Monsanto Petition (15-113-01p) for Determination of Nonregulated Status of Dicamba and Glufosinate Herbicide-Resistant MON 87419 Maize

OECD Unique Identifier: MON-87419-8

Preliminary Plant Pest Risk Assessment

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TABLE OF CONTENTS **PAGE**

A. Introduction

Monsanto Company (hereafter referred to as Monsanto) has petitioned the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) for a determination that genetically engineered (GE) dicamba and glufosinate herbicide-resistant[1] maize event MON 87419 and OECD Unique Identifier MON-87419-8 (hereafter referred to as MON 87419 maize) is unlikely to pose a plant pest risk and, therefore, should no longer be a regulated article under APHIS' 7 Code of Federal Regulations (CFR) part 340 (Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There Is Reason To Believe Are Plant Pests) (7 CFR part 340). This petition was assigned the number 15-113-01p and is hereafter referenced as Monsanto 2015 (Monsanto 2015a). APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act (PPA) of 2000 (7 USC 7701 *et seq.*)[2]. This plant pest risk assessment (PPRA) was conducted to determine if MON 87419 maize is unlikely to pose a plant pest risk.

APHIS regulations in 7 CFR part 340 regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is no longer subject to the plant pest provisions of the PPA or to the regulatory requirements of 7 CFR part 340 when APHIS determines that it is unlikely to pose a plant pest risk. A GE organism is considered a regulated article under 7 CFR part 340 if the donor organism, recipient organism, or vector, or vector agent used in engineering the organism belongs 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[3]. MON 87419 maize was produced by *Agrobacterium tumefaciens*-mediated transformation of immature embryos from inbred line LH244 using plasmid PV-ZMHT507801 (pp. 33-34,

[1] Monsanto has described the phenotype of MON 87419 maize as "herbicide tolerant" and historically APHIS has also referred to GE plants with reduced herbicide sensitivity as herbicide tolerant. However, the phenotype would fall under the Weed Science Society of America (WSSA) definition of "herbicide resistance" since MON 87419 maize has an "inherited ability to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type". By the WSSA (1998) definition, "resistance (to an herbicide) may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis." Herbicide tolerance, by the WSSA definition, only applies to plant species with an "inherent ability to survive and reproduce after herbicide treatment. This implies that there was no selection or genetic manipulation to make the plant tolerant; it is naturally tolerant."

[2] Plant Protection Act in 7 USC 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."

[3] 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).

Monsanto 2015a). Portions of the inserted genetic material were derived from plant pest organisms listed in 7 CFR 340.2 (i.e., coding sequence from *Streptomyces viridochromogenes*, promoter sequence from peanut chlorotic streak caulimovirus and T-DNA border sequences from *Agrobacterium tumefaciens*) (Table IV-1, pp. 49-50, Monsanto 2015a). Therefore, MON 87419 maize is considered a regulated article under APHIS regulations at 7 CFR part 340. Monsanto has conducted field releases of MON 87419 maize as a regulated article under APHIS authorizations since 2011 (Appendix A, pp. 189-191, Monsanto 2015a), in part, to gather information to support that MON 87419 maize is unlikely to pose a plant pest risk.

Potential impacts in this plant pest risk assessment are those that pertain to plant pest risk associated with MON 87419 maize and its progeny and their use in the absence of confinement relative to the unmodified recipient line and/or other appropriate comparators. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if MON 87419 maize is unlikely to pose a plant pest risk. APHIS regulations in 7 CFR 340.6(c) specify the information needed for consideration in a petition for nonregulated status. APHIS will assess information submitted by the applicant about MON 87419 maize 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 Office of Pesticide Programs of the U.S. Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA). Depending on its characteristics, certain biotechnology-derived products are subjected to review by one or more of these agencies.

Under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 USC 136 et seq.), EPA regulates the distribution, sale, use and testing of pesticidal substances produced in plants and microbes, including those pesticides 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 USC 301 et seq.). Prior to registration for a new use 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 nontarget species when used in accordance with label instructions. EPA must also approve the language used on the pesticide label in accordance with Data Requirements for Pesticides (40 CFR part 158). Other applicable EPA regulations include Pesticide Registration and Classification Procedures (40 CFR part 152) and Experimental Use Permits (40 CFR part 172).

Dicamba was first registered in 1967 and glufosinate in 1989 for use as pesticides (herbicides) in the United States (US-EPA 2009; BASF 2010; Bayer CropScience 2014; US-EPA 2015). Dicamba and glufosinate herbicides are currently approved for preplant and postemergence labeled uses on maize. Glufosinate use on MON 87419 will not change from current labeled uses of glufosinate. However, the postemergence use of dicamba is currently limited in maize due to the plant's sensitivity to the herbicide (BASF 2010; Cao et al. 2011; Table VIII-4, pp. 139-141, Monsanto 2015a). To allow for more effective use rates of dicamba on maize for control of problem weed species, Monsanto will submit an application to amend EPA Registration Number 524-582 to register a new use pattern for dicamba on MON 87419 which would allow an increase in the maximum use rate of dicamba in maize from 0.5 lbs. to 1.0 lbs. a.e. per acre for preemergence applications and up to two applications of 0.5 lbs. a.e. of dicamba per acre for postemergence applications through the V8 growth stage or maize height of 30 inches, whichever comes first. Monsanto states the combined maximum annual application rate of dicamba on MON 87419 would be 2.0 lbs. a.e. dicamba per acre per year (p. 29, Monsanto 2015a). Monsanto will also request that EPA amend 40 CFR part 180 to revise tolerances for residues of dicamba and its relevant metabolites in or on maize. EPA's assessment will analyze risks to nontarget organisms to determine if the label is sufficient to meet EPA's standards for registration: "reasonable certainty of no harm to humans" and "no unreasonable adverse effects on the environment." If these standards are not met, EPA will apply appropriate risk mitigation strategies and propose label modifications to address the specific concerns. After EPA has completed its assessments and provided these to APHIS, APHIS will update this PPRA if needed.

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 (US-FDA 2006) and a more comprehensive voluntary consultation process prior to commercial distribution of food or feed (57 FR 22984 1992). Monsanto has initiated a consultation with the FDA (Biotechnology Notification File [BNF] No. 148) on the food and feed safety and compositional assessment of MON 87419 maize (p. 28, Monsanto 2015a). After the outcome of the consultation has been made available to APHIS, APHIS will update this PPRA if needed. Outcomes of completed consultations are 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 2015).

B. Development of Dicamba and Glufosinate Herbicide-resistant MON 87419 Maize

Maize (*Zea mays* ssp. *mays*), commonly referred to as corn in English-speaking countries, is the most widely cultivated grain crop in the U.S. and the U.S. is by far the world's largest producer (FAOSTAT 2015; USDA-ERS 2015). Maize is primarily grown

for animal feed grain in the U.S, accounting for more than 95% of the total feed grain production in 2014, when over 14 billion bushels were produced on 83 million acres (USDA-NASS 2015b) across almost every state, generating a crop value of $51.9 billion (USDA-NASS 2015a). To optimize yield and economic return, growers select maize lines adapted to the local environmental and climatic conditions and grow them as annual row crops using appropriate cultivation practices such as seedbed preparation, planting timing and density, and integrated pest management to handle weed and disease pressure (Hoeft et al. 2000; OECD 2003). Crop productivity worldwide varies from year to year and is impacted by losses due to abiotic factors (light, water, temperature and nutrients) and biotic factors (weeds, pests and pathogens). Plant pests can have a considerable influence on yield and productivity of crops; total losses in maize due to biotic factors were estimated for three time periods, from 1964 to 1965 at 34.8%, from 1988 to 1990 at 38.3%, and from 2001 to 2003 at 31.2% (Oerke 2006). Losses in maize productivity due to biotic factors have been reduced through practices that include the increased use of herbicides, pesticides and hybrids resistant to pests and diseases. Global grain production has doubled since the 1960s, and much of the increase in crop yield per unit area has been attributed to efficient control of biotic stress rather than to an increase in yield potential (Oerke 2006).

The presence of weeds in maize fields can cause greater production losses than either insects or diseases (Aref and Pike 1998; Gibson et al. 2005; Oerke 2006). Before the development and widespread use of effective herbicides, cultural practices such as tillage, use of weed-free seed, row spacing and crop rotation were the primary ways to control weeds. Herbicide use began in the 1940s and 1950s, and rapidly accelerated in the 1960s as a series of more selective herbicides were introduced into the market. Since weed communities evolve over time in response to control practices imposed on them, herbicide-resistant weeds began appearing in the early 1970s in conventional cropping systems. Repeated and intensive use of herbicides with the same mechanisms of action can rapidly select for tolerant, difficult-to-control weeds and for herbicide-resistant weeds, especially in the absence of concurrent use of herbicides with different mechanisms of action and/or use of different mechanical or cultural practices for weed control (Vencill et al. 2012).

The commercial introduction of several herbicide-resistant crops in the 1990's, including soybean, cotton and maize resistant to glyphosate, led to significant changes in herbicide usage and tillage practices, resulting in weed biotype and species shifts and the evolution of glyphosate-resistant weeds (Young 2006; NRC 2010). There are currently at least 32 glyphosate-resistant biotypes of weed species known worldwide, with at least 11 reported in maize crops in the U.S.: two *Amaranthus* species (Palmer amaranth and tall waterhemp), two *Ambrosia* species (common and giant ragweed), two *Conyza* species (hairy fleabane and horseweed/marestail), *Echinochloa colona* (junglerice), *Eleusine indica* (goosegrass), *Kochia scoparia* (Kochia), *Lolium perenne ssp. multiflorum* (Italian ryegrass) and *Sorghum halapense* (Johnsongrass) (Table VIII-6, p. 146, Monsanto 2015a); (Heap 2016). For more than a decade, growers have been looking for more options for over-the-top herbicide applications for their no-till maize crops to use in conjunction with cultural and mechanical best management practices to mitigate the

evolution of herbicide-resistant weeds, especially glyphosate-resistant weeds (Service 2007).

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a selective pre- and post-emergent herbicide used to control more than 95 annual and biennial weed species, and suppress over 100 perennial broadleaf and woody plant species (BASF 2010). It is a synthetic auxin herbicide that acts similar to endogenous auxin (indole-3-acetic acid); low concentrations induce cellular elongation and turgor as well as cellular differentiation and division, while increased concentrations lead to abnormal cell division and growth, and higher concentrations inhibit cell division and growth to the point of plant death (Kelley and Riechers 2007; Bunch et al. 2012; WSSA 2012). Glufosinate (2-amino-4-(hydroxyl-methylphosphinyl) butanoic acid) is a broad-spectrum pre- and post-emergence contact herbicide that provides nonselective control of approximately 120 broadleaf and grass weeds (Bayer CropScience 2014). It is a glutamine synthetase inhibitor herbicide that reduces the formation of glutamine and increases the accumulation of ammonia in plant cells, causing cell membrane damage and inhibition of photosynthesis to the point of plant death (OECD 2002a; WSSA 2012). Because dicamba and glufosinate together are effective on broadleaf and grass weeds which are hard-to-control with glyphosate or are glyphosate-resistant, Monsanto intends to stack MON 87419 maize with glyphosate-resistant Roundup Ready® Corn 2 utilizing traditional breeding methods and then commercialize GE maize resistant to dicamba, glufosinate, and glyphosate so maize growers will have greater weed control options using any of these modes of herbicide action (pp. 27-28, Monsanto 2015a).

