Monsanto Petition (14-213-01p) for Determination of Non-regulated Status for Increased Ear Biomass MON 874Ø3 Maize.

OECD Unique Identifier:
MON-874Ø3-1

Plant Pest Risk Assessment

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A. Introduction

Monsanto Company (hereafter referred to as Monsanto) has petitioned the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) for a determination that the genetically engineered (GE**)** maize with increased ear biomass and OECD Unique Identifier MON 874Ø3-1 (hereafter referred to as MON 874Ø3 maize) is unlikely to pose a plant pest risk and, therefore, should no longer be a regulated article under the APHIS' 7 Code of Federal Regulations (CFR) part 340 (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). This petition was assigned the number 14-213-01p, and is hereafter referred to as Monsanto, 2014. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act (PPA) of 2000 (7 U.S.C. 7701 *et seq*.)[1] This plant pest risk assessment was conducted to determine if MON 874Ø3 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 part 340.2 and meets the definition of plant pest, or is an unclassified organism and/or an organism whose classification is unknown, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the Administrator determines is a plant pest or has reason to believe is a plant pest[2]. MON 874Ø3 maize event was produced by the *Agrobacterium*-mediated transformation of immature maize embryos from inbred line LH244 using plasmid PV-ZMAP5714 (Monsanto, 2014, pp. 31-38). Portions of the introduced genetic sequences were derived from plant pest organisms listed in 7 CFR part 340.2 (i.e., the T-DNA left and right border sequences derived from *Agrobacterium tumefaciens* and regulatory sequences derived from *Cauliflower mosaic caulimovirus*) (Monsanto, 2014, Table IV-1, pp. 45-46). Therefore, MON 874Ø3 maize is considered a regulated article under APHIS regulations at 7 CFR part 340. Monsanto has conducted introductions of MON 874Ø3 maize as a regulated article under APHIS authorizations since 2007 (Monsanto, 2014, Appendix A, pp. 147 - 152), in part, to gather information to support that MON 874Ø3 maize is unlikely to pose a plant pest risk.

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

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

Potential impacts addressed in this Plant Pest Risk Assessment (PPRA) are those that pertain to plant pest risk associated with MON 874Ø3 maize and its progeny and their use in the absence of confinement, relative to the unmodified recipient variety and/or other appropriate comparators. APHIS used data and information submitted by the applicant, in addition to current literature, to determine if MON 874Ø3 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 assessed information submitted by the applicant about MON 874Ø3 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 Food and Drug Administration (FDA), and the Office of Pesticide Programs of the U.S. Environmental Protection Agency (EPA). Depending on its characteristics, certain biotechnology-derived products are subjected to review by one or more of these agencies.

EPA regulates under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*) the distribution, sale, use and testing of pesticidal substances produced in plants and microbes, including those pesticides that are produced by an organism through techniques of modern biotechnology. EPA also sets tolerance limits for residues of pesticides on and in food and animal feed, or establishes an exemption from the requirement for a tolerance, under the Federal Food, Drug and Cosmetic Act (FFDCA) (21 U.S.C. Chapter 9). Prior to registration for a new use, or for a new or previously registered pesticide, EPA must determine through testing that the pesticide does not cause unreasonable adverse effects on humans, the environment, and non-target species when used in accordance with label instructions. EPA must also approve the language used on the pesticide label in accordance with 40 CFR part 158 (Data Requirements for Pesticides). Other applicable EPA regulations include 40 CFR part 152 (Pesticide Registration and Classification Procedures), part 174 (Procedures and Requirements for Plant Incorporated Protectants (PIPs) and part 172 (Experimental Use Permits). Maize event MON 874Ø3 is not engineered to express substances to protect it against plant pests, and is therefore not subject to EPA review.

The FDA under the FFDCA is responsible for ensuring the safety and proper labeling of all plant-derived foods and feeds, including those developed through modern biotechnology. To help sponsors of foods and feeds derived from genetically engineered crops comply with their obligations, the FDA encourages them to participate in its voluntary early food safety evaluation for new non-pesticidal proteins produced by new

plant varieties intended to be used as food (FDA, 2006), and a more comprehensive voluntary consultation process prior to commercial distribution of food or feed (57 FR 22984, 1992). Monsanto states in the petition (Monsanto, 2014, p. 26) that they would be submitting a "food/feed safety and nutritional assessment summary document to FDA in the near future", and this document was submitted on October 24, 2014.

