Monsanto and Forage Genetics International Petition (12-321-01p) for Determination of Non-regulated Status of Event KK179 Alfalfa

OECD Unique Identifier: MON-ØØ179-5

Plant Pest Risk Assessment

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Agency Contact
Cindy Eck
Biotechnology Regulatory Services
4700 River Road
USDA, APHIS
Riverdale, MD 20737
Fax: (301) 734-8669

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A. Introduction

Monsanto Company (referred hereafter as Monsanto) and Forage Genetics International, LLC (hereafter referred to as FGI) have petitioned the Animal and Plant Health Inspection Service (APHIS) of the United States Department Agriculture (USDA) for a determination that the genetically engineered (GE)) reduced lignin alfalfa (*Medicago sativa* L.) event KK179 (hereafter referred to as KK179 alfalfa) is unlikely to pose a plant pest risk and, therefore, should no longer be a regulated article under the APHIS' 7 Code of Federal Regulations (CFR) part 340. This petition was assigned the number 12-321-01p-a1, and is hereafter referenced as Monsanto and FGI 2013. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act (PPA) of 2000[1] (7 U.S.C. 7701 et seq.). This plant pest risk assessment was conducted to determine if KK179 alfalfa is unlikely to pose a plant pest risk.

APHIS regulations in 7 CFR Part 340 regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is no longer subject to the plant pest provisions of the PPA or to the regulatory requirements of 7 CFR part 340 when APHIS determines that it is unlikely to pose a plant pest risk. A GE organism is considered a regulated article under 7 CFR part 340 if the donor organism, recipient organism, or vector, or vector agent used in engineering the organism belongs any genera or taxa designated in 7 CFR § 340.2 and is also considered a plant pest. KK179 was produced by *Agrobacterium tumefaciens*-mediated transformation and includes introduced genetic sequences derived from plant pest organisms listed in 7 CFR § 340.2 (Monsanto and FGI 2013). Monsanto has conducted introductions of KK179 as a regulated article under APHIS-authorized notifications since 2007 (Table A-1, pages 231-235 in Monsanto 2013), in part, to gather information to support that it is unlikely to pose a plant pest risk.

Potential impacts in this Plant Pest Risk Assessment are those that pertain to plant pest risk associated with KK179 alfalfa and its progeny and their use in the absence of confinement. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if KK179 alfalfa is unlikely to pose a plant pest risk. APHIS regulations in 7 § CFR 340.6(c) specify the information needed for consideration in a petition for nonregulated status. APHIS will evaluate information submitted by the applicant related to plant pest risk characteristics, expression of the gene product, new enzymes, or changes to plant metabolism, weediness of the regulated article, impact on the weediness of any other plant with which it can interbreed, changes to agricultural or cultivation practices that may impact diseases and pests of plants, effects of the regulated

[1] Section 403 (14) of the Plant Protection Act (7USC Sec 7702(14)) defines plant pest as:"Plant Pest - The term "plant pest" means any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product: (A) A protozoan. (B) A nonhuman animal. (C) A parasitic plant. (D) A bacterium. (E) A fungus. (F) A virus or viroid. (G) An infectious agent or other pathogen. (H) Any article similar to or allied with any of the articles specified in the preceding subparagraphs."

article on nontarget organisms, indirect plant pest effects on other agricultural products, and transfer of genetic information to organisms with which it cannot interbreed.

APHIS may also consider information relevant to reviews conducted by other agencies that are part of the 'Coordinated Framework for the Regulation of Biotechnology' (51 FR 23302, June 26, 1986). Under the Coordinated Framework, the oversight of biotechnology-derived plants rests with the APHIS, the Food and Drug Administration (FDA), and the Office of Pesticide Programs of the U.S. Environmental Protection Agency (EPA). Depending on its characteristics, certain biotechnology-derived products are subjected to review by one or more of these agencies. The EPA under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), regulates the distribution, sale, use and testing of pesticidal substances produced in plants and microbes, including those pesticides that are produced by an organism through techniques of modern biotechnology. The EPA also sets tolerance limits for residues of pesticides on and in food and animal feed, or establishes an exemption from the requirement for a tolerance, under the Federal Food, Drug and Cosmetic Act (FFDCA). The FDA under the FFDCA is responsible for ensuring the safety and proper labeling of all plant-derived foods and feeds, including those developed through modern biotechnology. KK179 falls within the scope of the 1992 FDA policy statement concerning regulation of products derived from new plant varieties, including those developed through biotechnology (U.S. FDA, 1992). In compliance with this policy, Monsanto and FGI have initiated a consultation with the FDA on KK179, identified under BNF No. 138. A feed/food safety and nutritional assessment summary document was submitted in August 2012.

