Appendix U. Character and Quality of Glyphosate-Tolerant Alfalfa Traits

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Character and Quality of Glyphosate-Tolerant Alfalfa Traits

1.0 Introduction

Monsanto Company (Monsanto) and Forage Genetics International (FGI) have developed varieties of Roundup Ready® alfalfa using *Agrobacterium*-mediated transformation to stably incorporate into the alfalfa genome a coding sequence that produces a glyphosate-tolerant (GT) form of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). The production of the CP4 EPSPS protein in plant tissues is the basis of the Roundup Ready®, GT trait in alfalfa varieties. GT alfalfa is tolerant to foliar applications of glyphosate allowing for post-emergent control of most weeds in alfalfa fields. The methodology for plant transformation and subsequent product characterization data are described in detail in Petition Number 04-110-01p (http://www.aphis.usda.gov/brs/ aphisdocs/04_11001p.pdf). GT alfalfa varieties have been commercialized using a combination of the two different *cp4 epsps* insertion events (J101 and J163) combined through a conventional breeding process.

1.1 Weed Potential (Phenotypic Assessment)

Monsanto and FGI have performed a characterization of GT alfalfa populations containing events J101 and J163. Information was developed to assess whether the trait, the transformation process, or ensuing tissue culture process produced alfalfa events that would impact the plant pest characteristics of alfalfa differently than those observed for the control or conventional alfalfa varieties. Phenotypic, agronomic and compositional information also were provided to assess whether other plant characteristics were affected by the transformation as a means to detect if any unintended effects may be involved. For alfalfa, characteristics that may impact plant pest risk include enhanced growth, vigor or stand longevity; changes in susceptibility to plant pests and diseases; increases in seed yield; and changes in seed dormancy. The overall conclusions from the characterization were that there are no biologically meaningful differences between alfalfa populations that contain event J101 or J163 and the nontransformed alfalfa control or alfalfa reference variety populations (USDA EA for petition 04-110-01p, http://www.aphis.usda.gov/brs/ aphisdocs2/04_11001p_com.pdf). Crop compositional data and other phenotypic and agronomic data also led to the conclusion that alfalfa populations containing event J101 or J163 were not different from the nontransformed control or conventional alfalfa populations (USDA EA for petition 04-110-01p, http://www.aphis.usda.gov/brs/ aphisdocs2/04_11001p_com.pdf). The phenotypic evaluation was based on both laboratory experiments and replicated, multi-site field trials conducted over five years (1999-2003) by agronomists and scientists who are familiar with the production and evaluation of alfalfa. In each of these assessments, event J101 and event J163 were compared to an appropriate alfalfa control. Detailed information may be found in section VI of Petition Number 04-110-01p. As a result of this assessment, it was concluded that there were no meaningful changes in the phenotype of J101 and J163 and no increased pest potential (USDA

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EA for petition 04-110-01p, http://www.aphis.usda.gov/brs/ aphisdocs2/04_11001p_com.pdf). Broad data categories evaluated as part of the phenotypic and familiarity assessment are presented in table 1. The vast majority of this information was previously submitted to USDA in Petition Number 04-110-01p. A summary of the characterization data developed to support the food, feed and environmental safety assessment as well as additional relevant information developed after deregulation is provided in the following sections.

Table U-1. Data Categories Evaluated for Assessment of Weed Potential and Familiarity of Glyphosate-Tolerant Alfalfa