MON 87419 maize was developed from LH244, a high-yielding inbred line of *Zea mays* ssp. *mays* that was genetically engineered to be resistant to dicamba by expressing a mono-oxygenase gene (*dmo*) from *Strenotrophomonas maltophilia* that rapidly demethylates dicamba, rendering it inactive. The same DMO protein is found in MON 87708 soybean and MON 88701 cotton, both of which have been deregulated by USDA-APHIS (USDA-APHIS 2016) and have undergone consultations with the FDA (FDA BNF 125 and BNF 135, respectively, US-FDA 2015). Additionally, MON 87419 maize contains the phosphinothricin N-acetyltransferase (*pat*) gene from *Streptomyces viridochromogenes* that expresses the PAT protein to confer resistance to the herbicide glufosinate. The PAT protein is produced in numerous deregulated commercial soybean, canola, and corn products, and the safety of PAT proteins present in biotechnology-derived crops has been extensively assessed (US-FDA 2015; USDA-APHIS 2016).

The recipient inbred line LH244 is a medium-season, yellow dent maize line adapted to the central part of the U.S. corn belt in the Midwest. The near isogenic conventional control materials used as comparators in the MON 87419 safety assessments include the recipient line LH244 for molecular characterization studies and the F1 hybrid maize line NL6169 (HCL645× LH244) in compositional analysis studies and in phenotypic, agronomic and environmental interactions assessments (Figure IV-3, p. 52, Monsanto 2015a). Reference hybrids consisting of commercial hybrid maize lines were used where appropriate to establish a range of variability or responses representative of commercial maize in the United States (p. 31, Monsanto 2015a).

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 related to the transformation process; the source of the inserted genetic material and its function in both the donor organism and the GE crop event; and 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 87419 maize compared to the conventional control. The assessment encompasses a consideration of the expressed mono-oxygenase gene (*dmo*) from *Strenotrophomonas maltophilia*, the expressed phosphinothricin N-acetyltransferase (*pat*) gene from *Streptomyces viridochromogenes*, and any observed or anticipated effects on plant metabolism including, for example, any relevant changes in levels of metabolites, antinutrients, or nutrients in harvested grain or forage derived from MON 87419 maize compared to those in the conventional controls.

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 the GE crop event; or for expression of inserted DNA, new proteins or enzymes, or changes in metabolism to affect plant pests or diseases, nontarget beneficial organisms, weediness, agricultural practices that impact pests or diseases or their management, or plant pest risks through horizontal gene flow.

Description of the genetic modification and inheritance of inserted DNA

MON 87419 was developed using disarmed *Agrobacterium tumefaciens* strain ABI to transform conventional LH244 immature embryos (Sidorov and Duncan 2009), using the plasmid vector PV-ZMHT507801 (Figure III-1, p. 36, Monsanto 2015a). PV-ZMHT507801 is a two-T-DNA vector, with a Transfer DNA (T-DNA I) sequence containing the *dmo* and *pat* expression cassettes, a second Transfer DNA (T-DNA II) containing the *cp4 epsps* expression cassette which confers resistance to glyphosate, and plasmid backbone sequences necessary for maintenance or selection of the plasmid vector in bacteria but which are not expected to be transferred to the maize embryo (Table III-1, pp. 41-44, Monsanto 2015a).

After incubation with the *Agrobacterium* vector, the immature embryos were placed on selection medium with glyphosate to select for transformed lines and with the antibiotic carbenicillin disodium salt to eliminate *A. tumefaciens*. Monsanto conducted subsequent breeding, segregation, selection and screening to segregate T-DNA I from T-DNA II and generate marker-free plants that did not contain T-DNA II, plasmid backbone and other unintended plasmid sequences (Figure III-2, p. 37, Monsanto 2015a).

The inserted DNA in MON 87419 maize, with both the *dmo* and *pat* gene expression cassettes from T-DNA I for production of DMO and PAT proteins, is described as containing the following genetic elements (Table IV-1, pp. 49-50, Monsanto 2015a):

- Flanking DNA containing 1246 base pairs.
- **B-Right Border Region**: A specific DNA region from *A. tumefaciens* containing the 71 base pair right border sequence used for transfer of the T-DNA (Depicker et al. 1982; Zambryski et al. 1982).
- Intervening sequence: Short 125 base pair segment used in DNA cloning.
- **P-*Ubq*: Promoter Sequence** of 1644 base pairs from ubiquitin gene (*Ubq*) of *Andropogon gerardii* (big bluestem grass) that directs transcription in plant cells (Joung and Kamo 2006).
- **L-*Ubq*: Leader Sequence,** 99 base pair 5' UTR, for the ubiquitin gene (*Ubq*) from big bluestem grass that regulates gene expression (Joung and Kamo 2006).
- **I-*Ubq*: Intron Sequence**, 1042 base pair intron from *Ubq* from big bluestem grass (Joung and Kamo 2006).
- Intervening sequence: Short 5 base pair segment used in DNA cloning.
- **CS-*pat*: Coding Sequence,** 552 base pairs, for phosphinothricin N-acetyltransferase (PAT) protein from *Streptomyces viridochromogenes* that confers tolerance to glufosinate (Wohlleben et al. 1988; Wehrmann et al. 1996).
- Intervening sequence: Short 8 base pair segment used in DNA cloning.
- **T-*Ara5*: Terminator Sequence**, 213 base pair 3' UTR of the RA5B precursor gene from *Oryza sativa* (rice), encoding alpha-amylase/trypsin inhibitor (*Ara5*) that directs polyadenylation of mRNA (Hunt 1994).
- Intervening sequence: 147 base pair segment used in DNA cloning.
- **P-*PCISV*: Promoter Sequence**, 433 base pairs, for the Full-Length transcript (FLt) of peanut chlorotic streak caulimovirus (PCISV) that directs transcription in plant cells (Maiti and Shepherd 1998).
- Intervening sequence: Short 5 base pair segment used in DNA cloning.
- **L-*Cab*: Leader Sequence** and 5' UTR, 61 base pairs, from chlorophyll a/b binding (CAB) protein of *Triticum aestivum* (wheat) (Lamppa et al. 1985).
- Intervening sequence: Short 16 base pair segment used in DNA cloning.
- **I-*Ract1*: Intron Sequence** and flanking UTR, 480 base pairs, of *act1* gene encoding Actin 1 protein from rice (McElroy et al. 1990).
- Intervening sequence: Short 9 base pair segment used in DNA cloning.
- **TS-*CTP4*: Targeting Sequence** and 5' UTR leader, 216 base pairs, of *ShkG* gene from *Petunia hybrida* encoding the EPSPS transit peptide that directs the protein to the chloroplast (Herrmann 1995; Gasser et al. 1998).
- **CS-*dmo*: Coding Sequence**, 1023 base pairs, for dicamba mono-oxygenase (DMO) from *Stenotrophomonas maltophilia* that confers resistance to the herbicide dicamba (Wang et al. 1997; Herman et al. 2005).
- Intervening sequence: Short 30 base pair segment used in DNA cloning.
- **T-*Hsp17*: Terminator Sequence**, 210 base pairs, 3" UTR from heat shock protein gene, *hsp17*, of *Triticum aestivum* (wheat) that directs polyadenylation of mRNA (McElwain and Spiker 1989).

- Intervening sequence: Short 162 base pair segment used in DNA cloning.
- **B-Left Border Region:** A specific DNA region from *A. tumefaciens* containing the 211 base pair left border sequence used for transfer of the T-DNA (Barker et al. 1983).
- Flanking DNA containing 1251 base pairs.

Monsanto confirmed the insertion of the genetic elements listed above by conducting a detailed molecular characterization of the inserted DNA and associated native flanking sequences in MON 87419 compared to the DNA sequence of the recipient maize line LH244 and the plasmid vector. Monsanto used a combination of next-generation sequencing (NGS) (Shendure and Ji 2008; Zhang et al. 2011) and Junction Sequence Analysis (JSA) bioinformatics (Kovalic et al. 2012; DuBose et al. 2013) with directed sequencing (locus-specific polymerase chain reaction (PCR) analysis and DNA sequencing) in a multistep approach (Figure IV-1, p. 46, Monsanto 2015a) to determine the number of insertion sites, the presence/absence of plasmid backbone, insert copy number at each insertion site, DNA sequence of each inserted DNA, and sequence of the native locus/adjacent flanking genomic DNA at each insertion site in MON 87419. APHIS reviewed the molecular characterization data and methods provided in Section IV and Appendix B of the petition (Monsanto 2015a):

- The T-DNA inserted into the MON 87419 maize genome is present at a single locus, as demonstrated by the existence of only two junction sequence classes identified in MON 87419 containing partial T-DNA border sequence joined to maize genomic flanking sequence (Table IV-2, p. 54; Figure IV-4, p. 56; Figure B-3, p. 197).
- MON 87419 does not contain any sequence from the plasmid PV-ZMHT507801 backbone or from T-DNA II, as demonstrated by the evidence that none of the millions of 100 base pair sequence reads generated by whole-genome sequence analysis had mapped to plasmid backbone or T-DNA II sequence, while thousands of sequence reads had mapped to the plasmid T-DNA I sequence (Figure IV-2, p. 51; Section IV.A.2.3, p. 55).
- The T-DNA sequence in MON 87419 is 6,762 base pairs and the organization of genetic elements is identical to the corresponding T-DNA I sequence and gene order in the plasmid PV-ZMHT507801, as confirmed by directed DNA sequence analysis of the DNA insert compared to the plasmid sequence (Section IV.B, p. 57; Figure IV-5, p. 58); no DNA rearrangement occurred at the insertion site in MON 87419, although a deletion of 602 base pairs of genomic DNA occurred (Section IV.C, p. 59; Figure IV-6, p. 60);
- The T-DNA sequence inserted into MON 87419 was stably inherited at a single locus across five breeding generations (Section IV.D, p. 61; Table IV-3, p. 62), according to Mendelian principles of inheritance (as determined by chi-square analysis for three segregating generations, Section IV.E, pp. 63-66), consistent with the molecular characterization data that indicate MON 87419 maize contains a single, intact copy of the *dmo* and *pat* expression cassettes that were inserted into the maize genome at a single locus (Section IV.F, p. 67).

Insertion of foreign genetic material tends to induce mutations at sites of insertion (generally referred to as insertional mutations) in recipient genomes (Nacry et al. 1998; Laufs et al. 1999). Monsanto examined the T-DNA insertion site in MON 87419 maize compared to the conventional maize control LH244 using PCR and sequence analyses and discovered that 602 bases of maize genomic DNA were deleted during integration of the T-DNA I (Section IV.C, p. 59, Monsanto 2015a). According to Monsanto, the observed insertion and insertion-deletion mutation (indel mutation) presumably resulted from double-stranded break repair mechanisms in the plant during the *Agrobacterium*-mediated transformation process (Salomon and Puchta 1998). As discussed later in this document, none of these mutations altered the function of the *dmo* or *pat* genes or exhibited deleterious phenotypes in MON 87419 maize.