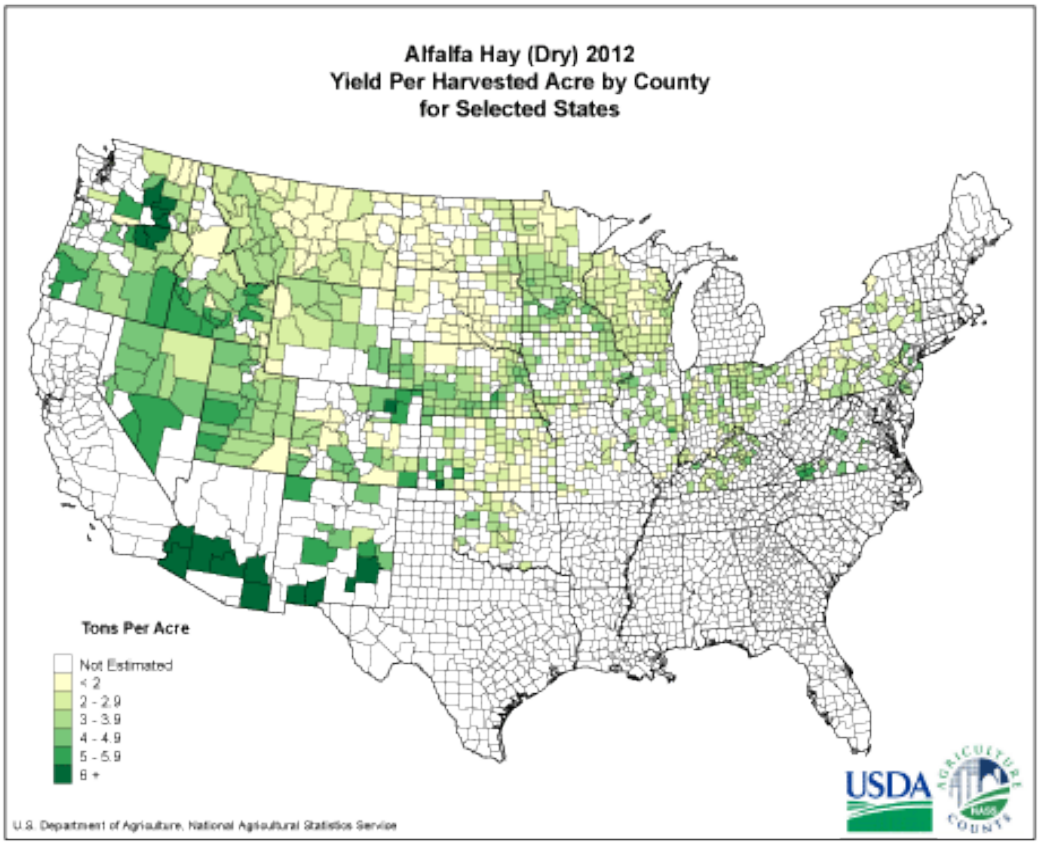
B. Development of KK179 Alfalfa

Alfalfa is widely planted throughout the world as a forage legume for hay, pasture and silage for its highly nutritious forage and broad adaptability (Li and Brummer 2012). In the U.S., alfalfa is the fourth most common crop by both acreage (~24 million acres) and value (~$10 million) with approximately 20 to 24 million acres of alfalfa hay harvested annually in the United States (USDA-NASS 2012). Alfalfa is grown for forage in all 50 states (Figure 1, next page) and Canada. In the year 2012, the price per ton was $187 (USDA-NASS 2012).

Alfalfa originated in southwestern Asia, and is believed to have been first domesticated near present day Caucasus regions and other countries in Asia Minor, and now has a worldwide distribution due to its popularity as an agricultural species (California Alfalfa and Forage Association 2001). It was introduced into the United States in 1736 in Georgia, but it was not until around 1850 that it began to be more widely planted. It is naturalized in many areas and distributed in temperate zones of the world, e.g. U.S., southern Canada, Europe, China, southern Latin America, and South Africa.

Alfalfa is grown as a perennial forage crop and includes field preparation, planting, stand establishment (first year), established stand maintenance (2-8 years), and stand termination (Orloff et al., 1997). If a crop is being harvested for hay, it is harvested multiple times per growing season, from 2-11 times depending on region. Seed crops can be harvested once at the end of each growing season (Putnam et al., 2008).

Figure 1: Alfalfa Hay (Dry) USDA-NASS (2012)



Alfalfa forage products are valued for their high protein content and highly digestible fiber for ruminants and horses (USDA-APHIS FEIS 2010). The principal commercial product is hay, which is forage that has been dried. Lignin deposition in maturing plants has a significant impact on the overall quality of alfalfa (Coors et al., 1986; Marten et al., 1988). Along with cellulose and hemicellulose constituents, lignin is a cell wall component that accumulates in the plant, particularly in the stem. At alfalfa crop maturity, lignin comprises 5-15% of dry matter (Putnam et al., 2008). Improved forage quality is associated with lower lignin levels (Guo et al. 2001) While a certain amount of lignin is essential for healthy alfalfa plants, lignin is indigestible and slows down the digestion of cellulose in the rumen of livestock. Therefore, forage producers and commodity purchasers desire alfalfa with lower lignin levels but without loss of nutritional components, including protein and fiber.

As described above, alfalfa forage may be harvested multiple times in a season. Alfalfa harvested at an immature growth stage (shorter interval between cuttings) gives low yield but high forage quality. If alfalfa is cut at a mature growth stage (long intervals between cuttings) it results in high yield but low forage quality (Orloff and Putnam 2008). Deciding when to harvest forage is a critical decision made by the grower that determines

both forage yield and quality. Growers must obtain a balance between obtaining high yield and high quality because the quality of forage declines rapidly as lignin accumulates in maturing plants. As a result, the interval of time during which forage quality and yield is optimized is relatively narrow and varies depending on which objective, quality or yield, is the priority for the grower (Orloff and Putman 2008). Alfalfa forage production fields remain economically viable for approximately three to five years after initial planting.

Monsanto and FGI have developed biotechnology-derived reduced lignin alfalfa, KK179, which has reduced levels of a specific lignin subunit when compared to conventional alfalfa at the same stage of growth. This leads to reduced overall accumulation of total lignin in alfalfa forage, the principal feed product derived from alfalfa. The levels of lignin in KK179 forage are generally similar to those found in conventional forage harvested several days earlier under similar production conditions. The reduced lignin alfalfa increases forage quality compared to conventional forage of the same age, maximizes forage yield by delaying harvest for several days, and gives farmers more flexibility in forage harvest timing to: 1) maximize forage quality by producing lower lignin levels in the forage; 2) maximize forage yields by allowing farmers to delay harvest for several days accumulating more forage biomass; and 3) allow more flexibility in harvest schedules during the alfalfa growth cycle due to untimely weather, labor availability, and dairy herd management (Monsanto & FGI 2013).

Description of Genetic Modifications

Monsanto and FGI have developed biotechnology-derived low lignin KK179 alfalfa designed to have lower quantities of guaiacyl lignin (commonly referred to as G lignin), one of the major subunits of total lignin, when compared to conventional alfalfa at the same stage of growth. This reduction in G lignin leads to reduced accumulation of total lignin in alfalfa forage.

Lignin provides strength to plants and allows the plant vascular system to transport water in the plant without leakage. Lignin increases with advanced maturity in alfalfa (Undersander 2010). However, lignin is indigestible and reduces fiber digestibility in ruminants. Thus reducing lignin content should increase fiber digestibility at any maturity stage. Lignin molecule fills the spaces between cellulose, hemi-cellulose and pectins as the plant ages and binds with the hemicellulose. Lignin coating the cellulose allows water to move up the plant stem without leakage but also reduces digestion of the cellulose in the rumen of forage animals (Undersander 2010).

KK179 reduces lignin in forage through the suppression of caffeoyl CoA 3-*O*-methyltransferase (CCOMT), a key enzyme in the lignin biosynthetic pathway. KK179 was produced by insertion of *CCOMT* gene segments, derived from alfalfa, assembled to form an inverted repeat DNA sequence. The inverted repeat sequence produces double-stranded RNA (dsRNA) which suppresses endogenous *CCOMT* gene expression via the RNA interference (RNAi) pathway. Suppression of the *CCOMT* gene expression leads to lower CCOMT protein expression resulting in reduced synthesis of G lignin subunit compared to conventional alfalfa at the same stage of growth. The

reduction in G lignin subunit synthesis leads to reduced accumulation of total lignin.

RNA-based suppression of the *CCOMT* gene, leading to the intended reduction of G lignin and total lignin in KK179, is mediated by dsRNA molecules. These dsRNA molecules, which are produced from assembled gene transcripts in KK179 composed of an inverted repeat sequence, suppress endogenous *CCOMT* gene via the naturally operating endogenous RNAi pathway. Double-stranded RNAs are commonly found in eukaryotes, including plants, for endogenous gene suppression and are composed of nucleic acids (Siomi and Siomi, 2009).