Phenotype/agronomic	Comparative information on the growth and development characteristics listed below was collected on test or control alfalfa populations to determine whether the trait or transformation process altered alfalfa's weed potential. Except where noted, data were collected from alfalfa established at four locations over three seasons using a randomized complete block design with four replicates
Seedling emergence	Number of emerged seedlings two to three weeks after establishment. (Faster seedling emergence may result in greater weediness potential)
Seedling vigor	Evaluation of seedling vigor three to four weeks after establishment. (Greater seedling vigor may result in greater weediness potential)
Spring vigor	Evaluation of spring re-growth in the second and third season at the 4-6 inch stage. (Higher spring vigor may result in greater weediness potential)
Spring stand	Determination of the percentage of plants surviving winter at the beginning of the second and third season. (Better spring stand may result in greater weediness potential)
Forage/seed yield	Forage yield taken at approximately 4-6 week intervals throughout the season. Seed yield taken from greenhouse and field grown plants. (Higher forage yield (faster growing plant) and higher seed yield (more and or larger seeds) may result in greater weediness potential)
Crop growth at cutting	For selected cuttings, crop growth stage was determined using the Mean Stage by Count (MSC) method (Kalu and Fick 1981). (Changes in plant growth and structure may result in greater weediness potential)
Regrowth after cutting	Regrowth at 10 to 15 days after each cutting in the second and third season. (Faster regrowth may result in greater weediness potential)
Fall plant height	Fall plant height measurements taken in the second and third seasons as an indicator of fall dormancy. (Changes in fall plant height may result in greater weediness potential)
Fall growth habit	Categorical score of selected plants for fall growth habit as upright, prostrate, or a mixture of both types. (Changes in fall growth habit may result in greater weediness potential)
Biotic and abiotic stressors	Observation of alfalfa response to insects, diseases, weeds and abiotic stressors during growing season. (Greater tolerance to stressors may result in greater weediness potential)
Reproductive characteristics	Assessment of flower morphology, pollen morphology, and pollen viability in greenhouse grown plants. (Changes in reproductive characteristics may result in greater weediness potential)
Stand longevity	Census of plants remaining in the stand taken at the end of each season (three seasons, one location). (Greater stand longevity may result in greater weediness potential)
Allelopathy	Evaluation of allelopathic potential of alfalfa through laboratory analyses of plant root exudates and field observations of rotational crop growth and development. (Higher level of allelopathy may result in greater weediness potential)

Table U-1. Data Categories Evaluated for Assessment of Weed Potential and Familiarity of Glyphosate-Tolerant Alfalfa

Crop composition	Compositional evaluation of forage as a measure of lack of unintended effects (McCann et al. 2006; Steckel and Hayes 2006))
Dormancy/ germination	Germination and dormancy potential measured over multiple temperatures using seed industry standard testing methods (AOSCA 2003). (Faster germination and changes in seed dormancy may result in greater weediness potential)
Gene flow	Determination of amount of gene flow using cp4 epsps as a marker (Fitzpatrick et al. 2003)
Plant biogeography	Census of feral alfalfa populations present in six U.S. alfalfa producing states (Kendrick et al. 2005)
Symbiotic organisms	Measurement of nodule numbers and morphology in greenhouse and field grown plants to assess symbiotic relationship between M. sativa and Sinorhizobium meliloti

2.0 Crop Compositional Assessment

Crop compositional data were reviewed and analyzed by United States Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) as part of the familiarity assessment and to confirm that there were no unintended effects. These data also were provided to the Food and Drug Administration (FDA) to confirm food and feed safety comparable to conventional alfalfa varieties. A brief summary of the information reviewed by USDA and FDA follows. GT Alfalfa varieties were grown at five replicated field sites across the alfalfa-producing regions of the United States during the 2001 field season. Field sites were located in the states of California, Illinois, New York, Washington and Wisconsin. Plots containing GT alfalfa lines were treated with a Roundup® agricultural herbicide at 1.5 lbs a.e./acre/application. Multiple applications were made to each field site (up to 7.5 lb a.e./acre). Forage samples were collected from all plots and analyzed for nutritional components. Compositional analyses of the forage samples included proximates (protein, fat, ash, and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin, amino acids, and minerals (calcium, copper, iron, magnesium, manganese, phosphorous, potassium, sodium, and zinc), as well as carbohydrates by calculation. In all, 35 different components were analyzed to assess the composition of GT alfalfa.

