Monsanto Petition (13-290-01p) for Determination of Non-regulated Status of MON 87411 Corn

OECD Unique Identifier: MON-87411-9

Plant Pest Risk Assessment

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A. Introduction

Monsanto Company (hereafter referred to as Monsanto) has petitioned the Animal and Plant Health Inspection Service (APHIS) of the United States Department Agriculture (USDA) for a determination that the genetically engineered (GE**)** corn rootworm protected and glyphosate tolerant corn event MON 87411 (hereafter referred to as MON 87411 corn) 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 13-290-01p, and is hereafter referenced as Monsanto 2013. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act (PPA) of 2000 (7 U.S.C. 7701 *et seq*.)[1]. This plant pest risk assessment was conducted to determine if MON 87411 corn 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 Part 340 when APHIS determines that it is unlikely to pose a plant pest risk. A GE organism is considered a regulated article under Part 340 if the donor organism, recipient organism, or vector, or vector agent used in engineering the organism belongs to any genera or taxa designated in 7 CFR 340.2 and meets the definition of plant pest, or is an unclassified organism and/or an organism whose classification is unknown, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the Administrator determines is a plant pest or has reason to believe is a plant pest[2]. MON 87411 corn event was produced by the *Agrobacterium*-mediated method of transformation of corn immature embryos from line LH244 (Sidorov and Duncan, 2009) by utilizing plasmid PV-ZMIR10871 (Monsanto, 2013, p. 41), and some of the introduced genetic sequences come from plant pest organisms listed in 7 CFR 340.2. The DNA left and right border sequences were derived from *Agrobacterium tumefaciens.* (Monsanto, 2013). Therefore, MON 87411 corn is considered a regulated article under APHIS regulations at 7 CFR part 340. Monsanto has conducted releases into the environment of MON 87411 corn as a regulated article under APHIS-authorized notifications since 2010 (Monsanto, 2013), in part, to obtain information to support that MON 87411 corn is unlikely to pose a plant pest risk.

Potential impacts in this Plant Pest Risk Assessment (PPRA) are those that pertain to plant pest risk associated with MON 87411 corn and its progeny and their use in the absence of confinement, relative to the unmodified recipient and/or other appropriate

[1] Plant Protection Act in 7 U.S.C. 7702 § 403(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."

[2] Limited exclusions or exemptions apply for certain engineered microorganisms and for interstate movement of some organisms, as in 7 CFR 340.1 and 340.2.(b).

comparators. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if MON 87411 corn 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 assessed information submitted by the applicant about MON 87411 corn related to: plant pest risk characteristics; expression of the gene product, new enzymes, or changes to plant metabolism; disease and pest susceptibilities and indirect plant pest effects on other agricultural products; effects of the regulated article on nontarget organisms; 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; 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, 1986; 57 FR 22984, 1992). Under the Coordinated Framework, the oversight of biotechnology-derived plants rests with 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.

EPA regulates under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq)* 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. 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) (21 U.S.C. Chapter 9). Prior to registration for a new use, or for a new or previously registered pesticide, EPA must determine through testing that the pesticide does not cause unreasonable adverse effects on humans, the environment, and non-target species when used in accordance with label instructions. EPA must also approve the language used on the pesticide label in accordance with 40 CFR part 158. Other applicable EPA regulations include 40 CFR part 152 - Pesticide Registration and Classification Procedures, part 174 - Procedures and Requirements for Plant Incorporated Protectants (PIPs) (EPA-FIFRA-SAP, 2006), and part 172 Experimental Use Permits. EPA has issued an experimental use permit for MON 87411 corn throughout the 2015 season.

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. To help sponsors of foods and feeds derived from genetically engineered crops comply with their obligations, the FDA encourages them to participate in its voluntary early food safety evaluation for new non-pesticidal proteins produced by new plant varieties intended to be used as food (FDA, 2006), and a more comprehensive voluntary consultation process prior to commercial distribution of food or feed (57 FR 22984, 1992). Monsanto initiated a consultation with the FDA (Biotechnology Notification File [BNF] No. 145) on the food and feed safety and compositional assessment of MON 87411 corn on December 5, 2013. Monsanto received a completed consultation letter from

the FDA on October 17, 2014. A copy of the text of this letter responding to BNF 145, as well as a copy of the text of FDA's memorandum summarizing the information in BNF 145, is available via the FDA webpage "Biotechnology Consultations on Food from GE Plant Varieties" at http://www.accessdata.fda.gov/scripts/fdcc/?set=Biocon (US-FDA, 2011).

B. Development of MON 87411 Corn

Corn, or maize (*Zea mays* L.), is one of the most widely produced crops in the world (USDA-FAS, 2013). The United States is currently the largest producer, planting more than 95 million acres (NCGA, 2013; USDA-NASS, 2014), producing close to 40% of all the corn of the world (CRA, 2014). Many factors can affect corn production: weeds reduce its maximum potential by 11%; animal pests contribute 10% losses; and pathogens and viruses cause an additional reduction of 11% (Oerke, 2006). GE corn varieties have been developed to assist growers to better manage weeds and pests. In the past 10 years, close to 75% of the U.S. corn acreage was planted with GE hybrids that are herbicide tolerant, or with corn hybrids expressing proteins from the soil bacterium *Bacillus thuringiensis* (Bt) to protect them from certain pests, or a combination of both traits (USDA-ERS, 2013). Adoption of GE corn in the United States has had an impact of reducing 59 millions of pounds of pesticide active ingredient per year (NCFAP, 2008). For example, an acre of glyphosate-resistant corn is usually sprayed with 1 pound of active ingredient less than an acre of non-GE corn that is managed with a conventional weed control program (Gianessi, 2005), a difference representing a reduction of 10% of the herbicide use in the country (Brookes and Barfoot, 2013).

The first corn event containing a glyphosate tolerance trait produced through the use of biotechnology was granted nonregulated status in 1995 and since that time ten other corn lines containing glyphosate tolerance, fourteen resistant to lepidopteran insects and six to coleopteran insects, and combinations of these traits in a single corn hybrid have also been granted nonregulated status by APHIS-USDA (ISB, 2014). APHIS BRS completed plant pest risk assessments and associated environmental assessments for glyphosate tolerant and insect resistant corn in response to a number of petitions (USDA-APHIS-BRS, 2014b), and APHIS concluded in all of these instances that the corn varieties do not pose a plant pest risk and that impacts from making a determination of nonregulated status would not pose a significant impact to the environment. To date, regulatory authorities in eight countries have approved the environmental (commercial) release of herbicide-tolerant corn that contains non-plant derived EPSPS proteins, and 16 countries insect-resistant corn (James, 2011).