The suppression cassette in KK179 functions by reducing the level of G lignin subunits, which are oxidatively coupled to other lignin subunits to form complex lignin molecules (Boerjan et al., 2003). This specific reduction in G lignin is achieved through use of endogenous alfalfa gene segments configured to suppress the *CCOMT* gene in order to lower CCOMT protein expression and thereby decrease the synthesis of G lignin (Figure I-2, Monsanto and FGI 2013). KK179 contains *CCOMT* gene segments under the control of the *Pal2* promoter from the phenylalanine ammonia-lyase gene in bean (*Phaseolus vulgaris*). PAL expression responds to endogenous cues and displays a pattern of expression that corresponds with sites of lignin deposition in maturing plants (Guo et al. 2001; Leyva et al., 1992). Thus, KK179 transgene expression correlates with tissues where higher lignin deposition is observed.

KK179 alfalfa was developed through *Agrobacterium tumefaciens*-mediated transformation with conventional alfalfa, R2336, with the plasmid vector PV-MSPQ12633. The PV-MSPQ12633 plasmid contains two separate transfer DNAs (T-DNAs), each delineated by Left and Right Border sequences (See Table 1, next page).

The first T-DNA, designated T-DNA1, contains the caffeoyl CoA O-methyltransferase (*CCOMT*) suppression cassette, the phenylalanine ammonia-lyase (*Pal2*) promoter and the nopaline synthase (*nos)* 3´ untranslated region (UTR) regulatory elements. T-DNA1 also contains the RNA-based suppression of *CCOMT* in KK179 is mediated by double stranded RNA (dsRNA) molecules transcribed from the suppression cassette, which decrease the level of endogenous *CCOMT* RNA transcripts resulting in reduced levels of G lignin.

The second T-DNA, designated T-DNA II, contains the neomycin phosphotransferarse II (*nptII)* expression cassette under the regulation of the 35S promoter and the *nos* 3´ UTR. During transformation, both T-DNAs were inserted into the alfalfa genome where T-DNA II, containing the *nptII* expression cassette functioned as a marker gene for the in vitro selection of transformed plantlets (Monsanto & FGI, 2013). This second T-DNA, however, was subsequently bred out of the alfalfa line through conventional breeding and is not present in KK179 alfalfa.

Table 1. The number of inserts and a brief description of the nature of the inserts in KK179 alfalfa

T-DNA I Genetic Components

Genetic Element	Function (Reference)
B1-Left Border Region	DNA region from *Agrobacterium tumefaciens* containing the Left Border sequence used for transfer of the T-DNA (Barker et al., 1983)
P2-*Pal2*	Promoter of the *Pal2* gene from *Phaseolus vulgaris* encoding the phenylalanine ammonia-lyase that directs transcription in plant cells (Cramer et al., 1989)
CCOMT*	Partial coding sequence of the *Medicago sativa CCOMT* gene that encodes the caffeoyl CoA 3-*O*-methyltransferase protein (Inoue et al., 1998) that forms part of the suppression cassette
CCOMT*	Partial coding sequence of the *Medicago sativa CCOMT* gene that encodes the caffeoyl CoA 3-*O*-methyltransferase protein (Inoue et al., 1998) that forms part of the suppression cassette
T3-*nos*	3′UTR sequence of the *nopaline synthase* (*nos*) gene from *Agrobacterium tumefaciens* pTi encoding NOS that directs polyadenylation (Bevan, 1984; Fraley et al., 1983)
B-Right Border Region	DNA region from *Agrobacterium tumefaciens* containing the Right Border sequence used for transfer of the T-DNA (Depicker et al., 1982; Zambryski et al., (1982).

Vector Backbone

Genetic Element	Function (Reference)
aadA	Bacterial promoter, coding sequence, and 3′UTR for an aminoglycoside-modifying enzyme, 3"(9)-Onucleotidyltransferase from the transposon Tn7 (Fling et al., 1985) that confers spectinomycin and streptomycin resistance.
OR4-ori-pUC	Origin of replication from plasmid pUC for maintenance of plasmid in E. coli (Vieira and Messing, 1987)
CS5-rop	Coding sequence for repressor of primer protein from the ColE1 plasmid for maintenance of plasmid copy number in E. coli (Giza and Huang, 1989)
OR-oriV	Origin of replication from the broad host range plasmid RK2 for maintenance of plasmid in Agrobacterium (Stalker et al., 1981)

Description of T-DNA II Genetic Components

Genetic Elements	Function
B-Left Border Region	DNA region from Agrobacterium tumefaciens containing the Left Border sequence used for transfer of the T-DNA (Barker et al., 1983)
P-35S	Promoter and leader from the 35S RNA of cauliflower mosaic virus (CaMV) (Odell et al., 1985) that directs transcription in plant cells
CS-nptII	Coding sequence of the neo gene from transposon Tn5 of E. coli encoding neomycin phosphotransferase II (NPT II) (Beck et al., 1982) that confers neomycin and kanamycin resistance (Fraley et al., 1983)
T-nos	3′ UTR sequence of the nopaline synthase (nos) gene from Agrobacterium tumefaciens pTi encoding NOS that directs polyadenylation (Bevan, 1984; Fraley et al., 1983)
B-Right Border Region	DNA region from Agrobacterium tumefaciens containing the Right Border sequence used for transfer of the T-DNA (Depicker et al., 1982; Zambryski et al., 1982)

The transformed plant was crossed with MS 208, an elite, male sterile, conventional alfalfa plant, in which the unlinked insertions for T-DNA I and T-DNA II were segregated to produce KK179 and confirmed by PCR analysis. The segregation of the two markers was used to isolate a subset of transformed plants that contained the CCOMT suppression cassette (T-DNA I) but did not contain the *nptII* expression cassette (T-DNA II). This resulted in the subsequent identification of a single marker-free plant line of KK179 (Monsanto and FGI 2013).

Genetic elements outside the T-DNA border regions are those that are essential for the maintenance or selection of PV-MSPQ12633 in bacteria and are referred to as the plasmid vector backbone. In addition to the above mentioned genetic elements, the inserted T-DNA also contains short non-coding intervening DNA sequences. The intervening sequences contain restriction enzyme recognition sites and are used for cloning purposes.

Monsanto and FGI provided evidence demonstrating that,

- KK179 contains a single copy of T-DNA I at a single insertion site in the alfalfa genome and is devoid of T-DNA II or backbone sequences from PV-MSPQ12633 (Figures IV-2 through IV-8, Monsanto and FGI 2013).

- KK179 genetic elements were confirmed through PCR and DNA sequence analyses of elements within the insert, and determined the 5´ and 3´ insert to plant junctions (Figure IV-9 and Figure IV-10, Monsanto and FGI 2013).

- Segregation analysis of heritability and generational stability of T-DNA I was maintained through four generations of the breeding history (Figure IV-12), and establishes that T-DNA I in KK179 is inherited according to Mendelian principles of inheritance (Figure IV-13 and Table IV-3, Monsanto and FGI 2013).