Components measured are consistent with those suggested by the Organization for Economic Cooperation and Development (OECD) consensus document (OECD 2005). To confirm the nutritional equivalence and evaluate whether the trait or transformation process had any unintended effects on alfalfa, the following components were measured: proximates (moisture, protein, fat and ash; carbohydrates), concentration of individual amino acids, ADF, NDF, lignin, and minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc). Components were selected after consideration of the role alfalfa plays as a source of nutrients in the diet and on the basis of what animal nutritionists consider is important from a feeding value perspective. Protein, ADF, NDF, fat, ash and lignin are used to determine the feeding value of alfalfa. Lignin is also usually the major factor limiting digestion of cell walls which in-turn impacts digestibility. High concentrations of lignin may reduce digestibility and can be an anti-quality factor. The feeding value of alfalfa exceeds that of other perennial grasses primarily because alfalfa has higher intake potential associated with faster digestibility. Minerals present in alfalfa can contribute significantly to meeting the mineral requirements of animals. The mineral content of alfalfa is taken into account by livestock nutritionists when formulating diets to meet or exceed National Research Council guidelines for maintenance, growth and lactation. Measurement of individual amino acids was included because CP4 EPSPS is involved with the synthesis of aromatic amino acids, which are important as a source of protein in alfalfa animal feed.

Statistical evaluation of the composition data involved comparison of the forage from the alfalfa test lines to the nontransgenic control. Statistically significant differences were determined at the 5 percent level of significance ($p \leq 0.05$). Compositional data on forage derived from the GT alfalfa plants containing event J101, J163, or the confirmatory synthetic population, J101XJ163, were reviewed and analyzed by USDA APHIS (data found in section VI, subsection H of Petition Number 04-110-01p). It was concluded that forage derived from J101 and J163 was

compositionally equivalent to forage produced by conventional alfalfa (USDA EA for petition 04-110-01p http://www.aphis.usda.gov/brs/aphisdocs2/04_11001p_com.pdf).

Additional compositional analyses conducted after deregulation of GT alfalfa support these same conclusions. McCann et al. (2006) summarized compositional data derived from GT alfalfa harvested in the establishment year and in the third year of the stand. These data are the same as those reported in Petition Number 04-110-01p. Forage analyses of samples collected from the third year included coumestrol, an important phytoestrogen. In this study, GT alfalfa had been treated multiple times with Roundup Agricultural herbicide at 1.5 lbs a.e./acre with cumulative doses of up to 7.5 and 16.5 lbs a.e./acre for the 2001 and 2003 growing seasons, respectively. It was concluded that forage derived from the 2001 and 2003 plots was compositionally equivalent to that derived from the control and conventional alfalfa plots, thus confirming compositional equivalence between GT alfalfa and conventional alfalfa forage. Furthermore, cumulative glyphosate treatments had no effect on the quality of forage harvested from GT alfalfa. Similarly, Steckel and Hayes (2007) concluded that forage quality and yield were unchanged over a three year period where GT alfalfa was treated numerous times at various single application rates (0.75 to 3 lbs a.e./acre) with a cumulative dose of 9 lbs a.e./acre.

3.0 Nutrient/Water Uptake

Agriculture is a major user of ground and surface water in the United States, accounting for over 80 percent of fresh water in the United States and exceeding 90 percent in some western states, which is the major area for irrigated agriculture (USDA-ERS 2007). Where agricultural lands are irrigated, alfalfa is one of the crops contributing to the use of water. As noted in the Environmental Assessment (EA) (USDA EA for petition 04-110-01p http://www.aphis.usda.gov/brs/aphisdocs2/04_11001p_com.pdf) and the petition, there are no phenotypic or compositional changes in GT alfalfa; therefore, it is expected that the impact on water use due to cultivation of GT alfalfa would be no different than the water used in cultivating conventional alfalfa. In the petition, data were not developed to specifically address water uptake.