Recently, the widespread use of glyphosate in corn, soybean and cotton cultivation in the United States, and the common practice of using herbicides exclusively for weed control, has led to the emergence of glyphosate-resistant weeds (USDA-ARS, 2013). The repeated and intensive use of the same mechanisms of herbicide action has rapidly selected for tolerant, difficult-to-control weeds and for herbicide-resistant weeds, especially in the absence of the concurrent use of herbicides with different mechanisms of action and/or use of different mechanical or cultural practices for weed control (Vencill and Nichols, 2012). As a result of the common use of glyphosate, at least ten

glyphosate-resistant weeds are reported in corn fields in the United States (Kruger *et al.*, 2009).

Corn growers have reported that on average, half of the insecticide applications are no longer necessary in Bt-expressing corn hybrids in order to control certain arthropod pests, compared with conventional non-Bt corn (Hunt and Buschman, 2007). However, these benefits can be reduced if corn pests become resistant to Bt-expressing and insect-protected GE corn, as it has been the case of certain weeds that have become resistant to glyphosate. The EPA has established requirements to delay the development of resistance of insect pests to GE corn (EPA, 2006), through effective measures that have maintained the effectiveness of GE corn against the European corn borer (Hutchison *et al.*, 2010), and other important corn pests such as the fall armyworm and corn earworm (Shelton, 2012). Unlike the success that GE corn has had controlling lepidopteran pests, the corn rootworm (CRW) complex (*Diabrotica* spp., Coleoptera) is only partially controlled by some GE corn hybrids (EPA, 2013). The Bt toxins Cry3Bb1, Cry34/35 and Cry3A currently expressed in GE corn hybrids provide incomplete control of the corn rootworm, providing the opportunity for the surviving larvae that may become resistant to this technology (EPA, 2013). Additional modes of action to control this pest complex would enhance the effectiveness of the GE crop and delay the evolution of Bt-resistance (Bravo and Soberón, 2008).

Monsanto developed MON 87411 corn to confer protection against corn rootworms and tolerance to the herbicide glyphosate. MON 87411 corn was developed using recombinant DNA techniques, and contains three different genes: DvSnf7 dsRNA and Cry3Bb1, which confer resistance to corn rootworm; and cp4 epsps, which confers tolerance to the herbicide glyphosate.

DvSnf7 confers resistance to corn rootworm by means of RNA interference (RNAi) technology. The RNAi technique can be used to silence genes in susceptible insects following ingestion of double stranded RNA (dsRNAs) from the plant (Baum *et al.*, 2007a; Whyard *et al.*, 2009; Terenius *et al.*, 2011). DvSnf7 was designed to match a genetic sequence in western corn rootworm (WCR, *Diabrotica virgifera virgifera*). The expression of DvSnf7 results in the formation of dsRNA transcript containing 240 base pairs of the WCR Snf7 gene in plant tissues. When the pest ingests plant tissues, DvSnf7 suppresses WCR Snf7 in the pest, which leads to significant effects on growth, development and survival of the insects.

The Cry3Bb1 is derived from *Bacillus thuringiensis* (subsp. *kumamotoensis*) and it controls corn rootworms. The Cry3Bb1 is identical to those present in MON 88017 and MON 863 corn (Monsanto, 2013, p. 35) that were granted non-regulated status by USDA-APHIS in 2006 (USDA-APHIS-BRS, 2014a). The EPA has also approved commercial use of the Cry3Bb1 as expressed in corn and has established an exemption from the requirement of a tolerance for residues of the Cry3Bb1 protein and the genetic material for its production in corn (40 CFR 174-180, 2007; Monsanto, 2013, pp. 91-92).

MON 87411 corn also expresses the cp4 epsps gene derived from *Agrobacterium* sp. The CP4 encodes for the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) protein, which confers tolerance to glyphosate, the active ingredient in Roundup® agricultural herbicides. The enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), catalyzes one of the enzymatic steps of the shikimic acid pathway, and is the target for the broad-spectrum herbicidal mode of action of glyphosate (Kishore *et al.*, 1988; Herrmann and Weaver, 1999). The same cp4 epsps is expressed in MON 88017 corn (USDA-APHIS-BRS, 2014b) and numerous other 'Roundup Ready' crops (corn, cotton, soybean, canola, alfalfa, sugar beet). The U.S. EPA has established an exemption from the requirement of a tolerance for residues of CP4 EPSPS protein and the genetic material necessary for its production in all plants (40 CFR part 174.523, 2007; Monsanto, 2013, p. 92).

C. Description of Inserted Genetic Material, Its Inheritance and Expression, Gene Products, and Changes to Plant Metabolism

To inform the potential hazards resulting from the genetic modification and potential routes of exposure related to the inserted DNA and its expression products, APHIS assessed data and information presented in the petition (Monsanto, 2013) related to the transformation process, the sources of the inserted genetic material and its function in both the donor organism and MON 87411 corn event, including the integrity, stability and mode of inheritance of the inserted genetic material through sexual or asexual reproduction based on the location of the insertion (e.g. nucleus or organelle), and the number of loci inserted.

APHIS also assessed data presented in the petition on whether the genetic modification results in expression of new genes, proteins, or enzymes or changes in plant metabolism or composition in MON 87411 corn relative to the original transformed line LH244. The assessment encompasses a consideration of the expressed dsRNA transcript of *Diabrotica virgifera virgifera Snf7* gene (DvSnf7), a *B. thuringiensis*' Cry3Bb1 protein, and the 5-enolpyruvylshikimate-3-phosphate (EPSPS) protein, and any observed or anticipated effects on plant metabolism, including any relevant changes in levels of metabolites, antinutrients, or nutrients in grain and forage derived from the MON 87411 corn event compared to those in the original transformation corn line LH244.

This information is used later in this risk assessment to inform whether there is any potential for plant pest vectors or sequences to cause disease or greater plant pest risks in MON 87411 corn; or for expression of inserted DNA, new proteins or enzymes, or changes in metabolism to affect plant pest or diseases, nontarget beneficial organisms, weediness, agricultural practices that impact pest or diseases or their management, or plant pest risks through horizontal gene flow.