C. Expression of the Gene Product and Changes to Plant Metabolism

USDA-APHIS assessed whether changes in plant metabolism in KK179 alfalfa is likely to alter their plant pest risk relative to the untransformed control. The assessment encompasses (1) a consideration of the specific effects on plant metabolism or composition of KK179 due to silencing CCOMT expression; and (2) evaluation of whether the nutrient and anti-nutrient levels are comparable to those in the respective non-transformed alfalfa and its effect on plant metabolism and if this could lead to an increase plant pest risk.

Analysis of KK179 DNA segments encoding dsRNA indicate that production of a protein from the dsRNA encoded by the insert in KK179 is highly unlikely. This is supported by evidence that eukaryotic dsRNA molecules are resistant to translation due to the inability of 40s ribosomal subunits to melt double-stranded regions, even ones as short as 18 nucleotides (Kozak, 1989). As a consequence, it is highly unlikely for the dsRNA produced by the transgene in KK179 to yield a translation product. Bioinformatic

analyses of the KK179 DNA insert and flanking sequences provided no evidence for concern regarding safety implications of putative polypeptides. Based on this information, the inserted DNA and resulting dsRNA are unlikely to produce a protein or polypeptide.

The dsRNSs are commonly found in eukaryotes, including plants, and function to suppress endogenous gene expression. RNA is composed of nucleic acids which have a long history of safe consumption and are generally recognized as safe (GRAS) by the U.S. FDA (U.S. FDA 1992). Several biotechnology-derived plant products previously reviewed by the US FDA, and deregulated by USDA-APHIS, were developed using RNA-based suppression mechanisms, including improved fatty acid profile soybean MON 87705(09-201-01p), plum pox virus-resistant plum trees (04-264-01p), virus-resistant papaya (96-051-01p), virus-resistant squash (95-352-01p), and delayed-ripening tomatoes(95-324-01p). The hairpin secondary structure of the ds-RNA produced by the *CCOMT* suppression cassette precludes translation initiation and protein synthesis; thus synthesis of the CCOMT protein or a putative polypeptide is highly unlikely. Based on this information, it is concluded that the inserted DNA and resulting dsRNA are safe and unlikely to produce a protein. As a result, the RNA-based suppression technology used in KK179 poses no novel risks from a feed, food or environment perspective.

Compositional Analysis

Compositional analyses were conducted to assess whether the composition and nutrient levels in grain and forage derived from Event KK179 alfalfa were comparable to the negative segregant, which has background genetics very similar to Event KK179 alfalfa, but without the *CCOMT* suppression gene cassette. Fourteen different conventional commercial alfalfa reference varieties were included across the field production sites in six U.S. sites: California (CAPR), Iowa (IARL), Illinois (ILCY), Kansas (KSLA), Texas (TXCL), and Wisconsin (WIDL) to provide data on the natural variability of compositional component analyzed (Appendix E).

Compositional analyses were based on OECD consensus document for alfalfa (OECD, 2005) to compare levels of key nutrients, anti-nutrients and secondary metabolites in KK179 to levels in the conventional control. Forage samples were analyzed for the following nutrients: proximates (ash, fat, moisture, and protein), carbohydrates, acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), minerals (Ca, Cu, Fe, Mg, Mn, P, K, Na, and Zn), and amino acids (essential and non-essential) (Appendix E). Anti-nutrient and secondary metabolites saponins, and canavanine (Section I.B.3, Appendix E). In *p*-coumaric acid, ferulic acid, sinapic acid, total polyphenols, and free phenylalanine were also analyzed to evaluate the potential effect of CCOMT suppression on the lignin biosynthetic pathway and cell wall associated metabolites (Appendix E).

Assessment of the results demonstrated that, with the exception of three compositional constituents (ash, canavanine, and ferulic acid), there were no statisitically significant differences in the constituents statistically compared (Appendix E). In the case of ash and ferulic acid, the relative magnitudes of the differences were under 10%. The mean levels

of all three analytes with observed statistical differences were within the 99% tolerance interval established from the population of conventional commercial reference varieties and within the range of values found in the published literature (Table VI-5, Monsanto & FGI 2013).

For the three constituents where significant differences were detected, an analysis, including the magnitudes of the differences and comparisons of mean values to the 99 % tolerance interval and literature values, indicated that they were not biologically meaningful from a feed/food safety or nutritional perspective. These results support the overall conclusion that, with the exception of the intended change in reduced G lignin and total lignin levels compared to conventional alfalfa at the same forage growth stage (Section I.B.3, Monsanto & FGI 2013) KK179 is compositionally equivalent to conventional alfalfa with regard to levels of nutrients, anti-nutrients, and secondary metabolites.

Although the mean level of total lignin (ADL) in KK179 was not significantly lower as reported in Section I.B.3., it was numerically lower in KK179 forage compared to the conventional control in the combined-site analysis (Table VI-2). The absolute difference in magnitude was 0.32% dw, which is a relative difference of -4.89%. The use of different methods in different laboratories likely contributed to the variability in total lignin (ADL) values between labs. Both methods did confirm a decrease in total lignin in KK179, with one lab reporting a significant decrease of 22.15%, and another lab a nonsignificant decrease of 4.89%.

Overall, a comprehensive evaluation of KK179 alfalfa and the controls showed no biologically meaningful differences for KK179 alfalfa and forage composition for the nutrients or key anti-nutrients, and secondary metabolites. The few differences detected differences were either small in magnitude or the mean component values of KK179 alfalfa and the controls were within the 99% tolerance level. Therefore based on the data presented by Monsanto and FGI on KK179, APHIS therefore concludes that feed derived from KK1179 alfalfa can be considered compositionally and nutritionally equivalent to those derived from conventional alfalfa.

Based on all the above noted considerations, APHIS concludes that KK179 alfalfa poses no more of a plant pest risk from new gene products, changes to plant metabolism or composition than conventional alfalfa varieties.

D. Potential Impacts on Disease and Pest Susceptibilities

USDA-APHIS assessed whether Event KK179 alfalfa is likely to have significantly increased disease and pest susceptibility because of the introduced gene silencing constructs compared to the non-transformed alfalfa. This assessment encompasses a through consideration of the transformation process, introduced genes, and their genetic elements, and their expression products to cause interactions with pests and diseases. Changes assessed include those which would: (1) affect not only the new GE crop, but that would also result in significant introduction or spread of a damaging pest or disease

to other plants; (2) result in the introduction, spread, and/or creation of a new disease; or (3) result in a significant exacerbation of a pest or disease for which APHIS has a control program. Any increase in pest or disease susceptibility is evaluated with respect to the context of currently cultivated varieties, the ability to manage the pest or disease, and the potential impact on agriculture.

Plant Protection and Quarantine (PPQ) is an APHIS program that safeguards agriculture and natural resources from the entry, establishment, and spread of animal and plant pests and noxious weeds into the United States of America; and supports trade and exports of U.S. agricultural products. PPQ responds to many new introductions of plant pests to eradicate, suppress, or contain them through various programs in cooperation with state departments of agriculture and other government agencies. These may be emergency or longer term domestic programs that target a specific pest. A variety of insect, plant disease, mollusk, or weed programs exist (see http://www.aphis.usda.gov/plant_health/plant_pest_info/index.shtml); however none specifically target pests of alfalfa.