Compositional data included the measurement of micronutrients in forage and, with the exception of iron, there were no biologically meaningful differences between GT alfalfa and control or conventional alfalfa. See section VI, subsection H of Petition Number 04-110-01p and McCann et al. (2006). According to McCann et al. (2006), the increase levels of iron were not associated with nutrient uptake because they were concluded to be due to environmental contamination. Previous reports on the yellow flash in soybean (Gordon, 2005; Ebelhar et al., 2005) suggest that application of glyphosate to GT soybean may effect manganese uptake or metabolism in soils with low levels of manganese, that are on bottomlands, are sandy, or that have high pH levels (pH 6.5 or greater). GT alfalfa varieties were not specifically tested under these conditions; however, levels of manganese were measured in forage and were not significantly different from the control. Since soybean and alfalfa are both legumes, an assumption can be made that yellow flash may occur in alfalfa under special circumstances; but as with soybean, this condition can be expected to not be considered serious with alfalfa.

Alfalfa is a plant that provides nitrogen to the soil due to a *Rhizobium*-legume symbiotic relationship, and high rates of nitrogen fixation are typically observed in alfalfa (Vance et al., 1988). Information reviewed and analyzed by USDA APHIS found in Petition Number 04-110-01p (section VI, subsection I) showed that there were no differences in the number of nodules or gross morphology of nodules associated with GT alfalfa compared to control alfalfa. Furthermore, when glyphosate agricultural herbicides were applied to GT alfalfa, no differences in nitrogen fixation indicators including total protein levels, amino acids asparagine and aspartate, and forage yield, were observed in GT alfalfa compared to control. Thus, it is not expected that cultivation of GT alfalfa with subsequent applications of glyphosate will impact nitrogen fixation and subsequent nitrogen credits. In soybean, a temporary decrease in the level of *Rhizobia* has been noted after glyphosate application (King et al., 2001, USDA EA GT soybean). Since soybean and alfalfa are both legumes, an assumption can be made that a temporary decrease in *Rhizobia* may occur in alfalfa under special circumstances, but as with soybean, this condition can be expected to not be considered serious with alfalfa.

3.1 Disease and Pest Susceptibility

During confined release field trials and after commercialization of GT alfalfa in 2005, GT alfalfa was grown and tested at a broad geographic distribution of sites in the United States exposing the

varieties to a wide range of naturally occurring diseases and disease complexes. The major diseases of economic importance in the United States are those alfalfa pathogens that impact the foliar, crown, root, vascular and seedling health of alfalfa plants. The majority of alfalfa diseases are caused by fungi. However, nematodes, bacteria, viruses and other microbes also incite economic losses in alfalfa production (Leath et al., 1988). The major economic diseases that occurred in the test locations included, but were not limited to: seedling damping-off (e.g., fungal genera such as *Pythium, Phytophthora, Aphanomyces*); foliar diseases (e.g., fungal genera such as *Leptosphaerulina, Colletotrichum, Peronospora, Phoma, Stemphylium, Cercospora*, and stem nematodes like *Ditylenchus*); and root rots, vascular wilts and crown diseases (e.g., fungal genera such as *Phytophthora, Verticillium, Fusarium, Phoma*, and bacterial wilt caused by *Clavibacter*).