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

Dicamba herbicide resistance in MON 87419 maize is derived from a bacterial oxygenase gene (*dmo*) from *Stenotrophomonas maltophilia* isolated from a stormwater retention pond outside a dicamba manufacturing plant (Krueger et al. 1989). *S. maltophilia* is an aerobic, ubiquitous bacterium that can be found in a variety of environments, including associated with plants used as food or feed (pp. 75-76, Monsanto 2015a). It has been used as a biocontrol agent for fungal plant pathogens, but it is not considered a plant pathogenic bacterium (Berg et al. 1999; ISPP 2016). Originally classified as *Pseudomonas maltophilia*, *S. maltophilia* was also grouped in the genus *Xanthomonas* before eventually becoming the type species of the *Stenotrophomonas* genus in 1993 (Palleroni and Bradbury 1993). The bacterium can utilize dicamba as a sole carbon source through the action of a multicomponent demethylase system comprised of a reductase, a ferredoxin and an oxygenase (Figure 1, Chakraborty et al. 2005), which work together in a redox system similar to many other oxygenases to degrade aromatic compounds by catalyzing the incorporation of oxygen into organic substrates. In MON 87419 maize, the reductase and ferredoxin are endogenous in the maize chloroplast where they associate with the transgenic DMO to transport electrons from nicotinamide adenine dinucleotide (NADH) to oxygen to catalyze the demethylation of dicamba to form the non-herbicidal metabolites DCSA (3,6-dichlorosalicyclic acid) and formaldehyde (Behrens et al. 2007; Dumitru et al. 2009).

Dicamba has been registered and used as an herbicide for almost 50 years; hence the safety of dicamba and its degradate DCSA as residues in or on commodities labeled for dicamba herbicide use has been evaluated by the EPA (US-EPA 2009; 40 CFR 180.227), prior to the development of genetically engineered dicamba-resistant crops, where DCSA is generated from dicamba through the catalytic activity of the transgenic DMO protein. The structure of DCSA is similar to salicylic acid (2-hydroxybenzoic acid), an endogenous plant benzoic acid (Frear 1976; p. 210, Monsanto 2015a). Endogenous salicylic acid compounds are known to be involved in plant responses to stress, including to pests and pathogens (Vlot et al. 2009; Thaler et al. 2010; An and Mou 2011; Balmer et al. 2013; Kumar et al. 2015; Tzin et al. 2015; Züst and Agrawal 2016). MON 87708 soybean and MON 88701 cotton, both of which contain the same DMO protein as MON 87419 maize, were previously found to have no differences in composition, phenotypic, agronomic or environmental interaction characteristics when treated or not treated with

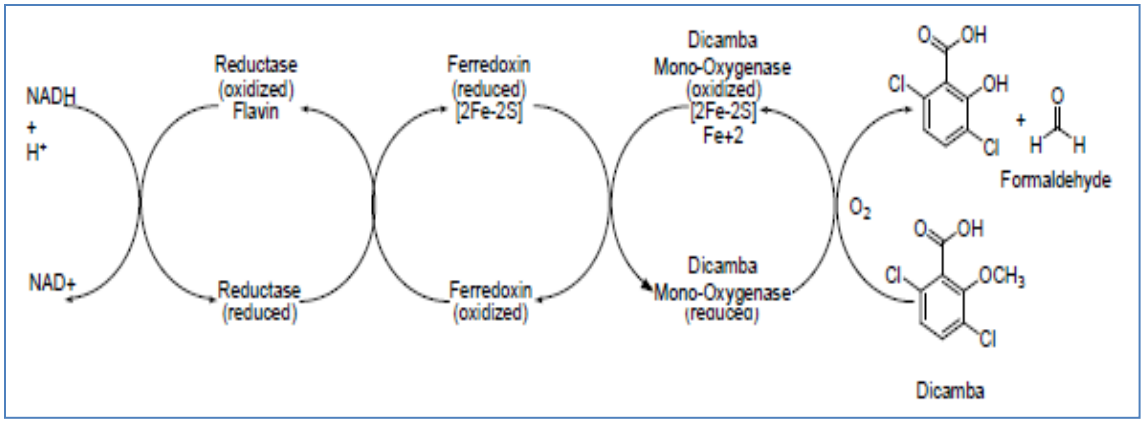

Figure 1. Demethylation of dicamba (3,6-dichloro-2-methoxybenzoic acid) catalyzed by dicamba mono-oxygenase to form the metabolites 3,6-dichlorosalicylic acid and formaldehyde (Chakraborty et al. 2005; Figure V-1, p. 74, Monsanto 2012a)

dicamba compared to the conventional control (Monsanto 2012a, 2012b; USDA-APHIS 2016). This supports the conclusion that the formation of DCSA does not make dicamba-resistant crops any more of a plant pest risk than their conventional counterparts. Nevertheless, the extent to which dicamba herbicide treatment, and hence the formation of DCSA, affects the composition, phenotypic and agronomic characteristics of MON 87419 maize will be examined herein.

Formaldehyde, the other breakdown product of dicamba by MON 87419 maize DMO, is found naturally in plants at levels up to several hundred parts per million (Adrian-Romero et al. 1999), as well as in agricultural commodities (WHO-IPCS 1989). Formaldehyde production in plants has been shown to change in response to abiotic and biotic stresses, although the mechanism and function are unclear (Sardi et al. 1996; Szabó et al. 2003; Szende and Tyihák 2010). The EPA has determined that formaldehyde is not a metabolite of concern for dicamba residue and safety studies because it is not metabolically stable and is quickly incorporated into the one-carbon pool reactions essential to all organisms (US-EPA 1996; Hanson and Roje 2001). In plants, these reactions supply the C1 units needed to synthesize proteins, nucleic acids, pantothenate, and a great variety of methylated molecules (Kalasz 2003). C1 pathways are particularly active in tissues that produce methylated compounds such as lignin, alkaloids, and betaines because the C1 demands for these physiologically and economically important secondary metabolites can dwarf those of primary metabolism. Formaldehyde was not measured in the residue studies when dicamba was applied to MON 87419 maize, MON 87708 soy or MON 88701 cotton; the levels are expected to be small and transient, similar to naturally produced endogenous formaldehyde, even when the maximum proposed labelled rate of dicamba is 100% intercepted and instantaneously metabolized by the transgenic crop (pp. 251-252, Monsanto 2012a; p. 273, Monsanto 2012b; pp. 210-211, Monsanto 2015a).

Glufosinate herbicide resistance in MON 87419 is derived from a bacterial acetyl transferase gene (*pat*) from *Streptomyces viridochromogenes* (Wohlleben et al. 1988), a

saprophytic bacterium that is widespread in the environment and is not pathogenic to plants (Kämpfer et al. 2014). A homologous protein is encoded by the *bar* gene of *S. hygroscopicus* (Wehrmann et al. 1996). The PAT protein expressed in MON 87419 is virtually identical to the wild type PAT protein encoded by *S. viridochromogenes* except that post-translational cleavage of its first methionine resulted in the MON 87419 PAT protein containing 182 amino acids in length instead of 183, with an apparent molecular weight of 25.2 kDa. N-terminal methionine cleavage is common and naturally occurs in the vast majority of proteins (Meinnel and Giglione 2008) and is not expected to affect the structure, activity, or specificity of the MON 87419 PAT protein. The MON 87419 PAT protein was found to be expressed throughout the life-cycle and tissues of the plant, including roots and seed, with the highest levels of expression in leaves and forage samples (Table V-2, p. 74, Monsanto 2015a).

PAT is an enzyme that confers herbicide resistance by catalyzing the acetylation of L-phosphinothricin, the active component in glufosinate, to the non-herbicidal compound N-acetyl L-phosphinothricin (OECD 1999, 2002a; p. 70, Monsanto 2015a) PAT proteins are highly specific for L-phosphinothricin. Other L amino acids, including the L-phosphinothricin analogue L-glutamate, are unable to be acetylated by PAT and do not inhibit acetylation of L-phosphinothricin in competition assays (Wehrmann et al. 1996). Therefore, the PAT protein is unlikely to affect the metabolic system of MON 87419. The PAT proteins expressed in glufosinate-resistant maize events previously reviewed and granted nonregulated status by APHIS (USDA-APHIS 2016) and several other countries (OECD 1999, 2002a; ILSI-CERA 2011) have an extensive history of safe use without affecting plant pest or disease risks, nontarget beneficial organisms, weediness, agricultural practices that impact pests or diseases or their management, or plant pest risks through horizontal gene flow. The safety of the PAT protein has been established in the scientific literature (Hérouet et al. 2005).

Compared to glufosinate-resistant crops, dicamba-resistant crops are a relatively new development undergoing regulatory review. The physicochemical characteristics and functional activity of the MON 87419 maize DMO protein that confers resistance to dicamba were determined using a panel of analytical techniques, including: 1) N-terminal amino acid sequence analysis to determine post-translational processing efficiency, 2) western blot analysis to establish identity and immunoreactivity of MON 87419 DMO using an anti-DMO antibody, 3) matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to generate a tryptic peptide map of the MON 87419 DMO, 4) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to establish the apparent molecular weight of MON 87419 DMO, 5) glycosylation status of MON 87419 DMO, and 6) MON 87419 DMO specific activity to demonstrate functional activity (Sections V.A and V.B. pp. 69-72, and Appendix C, pp. 210-230, Monsanto 2015a).

- MON 87419 expresses a single DMO precursor protein that undergoes incomplete post-translational processing after transport to the chloroplast to create two forms of the DMO protein, referred to as MON 87419 DMO +12 and MON 97419 DMO +7. Twelve amino acids are incompletely cleaved from the chloroplast transit peptide coding sequence (*CTP4*) at the N-terminus of the

MON 87419 DMO+12. MON 87419 DMO+7 does not contain the first five amino acids of MON 87419 DMO+12. In addition, a leucine was inserted at position 2 of the DMO protein sequence (confirmed by N-terminal sequencing, peptide analysis and western blot analysis by probing with an antibody specific for both forms of the MON 87419 DMO protein; see V.A.1, p. 69 and Appendix C.2.5-2.7, pp. 215-224).

- The difference in molecular weight between the two forms of MON 87419 DMO is small and results in only one single band observable by Coomassie stain of SDS-PAGE and western blot, with an apparent molecular weight of 39.5 kDa (see V.A.1, p. 69 and Appendix C.2.8, pp. 224-226); neither form of MON 87419 is glycosylated (Appendix C.2.9, p. 227-228).
- The specific activity of purified MON 87419 maize DMO (i.e., how much dicamba the naturally expressed DMO+12 and DMO+7 mixture converts to DCSA) was determined to be 232.5 nmoles DCSA/min/mg of MON 87419 DMO, which is higher than the specific activities measured previously for dicamba-resistant soybean (62.21 nmoles DCSA/min/mg of MON 87708 DMO) and dicamba-resistant cotton (5.48 nmoles DCSA/min/mg of MON 88701 DMO) (Table C-4, p. 312, Monsanto 2012a; Table C-5, p. 297, Monsanto 2012b; Table C-5, p. 230, Monsanto 2015a).

Since these studies showed that DMO purified from MON 87419 maize flour contains a mixture of both DMO+12 and DMO+7, and it is known that the active form of DMO necessary to demethylate dicamba and confer resistance to the herbicide is a trimer comprised of three DMO monomers (Chakraborty et al. 2005), the petitioner refers to both forms of the protein (DMO+12 and DMO+7) and all forms of the trimer (DMO+12, DMO+7, or a combination of both) as MON 87419 maize DMO (Section V, p.69, Monsanto 2015a). Except for the amino acids derived from the CTP4 (+7 or +12) and an additional leucine at position two, the MON 87419 DMO protein is virtually identical in sequence to the wild-type DMO protein from the DI-6 strain of *S. maltophilia* (Herman et al. 2005), as well as to soybean MON 87708 DMO and cotton MON 88701 DMO, which both have similarly small, incompletely cleaved transit peptide sequences at their N-termini (Figure C-1, p. 213, Monsanto 2015a). The active binding pocket of DMO with dicamba occurs through hydrogen-bonding and steric interactions at locations not involving the N-terminus of DMO (D'Ordine et al. 2009; Dumitru et al. 2009), which supports the petitioner's assertion that the differences in amino acid sequence between the wild-type DMO and both DMO+12 and DMO+7 will not affect the involvement of both forms of MON 87419 DMO in the formation of active trimers.