APHIS considered the potential for the transformation process to cause or aggravate disease symptoms in Event KK179 alfalfa or other plants or to cause the production of plant pathogens. Wild type *A. tumefaciens* carries a tumor-inducing (Ti) plasmid that can be transferred to broadleaf plants and cause crown gall disease. *Agrobacterium tumefaciens* strain contains a plasmid PV-MSPQ12633 contains a disarmed plasmid that is incapable of inducing tumor formation due to the deletion of the phytohormone genes originally present (Koncz and Shell 1986). *Agrobacterium*-mediated plant transformation has been used widely for decades, and has not been implicated in causing plant disease, and is highly unlikely to pose a plant pest risk.

The major alfalfa diseases of economic importance in the United States are caused by those pathogens that impact the foliar, crown, root, vascular, and seedling health of alfalfa plants. Alfalfa diseases are primarily caused by fungi; however, nematodes, bacteria, viruses, and other microbes also cause economic losses in alfalfa production (Leath et al., 1988). Field tests were visually monitored for incidence of plant disease and pests on KK179 and compared with the conventional alfalfa comparators (an identical breeding path was used for development of KK179 and non- transgenic reference lines). Event KK179 low lignin alfalfa was released in field trials from 2007 through 2012 in 11 states including Wisconsin, Pennsylvania, New York, Texas, Oklahoma, Kansas, Iowa, Illinois, Idaho, California and Washington (133 regulated releases) across a diverse range of environmental conditions representative of where KK179 is expected to be grown (pp. 231-235 Monsanto & FGI 2013). All plots were uniformly treated with pest control measures at each location based on local protocol. The experimental design was a randomized complete block with four replications. The evaluations for plant pests and insects were conducted at nine field sites in the U.S. and one field site in Canada to provide a diverse range of environmental and agronomic conditions representative of commercial alfalfa production areas in North America. Trials were managed according to standard, local agronomic practices for forage production in order to harvest forage at a growth stage of 1-10% bloom. For each growing season, assessments were made within each crop growth cycle and at each harvest. In addition to

phenotypic and agronomic characteristics, observations were also made for plant responses to abiotic stressors, diseases, and arthropod interactions. These studies were conducted over two complete growing seasons from 2010 to 2012. Monsanto and FGE used qualitative and quantitative techniques to observe insect and disease damage in the field: Anthracnose, Bacterial wilt, Black stem, Crown Rot, Downy mildew, *Fusarium* wilt, Leaf spots, *Phytophthora* root rot, *Sclerotinia* crown and stem rot, *Verticillium* wilt. No differences in the range of plant damage responses to disease stressors were observed for 129 comparisons between KK179 and the conventional control (Table G-15, Monsanto & FGI 2013). No differences were observed between KK179 and the conventional control during any observation for damage caused by any of the assessed disease stressors.

Environmental interactions were assessed qualitatively within each growing season over two years and included plant response to abiotic stressors, disease damage and arthropod damage. In the first year, no differences were observed between KK179 and the conventional control for any of the 93 comparisons of plant response to abiotic stressors, the 93 comparisons for plant damage caused by diseases, or the 96 comparisons for plant damage caused by arthropods. In the second year, no differences were observed between KK179 and the conventional control for any of the 129 comparisons of plant response to abiotic stressors, the 129 comparisons for plant damage caused by diseases, or the 129 comparisons for plant damage caused by arthropods.

Environmental interactions were assessed quantitatively within each growing season over two years and included assessments of alfalfa weevil damage and potato leafhopper damage and pest- and beneficial-arthropod abundance. For alfalfa weevil or potato leafhopper damage, no statistically significant differences were detected in combined site analyses for either insect in 2010 and in 2011. For arthropod abundance, four differences out of 69 comparisons were detected at individual sites in the first year and one out of 89 comparisons in the second year. At the sites where statistical differences were observed, the mean abundance values for pests and arthropods from KK179 were within the range of the conventional commercial reference varieties and/or the differences were not consistently detected across collection times or sites. Taken together, these data support the conclusion that compared to conventional alfalfa KK179 is no more susceptible to damage by alfalfa weevil or potato leafhopper and no more likely to promote increased abundance of these species.

The plant pest assessment was based on multiple lines of evidence developed from a detailed characterization of KK179 compared to conventional alfalfa, followed by a risk assessment on detected differences in insects observed (Section VII). Several insect orders were considered and observed in the field trial. Alfalfa weevil *(Hypera postica*) is one of the biggest pests in alfalfa, followed by several species of aphid (*Aphididae*), and army worms (*Spodoptera* spp.). Observations also included the Potato leafhopper (*Empoasca fabae*), grasshoppers and *Lygus* bug. There were no meaningful differences in observations over combined-site qualitative assessments on insect stressor evaluations for KK179 and the conventional control in the 2010 and 2011 year observations.

The environmental interactions evaluation (Section VII.C, Monsanto & FGI 2013) included qualitative assessment of plant response to insects and disease and quantitative of insect pests and abundance. The results of these assessments indicated that the presence of the trait for reduced G lignin and total lignin does not alter plant-insect interactions, nor does it alter disease susceptibility of KK179 compared to conventional alfalfa. The results also indicated that reduction of lignin to levels already present in the environment does not alter plant-insect interactions or disease susceptibility.

In 2010, no differences in the range of responses were observed between KK179 and the conventional control for any of the 93 comparisons of plant response to abiotic stressors, including drought, flood, frost, hail, heat, nutrient deficiency, soil compaction, and wind (Table VII-8 and Table G-8). Additionally, no differences in the range of responses were observed between KK179 and the conventional control for any of the 93 comparisons for plant damage caused by diseases, including Anthracnose, bacterial wilt, black stem, damping-off, downy mildew, Fusarium wilt, leaf spots, root rot, Sclerotinia crown and stem rot, stem nematode, and Verticillium wilt (Table VII-8 and Table G-9). Finally, no differences in the range of responses were observed between KK179 and the conventional control for any of the 96 comparisons for plant damage caused by arthropods, including alfalfa caterpillar, alfalfa weevil, aphid, armyworm, blister beetle, cutworm, grasshopper, meadow spittlebug, plant bug, potato leafhopper, spider mite, and thrips (Table VII-8 and Table G-10).