The major insect pest species that are economically important in alfalfa vary widely among regions in the United States. The broad geographic distribution of the GT alfalfa test sites in the United States and even broader exposure since deregulation in 2005 has exposed GT alfalfa to a wide range of naturally occurring insect pests. The major economic insects included, but were not limited to: potato leafhoppers (*Empoasca fabae*), aphids [pea (*Acyrthosiphon pisum*), blue (*A. kondoi*) and spotted alfalfa aphids (*Therioaphis maculata*)], alfalfa weevil (*Hypera postica*), lygus bugs (*Lygus* species), other plant bug species (family *Miridae*) and alfalfa caterpillars (various Lepidopteran species). The results of the disease and pest susceptibility observations were provided in the final reports submitted to USDA APHIS at the conclusion of the notification period for each field trial. The results from these observations consistently showed no meaningful differences in the disease and insect susceptibility between events J101 and J163 or synthetic populations developed using both events and the conventional control lines or commercial reference varieties. While occasional differences were noted at some field sites, there were no concurrent trends of differences across field sites or years, which indicate that these differences were likely due to random variation. Additional disease ratings taken as part of the phenotypic comparative studies (section VI, subsection D, Petition Number 04-110-01p) support these same conclusions that diseases and pest incidence are unchanged in GT alfalfa compared to the control and that GT alfalfa is not more or less susceptible to pests or diseases.

Commercial experience and additional research conducted since the 2005 deregulation decision further supports previous observations made during the regulated period before 2005. Rhodes (2007) showed that treatment of GT alfalfa with Roundup had no impact on its susceptibility to foliar diseases such as downey mildew (*Peronospora destructor*) when compared to the untreated GT alfalfa control. Monsanto, as part of its product stewardship efforts on commercial products, assesses and addresses product performance issues reported by growers. No reports of changes in susceptibility to diseases or pests have been noted for GT alfalfa since it was introduced.

Given the limited experience with GT alfalfa compared to other GT crops, it is worthwhile considering disease and pest susceptibility information reports from other commercialized GT crops. Several reports suggest that currently available GT crops may be more susceptible to infestation by soil-borne plant pathogens than untreated conventional soybeans (Kremer et al. 2000; Termorshuizen and Lotz 2002). While these preliminary studies show that some changes in population levels of pathogens in soil can occur following glyphosate treatment, there is no evidence of increased incidence of disease in the crop. Other reports in the literature indicate

that stress imposed by herbicide application or other common factors experienced in the field can indirectly lead to increased disease in crops (Kawate et al., 1997; Altman and Rovira, 1989; Giesler et al., 2002; Dissanayake et al., 1998; Johal and Huber, 2009). Potential differences in the incidence and severity of plant diseases in glyphosate-treated and untreated GT soybeans compared to untreated varieties have also been investigated (Sanogo et al., 2000; Sanogo, 2001; Lee et al., 2000). Results indicate that the disease susceptibility of GT soybeans is no different than that of conventional varieties following application of selected herbicides (Lee et al., 2000; Sanogo et al., 2000).

On the basis of pest and disease susceptibility data reviewed by USDA APHIS, GT alfalfa populations were not different from control or conventional alfalfa populations in the prevalence of disease or pests or in their response to pests or diseases (USDA APHIS, 2009). After the deregulation of GT alfalfa in 2005, 200,000 to 300,000 acres of GT alfalfa were established throughout the USA (USDA APHIS, 2007). No reports were received or could be found that indicated a change in disease and pest interactions in GT alfalfa compared to conventional alfalfa.

3.2 Yield and Quality

Forage yield and quality is not significantly different between conventional and GT alfalfa systems (Sheaffer et al., 2007; UC Davis, 2009). In Sheaffer's analysis, the forage quality and yield of GT alfalfa established with glyphosate and conventional alfalfa established with conventional herbicide (imazamox) was compared (2007). Yield and forage quality in the seeding year and in the year following seeding was comparable between the two types. Public data available from University of California (UC) Davis, and supported by additional data provided by the North American Alfalfa Improvement Conference, shows no significant difference in yield between conventional and GT alfalfa when grown using conventional weed management options (non-glyphosate) (UC Davis 2009; NAAIC 2010). These studies were conducted to analyze the innate yield potential of the GT alfalfa when compared to conventional alfalfa, and clearly showed no genetic yield advantage of the GT alfalfa. For instance, when the yields for multiple varieties of alfalfa were averaged over 2007 to 2009, only three of the top-ten-producing varieties were GT cultivars. In these studies, the exact quality and yield of the hay depended on time of harvest, companion cropping, and the location of the field, which indicates that the exact impact of using GT alfalfa on forage quality and yield is dependent on factors such as those. Under field conditions, however, with weed pressures many growers experience increased yields with GT alfalfa due to better weed control.