Description of the genetic modification and inheritance of inserted DNA

MON 87411 corn was developed by using *Agrobacterium*-mediated transformation of immature corn embryos (Sidorov and Duncan, 2009). The disarmed *Agrobacterium tumefaciens* strain ABI, a designated plant pest, was used for the transformation. The

disarmed *A. tumefaciens* carried a binary plasmid vector PV-ZMIR 10871 (Monsanto, 2013, figure III-1, p. 43). The transformed events were selected on media containing glyphosate and carbenicillin in order to eliminate the presence of *Agrobacterium* (Monsanto, 2013, p. 41). The vector has not imparted any plant pest sequences or characteristics to MON 87411. The size of the T-DNA was 16,497 base pairs and contained a single T-DNA delineated by left and right border regions in which there were three expression cassettes: DvSnf7 suppression cassette, *cry3Bb1* expression cassette, and *cp4 epsps* expression cassette. In addition the T-DNA has 3-149 base pairs intervening sequences to facilitate DNA cloning (Monsanto, 2013, table III-1, pp. 47-49).

The DvSnf7 suppression cassette consisted of the following genetic elements (Monsanto, 2013, table III-1, p. 47):

- Promoter from the 35S RNA of cauliflower mosaic virus (CaMV) (Odell *et al.*, 1985) containing the duplicated enhancer region (Kay *et al.*, 1987).

- Intron and flanking exon sequence of the *hsp70* gene from *Zea mays* (corn) encoding the heat shock protein 70 (HSP70) (Rochester *et al.*, 1986) that is involved in regulating gene expression (Brown and Santino, 1997).

- Partial coding sequence of the *Snf7* gene designed to match that from *Diabrotica virgifera virgifera* (Baum *et al.*, 2007a; Baum *et al.*, 2007b) encoding the SNF7 subunit of the ESCRT-III complex (Babst *et al.*, 2002).

- 3′ UTR of the rbcS gene family from *Pisum sativum* (pea) encoding the small subunit of ribulose bisphosphate carboxylase protein (Coruzzi *et al.*, 1984).

The cry3Bb1 expression cassette consisted of the following genetic elements (Monsanto, 2013, table III-1, p. 48):

- Promoter sequence of the pIIG gene encoding the physical impedance induced protein from *Zea mays* (Huang *et al.*, 1998) that directs transcription in plant cells.

- 5' UTR leader sequence from chlorophyll a/b-binding (CAB) protein of *Triticum aestivum* (wheat) (Lamppa *et al.*, 1985).

- Intron and flanking UTR sequence of the *act1* gene from *Oryza sativa* (rice) encoding rice Actin 1 (McElroy *et al.*, 1990).

- Codon-optimized coding sequence from Cry3Bb1 protein of *Bacillus thuringiensis* (English *et al.*, 1997).

- 3' UTR sequence from a heat shock protein, Hsp17, of *Triticum aestivum* (McElwain and Spiker, 1989).

The cp4epsps expression cassette consisted of the following genetic elements (Monsanto, 2013, table III-1, pp. 48-49):

- Promoter, 5′UTR leader and intron sequences of the *OsTubA* gene family from *Oryza sativa* encoding α-tubulin (Jeon *et al.*, 2000).

- Targeting sequence of the *ShkG* gene from *Arabidopsis thaliana* encoding the EPSPS transit peptide region that directs transport of the protein to the chloroplast (Klee *et al.*, 1987; Herrmann, 1995).

- Codon optimized coding sequence of the *aroA* gene from *Agrobacterium* sp. strain CP4 encoding the native CP4 EPSPS (Padgette *et al.*, 1996; Barry *et al.*, 2001).

- 3' UTR sequence of the *OsTubA* gene family from *Oryza sativa* encoding α-tubulin (Jeon *et al.*, 2000), that directs polyadenylation of mRNA.

Data provided from Monsanto and reviewed by APHIS BRS demonstrated that:

- The final product does not contain any of the backbone sequences outside of the T-DNA borders from the transformation vector PV-ZMIR10871.

- Data from Next Generation sequencing and Junction Sequence analyses provided and reviewed by APHIS, demonstrated that a single, intact T-DNA PV-ZMIR10871 (Monsanto, 2013, figure III-1, p. 41, tables IV-4 and IV-5, pp. 65 and 73, appendix B) was inserted into the genome of MON 87411 corn and that none of the sequences from the backbone of plasmid PV-ZMIR10871 was inserted (Monsanto, 2013, tables IV-2 and IV-3, p. 63). The stability of the introduced genes was determined by event-specific and locus specific PCR, DNA sequencing analyses for several generations (Monsanto, 2013, figures IV-9 and IV-10, pp. 75-76). Analysis of phenotypic and genotypic data of MON 87411 corn segregating progeny indicated that the MON 87411 T-DNA resides at a single locus within the maize corn genome and is inherited according to Mendelian ratio (Monsanto, 2013, tables IV-6 and IV-7, p. 80).

- This sequence assessment indicated that the integration site in the MON 87411 genome included a 118 bp deletion of genomic DNA but is otherwise identical to the native sequence (Monsanto, 2013, pp. 55 and 68-72, appendix B). These types of deletions occur during plant transformation process and may be as a result of double-stranded break repair mechanisms in the plant during the *Agrobacterium*-mediated transformation process (Salomon and Puchta, 1998).

Expression of inserted DNA and changes in gene expression, new proteins or metabolism

MON 87411 corn was developed based on the current Bt protein-based CRW control technology by incorporating a new mode-of-action based on RNA-mediated gene suppression (RNAi), that offers increased control of target insect pests and may prolong the durability of existing CRW-controlling Bt technologies (Monsanto, 2013, p. 30).

In MON 87411 corn the assembled gene transcript has an inverted repeat DvSnf7 that produces dsRNA that, via the RNA interference pathway, suppresses endogenous genes. The expression of the suppression cassette results in the formation of a dsRNA transcript containing a 240 base pair fragment of the WCR *Snf7* gene DvSnf7. Upon consumption of MON 87411 by the WCR, DvSnf7 dsRNA is recognized by the pest's RNAi machinery, resulting in the down-regulation of the targeted DvSnf7 gene leading to WCR mortality (Bolognesi *et al.*, 2012). MON 87411 corn plant material collected from field trial sites in 2011 and 2012 were used for DvSnf7 RNA expression in 19 plant tissue types, and the results indicate that the DvSnf7 was expressed at very low levels (Monsanto, 2013, table VI-1, pp. 102-103).