In 2011, no differences in the range of responses were observed between KK179 and the conventional control for any of the 129 comparisons of plant response to abiotic stressors, including drought, frost, hail, heat, heaving, nutrient deficiency, soil compaction, wet soil, wind, and winter injury kill (Table VII-9 and Table G-14). Additionally, no differences in the range of responses were observed between KK179 and the conventional control for any of the 129 comparisons for plant damage caused by diseases, including Anthracnose, bacterial wilt, black stem, crown rot, downy mildew, Fusarium wilt, leaf spots, root rot, Sclerotinia crown and stem rot, and Verticillium wilt (Tables VII-9 and G-15 Monsanto &FGI 2013). Finally, no differences in the range of responses were observed between KK179 and the conventional control for any of the 129 comparisons for plant damage caused by arthropods, including alfalfa caterpillar, alfalfa leafminer, alfalfa weevil, aphid, armyworm, bean leaf beetle, blister beetle, cutworm, grasshoppers, green cloverworm, Japanese beetle, Lygus bug, meadow spittlebug, plant bug, potato leafhopper, southern corn rootworm beetle, spider mite, and thrips (Table VII-9 and Table G-16, Monsanto & FGI 2013).

The lack of differences observed between KK179 and the conventional control for plant responses to abiotic stressors, disease damage, and arthropod-related damage in multiple environments across the U.S. and Canada supports the conclusion that the introduction of the trait for reduced G lignin and total lignin is not expected to cause a biologically meaningful change in terms of plant pest/weed potential or to have an effect on the environment for KK179 compared to the conventional control (see Section VII.B.2.). Based on all the above noted considerations, APHIS concludes that KK179 poses no more of a plant pest risk from changes in susceptibility to disease or pests.

E. Potential Impacts on Nontarget Organisms Beneficial to Agriculture

APHIS evaluated the potential for Event KK179 alfalfa to have damaging or toxic effects directly or indirectly on non-target organisms. The non-target organisms considered were representatives of the exposed species in the agricultural environment.

Alfalfa is predominantly cross-pollinated and the flowers depend entirely on bees for cross-pollination. Wind cross-pollination in alfalfa does not occur (Viands et al., 1988). There are two types of pollen mediated gene flow in alfalfa that may involve pollinators: 1) hay to hay field gene flow and, 2) hay to seed field gene flow for alfalfa seed production.

In alfalfa hay-to-hay field gene flow forage producers cut hay at regular intervals prior to or near early bloom for optimum forage quality and yield. Forage quality of the crop deteriorates rapidly after this stage of crop maturity. Since flowering is not advantageous to the nutritional quality of alfalfa, the crop is usually cut before flowering. Pollinators are not usually found in the field before flowering.

For hay to seed alfalfa of the growers in the eleven (11) western U.S. states that are eligible to grow alfalfa for seeds, only those in the Desert Southwest States (CA, AZ, etc.) rely predominately on commercially managed honey bees as pollinators. In other geographies where wild or commercial honey bees may be present (e.g. the Dakotas) alfalfa seed increase will either not be licensed and/or seed producers stock non-*Apis* bee species. There are approximately 44,000 acres of alfalfa seed in the southwestern United States, of which approximately 30,000 acres (Van Deynze et al., 2008; McCaslin, 2007) is produced for export—this is a market sector that is sensitive to biotechnology-derived traits and unlikely to adopt GE varieties at this time (McCaslin, 2007; Van Deynze et al., 2008).

As stated above, although bees serve no direct agronomic (pollination) purpose in hay production fields, some alfalfa hay growers opt to allow their late-summer hay crop to flower extensively, precluding the harvest of maximum hay quality or yield. Working with honey bee keepers, these non-irrigated hay growers may allow their hay crop to flower and be used as an intentional source of honey bee forage instead of cutting for other livestock feeding purposes. In this situation, the bees are not "pollinators" used to produce the alfalfa crop *per se*; rather they are placed there as "foragers" to produce a honey crop and bee progeny (not seed). While it is theoretically possible for exposure of honey-producing bees to alfalfa flowers found in fields where harvesting was delayed due to weather or on unmanaged feral plants to occur intermittently in some geographies, because of factors of scale, hay harvest routines and the seed purchase license restrictions, the extent and duration of any unintentional exposure is expected to be minor in comparison to intentional seed crop pollination use (see Van Deynze et al. 2008; Kendrick et al.; 2005) (Monsanto & FGI 2013).

Event KK179 alfalfa is not expected to be toxic or allergenic to plants or animals. The RNAi suppression cassette that precludes translation initiation and protein synthesis, thus

synthesis of the CCOMT protein or a putative polypeptide is highly unlikely. Based on this information, it is concluded that the inserted DNA and resulting ds-RNA are unlikely to produce a protein.

Data submitted by Monsanto and FGI indicated that the presence of the trait for reduced G lignin and total lignin does not alter plant-insect interactions, including beneficial arthropods and pests, between alfalfa KK179 populations comparable to non-transgenic control populations for disease and insect disease observations. If deregulated KK179 would require the same management practices for disease and other pests as for conventional alfalfa.

F. Potential for Enhanced Weediness of KK179 Alfalfa

APHIS assessed whether KK179 alfalfa are likely to become more weedy (i.e. more prevalent, competitive, damaging or difficult-to-control) than the nontransgenic progenitors from which they were derived.

Alfalfa is a widely adapted crop that can be found in all parts of the continental United States, Alaska, and Hawaii, preferring fertile, well-drained soils. Because of its adaptability, it also survives outside of cultivation. Little evidence exists to suggest that alfalfa behaves as a weed, other than as a volunteer in agricultural settings (USDAAPHIS, 2010). Weed control experts from states where alfalfa is cultivated extensively, including Arizona, California, Idaho, Oregon, Pennsylvania, South Dakota, Washington, and Wisconsin, have communicated that they do not consider alfalfa a weed (Rogan and Fitzpatrick, 2004; USDA-APHIS, 2010). Out of 12 weed lists available in the USDA PLANTS Database (USDA-NRCS, 2012), *Medicago sativa* is found in only one weed identification guide by the Southern Weed Science Society (SWSS), however the author of the SWSS entry for alfalfa has clarified that alfalfa is not an invasive weed and does not displace native species but alfalfa does colonize disturbed areas (USDA-APHIS, 2010, Brett Serviss, Docket No. 04-085-1 #480).

Though not considered a weed, alfalfa does exist in a feral state outside of agricultural settings. These plants originated from introduced varieties and can be found in sparse populations throughout the U.S. (USDA-APHIS, 2010). Surveys have confirmed that minor feral populations exist in six major alfalfa-producing states, in areas where alfalfa seed or forage is produced (Rogan and Fitzpatrick, 2004).

Seed aging, weathering or mechanical scarification makes the seed coat permeable to water and allows rapid germination under favorable conditions. Apart from an impervious seed coat, alfalfa has no physiological seed dormancy mechanism to delay germination (Bass et al., 1988). The viability of most alfalfa seed in soil declines over time (Bass et al., 1988). A portion of the residual alfalfa seed can persist in the soil for several years, and if it remains viable may germinate as volunteers (Bass et al., 1988; Mueller, 2008). A decrease in the percent of hard seed was observed in KK179 relative to the conventional control, however, this difference is not considered a characteristic associated with increased weediness or plant pest potential. No other changes in the seed dormancy or germination characteristics were observed in either scarified or non-

scarified seed that would be indicative of increased plant weediness or plant pest potential of KK179 compared to the conventional control as described in Section VII.C.1 (Monsanto & FGI 2013).