The petitioner provided data that demonstrated that DMO was expressed throughout MON 87419 maize, that it conferred the dicamba herbicide resistance phenotype, and that it has a high specificity for dicamba as a substrate (Section V.C-V.E, pp. 71-77, and Appendices C and D, pp. 230-250, Monsanto 2015a):

- The MON 87419 DMO protein was found to be expressed throughout the life-cycle and tissues of the plant, including roots and grain, with the highest level of expression in leaves (Table V-1, p. 73). This is expected since expression of the

dmo gene in MON 87419 is driven by the constitutive PC1SV promoter from peanut chlorotic streak caulimovirus (Maiti and Shepherd 1998).

- In addition to dicamba (formulation Clarity®, BASF 2010), three herbicides with distinct mechanisms-of-action active against conventional maize were applied at the labelled use rate and at least two times the maximum labelled use rate to MON 87419 maize and conventional maize control plants at the V2-VS growth stage. As expected, MON 87419 maize sprayed over-the-top with dicamba exhibited negligible injury whereas the conventional maize control sustained injury. However both MON 87419 and the conventional control sustained similar levels of injury to the other three herbicides, demonstrating that the DMO protein expressed in MON 87419 is highly specific to dicamba and that the other herbicides do not serve as a substrate for MON 87419 DMO.
- The possibility that MON 87419 DMO can metabolize plant endogenous substrates in soybean, cotton and maize that are structurally similar to dicamba was tested *in vitro*. None of the five potential substrates (o-anisic acid, vanillic acid, syringic acid, ferulic acid and sinapic acid; Figure C-6, p. 234) were metabolized by *E. coli*-produced DMO (Figures C-7 to C-12, pp. 237-242).

The petitioner carried out a compositional assessment of grain and forage samples using the principles and analytes outlined in the OECD consensus document for maize composition (OECD 2002b) to assess whether levels of key nutrients, anti-nutrients, and secondary metabolites in MON 87419 maize were equivalent to levels in the conventional control (Section VI, pp. 81-100, and Appendix E, pp. 251-285, Monsanto 2015a). The samples for compositional assessment were collected in the 2013 growing season from five replicated sites chosen to represent the typical maize growing regions of the U.S. In addition to the conventional weed control programs, MON 87419 maize plots were either treated with dicamba and glufosinate at least a week apart between the V2 and V4 growth stage at common agronomic use rates, or not treated with dicamba and glufosinate (Section G.5, p. 294, Monsanto 2015a).

Components analyzed in grain included 49 nutrients (3 proximates, 18 amino acids, 9 fatty acids, carbohydrates by calculation, 3 fiber types, 8 minerals, and 7 vitamins), 2 anti-nutrients, and 2 secondary metabolites. Components analyzed in forage included 3 proximates, carbohydrates by calculation, 2 fiber types, and 2 minerals. In total, 61 components were statistically analyzed for MON 87419 maize treated with dicamba and glufosinate, and the same 61 components were statistically analyzed for MON 87419 maize not treated with dicamba and glufosinate.

Only seven out of 122 statistical tests showed a significant difference ($p<0.05$) between MON 87419 and the conventional control: manganese in grain in both MON 87419 maize treated and untreated with dicamba and glufosinate; and five amino acids (glycine, histidine, proline, serine, and threonine) in MON 87419 maize not treated with dicamba and glufosinate. However, the mean differences between the comparisons for all seven of these components were less than the range values of the conventional control, indicating that MON 87419 does not impact these components more than natural variation within the conventional control grown at multiple locations. Also, the mean values for these

seven components in MON 87419 were all within the range of values observed in the ILSI Crop Composition Database and the scientific literature for maize (ILSI-CERA 2014; Table VI-8, pp. 98-99, and Table E-9, pp. 280-281, Monsanto 2015a). These results support the overall conclusion that MON 87419 was not a major contributor to variation in levels of key nutrients, anti-nutrients, and secondary metabolites in maize grain and forage, and confirmed the compositional equivalence of MON 87419 to the conventional control, whether treated or untreated with dicamba and glufosinate.

The results of the petitioner's DMO characterization studies support the conclusion that the functional activity, specificity and expression levels of the MON 87419 maize DMO confer the intended phenotype of dicamba resistance to MON 87419 maize. Based on the data presented by the petitioner on the composition of key nutrients, anti-nutrients, and secondary metabolites in maize grain and forage for both dicamba-treated and untreated MON 87419 maize, it is reasonable to conclude that neither the dicamba and glufosinate herbicide-resistance trait nor the dicamba or glufosinate herbicide treatments has a meaningful impact on the composition of seed or forage derived from MON 87419 maize compared to other commercial maize hybrids. Based on all the above noted considerations, APHIS concludes that MON 87419 maize poses no more of a plant pest risk from new gene products, changes to plant metabolism or composition than conventional maize hybrids.

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 87419 maize 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 87419 maize is likely to have significantly increased disease and pest susceptibility based on data and observations from field trials 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 the new GE crop 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 U.S. and supports trade and exports of U.S. agricultural products. PPQ responds to many new introductions of plant pests to eradicate, suppress, or contain them through various programs in cooperation with state departments of

agriculture and other government agencies. These may be emergency or longer term domestic programs that target a specific pest. A variety of insect, plant disease, mollusk, nematode or weed programs exist (USDA-APHIS 2015).

Currently, PPQ has several active pest management programs that target insect pests and a noxious weed that can affect maize. These include programs for grasshoppers (Order Orthoptera) on rangelands, the light brown apple moth (*Epiphyas postvittana*) in California, and of more relevance, the Japanese beetle (*Popillia japonica*), the Old World bollworm (*Helicoverpa armigera*), and witchweed (*Striga asiatica*) (for more information on each of these programs, see USDA-APHIS 2015). The Japanese beetle can cause significant damage feeding on many plant species; when adults feed on maize silk it affects pollination and kernel formation. A recently established program targets the Old World bollworm. This pest can affect 180 species of plants, with maize listed as one of its preferred hosts. It is closely related to the corn earworm (*H. zeae*). It was first detected in western Puerto Rico in September 2014, and at this time it is not present in the continental United States.

Maize itself is not considered a plant pest in the U.S. (7 CFR 340.2). The plant pest derived vector DNA and the plant pest vector used to insert the DNA do not pose a plant pest risk to MON 87419 maize. The binary plasmid vector PV-ZMHT507801 proved to be disarmed; the T-DNA inserted into MON 87419 maize contained only the intended sequences, along with the typical insertion site mutations, and lacked sequences from Tumor-inducing (Ti) plasmids normally responsible for the formation of crown gall tumors upon *A. tumefaciens* infection (Hoekema et al. 1983; Hellens et al. 2000). The sequences derived from plant pathogens retained in MON 87419 maize (i.e., promoter sequence from peanut chlorotic streak caulimovirus and T-DNA border sequences from *A. tumefaciens*) are non-coding sequences which do not cause plant disease. Furthermore, following transformation, the R0 plant tissue was treated with the antibiotic carbenicillin to eliminate *A. tumefaciens* (Nauerby et al. 1997; p. 34, Monsanto 2015a).

MON 87419 maize was grown within confined field trials in the U.S. from 2011 through 2014 in at least 160 locations across 19 states and territories covering a diverse range of environmental conditions representative of where maize is currently grown and bred, and where MON 87419 is expected to be grown (Appendix A, Table A-1, pp. 190-191, Monsanto 2015a). In addition to the observational data that Monsanto annually reported to USDA-APHIS from these product development trials, which would have included reports of unusual pest and/or disease incidence, Monsanto also assessed phenotypic, agronomic and environmental interaction characteristics for MON 87419 maize compared with the conventional control and commercial reference hybrids in 2013 and 2014 (Section VII and Appendix G, Monsanto 2015a). MON 87419 maize was grown untreated with dicamba and glufosinate for all 30 phenotypic, agronomic and environmental interaction characteristics, which allowed for the assessment of the effect of the inserted *dmo* gene on the potential for enhanced weediness and plant pest and disease impacts. To also allow for the assessment of the effect of the dicamba and glufosinate herbicide treatment, 14 of the 22 characteristics that were evaluated in field settings (i.e., all field-evaluated characteristics except the eight (8) environmental

interactions) were also assessed for MON 87419 maize treated with dicamba and glufosinate.

Out of the 30 phenotypic and agronomic characteristics assessed, 22 characteristics (related to germination, dormancy and emergence of seed; vegetative growth; reproductive growth; and lodging and seed retention) will be discussed in the later section on *Potential for Enhanced Weediness of MON 87419 Maize*. The eight (8) environmental interactions (interactions between the crop plants and their receiving environment, including responses to abiotic stress, general disease damage plus both stalk rot and ear/kernel rot disease damage, general arthropod-related damage plus corn earworm and European corn borer damage, and pest and beneficial arthropod abundance) will be discussed in this section on *Potential Plant Pest and Disease Impacts*.

Qualitative data on environmental interactions for responses to abiotic stress, general disease damage, stalk rot disease damage, ear/kernel rot disease damage, and general arthropod-related damage were collected in 2013 on MON 87419 maize at eight field sites. Quantitative data on environmental interactions for corn earworm damage, European corn borer damage, and pest and beneficial arthropod abundance were collected in 2013 on MON 87419 maize at three of these eight field sites (Section VII, pp. 101-126, and Appendix G, pp. 293-397, Monsanto 2015a).

- These eight locations provided a diverse range of environmental and agronomic conditions representative of commercial maize production areas in North America (Table VII-3, p. 115; Appendix G.3, p. 293; Table G-3, p. 297). Four commercial reference hybrids were grown concurrently with MON 87419 maize and the conventional control at each site to establish a range of natural variability for the assessed stressors (Table G-1, p. 295).
- The researchers at each field site were expected to be familiar with the growth, production, and evaluation of maize characteristics, and to use well-established qualitative and/or quantitative techniques to observe and evaluate environmental interactions. They chose abiotic stressors, diseases and arthropod pests that were either actively causing injury or were likely to occur in maize during the given observation period. The assessed stressors were present at natural levels, as no artificial infestation or imposed abiotic stress was used.
- For plant responses to abiotic stress, disease damage and arthropod-related damage, at least three abiotic stressors, three diseases and three arthropod pests were evaluated up to four times during the growing season at all 26 sites. The researcher at each field site chose abiotic stressors, diseases, and arthropod pests that were either actively causing plant injury in the study area or were likely to occur in maize during the given observation period. Therefore, the stressors typically varied between observations at a site or among sites (Appendix G.7.1, p. 299).
- Arthropod abundance was measured only at three of the eight sites, which were designed to contain plots suited for the purpose of collecting robust arthropod abundance data (Appendix G.3, p. 293; Appendix G.7.2, p. 300).
- Qualitative data on observations of plant response to abiotic stress, disease damage, and arthropod damage were collected from each plot using a categorical

scale (none, slight, moderate and severe). Qualitative categorical data were not statistically analyzed; they were considered different in susceptibility or tolerance on a particular observation date at a site if the range of injury symptoms to MON 87419 maize did not overlap with the range of injury symptoms to the control across all four replications. Qualitative numerical data for arthropod damage and quantitative data for arthropod abundance underwent statistical analysis ($\alpha = 0.05$) (Table VII-7, p. 121, and Appendix G.9.2, p. 302).