MON 87411 corn expresses CTP2, the transit peptide for chloroplast targeting and CP4 EPSPS protein containing 531 amino acids. This protein is identical to that expressed in MON 88017 corn (USDA-APHIS-BRS, 2014b), and other Roundup Ready crops such as cotton, soybean, canola, alfalfa, and sugar beet. The CP4 EPSPS protein is structurally similar and functionally identical to endogenous plant EPSPS enzymes, but has a much reduced affinity for glyphosate relative to endogenous plant EPSPS (Padgette *et al.*, 1996). In MON 87411, as in other Roundup Ready plants, aromatic amino acids and other metabolites necessary for plant growth and development are produced by the continued action of the CP4 EPSPS enzyme in the presence of glyphosate (Padgette *et al.*, 1996).

MON 87411 corn also expresses the Cry3Bb1 protein consisting of 653 amino acids with a molecular weight of 77 kDa (Monsanto, 2013, table C-4, p. 256),which is present in MON 88017 corn that was granted non-regulated status by USDA-APHIS in 2006 (USDA-APHIS-BRS, 2014b). The amino acid sequence deduced from the Cry3Bb1 expression cassettes of MON 87411 and MON 88017 corn is also 99.8% identical to the deduced amino acid sequence for Cry3Bb1 protein in MON 863 (Monsanto, 2013, p. 35), a corn event that was granted non-regulated status by USDA-APHIS in 2002 (USDA-APHIS-BRS, 2014b). The use of Bt-expressing crops in United States has been widespread and the mode-of-action and specificity of these proteins has been studied and is well understood (Gill *et al.*, 1992; Bravo *et al.*, 2007).

Monsanto performed bioinformatics analyses to assess the potential for allergenicity and toxicity or biological activity of Cry3Bb1 and CP4 EPSPS. The data indicated that neither protein have amino acid sequence similarities with known allergens, gliadins, glutenins and toxins that may have adverse effects on human or animal health (Monsanto, 2013, pp. 93-95).

Monsanto also performed compositional analyses on MON 87411 corn grain and forage, the original transformation line LH244, and 20 different commercial reference hybrids grown at eight representative agricultural sites in 2011/2012 in Argentina. The compositional analyses were done for a total of 78 components (nine in forage and 69 in grain) (Monsanto, 2013, appendix G, pp. 298-309). Of the 78 components assayed, 18 had more than 50% of observations that were below the assay limit of quantitation and were therefore excluded from statistical analysis. Of the 60 remaining components statistically assessed, only 12 components (protein, histidine, tyrosine, oleic acid, neutral

detergent fiber, copper, iron, manganese, zinc, niacin, vitamin B1 in grain, and ash in forage) showed a statistically significant difference between MON 87411 corn and the original transformation line LH244, and the mean difference was less than the natural variation found between the original transformation line LH244 and reference corn hybrid values. Additionally, MON 87411 corn mean component values were within the tolerance intervals of the reference hybrids, the values for corn observed in the literature, and/or the International Life Sciences Institute Crop Composition Database values (ILSI, 2010; Monsanto, 2013, tables VII-1-VII-8, pp. 122-137). These results suggest that MON 87411 corn has a compositional equivalence of grain and forage equivalent to its original transformation line LH244 and to other conventional corn hybrid.

D. Potential Plant Pest and Disease Impacts

APHIS assessed whether potential plant pest or disease impacts are likely to result from the transformation process, from DNA sequences from plant pests, or from any other expression products, new enzymes, proteins or changes in plant metabolism or composition in MON 87411 corn that are known or anticipated to cause disease symptoms, or to affect plant pests or diseases or plant defense responses (as identified from the previous section). APHIS also assessed whether MON 87411 corn is likely to have significantly increased disease and pest susceptibility based on data and observations from field trials and laboratory experiments on specific pest and disease damage or incidence and any agronomic data that might relate to such damage. Impacts or changes are assessed to determine if they would (1) affect MON 87411 corn and/or 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; and/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; and supports trade and exports of U.S. agricultural products. PPQ responds to 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, nematode or weed programs exist (USDA-APHIS-BRS, 2014a), however, none specifically target pests of MON 87411 corn.

Corn itself is not considered a plant pest in the United States. (7 CFR part 340.2, 2009). MON 87411 corn contains a suppression cassette that expresses an inverted repeat sequence of DvSnf7 designed to match the sequence in WCR resulting in the formation of dsRNA transcript, that orally-administrated and uptake in sufficient quantities, suppresses the target mRNA leading to significant effects on insect growth, development and survival. MON 87411 corn also produces Cry3Bb1 protein from *B. thuringiensis*,

providing an extra protection from corn rootworm larvae and a different mode-of-action against this pest. Additionally, MON 87411 corn expresses the cp4 *epsps* gene from *Agrobacterium* sp., conferring tolerance to glyphosate, the active ingredient in Roundup® agricultural herbicides. None of these insertions are considered a plant pest (USDA-NRCS; USDA-APHIS-FRSMP, 2014).

The introduced genes did not significantly alter the observed insect pest infestation as observed in nine agricultural fields over two years encompassing fourteen arthropod pests and the occurrence of 16 corn diseases, resulting on no damage of MON 87411 corn compared with its original transformation line LH244 and 22 corn reference hybrids (Monsanto, 2013, appendix I). There were no significant changes in MON 87411 composition that would render MON 87411 corn more susceptible to pests and diseases over its control or reference corn varieties (Monsanto, 2013). The observed agronomic traits also did not reveal any significant changes that would indirectly indicate that MON 87411 corn is or could be relatively more susceptible to pests and diseases over the original transformation isoline LH244 or reference varieties (Monsanto, 2013, appendix I). Thus MON 87411 corn is unlikely to be more susceptible to plant pathogens and insect pests than conventional corn. For this reason, MON 87411 corn is unlike to differ from conventional corn in its ability to harbor or transmit plant pathogens or pests and cause indirect plant pest effects on other agricultural products.