The viability of most alfalfa seed in soil declines over time (Bass et al., 1988). A portion of the residual alfalfa seed can persist in the soil for several years, and if it remains viable may germinate as volunteers (Bass et al., 1988; Mueller, 2008). Alfalfa that has germinated and emerged unintentionally in a subsequent crop, also known as volunteer alfalfa, may compete with the succeeding rotational crop. However, problems controlling volunteer alfalfa are not common (Van Deynze et al., 2008). Volunteers, including ones with herbicide-tolerant traits, can be managed with pre-plant or selective post-emergent herbicide applications or by mechanical means as described in Section VIII.G (Monsanto & FGI 2013).

Grasses controlled early have a low lignin content, and residue breakdown and soil root release occurs far more quickly (Victoria Department of Environment and Primary Industries 2011). Low lignin grasses with higher nutritional values would allow a return to taller more competitive varieties that are more competitive with weeds and close canopy over them, lowering the amount of herbicide needed to control weeds (Gressel 2002).

Specific characteristics that are related to weediness, *e.g.*, lodging, and split pods, were used to assess whether there is a potential increase in weediness of KK179 compared to conventional alfalfa. In the combined-site analysis of the phenotypic characteristics assessed in the second year (2011-2012), no statistically significant differences were detected between KK179 and the conventional control for any of the assessed characteristics, including lodging, crop growth stage, forage yield or regrowth after the first four cuttings; lodging, crop growth stage, or forage yield for a fifth cutting; fall plant height, total forage yield; spring vigor, spring stand recovery, or spring stand count (Table VII-6). An additional, combined-year analysis was conducted for the characteristics measured in both growing seasons, which were fall plant height, total forage yield, spring vigor, spring stand recovery, and spring stand count. No statistically significant differences were detected between KK179 and the conventional control for any of the assessed characteristics in the combined-year analysis (Table VII-7 Monsanto & FGI 2013). Lodging was a characteristic used to specifically assess the potential weediness of KK179. No differences were observed between KK179 and the conventional control for lodging within any crop growth cycle assessed over the two years. Based on the assessed phenotypic and agronomic characteristics within each individual year and across years, the results demonstrate that there were no unexpected changes in phenotype indicative of increased weed potential of KK179 compared to the conventional control (See Figure VII-1, Monsanto & FGI 2013).

The results of the assessments of agronomic and phenotypic characteristics of KK179 managed under conditions for both forage and seed production demonstrated that the introduction of the trait for reduced G lignin and total lignin did not meaningfully alter the weed potential of KK179 compared to conventional alfalfa. Furthermore,

the lack of meaningful differences in environmental interactions also support the conclusion that the introduction of the trait is not expected to result in increased weed potential or to have an effect on the environment for KK179 compared to conventional alfalfa. Results of these evaluations show that there are no biologically significant differences between KK179 and the conventional control for traits potentially associated with weediness therefore KK179 is unlikely to be more weedy than their respective parent varieties.

G. Potential Impacts on the Weediness of Any Other Plants with which It Can Interbreed

Alfalfa is self-incompatible and predominately outcrossing plant and is not capable of natural hybridization with any other species found in North America (USDA-APHIS 2010). Alfalfa belongs to the genus *Medicago* and the tribe Trifolieae, which includes genera *Trifolium* (true clovers), *Melilotus* (sweetclover), and *Trigonella* (fenugreek). *Medicago* species do not hybridize (interbreed to form hybrid offspring) with these or other genera. The *M. sativa* complex has been successfully hybridized with 12 other perennial *Medicago* species (McCoy and Bingham, 1988). However, many of these interspecific hybrids have been successful only by using embryo culture of the hybrid in the greenhouse or laboratory (McCoy and Smith, 1986), making them highly unlikely to occur in nature. Moreover, no perennial *Medicago* species occur naturally in the U.S. (Xu et al., 2004). There are no sexually compatible relatives of *Medicaga sativa* in North America, thus, the risk for cross pollination and subsequent transgene introgression is limited to cultivated and feral alfalfa populations growing in North America (USDA-APHIS 2010).

Gene introgression is a process whereby one or more genes successfully incorporate into the genome of a recipient plant. The baseline potential for gene flow and introgression in conventional alfalfa in agricultural and feral settings has been comprehensively reviewed by USDA-APHIS (USDA-APHIS, 2010) and is relevant to the environmental analysis of KK179. Gene flow is a measure of the exchange of genes between populations, and it occurs naturally between alfalfa populations. Review of information previously provided by Monsanto and FGI, information in the (USDA APHIS 2004, Petition 04-110-01p), as well as in the technical report, *The Potential for Gene Flow from Glyphosate-Tolerant Alfalfa (Medicago sativa L.) to Related Species* (appendix I) concludes that alfalfa does not naturally hybridize with any wild relatives in North America (USDA-APHIS 2010).

Gene Flow among Alfalfa Populations

The most commonly occurring alfalfa field interface is hay field-to-hay field; however, pollen-mediated gene flow is highly improbable between adjacent hay fields (Putnam, 2006; Van Deynze et al., 2008). Several factors in forage production limit potential gene flow from and into hay production fields: 1) harvest takes place at vegetative and early flower stages when little to no pollen is produced and few flowers are present; 2) few natural pollinators of the optimal type are present; 3) biomass with flowers is removed on a regular basis which prevents seed setting; and 4) the competition and natural

autotoxicity of the alfalfa prevents new seedlings resulting from rare outcrossing events to successfully grow within established stands (Canevari and Putnam, 2008). Thus, normal forage production practices significantly lower the risk of pollen-mediated gene flow between hay production fields and outside populations (Van Deynze et al., 2008).

It is also improbable that pollen from an adjacent seed field would result in gene flow into a hay field. Normal forage production practices, which include multiple harvests per year of the hay field, coupled with physical isolation distance requirements of certified alfalfa seed production fields keep the potential for gene flow from seed production fields to hay production fields very low (USDA-APHIS, 2010; Van Deynze et al., 2008).

Gene flow to feral alfalfa plants from large-scale seed or hay production fields of conventional alfalfa and biotechnology-derived alfalfa has been shown to occur (Hammon et al., 2006; St. Amand et al., 2000). However, typical conditions and practices for hay and seed production all but preclude the chance of gene flow into hay or seed production fields as previously described (USDA-APHIS 2010)

The data presented in this petition strongly suggest that the trait in KK179 would not confer a selective advantage to a feral alfalfa plant. Monsanto and FGI are not aware of a conceivable mechanism by which the introduced trait for reduced G lignin and total lignin could confer a selective advantage to feral alfalfa plants or be selected for in an unmanaged setting.