The petitioner provided data that demonstrated that the dicamba and glufosinate herbicide-resistance trait did not alter the assessed environmental interactions of MON 87419 maize compared to the conventional control (Section VII.C.2.2, pp. 119-123; Appendix G.10.3, pp. 304-305; Tables G-9 through G-14, pp. 320-326, Monsanto 2015a).

Abiotic stressors

- No differences were observed between MON 87419 maize and the conventional control for all 93 observations for injury from nine abiotic stressors (cold, drought, flooding, hail, heat, nutrient deficiency, soil compaction, and wind) (Table VII-7, p. 121, and Table G-9, p. 320).

Disease damage

- No differences were observed between MON 87419 maize and the conventional control for all 107 observations for disease damage from all 15 categories of diseases evaluated (anthracnose - *Colletotrichum graminicola*; bacterial blight - *Pseudomonas avanea* subsp. *Avenae*; crazy top - *Sclerophthora macrospora*; ear rot - can be caused by *Aspergillus* spp., *Cladosporium* spp., *Diplodia* spp., *Fusarium* spp., or several other genera; eyespot - *Kabatiella zeae*; *Fusarium* spp.; Goss's bacterial wilt - *Clavibacter michiganensis* subsp. *Nebraskensis*; gray leaf spot - *Cercospora zeae-maydis*; leaf blight (including northern and southern leaf blight) - *Exserohilum turcicum* and *Bipolaris maydis*; northern leaf spot *Cochiobolus carbonum*, *Helminthosporium carbonum* and other spp.; *Pythium* spp.; rust (includes common rust) - *Puccinia sorghi*; smut (includes common smut) - *Ustilago* spp. ; stalk rot - many causal organisms including different bacteria and fungi such as *Colletotrichum graminicola*, *Fusarium* spp., *Gibberella zeae*, *Diplodia maydis*, etc.; and Stewart's bacterial wilt - *Pantoea stewartii*) (Table VII-7, p. 121, and Table G-10, p. 321).

Arthropod damage

- No differences were observed between MON 87419 maize and the conventional control for all 91 observations for arthropod damage from all 15 categories of arthropods evaluated (aphids (Aphididae); armyworms (Noctuidae); billbugs (*Sphenophorus parvulus*); corn earworms (*Helicoverpa zea*); corn flea beetles (*Chaetocnema pulicaria*); corn rootworm beetles (*Diabrotica* spp.); cutworms (Noctuidae); European corn borers (*Ostrinia nubilalis*); grape colaspis (Chrysomelidae); grasshoppers (*Melanoplus* spp.); Japanese beetles (*Popillia japonica*); sap beetles (Nitidulidae); spider mites (*Tetranychus* spp.); Southwestern corn borers (*Diatraea grandiosella*); stink bugs (Pentatomidae)) (Table VII-7, p. 121, and Table G-11, p. 322).

- No statistically significant differences were detected between MON 87419 and the conventional control for quantitative damage from corn earworm (*Helicoverpa zea*) and European corn borer (*Ostrinia nubilalis*) for eight out of nine comparisons at three sites (Table VII-8, p. 123, and Table G-12, p. 323). A single difference was observed where MON 87419 had less damage from corn earworm infestation compared to the conventional control at one site. However, the mean damage rating for MON 87419 was within the range of the commercial reference hybrids at this site and no differences were detected at other sites. Thus, these differences were not indicative of a consistent response associated with the trait and are not considered biologically meaningful in terms of increased plant pest potential of MON 87419 compared to conventional maize.

Arthropod abundance (pests and beneficials)

- Using sticky traps to measure arthropod abundance, no statistically significant differences were detected between MON 87419 and the conventional control for 21 out of 23 comparisons (Table VII-8, p. 123, and Table G-13, pp. 324-325). Pest arthropods caught in the sticky traps were the herbivores corn flea beetles (Chrysomelidae: Coleoptera), corn rootworm beetles (Chrysomelidae: Coleoptera), sap beetles (Nitidulidae: Coleoptera), leafhoppers (Cicadellidae: Hemiptera), planthoppers (Delphacidae: Hemiptera) and spider mites (Tetranychidae: Acari). Beneficial arthropods caught in the sticky traps were the predators ladybird beetles (Coccinellidae: Coleoptera), minute pirate bugs (Anthocoridae: Hemiptera), parasitic wasps (Hymenoptera), lacewings (Chrysopidae: Neuroptera), syrphid flies (Syrphidae: Diptera), and spiders (Araneae). Significant differences were detected between MON 87419 and the conventional control for corn rootworm beetles (less abundant on MON 87419) and spiders (more abundant on MON 87419). The mean abundance values of MON 87419 for these arthropods were slightly outside of the respective ranges of the reference hybrids. However, these differences were not consistently detected across sites and/or collection methods (i.e., in visual counts; Table G-14). Thus, these differences were not consistent responses associated with the trait and are not considered biologically meaningful in terms of increased plant pest potential of MON 87419 maize compared to the conventional control maize.
- Using visual counts to measure arthropod abundance, no statistically significant differences were detected between MON 87419 and the conventional control for 10 out of 11 comparisons (Table VII-8, p. 123, and Table G-14, p. 326). Pest arthropods visually observed were corn flea beetles, corn rootworm beetles, sap beetles, and shining flower beetles (Phalacridae: Coleoptera). Beneficial arthropods visually observed were ladybird beetles, minute pirate bugs, and spiders. A significant difference was detected between MON 87419 and the conventional control for minute pirate bugs (less abundant on MON 87419). However, the mean abundance value for MON 87419 was within the range of the reference hybrids. Additionally, this difference was not consistently detected across sites or collection methods (i.e., not detected in sticky traps; Table G-13). Thus, this difference was not indicative of a consistent response associated with the trait and is not considered biologically meaningful in terms of increased plant pest potential of MON 87419 compared to conventional maize (Section VII.B.2).

The results of the petitioner's field studies on the assessed environmental interactions between MON 87419 maize and its receiving environment indicate that the dicamba and glufosinate herbicide-resistance trait is not expected to alter the response of MON 87419 to abiotic stress, diseases, or arthropod pests under natural levels of these stressors, nor cause pest arthropods to be more abundant around MON 87419 plots, compared to conventional maize.

In summary, the introduced genes did not significantly alter the observed insect pest infestation and disease occurrence or resulting damage on MON 87419 compared to the control and other reference lines. As discussed earlier, neither the dicamba and glufosinate herbicide-resistance trait nor the dicamba and glufosinate herbicide treatments (including the DCSA and formaldehyde metabolites produced as a result) significantly altered MON 87419 grain or forage composition that would render MON 87419 more susceptible to pests and diseases compared to the control and other reference lines. As presented later in this document, neither the dicamba and glufosinate herbicide-resistance trait nor the dicamba and glufosinate herbicide treatments (including the DCSA and formaldehyde metabolites produced as a result) significantly altered the observed agronomic and phenotypic traits and did not reveal any significant changes that would indirectly indicate that MON 87419 is or could be more susceptible to pests and diseases compared to the control or reference lines. Thus, MON 87419 is unlikely to be more susceptible to plant pathogens and insect pests than conventional maize and existing commercial hybrids, and it is unlikely to differ from conventional maize 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 87419 maize is not engineered for pest resistance, thus there are no 'target' species, and thus no 'nontarget' species either. APHIS assessed whether exposure or consumption of MON 87419 maize would have a direct or indirect adverse impact on species beneficial to agriculture. Organisms considered were representatives of the species associated with production of the regulated crop in the agricultural environment. The assessment includes an analysis of data and information on MON 87419 maize compared to the conventional control and other comparators used as a reference range for 1) any biologically relevant changes in the phenotype or substances produced (e.g., the MON 87419 DMO and PAT, nutrients, antinutrients, metabolites, etc.) that may be novel or expressed at significantly altered amounts and are associated with impacts on organisms beneficial to agriculture, and/or 2) any observations of beneficial organisms associated with the plants.

APHIS reviewed information Monsanto provided justifying the safety of MON 87419 maize (Sections V.D-V.F, pp. 75-80, Monsanto 2015a), as well as additional literature:

- The same DMO protein is found in MON 87708 soybean and MON 88701 cotton, both of which have been deregulated by USDA-APHIS (USDA-APHIS 2016) and have undergone consultations with the FDA (FDA BNF 125 and BNF 135, respectively)(US-FDA 2015). The donor organism for the *dmo* gene, *S. maltophilia,* is currently being reviewed as part of a safety and nutritional assessment of MON 87419 maize that Monsanto submitted to the FDA (BNF No. 148). *S. maltophilia* is an aerobic, ubiquitous, environmental, gram-negative bacterium, and although its close genetic relatives are plant pathogens, it is not classified as such. APHIS examined a recent review of *S. maltophilia* by Brooke (2012) that indicates that it has been isolated from soil, water, animals, invertebrates, plant matter including food, and hospital equipment, and can cause infections in humans, particularly immunocompromised and debilitated individuals; but there is no indication that the *dmo* from *S. maltophilia* plays a role in pathogenicity, virulence, antibiotic resistance, adhesion or other interactions with human, animals or invertebrates.
- The DMO enzyme present in MON 87419 maize shares sequence identity and many catalytic and domain structural similarities with a wide variety of oxygenases found in numerous species of microorganisms widely distributed and prevalent in the environment (Chakraborty et al. 2012). This includes oxygenases such as pheophorbide A oxygenase also found in plants such as rice, maize, canola and pea (Rodoni et al. 1997; Yang et al. 2004) that are consumed in a variety of food and feed sources which have a history of safe human consumption, establishing that plants, animals and humans are extensively exposed to these types of enzymes (Section V.E.1.1.2, p. 76).
- Bioinformatics analyses presented to US-FDA demonstrated that MON 87419 maize DMO does not share amino acid sequence similarities with known allergens, gliadins, glutenins or protein toxins which could have adverse effects to human or animal health (Section V.D, p. 75, and Section V.E.1.2, p. 77, US-FDA 2015; Monsanto 2015a).
- MON 87419 maize DMO is readily digestible in simulated gastric and intestinal fluids, according to Monsanto's submissions to US-FDA, making it highly unlikely that it would be absorbed in the small intestine and have any adverse effects on human or animal health (Section V.E.1.3, p. 77).
- An acute oral toxicity study previously conducted with DMO protein in MON 88701 cotton indicated no adverse effects in mice at the highest dose tested (283 mg/kg body weight), and by extrapolation using the Margin of Error approach, no meaningful risk to human or animal health from dietary exposure to MON 87419 maize DMO (Section V.E.1.4-5, p. 77).

As indicated earlier in this plant pest risk assessment, the petitioner's characterization of MON 87419 maize showed nutrient and anti-nutrient levels in grain and forage were comparable to the conventional control, and that the MON 87419 maize DMO protein makes up no more than approximately 0.00016% of the total protein in the grain that could be consumed, so there is unlikely to be nontarget effects resulting from changes in composition or from consumption of MON 87419 DMO. Also the study on

environmental interactions found that there were no changes in beneficial arthropod abundance in field plots of MON 87419 maize.