4.0 Herbicide Susceptibility

Considerable data were provided in the Petition to demonstrate that GT alfalfa was susceptible to herbicides that may be used to terminate alfalfa stands (See section VII, subsection F of Petition Number 01-110-04p). According to Crop Data Management System's (CDMS) Ag Product Label Service database indicated that 2,4-D, clopyralid, dicamba, glufosinate, glyphosate, and primsulfuron-methyl were labeled for control of alfalfa. Independent research has demonstrated that dicamba, 2,4-D, tank mixtures of dicamba and 2,4-D, and clopyralid were often more effective than glyphosate for terminating alfalfa stands (Endres, 1999; Mayerle, 2002; Manitoba Agriculture and Food, 2002). Additional data presented in the Petition (Addendum 1) demonstrated that early postemergence applications of herbicides used to control weeds in corn (Harness XTRA (acetochlor + atrazine), Degree (acetochlor), and Degree XTRA (acetochlor + atrazine) applied in tank mixtures with broadleaf herbicides Banvel (dicamba), 2,4-D, Marksman (atrazine + dicamba) and Hornet (clopyralid + flumetsulam)) effectively controlled GT alfalfa in a GT corn crop. As expected, J101 and J163 were susceptible to herbicides typically used to control alfalfa.

J101 and J163 are highly tolerant to glyphosate and show no yield loss or loss in forage quality when treated over multiple seasons over and above maximum glyphosate application rates (Pierson and Reyes, 2006). Recommended use rates are provided by Monsanto to growers in Regional Technical Bulletins. In season maximum application of glyphosate is up to 1.5 lbs a.e./acre at a single application, and total per year applications is 4.5 lbs a.e./acre. These application rates were used by Monsanto when conducting residue studies and for event selection purposes. In practice, these maximum rates will rarely be used since the vast majority of weeds are controlled using recommended rates and it would be uneconomical for growers to apply excess herbicide.

5.0 Impact on Agronomic Practices and Changes in Land Use

The impact on agronomic practices found in section VII of Petition 04-110-01p has been reviewed and analyzed by USDA APHIS. More than 20 million acres of alfalfa have been planted annually since 1950. Acreage peaked in the mid 1960's at 28 million acres and in the last five years has been relatively constant at approximately 22 million acres (USDA-NASS, 2006). Based on the adoption of other GT crops, it is expected that GT alfalfa will displace a significant portion of other alfalfa varieties, especially where alfalfa is highly managed (e.g., west and southwestern states). As GT alfalfa is adapted, it is expected that Roundup® herbicide will replace other forms of weed control currently used in alfalfa. It is not expected to be adopted in areas where alfalfa is minimally managed and where inputs are low. These areas include pastures, hay fields, and road sides with mixed stands of perennial grasses and other perennial forage legumes and in these same areas in which herbicides are generally not used to control weeds.

Changes in land and the impact of GT alfalfa on land use patterns found in section VII, and Subsections D and F of Petition Number 04-110-01p has been reviewed and analyzed by USDA APHIS. These sections of the petition are relevant to the potential impact of GT alfalfa on land use patterns because they address the impact on agronomic practices and on rotational crops. The impact of GT alfalfa on the overall amount of land devoted to alfalfa cultivation is expected to be minimal. The decision for use of agricultural or other lands for alfalfa production is largely a market-driven decision and the availability of a new weed control option where other options already exist is not expected to impact land use decisions to any great extent. Due to the broad range of weeds controlled by glyphosate, farmers may choose to plant GT alfalfa on fields with greater weed pressure. Overall land devoted to alfalfa cultivation, however, will be affected largely by the price of alfalfa hay and not by the availability of GT technology.