B. Development of Increased Ear Biomass MON 874Ø3 Maize

Zea mays subsp. *mays* L., commonly referred to as maize or corn, belongs to the grass family Poaceae (OECD, 2003). It is the most widely cultivated cereal crop in the world and the three major producers are the United States, China and Brazil. In the United States maize is grown in all 48 states, and the major production area is the so called Corn Belt (Figure 1), which is usually defined as encompassing Iowa, Illinois, Indiana, Minnesota, Nebraska, Ohio, Wisconsin and South and North Dakota, but other states are sometimes included. The total estimated area planted to maize in the United States was 95.4 and 90.6 million acres in 2013 and 2014, respectively (USDA-NASS, 2015a). The average corn yield was estimated at 158.1 bushels per acre for 2013 and 171 bushels per acre for 2014 (USDA-NASS, 2015b) .

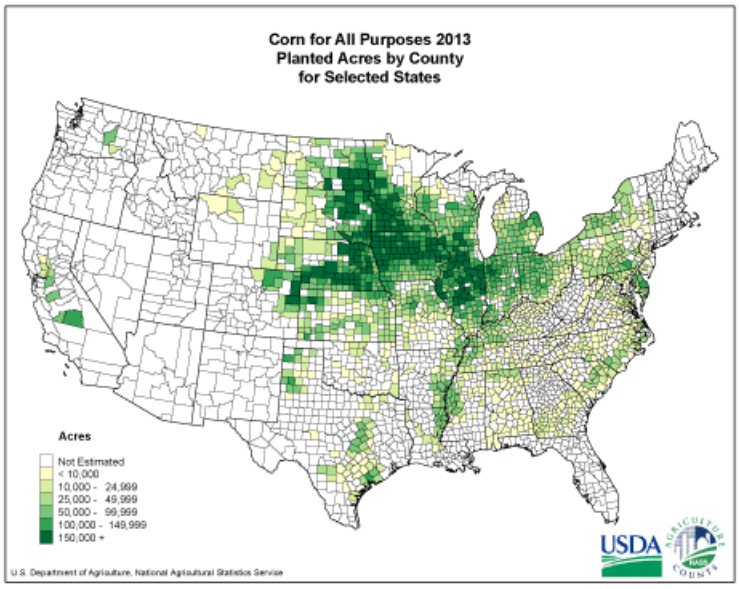

Figure 1. Maize (corn) production in the United States (USDA-NASS, 2015a).

Crop productivity worldwide varies from year to year and is impacted by losses due to abiotic factors (irradiation, 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 varieties resistant to pests and diseases. Increases in crop yield per unit of area have

been attributed to efficient control of biotic stress rather than to an increase in yield potential (Oerke, 2006).

Average maize yield in the United States changed little from 1866 to 1930 and then increased steadily from 1930 to 2000 (the hybrid era) (Tollenaar and Lee, 2004; Egli, 2008). Increases in grain yield during the hybrid era have been considered to be the result of the interaction of improvements in genetics of the plant obtained by plant breeding and improved agronomic practices (Tollenaar and Lee, 2004). Changes leading to genetic improvements through maize plant breeding and associated with higher yields include the use of hybrids, longer seed-filling periods, tolerance to high plant density, improved stay-green characteristics (delayed senescence) during seed filling, more upright leaves, decrease in protein concentration and increase in stress tolerance (Egli, 2008; Liu and Tollenaar, 2009). On the other hand, several changes in crop management practices have also led to increased maize productivity and higher yields: increased use of high yielding varieties and earlier planting; narrower rows and higher plant populations; increase in the area planted; higher rates of fertilizer; herbicide use and better weed control; and mechanization (Oerke, 2006; Egli, 2008). However, increased tolerance to high plant density resulted in an increase in the number of ears per hectare (Bruns and Abbas, 2003) and in a net increase in grain yields, but a decrease in both grain yield per plant (Liu and Tollenaar, 2009) and ear size at the individual plant level (Monsanto, 2014, p.25).