Honeybees were not among the arthropods sampled in the beneficial arthropod study and are not essential for maize pollination, with natural outcrossing rates in cultivated maize due predominantly to wind (Table IX-1, p. 166, Monsanto 2015a). Monsanto examined MON 87419 maize pollen and found there was no difference in pollen viability, size or visual morphology due to the dicamba and glufosinate herbicide-resistance trait (Section VII.C.3, pp.124-125). MON 87419 maize DMO is targeted to the chloroplast and is not expected to be found in nectar or pollen. Maize produces generous amounts of pollen that is visited by bees if there is no better forage available. Since the DMO protein has no known toxicity and is present at low levels in maize seed, no adverse effect on honeybees would be expected from such consumption.

As discussed in the previous section on *Potential Plant Pest and Disease Impacts*, Monsanto found that four beneficial arthropods (ladybird beetles, parasitic wasps, lacewings, syrphid flies, and spiders) were no more abundant on MON 87419 maize compared to the conventional control. Two beneficial arthropods had different abundances on MON 87419 compared to the control - spiders in greater amounts and minute pirate bugs in lesser amounts, but the differences were not consistently detected across collection methods (i.e., sticky traps and visual counts) and thus were not indicative of consistent responses associated with the trait and not considered biologically meaningful in terms of increased plant pest potential of MON 87419 compared to conventional maize.

Therefore, based on the above analysis of Monsanto's studies on MON 87419 maize food and feed safety, nutrient and anti-nutrient composition, levels of DMO in tissues, environmental interactions with beneficial arthropods, and pollen characteristics, APHIS concludes exposure to and/or consumption of MON 87419 maize are unlikely to have any adverse impacts on organisms beneficial to agriculture.

F. Potential for Enhanced Weediness of MON 87419 Maize

APHIS assessed whether MON 87419 maize is likely to become more weedy (i.e., more prevalent, competitive, damaging or difficult-to-control in situations where it is not wanted) than the nontransgenic progenitor from which it was derived, or other lines and hybrids of the crop currently under cultivation. The assessment considers the basic biology of the crop, the situations in which crop volunteers or feral populations are considered weeds, and an evaluation of MON 87419 maize compared to its progenitor maize or commercial reference hybrids evaluated under field conditions characteristic for the regions of the U.S. where maize is grown (and/or evaluated under laboratory or greenhouse conditions) for characteristics related to establishment, competiveness, reproduction, survival, persistence and/or spread that could influence weediness and the ability to manage the crop as a weed. For maize, such characteristics include, in particular, viable hard seed (dormant seed) and pre-harvest seed loss characteristics, stalk

and root lodging, and ear drop. The assessment also considers whether the engineered trait affects methods of control for the crop in situations where it is managed as a weed or volunteer in subsequent crops or in feral populations.

In the U.S., maize is not listed as a weed in the major weed references (Crockett 1977; Holm et al. 1979; Holm et al. 1997), nor is it designated as a noxious weed by the federal government (USDA-APHIS 2010), although it has been mentioned as an agricultural weed, arguably as volunteer plants, by the Southern Weed Science Society (USDA-NRCS 2016). Maize does not possess any of the attributes commonly associated with weeds (Baker 1965) such as long persistence of seed in the soil, the ability to disperse, invade, and become a dominant species in new or diverse landscapes, or the ability to compete well with native vegetation. Maize is unable to establish outside agriculture, as evidenced by the lack of reports of such behavior despite being one of the most widely cultivated grains in the world, and by data from controlled experiments where maize plantings left unharvested resulted in no feral plants within a year or two after planting (Monsanto 2009; Raybould et al. 2012; Sammons et al. 2014). Maize seeds are retained on the cob covered in a husk and are poorly dispersed, have no innate dormancy and are susceptible to low temperatures, although some seeds may overwinter and germinate when weather conditions allow; however germinating seedlings and plants are sensitive to cold and do not survive freezing winter conditions (Hoeft et al. 2000; OECD 2003; OGTR 2008; Andersson and de Vicente 2010). Although maize seed does not shatter, kernels are often scattered by harvest equipment or foraging wildlife, and some may survive to create volunteer plants the following year. Similar to conventional maize volunteers, herbicide-resistant maize volunteers can be managed by optimizing mechanical cultivation, crop rotation, and the careful selection of the modes of action for pre-emergent and post-emergent herbicides to balance competing herbicide sensitivities between volunteers and the rotational crop (Vencill et al. 2012).

To test the expectation that MON 87419 maize has not obtained characteristics that would increase its weediness, Monsanto conducted a combination of replicated laboratory, greenhouse and multi-site field experiments in 2013 and 2014, similar to the design of the compositional assessments previously discussed, which compared MON 87419 with the control to evaluate agronomic and phenotypic characteristics that may impact weediness (e.g., viable hard seed as an indication of seed dormancy, vegetative vigor as an indication of competitiveness, lodging and seed retention as indications of the potential for seed to occur on the soil following harvest and potentially volunteer in the subsequent crop) (Table VII-1, pp. 103-105, Monsanto 2015a). Multiple commercial reference hybrids were included in the assessment of weediness characteristics to provide a range of comparative values that are representative of existing commercial maize hybrids.

For seed germination and dormancy characteristics, the seed lots for 100 selfed F_2 grain from MON 87419 maize, the control, and eight reference hybrids were produced in replicated field trials during 2013 in Arkansas, Nebraska and Pennsylvania, which represent environmentally relevant conditions for maize production. The seed germination and dormancy characteristics analyzed included percent germinated, percent

viable hard seed, percent dead, and percent viable firm swollen seed. In addition to the Association of Official Seed Analysts recommended temperature range of 20/30°C (AOSA 2013), the seed was tested at six additional temperature regimes to assess seed germination properties, following the methods presented in Appendix F (pp. 286-292, Monsanto 2015a). The data were pooled among the three seed production sites in a combined site analysis (AOSA 2013; Table VII-2, p. 111, Monsanto 2015a):

- There were no viable hard seed detected at any germination temperature either for MON 87419 or the control.
- There were no biologically meaningful significant differences detected ($\alpha = 0.05$) between MON 87419 maize and the control for percent germinated, percent dead, and percent viable form swollen at any of the seven temperature regimes. No differences were outside the range of the eight commercial reference hybrids; all were small in magnitude.
- Germination rates were high for both MON 87419 and the control.

The presence of a hard seed coat is a characteristic that contributes to dormancy, and seed dormancy is an important characteristic often associated with plants considered to be weeds (Anderson 1996). MON 87419 maize seed as well as control seed exhibited no hard viable seeds and had high rates of germination under optimal conditions, which aligns with maize's long history of cultivation with no reports of seed dormancy or weediness.

Monsanto assessed 14 phenotypic and agronomic characteristics in 2013 across eight field sites with four replications to determine if MON 87419 maize was likely to be more weedy than the control, following the methods presented in Section V.II and Appendix G (pp. 101-126 and pp. 293-327, Monsanto 2015a). The same 14 characteristics were similarly assessed in 2013 and 2014 on dicamba and glufosinate herbicide-treated MON 87419 maize compared to the control, to assess MON 87419 under conditions of the intended agronomic use of the product.

- In the combined-site analyses, no statistically significant differences ($\alpha=0.05$) were detected between MON 87419 maize and the control or between dicamba and glufosinate herbicide-treated MON 87419 and the control for any of the 13 quantitatively assessed characteristics: early stand count, days to 50% pollen shed, days to 50% silking, stay green rating, ear height, plant height, dropped ears, stalk lodged plants, root lodged plants, final stand count, grain moisture, test weight, and yield (Tables VII-5 and VII-6, pp. 117-118).
- In the individual site analyses, a small number of comparisons (five out of 87 between MON 87419 and the control, and eight out of 97 between dicamba and glufosinate herbicide-treated MON 87419 and the control) were statistically significant, but none of these differences were detected in the combined-site analysis. Thus, none indicate consistent responses associated with the GE trait and therefore they are unlikely to be biologically meaningful (Tables G-7 and G-8, pp. 310-319).
- Plant vigor data were summarized as ranges within individual sites. MON 87419 and the control were considered different if the range of vigor values did not overlap across all four replications. There were no differences observed in plant

vigor at any site between MON 87419 maize compared to the control (Table G-7, p. 310) or between dicamba and glufosinate herbicide-treated MON 87419 maize compared to the control (Table G-8, p. 315).

The data show that neither the dicamba and glufosinate herbicide-resistance trait nor the dicamba and glufosinate herbicide treatment (including the DCSA and formaldehyde metabolites produced as a result) altered the weediness potential of MON 87419 maize compared to the conventional control based on the assessed phenotypic and agronomic traits.

APHIS evaluated information provided in the petition regarding dicamba and glufosinate herbicide use in the U.S. and control of volunteers of MON 87419 maize in rotational crops. Resistance of MON 87419 maize volunteers to dicamba and glufosinate would increase its survival compared to its conventional control in situations where it is treated with dicamba or glufosinate, e.g., in subsequent rotation with dicamba and or glufosinate-resistant maize or in fallow land or another crop such as soybean with a labeled application of dicamba. In crops that are normally treated with dicamba to control broadleaf weeds, should volunteers of MON 87419 maize appear, they could be controlled with other effective herbicides. Since Monsanto intends to commercialize MON 87419 maize as a stack with glyphosate-resistant Roundup Ready® Corn 2, glyphosate would also not be an effective herbicide for volunteer control. The petitioner proposed several labeled selective postemergence herbicides for the effective control of volunteer maize, including Assure II® (quizalofop), Fusilade® DX (fluazifop), Fusion® (fluazifop + fenoxaprop), Poast® (sethoxydim), and Select® 2EC (clethodim) (pp. 155-156, Monsanto 2015a). These herbicides are labeled for use in 12 vegetable rotation crops and 10 field crops that include soybean, hay (from grasses and alfalfa), and cotton. U.S. maize acreage is most frequently rotated the following year to soybean (57.1%), maize (29.7%), wheat (4.7%), cotton (2.0%), and alfalfa (1.4%), with other minor crops making up less than one percent of the estimated rotated acreage (p. 153, Monsanto 2015a). Pre-plant tillage and in-crop cultivation are also available to control volunteer maize. Controlling volunteer MON 87419 maize will be more challenging in replant maize fields than in typical rotational crops, mostly due to limited herbicide choices, but also due to reluctance to convert from no-till cropping to mechanical cultivation (Marquardt et al. 2012; PennState 2013). Maize to maize rotations increase the importance of early fall tillage to stimulate germination and emergence prior to winter to reduce emergence the following spring, and of later planting of the rotational crop to allow as much of the corn volunteers to germinate prior to the final pre-plant control measures (Monsanto 2010).

Based on the agronomic field data and literature survey concerning weediness potential of the crop, the dicamba and glufosinate herbicide-resistant MON 87419 maize is unlikely to persist as a troublesome weed or to have an impact on current weed management practices. Furthermore, extensive post-harvest monitoring of confined field trials from 2011 through 2014 in at least 160 locations across 19 states and territories covering a diverse range of environmental conditions representative of where maize is currently grown (Appendix A, Table A-1, pp. 190-191, Monsanto 2015a) did not reveal any differences in survivability or persistence relative to other hybrids of the same crop

currently being grown. These data suggest that MON 87419 is no more likely to become a weed than conventional hybrids of the crop. MON 87419 volunteers can be managed using a variety of currently available methods and alternative herbicides.