Information provided in Petition Number 04-110-01p section VII, subsection F.4 evaluated the impact of GT alfalfa on rotational crop practices including rotations with other GT crops. With the exception of cotton, non-glyphosate-based herbicides are readily available for control of GT alfalfa in common alfalfa rotations; therefore, it was concluded that there would be no impact on crop rotation practices. Cotton rotations with alfalfa may be managed using mechanical weed control and good stand termination practices.

There has been some speculation that weed-free stands may result in longer stand life resulting in a change in land use. Extended stand life provides positive economic and environmental benefits because a significant amount of the total production costs over the life of the stand are associated with the establishment year. These costs include those associated with seed bed preparation, seed, fertilizer, herbicides and pesticides (Ward, 2007). After the stand is successfully established, costs diminish, and extending a healthy stand will increase profitability. In addition, increased stand life would result in less tillage of agricultural lands and growth of additional alfalfa harvests. Since alfalfa is a plant that provides nitrogen to the soil due to a *Rhizobium*-legume symbiotic relationship resulting in high fixation rates of nitrogen that is usable by the following crop (Vance et al., 1988), growing alfalfa could possibly reduce the need for nitrogen fertilizers obtained through the utilization of fossil fuels.

The introduction of GT alfalfa may provide growers the opportunity to extend the life of a stand because Roundup® agricultural herbicides do not cause crop injury like some of the other herbicides currently used on alfalfa. Crop injury caused by an herbicide applied to a crop is described on the label. Herbicide labels are available online at www.cdms.net. For instance, the label for Pursuit® (imazathapyr), a commonly used herbicide for control of weeds in alfalfa specifically states that "occasionally, internode shortening and/or temporary yellowing may occur following Pursuit® application" and "if applied to alfalfa or clover under cool conditions (40º F or less), temporary stunting or yellowing of the crop may occur." Similar language is included in the label for Raptor® (imazamox). Eptam® (EPTC: S-ethyl dipropylthiocarbamate) another popular herbicide used in alfalfa also mentions possible crop injury and temporary crop stunting may occur under certain conditions and crop growth stages. Additional information on alfalfa's tolerance to popular herbicides may be found in state weed control guides (Loux et al., 2007). The tolerance of alfalfa to herbicides commonly used for weed control in alfalfa is presented in Monsanto's TUG. The majority of the herbicides are listed as good to fair and only two of the herbicides are listed as excellent. According to the manufacturer's labels for Pursuit® and Raptor®, any crop injury is temporary and plants recover with the assumption that there are no impacts on forage nutrition or quality. Thus, removal of weeds in the final years of the stand without crop injury provides the opportunity to maintain a healthy weed free stand. The decision to terminate an alfalfa stand ultimately depends on stand productivity and planned crop rotation practices. Weed pressure is certainly a factor in the decreased production of high quality alfalfa, but they are not the only factor leading to stand decline. Other factors impacting stand life include pest and disease pressure, abiotic stress (e.g., winter kill), harvest practices, rotational crop plans and natural plant senescence as noted in Petition Number 04-110-01p.

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6.0 Implications to Allergies/Animal and Human Health.