The physiological determinants responsible for genetic gain in maize grain yield have been associated with improved kernel number, enhanced post silking biomass production and biomass allocation to reproductive sinks (Liu and Tollenaar, 2009). Maize grain yield is a function of total assimilates (biomass) produced by the source tissue and the fraction of assimilates partitioned to the sink, the ear tissue (Lee and Tollenaar, 2007).
The maximum sink size is determined during early development at early reproductive stages and is influenced by environmental conditions and plant genetics. This potential can be fulfilled when plants are grown under conditions of adequate assimilate availability during the late grain filling stage (Jones *et al.*, 1996; Borrás and Westgate, 2006; Severini *et al.*, 2011; Monsanto, 2014, p.155). Early stages of maize kernel development are important for establishing kernel sink capacity and final kernel weight (Borrás and Westgate, 2006). Grain yields in maize may be improved by genetic improvements in photosynthate distribution by increased partitioning of dry matter to the ear at flowering to provide increases in grain yield (number and size) at harvest (Fisher and Palmer, 1983; Severini *et al.*, 2011). Severini *et al.* (2011) showed that in general, maize genotypes and plant density and source (leaf) treatments with higher kernel numbers per plant and more vigorous plant growth rates around flowering also had the highest ear biomass at 14 days after anthesis. Greater ear biomass during the early reproductive stages provides increased yield opportunity in maize (Monsanto, 2014, p.155) .

MON 874Ø3 maize was developed by Monsanto to have increased ear biomass at an early reproductive stage (R1) for increased yield opportunity. Recombinant DNA methods were used to generate MON 874Ø3 using LH244 as the recipient yellow dent inbred maize line. The *Agrobacterium*-mediated transformation method was used to

insert into LH244 gene expression cassettes that include the coding region of the full-length *ATHB17* gene from *Arabidopsis thaliana* (Monsanto, 2014, Section II.B, p. 29) ATHB17 is a protein of the class II family of the homeodomain-leucine zipper (HD-Zip) transcription factors. HD-Zip proteins are found in many plant species and are important in the modulation of plant growth and development and regulate gene expression (Ariel *et al.*, 2007; Monsanto, 2014, Section I.B., p. 24).

The conventional control materials used as comparators in safety assessment studies were chosen to have similar genetic backgrounds as MON 87403 and its advanced generation hybrids used in these studies (Monsanto, 2014, Fig. IV-5, p. 48). They include the original LH244 line used for transformation, and LH244 crossed to the same two conventional lines, LH287 and LH295 that were used to make F1 hybrids with advanced generations of MON 87403. For molecular characterization studies both near isogenic conventional control hybrids MPA640B (LH244 X LH287) and EXP257 (LH244 X LH295) were used. MPA640B was used as the conventional control in compositional analysis studies and in phenotypic, agronomic and environmental interactions assessments. Commercial hybrid maize materials (reference hybrids) were also used to establish a range of variability or responses representative of commercial maize in the U.S. (Monsanto, 2014, Section II.C, pp.29-30).

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 sources of the inserted genetic material and its function in both the donor organism and the MON 874Ø3 maize event; and the integrity, stability and mode of inheritance of the inserted genetic material through sexual reproduction based on the location of the insertion (i.e. in the nucleus) 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 874Ø3 maize relative to the nontransgenic counterparts. The assessment encompasses a consideration of the expressed protein ATHB17Δ113 and any observed or anticipated effects on plant metabolism including any relevant changes in levels of metabolites, antinutrients, or nutrients in grain or forage derived from the MON 874Ø3 maize event compared to the conventional controls, the original recipient line LH244, and near isogenic conventional control hybrids MPA640B and EXP257, as well as other comparators.

This information is used later in this risk assessment to inform whether there is any potential for plant pest vectors or sequences to cause disease or greater plant pest risks in MON 874Ø3; 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 pest or diseases or their management, or plant pest risks through horizontal gene flow.

Description of the genetic modification and inheritance of inserted DNA

MON 874Ø3 maize was developed using *Agrobacterium tumefaciens* for the transformation of immature maize embryos (Sidorov and Duncan, 2009) and insertion of the coding region of the full-length *ATHB17* gene from *Arabidopsis thaliana*.

Disarmed *Agrobacterium tumefaciens* strain ABI, a designated plant pest, carrying the plasmid vector PV-ZMAP5714 (Monsanto, 2014, Figure III-1, p. 33) (described below) was used for transformation of maize embryos (Monsanto, 2014, Figure III-2, p. 34). A full description of the genetic elements in PV-ZMAP5714 is also provided in the petition Table III-1 (Monsanto, 2014, pp. 36-38).

PV-ZMAP5714 (approx.11.7 kb) contains three cassettes: one T-DNA element containing the *ATHB17* expression cassette between the Left and Right border regions; the plasmid backbone with the *cp4 epsps* selectable marker cassette and the *aadA* expression cassette. Various short intervening sequences are also present in the vector to facilitate cloning (Monsanto, 2014, Table III-1, p. 36), however they are not included in the description below.