E. Potential Impacts on Nontarget Organisms Beneficial to Agriculture

MON 87411 corn was engineered for herbicide tolerance and pest resistance. APHIS assessed whether exposure to or consumption of MON 87411 corn and the plant incorporated protectants (PIPs) would have a direct or indirect adverse impact on species beneficial to agriculture**.** APHIS also evaluated the potential for MON 87411 corn to have damaging or toxic effects directly or indirectly on nontarget organisms that are considered representatives of the exposed species in the agricultural environment. The assessment includes an analysis of toxicity and specificity of the PIP and RNAi, and exposure to sensitive nontarget organisms in the agricultural environment of the GE plants.

Double stranded RNAs have been used in an increasing number of applications. RNA-mediated gene suppression has been used in a number of biotechnology-derived food crops that have previously been deregulated by USDA or other regulatory authorities including virus resistant papaya, squash, potato, common bean, and plum, as well as a delayed ripening tomato and a soybean with altered oil composition (USDA-APHIS-BRS, 2014b). As a tool for pest control, these molecules can be applied in a similar manner as pesticides usually are sprayed (EPA-FIFRA-SAP, 2006; Price and Gatehouse, 2008).

In the case of MON 87411 corn, the dsRNA DvSnf7 is expressed by the corn plant and the exposure of nontarget organisms is expected to occur primarily through ingestion of the plant material (EPA, 2013), limiting the number of organisms that are commonly found in corn fields. Monsanto (Monsanto, 2013) stated that because of the sequence-

specific gene silencing, the products of RNAi technology, included MON 87411 corn, will have the potential to selectively target closely related pest species and greatly reduce the likelihood of adverse effects on non-target organisms, including those beneficial to agriculture.

The activity spectrum of DvSnf7 RNA has been shown to be highly specific to corn rootworms (Baum *et al.*, 2007a; Bachman *et al.*, 2013; Monsanto, 2013). Bachman *et al.* used bioassays to test representative insect species having close taxonomic relatedness to corn rootworm. In total 14 representative insect species from 10 Families and 4 Orders (Hemiptera, Hymenoptera, Lepidoptera, and Coleoptera) were tested. In these bioassays activity was found only in the subfamily Galerucinae in the family Chrysomelidae within the order Coleoptera. Specifically, only the western corn rootworm and the southern corn rootworm were affected. The Colorado potato beetle, which is in another subfamily (Chyrsomelinae) of Chrysomelidae and which is known to be sensitive to ingested dsRNA, was not affected by DvSnf7 RNA.

In addition, Monsanto found no effect of DvSnf7 RNA on any of the other nontarget species tested including the following which are often considered beneficial to agriculture: the spotted ladybird beetle, ground beetle, honeybee, insidious flower bug, and earthworm. This, together with the results from the study using the 14 species described above and the sequence specific nature of RNAi support a conclusion that it is unlikely that DvSnf7 RNA will have an effect on nontarget organisms.

In previous registrations, USDA-APHIS has granted nonregulated status to six glyphosate-tolerant corn events (MON 802, GA21, MON 88017, 98140, VCO-Ø 1981-5, and MON 87427, (USDA-APHIS-BRS, 2014b). In each of these petitions, an analysis of the impact to nontarget organism was conducted without identifying a negative effect of the CP4 EPSPS protein (CERA, 2011). Cry3 proteins from *B. thuringiensis* can be very specific affecting only a limited number of species in a few insect Orders (MacIntosh *et al.*, 1990; Bravo *et al.*, 2007). Cry3Bb1 expressed by MON 87411 corn has demonstrated to affect a narrow spectrum of organism (Spencer *et al.*, 2003; Höss *et al.*, 2011). Also, from previous USDA-APHIS and U.S. EPA registrations, reviews were conducted concluding that Cry3Bb1 expressing corn has no impact on nontarget organisms (EPA, 2005; USDA-APHIS-BRS, 2005). Peer-reviewed reports have also established that the Bt protein Cry3Bb1 has not shown negative impacts on nontarget organisms (Bhatti *et al.*, 2005; Bitzer *et al.*, 2005; Flores *et al.*, 2005; Romeis *et al.*, 2006; Ferry *et al.*, 2007; Marvier *et al.*, 2007; Meissle and Romeis., 2009; Rauschen *et al.*, 2009a; Rauschen *et al.*, 2009b; Schmidt *et al.*, 2009; Li and Romeis., 2010; Rauschen *et al.*, 2010; Cheeke *et al.*, 2012; Devos *et al.*, 2012; Burns and Raybould, 2013).

Therefore, based on the above analysis of similar corn events that have been granted non-regulated status by APHIS, the peer-reviewed literature and the information provided in Monsanto 2013, APHIS concludes that exposure to and/or consumption of MON 87411 corn and the expressed PIPs, are unlikely to have any adverse impacts to nontarget organisms beneficial to agriculture.

F. Potential for Enhanced Weediness of MON 87411 Corn

APHIS assessed whether MON 87411 corn is any more likely to become a weed than the non-transgenic comparator corn line LH244 or other corn varieties currently under cultivation. The assessment encompasses a thorough consideration of the basic biology of corn and an evaluation of the unique characteristics of MON 87411 corn. Monsanto has conducted agronomic evaluations in both laboratory experiments and field trials on phenotypic, agronomic, and environmental interactions of MON 87411 corn (Monsanto, 2013), and data indicate that MON 87411 corn performs similarly to the original transformation line LH244 and the tested conventional corn hybrids. APHIS has granted non-regulated status to six corn events that are glyphosate-tolerant (USDA-APHIS-BRS, 2014b), and no adverse effects on the weediness of this crop has been reported.

In the United States., corn is not listed as a weed (Crockett, 1977; Muenscher, 1980), nor is it present on the Federal Noxious Weed List (7 CFR part 360, 1976). Corn is grown throughout the world without any report that it is a serious weed or that it forms persistent feral populations (Gould, 1968). Like many domesticated crops, corn seed from a previous year's crop can overwinter and germinate the following year. Manual or chemical measures are often applied to remove these volunteers, but the plants that are not removed do not typically result in feral populations in following years because corn is incapable of sustained reproduction outside of domestic cultivation and corn is non-invasive in natural habitats (Gould, 1968). Corn possesses few of the characteristics of those plants that are notably successful as weeds (Baker, 1965; Keeler, 1989). Compared to other corn varieties, MON 87411 corn has improved fitness in the presence of glyphosate herbicide and certain insect pests, but there are many available options for the control of MON 87411 corn if unwanted plants might be growing in a field.

