n
01-236-03n
01-243-10n
01-275-02n

2002 Field Trials

02-004-12n
02-004-13n
02-004-14n
02-004-15n
02-004-17n
02-007-08n
02-010-08n
02-010-09n
02-010-10n
02-010-11n
02-011-01n
02-011-02n
02-028-29n
02-028-30n
02-044-10n
02-044-11n
02-044-15n
02-046-16n
02-046-19n
02-046-22n
02-046-24n
02-046-25n
02-046-26n
02-051-11n
02-051-17n
02-051-20n
02-051-21n
02-051-23n
02-051-24n
02-051-26n
02-051-27n
02-052-06n
02-053-04n
02-056-08n
02-056-12n
02-060-08n
02-060-09n
02-077-14n
02-077-22n
02-078-04n
02-084-19n

2002 Field Trials
02-093-09n
02-099-01n
02-105-04n
02-170-02n
02-193-02n
02-205-02n
02-206-01n
02-212-04n
02-212-05n
02-214-09n
02-220-16n
02-247-07n
02-346-12n
02-346-14n
02-346-15n
02-346-16n
02-346-17n
02-346-18n
02-352-01n
02-352-02n

2003 Field Trials
03-021-15n
03-021-17n
03-021-18n
03-021-19n
03-021-21n
03-021-22n
03-021-23n
03-022-03n
03-022-04n
03-034-30n
03-043-09n
03-043-10n
03-052-19n
03-052-21n
03-062-03n
03-062-04n
03-098-02n
03-098-03n
03-098-04n
03-098-06n
03-121-05n
03-121-06n
03-184-03n
03-184-04n
03-184-05n
03-184-06n
03-191-01n
03-191-02n
03-191-03n
03-191-04n
03-191-05n
03-191-06n
03-202-10n
03-218-01n
03-247-01n
03-247-02n
03-247-04n
03-304-03n
03-304-04n
03-304-05n
03-310-02n
03-314-03n
03-318-04n
03-318-05n
03-324-01n

2003 Field Trials
03-325-01n
03-328-02n
03-345-01n
03-345-03n
03-350-01n

2004 Field Trials
04-005-01n
04-007-01n
04-013-02n
04-030-10n
04-030-14n
04-036-02n

Appendix C: Summary table of critical data submitted with petition 04-110-01p for alfalfa events J101 and J163

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Southern blots verifying stability of inheritance of the *epsps* gene over multiple generations	Fig. V-19 p.68
Western blot characterization of EPSPS protein in events J101 and J163	Fig. V-21 p.78, Fig. V-22 p.80, Fig. V-25 p.89, Fig. V-26 p.91
Statistical analysis of genetic segregation pattern of multiple generations of events J101 and J163	Table V-1, p.71
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Relative efficacy on a variety of weed species	Table VII-3 pp.271-272, Table VII-4 p.273, Table VII-5 pp.274-276
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