G. Potential Impacts on the Weediness of Any Other Plants with which MON 87419 Maize 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; Grant 1994; 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; Rieseberg 1997; Preston 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 (see Table 1 in Ellstrand *et al.* (1999). By providing fitness-related traits such as resistance to insects, diseases, herbicides or harsh growing conditions, gene flow from crops to their wild relatives could allow the hybrids to compete better, produce more seeds, and become more abundant (Snow 2002). Besides weediness, other concerns are the loss of herbicide resistance as a tool to protect crops from closely related weeds (Gepts and Papa 2003). This topic is covered in two sections: 1) the potential for gene flow, hybridization and introgression from MON 87419 maize 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 following introgression, based on the phenotypic changes that have been observed in the engineered plants.

Potential for gene flow, hybridization and gene introgression

Cultivated maize (or corn), *Zea mays* subsp. *mays*, is a member of the grass family Poaceae. The genus *Zea* has five species: *Z. mays*, *Z. diploperennis*, *Z. luxurians*, *Z. nicaraguensis*, and *Z. perennis*. *Zea mays* is further divided into four subspecies: *mays, huehuetenangensis, mexicana and parviglumis*. *Z. mays* subsp. *mays* is the only cultivated species of the genus *Zea*; the other species and subspecies are referred to as teosintes (OGTR 2008). Teosinte is a common name applied to several distinct wild, annual and perennial diploid and tetraploid taxa native to a region extending from Northern Mexico to Western Nicaragua and normally confined to the tropical and subtropical regions of Mexico, Guatemala, and Nicaragua (OGTR 2008; Andersson and de Vicente 2010).

Except for *Z. perennis*, teosintes can be crossed with cultivated maize to produce fertile first generation hybrids (Doebley 1990; OGTR 2008). There are barriers that reduce or prevent gene flow between maize and teosinte. For example, temporal and spatial factors isolate *Z. mays* subsp. *parviglumis* from maize, and there is some genetic incompatibility

between maize and *Z. luxurians* and *Z. mays* subsp *mexicana*. Experimental and molecular data suggests that maize and teosintes can hybridize when grown in close proximity, and hybridization occurs sporadically and at very low rates (Doebley 1990; Baltazar et al. 2005). On the other hand, *Z. mays* subsp *parviglumis* and maize can hybridize readily at higher rates (Ellstrand et al. 2007). Several features of teosinte inflorescences and pollen and the existence of incompatibility systems in teosintes may discourage pollination of teosintes by other taxa (Baltazar et al. 2005). Introgression between maize and teosintes is also limited by the geographical distribution of teosintes which have natural range limited to Mexico and certain parts of Central America.

A search of the Plants Database yielded results showing that *Zea mexicana* (Syn. *Z. mays* subsp *mexicana*) is listed as present in Florida, Alabama and Maryland, having been introduced from Mexico (USDA-NRCS 2015i); *Zea perennis* is listed in Texas and South Carolina (USDA-NRCS 2015f). *Zea diploperennis* and *Zea luxurians* are also listed, but there is no information about their location and status (USDA-NRCS 2015e, a). Experts familiar with the teosinte collections in the United States have been previously consulted and are not aware of the presence of any naturalized or native populations of teosintes currently growing in the United States (USDA-APHIS 2013). Therefore, introgression of MON 87419 maize into teosinte is unlikely in the U.S.

The genus most closely related to *Zea* is *Tripsacum*, a genus with 16 species. Plants in this genus are rhizomatous perennial grasses with geographical distribution extending from northern U.S. to Paraguay in South America. Some species are present as cultivated or wild species in the U.S.; *Tripsacum dactyloides*, *T. floridatum* and *T. laceolatum* occur in the continental U.S. (USDA-NRCS 2015b, h, g) and *T. fasciculatum* and *T. latifolium* occur in Puerto Rico (USDA-NRCS 2015d, c). *Tripsacum* species (2n=18) can be represented by diploid, triploid, tetraploid and higher ploidy levels. All species with the same ploidy levels can be crossed with *Zea* species (2n=20) under experimental lab conditions with difficulty and the hybrid offspring are sterile (Galinat 1988; OGTR 2008; Andersson and de Vicente 2010).

Maize is a predominantly outcrossing plant species via wind pollination. Insect pollination has not been reported. Maize cultivars and landraces are diploid plants (2n=20) that can crossbreed to a large degree. However, some evidence for genetic incompatibility exists within the species (e.g., popcorn x dent and Mexican maize landraces x Chalco teosinte crosses) (Wozniak 2002). There is a difference in floral synchrony between male (tassel) and female (silk) flowers on the same plant; the tassels begin shedding pollen before female flowers are receptive to fertilization. Typically tassels shed pollen for 2-14 days depending on environmental conditions. Because female flower development lags behind that of tassel and anthers with minimum overlap, the rate of self-pollination is only approximately 5% (Sleper and Poehlman 2006). Pollen viability has been variously described as lasting from 10-30 minutes (Coe et al. 1988) to up to 2 hours (Luna et al. 2001). Due to weight and diameter, most pollen grains are deposited within 60 feet of the source plant. Cross pollination between a donor field and receptor field can occur over a 7 day period (Coe et al. 1988; OGTR 2008). However,

adverse consequences of gene flow from MON 87419 maize to wild or weedy related species in the U.S. are highly unlikely.

Gene flow potential of MON 87419 maize was evaluated thoroughly. The introduced *dmo* and *pat* genes in MON 87419 maize are not expected to change the ability of the plant to interbreed with other plant species. Furthermore, the APHIS evaluation of data provided by Monsanto of agronomic and phenotypic properties of MON 87419 maize, including those characteristics associated with reproductive biology such as seed germination and dormancy, early stand count, days to 50% pollen shed, days to 50% silking, ear height, plant height, final stand count, grain moisture, test weight, yield and pollen morphology and viability indicated no unintended changes likely to affect the potential for gene flow from MON 87419 maize to sexually compatible species. The potential for gene flow to occur specifically between herbicide-resistant crop varieties and their sexually compatible relatives has been previously addressed (Mallory-Smith and Sanchez Olguin 2010). Gene flow does not differ whether the herbicide resistance trait is introduced via genetic engineering or via conventional breeding techniques, and gene flow has been occurring between non-GE maize and GE maize hybrids. Therefore, the potential for gene flow and introgression of the dicamba and glufosinate herbicide-resistant traits from MON 87419 maize to other maize hybrids and its consequences are anticipated to be similar to those as for existing commercial maize hybrids.

Many conditions have been identified that are required for gene flow and introgression to occur between a crop and its wild relatives (Carpenter et al. 2002; Jenczewski et al. 2003; Stewart et al. 2003; Owen 2005), including flowering synchrony, abundance and method of pollen spread, distance of pollen movement, genetic compatibility, and environmental conditions pertinent to cross-pollination, but the foremost condition is the presence of wild relatives within pollen or seed dispersal range from the crop. In the U.S., the lack of sexually compatible wild relatives of *Zea mays* ssp. *mays* precludes the opportunity for gene flow to occur between cultivated maize and its wild relatives. Based on the information presented in the petition and in relevant literature, APHIS has reached the following conclusions: The genetic modification in MON 87419 maize is not expected to increase the potential for gene flow, hybridization and/or introgression to sexually compatible taxa compared to the nontransgenic recipient or other hybrids of the crop commonly grown. Gene flow, hybridization and/or introgression of genes from MON 87419 to other sexually compatible relatives with which it can interbreed is not likely to occur in the U.S. and its territories.

Potential for enhanced weediness of recipients after hybridization and/or introgression

As described earlier, there is no indication that MON 87419 maize possesses a selective advantage that would result in increased weediness either in cultivated or unmanaged fallow fields. In the extremely unlikely event successful hybrids of cultivated maize and wild relatives were to occur in the U.S., the herbicide resistance trait would only provide selective advantage in situations in which the hybrid was in contact with the herbicide (i.e., in an agricultural or fallowed field or field edge). Any herbicide-resistant hybrid-derived populations are likely to be controlled using other available chemical or mechanical means. As discussed in the previous section, *Potential for Enhanced*

Weediness of MON 87419, many grass and/or broad spectrum herbicides that are effective for control of dicamba and glufosinate herbicide-resistant maize as volunteers would likely be effective for control of hybrids formed with other conventional maize or related species (p. 156, Monsanto 2015a).

APHIS concludes, based on the information presented in the petition and in relevant literature, that MON 87419 maize is not expected to increase the weed risk potential of other maize, nor of other species with which it can interbreed in the U.S. or its territories, as other sexually compatible species do not occur there. The genetic modification in MON 87419 maize is not expected to increase the potential for gene flow, hybridization and/or introgression to occur to sexually compatible taxa compared to the nontransgenic recipient or other hybrids of the crop commonly grown. It is highly unlikely that maize plants will be found outside of an agricultural setting. It is also highly unlikely that gene flow and introgression will occur between MON 87419 maize plants and sexually compatible relatives in a natural environment, since sexually compatible relatives do not occur in the U.S. Herbicides and other methods are available to control volunteer dicamba and glufosinate-resistant maize and other maize and *Zea* species with which it might cross.

H. Potential Changes to Agriculture or Cultivation Practices

APHIS assessed whether significant changes to agricultural or cultivation practices from adoption of MON 87419 maize 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.

The only agricultural or cultivation practices that are currently employed for maize production that are expected to change if MON 87419 is determined to be no longer subject to the regulatory requirements of 7 CFR part 340 are those related to weed management: in particular, choice of herbicide(s) or herbicide combinations, times of application, and potential crop choices or buffer zones for adjacent lands to avoid spray drift or volatilization to sensitive plants. Although dicamba and glufosinate spray drift and volatilization can potentially injure susceptible crops in proximity to MON 87419 maize, such impacts are not considered plant pest risks, but are assessed by the U.S. EPA as herbicide risks.

The current and proposed uses of dicamba and glufosinate in maize are described in the petition (Section VIII.F-J, pp. 133-158, Monsanto 2015a). Dicamba and glufosinate are currently approved for preplant and postemergence labeled uses on maize. Glufosinate use on MON 87419 will not change from current labeled uses of glufosinate. However, if EPA approves Monsanto's request to amend EPA Registration # 524-582, growers would be able to increase the maximum use rate of dicamba in maize from 0.5 lbs. to 1.0 lbs. a.e. per acre for preemergence applications and up to two applications of 0.5 lbs. a.e. of dicamba per acre for postemergence applications through the V8 growth stage or maize

height of 30 inches, whichever comes first, for a combined maximum annual application rate of 2.0 lbs. a.e. dicamba per acre per year on MON 87419 (p. 29, Monsanto 2015a). Monsanto will also request that EPA amend 40 CFR part 180 to revise tolerances for residues of dicamba and its relevant metabolites in or on maize. Issues related to herbicide drift and volatilization are further addressed in the Environmental Assessment (EA) APHIS prepared for this petition (USDA-APHIS 2016), consistent with its obligations under the National Environmental Policy Act (NEPA)(42 USC 4321-4370h).