The FDA is the lead U.S. regulatory agency for review of the food and feed safety of biotechnology-improved crops. FGI and Monsanto completed a consultation with the FDA in 2004 (FDA, 2004) on GT alfalfa. The following information relevant to the potential impact on allergies and human and animal health was taken from the FDA submission (appendix U-2) and from other information reviewed by the USDA (Petition Number 04-110-01p, Addendum 1). The safety assessment of the CP4 EPSPS protein produced in GT alfalfa events J101 and J163 included protein characterization, functional and structural comparisons to ubiquitous plant and microbial EPSPS synthetases with a history of safe consumption, in vitro digestibility in simulated gastric and intestinal fluids, no acute oral toxicity in mice, and amino acid comparison to known toxins and allergens. The CP4 EPSPS protein produced by J101 and J163 was isolated from forage and characterized using analytical methods capable of assessing the chemical and functional characteristics of the protein. As a result of the characterization it was concluded that the CP4 EPSPS protein produced by J101 and J163 was biochemically and functionally equivalent to CP4 EPSPSs produced by other GT crops and to the family of EPSPS proteins that naturally occur in crops and microbiologically based processing agents that have a long history of safe consumption by humans and animals. All of these data and information taken together demonstrate a history of safe experience with respect to GT crops which have been consumed in significant amounts, either directly or as processed products, by humans and animals since their initial commercialization in 1996.

Food uses of alfalfa are extremely minor. The majority of limited food uses include consumption of sprouts in many countries throughout the world. Outbreaks of food-borne illness associated with sprouts containing the microbial pathogens *Salmonella* spp, *Escherichia coli* O157:H7 or *Listeria monocytogenes* have been reported. These microbial toxins are associated with alfalfa seed used for sprouting purposes (FDA, 1999; CFIA, 2001) but are not specifically produced by alfalfa. Seed have been identified as the primary source of these microbial contaminants (Puohiniemi et al., 1997; CDC, 1997; Mahon et al., 1997). Therefore, the sprouting industry endorses the use of certified sprouting seed to avoid these outbreaks (International Specialty Supply, 2004).

Documented clinical reactions to alfalfa after ingestion have not been reported. However, respiratory disorders may be exacerbated by alfalfa pollen (Bener et al., 2002; Wilkins et al., 1999). Since alfalfa is not a reported allergenic food and may be a relatively minor contributor to some respiratory allergic diseases, the risk to human health is minimal. The statement that alfalfa is not a reported allergenic food is based on two observations. (1) Alfalfa is not one of the eight foods responsible for the vast majority of food allergic reactions. These foods include peanuts, tree nuts, soy, wheat, milk, eggs, fish, and crustacea (Metcalfe et al., 1996). (2) After searching literature databases such as PubMed using key words such as alfalfa and food allergy there were no reports of documented food allergic reactions to alfalfa.

7.0 Animal Feeding Studies since the 2005 Deregulation Decision

Dairy Cow Feeding Study. Combs and Hartnell (2007) examined the effect of GT alfalfa forage on feed intake, milk composition and milk production in 16 multiparous lactating Holstein dairy cows. GT or conventional control alfalfa hay was incorporated into diets at 40 percent of total dry matter. Milk production, milk composition, feed intake and feed efficiency were not different for dairy cows fed GT versus control alfalfa hay ($p > 0.05$). GT alfalfa hay has also been fed extensively without incidence since deregulation in 2005. These results confirm those derived from previously conducted compositional analyses for GT alfalfa where no differences were observed between GT alfalfa and the control, and further confirm the feed safety of glyphosate and the CP4 EPSPS protein.

The safety assessment conducted on J101 and J163 addressed the food, feed, and environmental safety of GT alfalfa. Studies conducted over several years showed that other than tolerance to glyphosate, GT alfalfa populations are no different from conventional alfalfa and are as safe for the environment as conventional alfalfa. The comparative analyses of the composition of GT alfalfa to its conventional counterparts, the safe use of the CP4 EPSPS protein in other GT crops, the practical experience with GT alfalfa by growers, the ongoing use of GT alfalfa as an animal feed, as well as data developed after the 2005 deregulation decision, confirms the environmental and feed safety of GT alfalfa. After reviewing the Monsanto/FGI submission to FDA and other information, APHIS agrees with the FDA assessment and concludes that no concerns exist for the food and feed safety of GT alfalfa vs. conventional alfalfa.

Appendix U-1. References

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