Seed dormancy, an important characteristic that is often associated with plants that are considered weeds (Anderson, 1996), was assessed with MON 87411 and five corn hybrids, showing no differences in germination between these five corn lines (Monsanto, 2013). Although dormancy is not associated with modern corn cultivars, corn seed dormancy tests can be used to determine whether MON 87411 corn is agronomically comparable to conventional corn and determine whether MON 87411 corn is more likely to pose a plant pest risk when compared to conventional corn. A set of different phenotypic characteristics, including seed dormancy and germination, and pollen morphology, were conducted under laboratory conditions (Monsanto, 2013). Corn stand count, days to 50% pollen shed and silking, stay green, ear and plant height, dropped ears, stalk and root lodging, final stand count, grain moisture, test weight, and yield were evaluated in nine fields using MON 87411 and its original transformation isoline LH244 and 22 commercial corn hybrids (Monsanto, 2013),. The comparisons indicate that there were no significant differences between MON 87411 and LH244, and the range of responses of these corn lines are between the parameters found in another 22 commercial corn hybrids.

In addition, the difference in susceptibility to 16 corn diseases was observed between MON 87411 corn and its original transformation isoline LH244, without finding

significant differences (Monsanto, 2013). The incidence of 14 arthropods was also measured between MON 87411, LH244 and 22 commercial corn hybrids in nine fields, not finding significant differences between MON 87411 and the original transformation line LH244 (Monsanto, 2013), and these susceptibilities and abundance of beneficial arthropods were within the range observed with 22 commercial hybrids (Monsanto, 2013).

Based on the agronomic field data and literature survey concerning weediness potential of the crop, MON 87411 corn is unlikely to persist as a troublesome weed or to have an impact on current weed management practices. Furthermore, extensive post-harvest monitoring of field trial plots planted with MON 87411 corn under USDA-APHIS notifications or permits (Monsanto, 2013, appendix A, pp. 212-215), did not reveal any differences in survivability or persistence relative to corn varieties. These data suggest that MON 87411 corn is no more likely to become a weed than conventional corn.

G. Potential Impacts on the Weediness of Any Other Plants with which MON 87411 Corn Can Interbreed

Gene flow is a natural biological process with significant evolutionary importance. A number of angiosperm taxa are believed to be derived from hybridization or introgression between closely related taxa (Grant, 1981; Rieseberg and Wendel, 1993; Soltis *et al.*, 1993; Hegde *et al.*, 2006), and even in the existing floras, the occurrence of hybridization or introgression is reported to be widespread (Stace, 1987; Rieseberg and Wendel, 1993; Peterson *et al.*, 2002). It has been a common practice by plant breeders to artificially introgress traits from wild relatives into crop plants to develop new cultivars (Khoury *et al.*, 2013). However, gene flow from crops to wild relatives is also thought of as having a potential to enhance the weediness of wild relatives, as observed in rice, sorghum, sunflower and a few other crops (Ellstrand *et al.*, 1999, table 1). This topic is covered in two sections: 1) the potential for gene flow, hybridization and introgression from MON 87411 corn to sexually compatible relatives, including wild, weedy, feral or cultivated species in the United States. and its territories, and 2) if so, the risk potential with respect to weediness of those taxa based on the phenotypic changes that have been observed in the engineered plants.

APHIS evaluated the potential for gene introgression to occur from MON 87411 corn to sexually compatible wild relatives and considered whether such introgression would result in increased weediness. Introgression is a process whereby gene(s) successfully incorporate into the genome of a recipient plant.

Corn belongs to the grass family Poaceae. The genus *Zea* has five species: *diploperennis* HH, *perennis, luxurians, mays*, and *nicaraguensis* (OGTR, 2008). *Zea mays* is further divided into four subspecies: *huehuetenangensis, mexicana, parviglumis*, and *mays*. The first three subspecies are teosintes. *Zea mays* ssp. *mays* occurs only where corn is cultivated in the United States. Occasionally it is found in abandoned fields or on roadsides. The closest wild relatives of corn are the teosintes (wild *Zea* spp.) (Ellstrand *et al.*, 2007), which are sexually compatible with *Zea mays* (Chavez et al., 2012). All

teosinte members can be crossed with cultivated corn to produce fertile first generation hybrids (Doebley, 1990). Teosintes are normally confined to the tropical and subtropical regions of Mexico, Guatemala, and Nicaragua. A fairly rare, sparsely dispersed feral population of teosinte has been reported in Florida (USDA-NRCS, 2014).

Tripsacum is a genus of grass in the Poaceae family. Although it is difficult, *Tripsacum* can be successfully hand crossed with corn to form hybrids. However these hybrids have a high degree of sterility (Doebley, 1990) and are generally unstable because of differences in chromosome number and lack of pairing between chromosomes (Eubanks, 1997). First generation hybrids are much less fit for survival and dissemination in the wild and typically show reduced reproductive capacity. Furthermore, gene flow from corn to *Tripsacum* is virtually impossible because of several factors including distribution, genetic incompatibility, temporal separation of flowering time, etc. (Galinat, 1988). These distinctions between related species affect the ability of cultivated corn to interbreed with wild relatives. Modern corn is highly domesticated and requires significant human intervention to grow and reproduce. As with all domesticated corn, the likelihood that MON 87411 corn would reproduce and sustain populations outside of cultivation is extremely small.

Corn is predominantly an outcrossing plant species. The rate of self-pollination is 5% (Sleper and Poehlman, 2006). The short viability period of pollen grains limits significant outcrossing. Since MON 87411 corn does not exhibit characteristics that can cause it to be any weedier than other cultivated corn, its potential for gene introgression into teosinte is not expected to be any different from that of other cultivated corn varieties.

Based also on the data presented in the petition, MON 87411 corn does not exhibit characteristics that cause it to be any weedier than other cultivated corn. Furthermore, none of the sexually compatible relatives of corn in the United States are considered to be weeds in the United States. (7 CFR part 360, 1976). Therefore, even in those instances of accidental gene flow between MON 87411 corn and wild relatives, the transgenes of MON 87411 are unlikely to transform corn wild relatives into more weedy species. Moreover, its potential impact due to the extremely limited potential for gene introgression into teosinte is not expected to be any different than that of other cultivated corn varieties. Based on the above considerations, MON 87411 corn is unlikely to adversely impact sexually compatible wild relatives or their weediness characters.