Hybridization with Annual Species of **Medicago**

Medicago sativa is very distantly related to the annual members of *Medicago* (Lesins and Lesins 1979). No annual species of this genus is native to North America. One annual species, *M. lupulina* (black medic), however, is found in the U.S. and is considered a weed in lawns and unmanaged areas, as well as in forage seed crops due to its seeds contaminating other small-seeded forage legume seed crops. Crosses between the annual and perennial species do not occur naturally, and even artificial cross-fertilization is unsuccessful (Fridriksson and Bolton, 1963; Sangduen et al., 1983). Significant biological barriers exist between annual and perennial species, which prevent successful unassisted hybridization. Annual species are self-pollinating, while perennial species outcross and require bees to facilitate pollination. Ploidy and karyotype differences between alfalfas and the annual *Medicago* species also prevent successful hybridization. Additional reproductive barriers include both pre- and post-fertilization abnormalities, such as abnormal pollen tube growth (Sangduen et al., 1983) and post-fertilization abortion of ovules (Fridriksson and Bolton, 1963). Successful hybridizations between *M. sativa* and *M. lupulina* were reported once several decades ago, but numerous attempts to repeat the crosses have failed and the ability of the two species to hybridize is disputed (USDAAPHIS, 2010). Expert opinion concludes that no annual species is known to naturally hybridize with *M. sativa* (McCoy and Bingham, 1988; Quiros and Bauchan, 1988). USDA-APHIS has also concluded, due to lack of confirmatory evidence, hybridization between *M. lupulina* and *M. sativa* is very unlikely to occur (USDA-APHIS, 2010). Recent research further supports that *M. lupulina* and *M. sativa* hybridization is unlikely (Chandra et al., 2011; Steele et al., 2010).

Hybridization with the Perennial Species of **Medicago**

The baseline potential for gene flow and introgression in conventional alfalfa in agricultural and feral settings has been comprehensively reviewed by USDA-APHIS (USDA-APHIS, 2010). The lack of differences between the KK179 and conventional control with respect to plant pest potential, described in Section VII.C. (Monsanto & FGI 2013) includes aspects related to gene flow and introgression such as pollen, flower, and seed characteristics.

No perennial *Medicago* species are present naturally in the Americas, Australia, New Zealand, or South Africa (Quiros and Bauchan, 1988; USDA APHIS, 2010) therefore; no risk of interspecific hybridization exists in the United States. This was confirmed by a search of survey databases for *Medicago* populations in the U.S. which only produced matches for *Medicago sativa* itself and for species that are sexually incompatible with *M. sativa* complex members (USDA-APHIS, 2010).

APHIS evaluated the potential for hybridization and gene introgression 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. 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*. 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).

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; (USDA APHIS 2010).

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 the lack of hybrids after 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). 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.

APHIS concludes that the potential of KK179 low lignin trait moving to other sexually compatible *Medicago* species in the United States is essentially non-existent.

H. Potential Changes to Agriculture or Cultivation Practices

The potential of KK179 alfalfa to impact current alfalfa cultivation practices and management are described in Section VIII of Monsanto and FGI 2013. Implementing the first production strategy, the cutting schedules and the timing of harvest remains the same as those used with conventional alfalfa. The second production strategy involves delaying harvest to maximize yield without forfeiting forage quality compared to conventional alfalfa. KK179 can be harvested later and still produce high quality forage that is comparable to earlier harvest timings with conventional alfalfa. A delayed harvest schedule with its longer cutting intervals could potentially lower production costs over the life of the KK179 alfalfa stand.

KK179 is similar to conventional alfalfa in its agronomic, phenotypic, ecological, and compositional characteristics, and has levels of resistance to insects and diseases comparable to conventional alfalfa. KK179 will utilize the same agronomic practices as conventional alfalfa production, including tillage operations, seedbed preparation, pest management, and harvesting procedures. Therefore, no impacts on current cultivation and management practices for alfalfa are expected following the introduction of KK179.

I. Potential Impacts from Transfer of Genetic Information to Organism with which KK179 Alfalfa Cannot Interbreed

APHIS examined the potential for the new genetic material inserted into KK179 alfalfa to be transferred to other organisms without sexual reproduction (horizontal gene transfer) and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants. KK 179 alfalfa does not contain any coding sequence from plant pathogenic organisms.

The horizontal gene transfer between unrelated organisms is one of the most intensively studied fields of science. Horizontal gene transfer and expression of DNA from a plant species to bacteria or animal species is unlikely to occur. A number of points support this conclusion:

- Many genomes (or parts thereof) from bacteria that are closely associated with plants have been sequenced including *Agrobacterium* and *Rhizobium* (Kaneko et al. 2000; Wood et al. 2001; Kaneko et al. 2002). There is no evidence that these organisms contain genes derived from plants. Also, *Agrobacterium tumefaciens* or

Arthrobacter globiformis species are generally common in soil and therefore various *epsps* genes have been available for long periods of time for horizontal transfer from *Agrobacterium tumefaciens* or *Arthrobacter globiformis* to plants or other soil microorganisms and decaying plant material. Therefore the likelihood of any impact or new horizontal gene transfer that is not already capable of taking place in the soil is extremely unlikely.

- No evidence has been identified for any mechanism by which alfalfa genes could be transferred to humans or animals, or any evidence that such gene transfer has occurred for any plant species during evolutionary history, despite animals and humans eating large quantities of plant DNA. In cases where review of sequence data implied that horizontal gene transfer occurred, these events are inferred to occur on an evolutionary time scale on the order of millions of years (Koonin et al. 2001; Brown 2003).

- Transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression. Thus, even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced.

- FDA has evaluated horizontal gene transfer from the use of antibiotic resistance marker genes, and concluded that the likelihood of transfer of antibiotic resistance genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is extremely unlikely (US-FDA 1998).

- APHIS also considered whether horizontal transfer of DNA from KK179 alfalfa to plant viruses was likely to occur and would lead to the creation or selection of a more virulent plant pathogen through recombination with other plant viruses. This issue has been considered before by other science review panels and government regulatory bodies (Keese 2008). Although sequences of the Cauliflower Mosaic Virus are contained within KK179 alfalfa, those sequences are limited to the regulatory elements. Regulatory elements such as promoters and terminators have not been implicated in viral recombination.

- Finally, under natural conditions, no transfer of an intact functional gene has been demonstrated to date (Miki and McHugh, 2004).

Therefore, APHIS concludes that horizontal gene transfer is unlikely to occur and thus poses no plant pest risk.

J. Conclusion

APHIS has prepared the plant pest risk assessment in order to determine if Event KK179 alfalfa is unlikely to pose a pest risk. Based on the information provided by the applicant and the from the inserted genetic material, weedy characteristics, responses to disease, insects or plant pests in the field, effects on non-targets or beneficial organisms in the agro-ecosystem, and horizontal gene transfer, APHIS has concluded that Event KK179 low lignin alfalfa is highly unlikely to pose a plant pest risk.

Based on all the above noted considerations, APHIS concludes that KK179 alfalfa poses no more of a plant pest risk from new gene products, changes to plant metabolism or composition than conventional alfalfa.

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