Upon integration of MON 87419 maize into the Roundup Ready® Corn 2 system, aside from the anticipated label changes requested, Monsanto expects that growers will have the ability to continue to use established maize production practices including crop rotation, tillage systems, labeled herbicides, pest and disease management, row spacing, and planting and harvesting machinery currently being utilized (Section VIII.F.5, p. 151, Monsanto 2015a). The anticipated label changes would facilitate more effective use rates for dicamba, and are expected to provide a more effective tool for improved control of problem grass and broadleaf weed species (including some with resistance to other herbicides such as glyphosate, acetolactase synthase (ALS) and protoporphrinogen oxidase (PPO) chemistries) that can be integrated into weed management programs using no-till or reduced tillage or conventional tillage. Monsanto's anticipated weed management recommendations for MON 87419 maize combined with glyphosate-resistant Roundup Ready® Corn 2 also include a pre-emergence (burndown at planting) application of a residual herbicide alone or combined with dicamba in conventional tillage, or a residual herbicide combined with glyphosate or, in addition, dicamba in conservation tillage (Table VIII-7, p. 149, Monsanto 2015a). The impacts of this system for reducing or managing weeds and the evolution of herbicide-resistant weeds are addressed in the EA which APHIS prepared for this petition (USDA-APHIS 2016). Greater weed control could potentially reduce disease and pest pressure in maize if diseases and pests of the weeds also use maize as a host.

Crop rotation practices are not expected to be impacted by the use of dicamba on fields planted to MON 87419 maize. Crop rotation practices in maize were analyzed in the petition (Section VIII.G, pp. 151-154, Monsanto 2015a; and Tables 1 through 6 in Monsanto's supplemental data, Monsanto 2015b). Crops are grown in rotation for many reasons such as to manage weed, insect, and disease pests, reduce soil erosion, and improve soil organic matter, but maize is often grown following soybean because the biologically fixed nitrogen from the legume increases maize yields by about 10-15% in the U.S. corn belt (Singer and Bauer 2009). U.S. maize acreage is most frequently rotated the following year to soybean (57.1%), maize (29.7%), wheat (4.7%), cotton (2.0%), and alfalfa (1.4%), with other minor crops making up less than one percent of the estimated rotated acreage (p. 153, Monsanto 2015a). Dicamba can be absorbed through leaves and roots and translocated, but is considered only moderately persistent in soil, with a half-life of six days for dicamba acid under aerobic soil conditions with formation of the non-persistent degradate DCSA, and a half-life of 141 days under anaerobic soil conditions (US-EPA 2009). Crop rotation restrictions range from 30 to approximately180 days, depending on the rate applied, inches of rainfall and the following crop, according to the

Clarity® label (BASF 2010), and these should be adequate for rotation to other crops the spring following harvest of maize.

Changes in agricultural practices related to weed control are unlikely to adversely impact pest and disease management practices in maize and may provide some benefit by providing another tool for in-crop control of broadleaf weeds that may serve as alternative hosts for pests and diseases. As described above (see *Potential Plant Pest and Disease Impacts Impacts*), field studies on MON 87419 maize demonstrated that the herbicide resistance traits did not appear to alter the response of MON 87419 to abiotic stress, diseases, or arthropod pests under natural levels of these stressors, nor were pest arthropods more abundant around MON 87419 plots. Agronomic practices used to prepare and maintain each study site were characteristic of those used in each respective geographic region and all maintenance operations were performed uniformly over the entire trial area. Although pest and disease susceptibility data was not presented for MON 87419 stacked with the glyphosate-resistant trait, a recent review indicates that nether the glyphosate resistance trait nor glyphosate use in glyphosate resistant crops increases crop disease (Duke et al. 2012), and there is no evidence that either increase susceptibility to insect pests. Therefore, changes in agricultural practices related to weed control in MON 87419 or MON 87419 stacked with the glyphosate resistance trait are unlikely to adversely impact pest and disease control practices or any other cultivation and management practices in maize.

In conclusion, MON 87419 maize is similar to conventional maize in its agronomic, phenotypic, environmental response and compositional characteristics and has levels of pests and diseases or their damage levels comparable to conventional maize. The only changes in agricultural or cultivation practices that are anticipated with adoption of MON 87419 maize (including the anticipated stack with glyphosate-resistant Roundup Ready® Corn 2) are related to weed management practices. Anticipated changes in herbicide use patterns in MON 87419 maize alone or stacked with glyphosate-resistant Roundup Ready® Corn 2 are unlikely to increase pests or diseases or adversely impact their management, nor will they impact APHIS pest control programs.

I. Potential Impacts from Transfer of Genetic Information to Organisms with which MON 87419 Maize Cannot Interbreed

APHIS examined the potential for the new genetic material inserted into MON 87419 maize to be horizontally transferred to other organisms without sexual reproduction 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 have been 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 (Brown 2003; Keeling and Palmer 2008; Keese 2008).

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

MON 87419 maize contains protein coding regions derived from the *pat* gene from the bacterium *Streptomyces viridochromogenes*, the *dmo* gene from the bacterium *Stenotrophomonas maltophilia* and the transit peptide from petunia (*Petunia hybrida*) for chloroplast targeting of the DMO protein in MON 87419 maize. It also contains non-protein-coding regions from *Agrobacterium tumefaciens*, grasses (big bluestem, rice, and wheat) and the plant virus peanut chlorotic streak caulimovirus. One example of HGT involves a class of enzymes similar to DMO. Chakraborty et al. (2012) propose that HGT contributed to the distribution of ring-hydroxylating oxygenase (*rho*) genes among prokaryotic phyla (proteobacteria, actinobacteria, cyanobacteria, and archaea), and note that homologues of *rho* genes are found in plants (in strains of *Arabidopsis, Zea mays, Oryza sativa, Physcomitrella patens and Amaranthus tricolor*). Ring-hydroxylating oxygenases (RHO) catalyze the addition of hydroxyl groups to aromatic ring compounds, initiating one of the major pathways for oxidative degradation of both natural and synthetic aromatic compounds in the environment (Peng et al. 2010). Dicamba mono-oxygenase is a unique type of RHO that initiates the degradation of dicamba by oxygenating the exocyclic methyl group, rather than the more conventional oxygenation of the aromatic ring of the substrate seen in most other RHOs (Dumitru et al. 2009). Chakraborty et al. (2012) suggest that distribution and diversification of *rho* genes can be explained by the mechanisms of gene duplication, transposition events and DNA rearrangements in most cases. In other cases, HGT is assumed to be the primary mechanism where occurrence of the genes was found to be limited to just one or two organisms within phyla (such as *rho* genes in some cyanobacteria, firmicutes and crenarchaeota), since the possibility of being remnants of a partially deleted *rho* operon is ruled out due to the absence of similar genes in any other member of these genera. Although it is widely accepted that HGT has generated novel degradation capabilities and increased metabolic diversity among bacterial communities exposed to an ever-evolving array of polycyclic aromatic compounds, such degradative capabilities are mostly indicative of divergent evolution from a common ancestor, not HGT (Peng et al. 2010).

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; Acuna 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 sp.* and *Rhizobium sp.* (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 (Koonin et al. 2001; Brown 2003; EFSA 2009). 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 (US-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 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 (US-EPA 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 U.S. (Fuchs and Gonsalves 2007).

The only virus sequence inserted in MON 87419 maize is the promoter for the peanut chlorotic streak virus (PCSV) involved in regulating gene expression. Maize is considered susceptible to PCSV (Brunt et al. 1996). PCSV belongs to the Caulimovirus family of pararetroviruses, double-stranded DNA viruses in which replication occurs in the cytoplasm via reverse transcription of an RNA intermediate. Caulimoviruses generally have a narrow host range (Hansen and Heslop-Harrison 2004). The only other Caulimovirus that maize is susceptible to is a maize chlorotic mottle caulimovirus (SbCMV) (Brunt et al. 1996). Neither of these viruses are considered widely prevalent in the United States (University of Georgia 2012); therefore exposure of either of these two viruses to the PCSV sequences in MON 87419 maize is expected to be low or unlikely. Moreover, recombination in Caulimoviruses occurs predominantly, if not exclusively, in the cytoplasm by template switching between RNA transcripts during the replication process, although a low level of recombination involving viral DNA may occur in the nucleus (Froissart et al. 2005). Since the Caulimovirus promoter sequences are not transcribed in transgenic plants, there is little or no opportunity for them to recombine with any related Caulimoviruses that may infect maize. Although TEV occurs in the United States and is considered widely prevalent (Froissart et al. 2005), since maize is not susceptible to this virus, it is unlikely that TEV would be exposed to sequences from TEV in MON 87419. Since the TEV sequence in MON 87419 is a 5' non-translated region, even if recombination were to occur with another related potyvirus that infects maize, it is unlikely to encode a peptide. Based on the foregoing, horizontal transfer of DNA from MON 87419 maize to plant viruses is unlikely to occur or is unlikely to lead to the creation or selection of plant viruses that are more virulent or have a broader host range.

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). Recently, 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 (Yoshida et al. 2010). 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. More recent 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 the GE crop, the DNA sequences were inserted into the nuclear genome, not the mitochondrial genome.

If the GE plant 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 the GE plant. 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 87419 maize to other organisms with which it cannot interbreed 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, public comments in response to the Federal Register notice concerning this petition and other relevant information to assess the plant pest risk of MON 87419 maize compared to the unmodified line from which it was derived and other maize reference hybrids. APHIS concludes that MON 87419 maize 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 87419: the *Agrobacterium* transformation vector was disarmed, transformed material was treated to kill the bacterium, and the plant pest sequences inserted do not cause disease or create an infectious agent.
- No increase in plant pest risk was identified from expression of the inserted genetic material, the new MON 87419 DMO or PAT proteins, or changes in metabolism or composition. The composition of MON 87419 grain and forage were determined to be substantially equivalent to other maize commercially grown and the mode of action and specificity of MON 87419 DMO and PAT raise no plant pest concerns.
- Disease and pest incidence and/or damage were not observed to be significantly increased or atypical in MON 87419 compared to the nontransgenic counterpart in field trials conducted in growing regions representative of where this maize is expected to be grown**.** The dicamba and glufosinate resistance traits did not significantly alter the response of MON 87419 to diseases or arthropod pests under natural levels of these stressors, and pest arthropods were not more abundant around MON 87419 plots compared to the control line. Observed agronomic traits also did not reveal any significant differences that would indirectly indicate that MON 87419

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 87419 maize is unlikely to have any adverse impacts on organisms beneficial to agriculture based on APHIS' analysis of studies on MON 87419 maize food and feed safety, nutrient and anti-nutrient composition, levels of DMO and PAT in tissues, environmental interactions with beneficial arthropods, and pollen characteristics.
- MON 87419 maize is unlikely to become more of a weed or volunteer problem than other conventional or commercial maize hybrids based on its observed agronomic characteristics, the low weediness potential of maize and current management practices available to control MON 87419 as a weed. MON 87419 volunteers, although resistant to dicamba and glufosinate, can still be controlled with other currently available weed control methods.
- MON 87419 is not expected to increase the weed risk potential of other maize, and other species with which it can interbreed do not naturally occur in the U.S. or its territories. The genetic modification in MON 87419 maize is not expected to increase its potential for gene flow, hybridization and/or introgression to sexually compatible taxa, nor is it likely to increase their weediness potential in the event that such species were to be introduced. Introgression of the dicamba and glufosinate resistant traits into other maize or related species will likely make them resistant to dicamba and glufosinate herbicides, but other currently available weed control methods could be used for their control.
- Changes to agricultural or cultivation practices (e.g., pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of MON 87419 maize (including the anticipated stack with glyphosate-resistant Roundup Ready® Corn 2) are only related to weed management practices and herbicide use patterns, and these are unlikely to increase pests or diseases or adversely impact their management, nor will they impact APHIS pest control programs.
- Horizontal gene transfer of the new genetic material inserted into MON 87419 maize 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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