H. Potential Changes to Agriculture or Cultivation Practices

APHIS assessed whether significant changes to agricultural or cultivation practices from adoption of MON 87411 corn are likely to impact plant diseases or pests or their management, including any APHIS control programs. This includes consideration of any changes in pesticide applications, tillage, irrigation, harvesting, etc. as they relate to plant pests and diseases.

Compared to currently-available glyphosate tolerant corn products containing EPSPS, no increased use of herbicides from the use of MON 87411 corn is expected. For the past 10

years herbicide-resistant corn varieties were grown in approximately 21% of all corn acres in the United States. Adoption of corn varieties that are only insect-resistant has had the same level (21%) of implementation by American farmers. The combination of these two traits in a corn variety has gradually increased in the past decade, from 40% in 2003 to 90% in 2013 (USDA-ERS, 2013). As these varieties were adopted, farmers have used glyphosate as an herbicide for their weed-management tactics, and relied in the effectiveness of Bt proteins to protect their corn from certain insect pests. In general, glyphosate is less toxic to humans than other common herbicides and not as likely to persist in the environment as many of the herbicides it replaces (USDA-ERS, 2006), while *Bacillus thuringiensis* is one of the safest insecticides available (Mendelsohn *et al.*, 2003), and specifically Cry3Bb1 has been granted tolerance exception since 2007 by EPA (40 CFR 174-180, 2007). APHIS does not foresee any increased glyphosate or insecticide use by the addition of MON 87411 corn to the market.

However, continuous use of one herbicidal mode-of-action to control weeds may select for weed resistance. In general, weed problems in fields planted with GE glyphosate-resistant crops will become more common as weeds evolve resistance to glyphosate or weeds less susceptible to glyphosate become established in areas treated exclusively with the same herbicide (Dill, 2005; Powles, 2008). A number of new genetically engineered, herbicide-resistant corn varieties are currently under development or have been granted nonregulated status (USDA-APHIS-BRS, 2014b), which may provide growers with other herbicides and weed management options. Growers need to consider other effective weed-management tools or use the alternative herbicides with different modes of actions. Such practices should be encouraged through collaborative efforts by federal and state government agencies, private-sector technology developers, universities, and farmer organizations to develop cost-effective resistant-management programs and practices that preserve effective weed control in herbicide-resistant crops (NRC, 2010).

Insect-resistant GE varieties have not had important instances of evolved resistance in the United States so far (Huang *et al.*, 2011). However, a few isolated populations of WCR had injured WCR-resistant corn (EPA, 2013), but these instances had to do primarily with the fact that some of the WRC-resistant corn hybrids do not express sufficient Bt protein to effectively arrest the damage of corn rootworm larvae (EPA-FIFRA-SAP, 2014).

APHIS could not identify any significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of MON 87411; therefore, no impact on plant diseases or pests or their management is likely to occur.

I. Potential Impacts from Transfer of Genetic Information to Organisms with which MON 87411 Corn Cannot Interbreed

APHIS examined the potential for the new genetic material inserted into MON 87411 corn to be horizontally transferred without sexual reproduction to other organisms and whether such an event could lead directly or indirectly to disease, damage, injury or harm

to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants. The horizontal gene transfer between unrelated organisms is one of the most intensively studied fields in the biosciences since 1940, and the issue gained extra attention with the release of transgenic plants into the environment (Dröge *et al.*, 1998). Potential risks from stable horizontal gene transfer (HGT) from genetically engineered organisms to another organism without reproduction or human intervention were recently reviewed (Keese, 2008). Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake and recombination and rearrangement, most notably through viruses and mobile genetic elements. HGT has been a major contributor to the spread of antibiotic resistance amongst pathogenic bacteria; emergence of increased virulence in bacteria, eukaryotes and viruses; and, in the long run, to major transitions in evolution.

Potential for horizontal gene transfer to bacteria, fungi, or invertebrates

Horizontal gene transfer and expression of DNA from a plant species to bacterial, fungal or invertebrate species is unlikely to occur based on the following observations. Although there are many opportunities for plants to directly interact with fungi and bacteria (e.g. as commensals, symbionts, parasites, pathogens, decomposers, or in the guts of herbivores) and with invertebrates as plant pests, there are almost no evolutionary examples of HGT from eukaryotes to bacteria or from plants to fungi or invertebrates (Keese, 2008). Examples of HGT between eukaryotes and fungi primarily involve gene acquisition or transfer by fungi to or from other distantly related fungi or bacteria (Keeling and Palmer, 2008; Keese, 2008) and HGT between plants and fungi is extremely rare (Richards *et al.*, 2009). Examples of HGT between plants and invertebrates are also extremely rare, and most examples of HGT in insects involve acquisition of genes from their pathogens or endosymbionts (Keese, 2008; Zhu *et al.*, 2011; Acuña *et al.*, 2012).

Horizontal transfer from and expression in bacteria of the foreign DNA inserted into the nuclear genome of the GE plant is unlikely to occur. First, many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Wood *et al.*, 2001; Kaneko *et al.*, 2002). There is no evidence that these organisms contain genes derived from plants. HGT from plants to bacteria is a very low frequency event, primarily because functional and selective barriers to HGT increase with genetic distance (Keese, 2008). Second, 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 (Brown, 2003; EFSA, 2009; Koonin *et al.*, 2011). Third, 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. Fourth, both the FDA (FDA, 1998) and the European Food Safety Authority (EFSA, 2009) have 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 very rare or remote.

Potential for horizontal gene transfer to viruses

APHIS examined the potential for the new genetic material inserted into MON 87411 corn to be horizontally transferred without sexual reproduction to other organisms and whether such an event could lead directly or indirectly to disease damage, injury to plants, including the creation of new or more virulent pests, pathogens or parasitic plants. APHIS also considered whether horizontal transfer of DNA from the GE plant to plant viruses was likely to occur and would lead to the creation or selection of plant viruses that are more virulent or have a broader host range. This issue has been considered before by other science review panels and government regulatory bodies (EPA-FIFRA-SAP, 2006; Keese, 2008). HGT is not unusual among plant viruses; however this is generally limited to exchange between viruses present in the same host organism in mixed infections, and most commonly involves homologous recombination, relying on sequence similarity at the point of crossover (Keese, 2008). HGT from virus sequences engineered into plants has been demonstrated with infecting or challenge viruses, including both DNA viruses (e.g. geminiviruses which replicate in the nucleus) (Frischmuth and Stanley, 1998) and RNA viruses (which typically replicate in the cytoplasm); however most have been under conditions that favor recombination to restore a defective virus (Fuchs and Gonsalves, 2007; Keese, 2008; Thompson and Tepfer, 2010). Populations of recombinants between virus transgenes expressed in transgenic plants infected with related viruses are similar to recombinants found in mixed infections of the same viruses in nontransgenic plants, indicating that there was no novel recombination mechanism in the transgenic plants and no increased risk is expected over what is expected from mixed infections (Keese, 2008; Turturo *et al.*, 2008). Nonhomologous recombination in HGT among viruses or between virus transgenes and infecting viruses can occur, but frequently results in gene deletions which can result in nonviable viruses (Morroni *et al.*, 2013). Depending on the particular virus and sequences involved, various hot-spots for recombination have been found in both coding and noncoding regions, and strategies implemented in design of transgenes to avoid recombination have been suggested. No recombinant or undesirable viruses with new properties have been detected for over at least 8-10 years in field tests or during commercial growth of deregulated virus resistant plum, squash, or papaya engineered with genes from viruses that have been deregulated in the United.States. (Fuchs and Gonsalves, 2007).

Potential for horizontal gene transfer to parasitic plants

Evidence for HGT from plants to other plants is limited to two specific scenarios: (1) exchange of genes between a parasitic plant and its host; and (2) exchange of genes between cells of two plants living in close proximity, such as in a graft junction. In both cases, this type of HGT requires physical contacts between the two plants. Most cases of HGT in plants involve transfer of mitochondrial genomes, which are primarily maternally inherited in plants (Barr *et al.*, 2005), to other mitochondria genomes, and mostly involve parasitic plants and their hosts (Richardson and Palmer, 2007). Through a comparative genomics analysis implicated HGT for the incorporation of a specific genetic sequence in the parasitic plant purple witchweed (*Striga hermonthica*) from its monocot host plant. According to this study, the incorporation of the specific genetic sequence (with an unknown function) occurred between sorghum and purple witchweed. However, this

HGT occurred before speciation of purple witchweed and related cowpea witchweed (*S. gesnerioides*) from their common ancestor. Furthermore, *S. hermonthica* is not found in the United .States. and *S. asiatica*, another related parasite of cereal crops, is only present in North Carolina and South Carolina (USDA-NRCS, 2013). Other studies demonstrated that in a few parasitic species of the Rafflesiaceae family, out of several genetic sequences examined, about 2.1% of nuclear (Xi *et al.*, 2012), and 24 –41% of mitochondrial (Xi *et al.*, 2013) gene transcripts appeared to be acquired from their obligate host species. However, all the above-mentioned instances of HGT between parasitic plants and their hosts were reported to be of ancient origins, on an evolutionary time scale spanning thousands to millions of years ago. Furthermore in MON 87411 corn, the DNA sequences were inserted into the nuclear and chloroplast genomes, not in the mitochondrial genome.

If MON 87411 corn becomes infected by a parasitic plant or is naturally grafted to another plant, there is a very low probability that HGT could result in the other plant acquiring DNA from MON 87411 corn. However, in both scenarios this newly introduced DNA would likely reside in somatic cells, and with little chance of reaching the germ cells, this introduced DNA could not persist in subsequent generations unless the recipient plant reproduced asexually from the affected cells.

Based on the above analysis APHIS therefore concludes that HGT of the new genetic material inserted into MON 87411 corn to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

J. Conclusion

APHIS has reviewed the information submitted in the petition, supporting documents, and other relevant information to assess the plant pest risk of MON 87411 corn compared to the original transformation corn LH244 from which it was derived. APHIS concludes that MON 87411 corn is unlikely to pose a plant pest risk based on the following findings.

- No plant pest risk was identified from the transformation process or the insertion of new genetic material in MON 87411 corn because MON 87411 corn was produced using disarmed *Agrobacterium tumefaciens* and the transformed plant tissues were treated with an antibiotic to devitalize *A. tumefaceins*. Therefore the inserted genetic material which was derived from plant pest does not result in the production of infectious agents or disease symptoms in plants.
- No increase in plant pest risk was identified in MON 87411 corn from expression of the inserted genetic material of new proteins, because MON 87411 corn can be considered compositionally or nutritionally equivalent to those derived from its original transformation corn line LH244.
- Disease and pest incidence and/or damage were not observed to be significantly increased or atypical in MON 87411 corn compared to the original transformation line LH244 or other corn hybrid comparators in field trials conducted in growing

regions representative of where MON 87411 is expected to be grown, as well from laboratory studies. Observed agronomic traits also did not reveal any significant differences that would indirectly indicate that MON 87411 corn is more susceptible to pests or diseases**.** Therefore, no plant pest effects are expected on these or other agricultural products and no impacts are expected to APHIS pest control programs.

- Exposure to and/or consumption of MON 87411 corn, Cry3Bb1 and dsRNA are unlikely to have adverse impacts on organisms beneficial to agriculture based on the analysis of the peer-reviewed studies and information provided in the petition.
- MON 87411 corn is no more likely to become a weed than conventional corn varieties based on its observed agronomic characteristics, corn weediness potential and current management practices available to control MON 87411 as a volunteer. Volunteers and feral populations of MON 87411 corn can be managed using a variety of currently available methods and alternative herbicides.
- MON 87411 corn is not expected to increase the weed risk potential of other species with which it can interbreed in the United .States. or its territories. Gene flow, hybridization and/or introgression of inserted genes from MON 87411 corn to other sexually compatible relatives with which it can interbreed is not likely to occur. These compatible relatives (teosinte and *Tripsacum* sp.) are not considered weedy or invasive. The new phenotypes conferred by genetic engineering are not likely to increase the weediness of these compatible relatives or affect the current ability to control them in situations where they are considered weedy or invasive.
- Significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of MON 87411 corn are not likely to increase plant diseases or pests or compromise their management.
- Horizontal gene transfer of the new genetic material inserted into MON 87411 corn to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

K. References

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