The T-DNA contains the *ATHB17* expression cassette with the following genetic elements:

- Right Border - DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA (Depicker *et al.*, 1982; Zambryski *et al.*, 1982).
- P -*e35s/Ract1* - Chimeric promoter consisting of the duplicated enhancer region from the *Cauliflower mosaic virus* (CaMV) 35S RNA promoter (Kay, 1987) combined with the promoter of the *act1* gene (encodes Actin 1) from *Oryza sativa* (rice) (McElroy *et al.*, 1990) to direct transcription in plant cells.
- L -*Cab* – The 5´ untranslated region (UTR) leader sequence of the chlorophyll a/b-binding (CAB) protein from *Triticum aestivum* (wheat) involved in regulating gene expression (Lamppa *et al.*, 1985).
- I -*Ract1* - Intron and flanking UTR sequence of the *act1* gene from *Oryza sativa* (rice) encoding the Actin 1 protein, involved in regulating gene expression (McElroy *et al.*, 1990).
- CS -*ATHB17* - Coding sequence of the *ATHB17* gene from *Arabidopsis thaliana* encoding a member of the class II homeodomain-leucine zipper gene family (HD-Zip II) that is thought to act as a transcription factor (Ariel *et al.*, 2007).
- T -*Hsp17* - The 3´ UTR from the gene for the heat shock protein Hsp17 of *Triticum aestivum* (wheat) (McElwain and Spiker, 1989) that directs polyadenylation of the mRNA.
- Left Border Region - DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker *et al.*, 1983).

The backbone region of PV-ZMAP5714 contains genetic elements important for the maintenance of the plasmid vector in bacteria: two origins of replication *ori-V* and *ori-pUC*), the expression cassette for a bacterial selectable marker gene (*aadA*) and a coding sequence (*rop*) for repressor of primer (ROP) protein to maintain plasmid vector copy number in *Escherichia coli*. The backbone also contains the *cp4 epsps* expression cassette. The backbone genetic elements in PV-ZMAP5714 are listed below:

- P -*Ract1* - promoter and leader of the *actI* gene from *Oryza sativa* (rice), encoding the rice Actin 1 protein (McElroy *et al*., 1990) that directs transcription in plant cells.
- I -*Ract1* - Intron and flanking UTR sequence of the *act1* gene from *Oryza sativa* (rice) encoding rice Actin 1 protein (McElroy *et al.*, 1990). This sequence is involved in regulating gene expression.
- TS -*CTP2* - Targeting sequence of the *ShkG* gene from *Arabidopsis thaliana* encoding the EPSPS transit peptide region that directs transport of the protein to the chloroplast (Klee, 1987; Herrmann, 1995).
- CS -*cp4-epsps* – Coding sequence of the *aroA* gene from *Agrobacterium sp*. strain CP4 encoding the CP4 EPSPS protein that provides glyphosate tolerance (Padgette *et al.*, 1996; Barry *et al.*, 2001).
- T -*nos* - 3' UTR sequence of the *nopaline synthase* (*nos*) gene from *Agrobacterium tumefaciens* pTi encoding NOS (Bevan *et al.*, 1983; Fraley *et al.*, 1983) that directs polyadenylation of the mRNA.
- OR -*ori V* - Origin of replication from the broad host range plasmid *RK2*, used for maintenance of plasmid in *Agrobacterium* (Stalker *et al.*, 1981).
- CS -*rop* - Coding sequence for repressor of primer protein from the ColE1 plasmid for maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989).
- OR -*ori-pUC* - Origin of replication from plasmid pUC for maintenance of plasmid in *E. coli (*Vieira and Messing, 1984).
- *aadA* - Bacterial promoter, coding sequence, and 3' UTR for an aminoglycoside-modifying enzyme, 3"(9)-*O*-nucleotidyltransferase from the transposon Tn7 (Fling *et al.*, 1985). This sequence confers spectinomycin and streptomycin resistance.

Marker-free plants were generated using the binary plasmid PV-ZMAP5714 in a tandem T-DNA approach for separate, unlinked insertion of the T-DNA carrying the *ATHB17* gene between the right and left T-DNA borders and the *cp4 epsps* selectable marker gene located outside the T-DNA in the plasmid backbone. After co-culturing the embryos with *A. tumefaciens* the embryos were placed on selection medium with glyphosate to select for transformed lines and with the antibiotic carbenicillin disodium salt to inhibit growth of excess *A. tumefaciens*. Transformed callus was placed on media conducive to shoot and root development and selected rooted plants (R_0) were transferred to soil. The *cp4 epsps* cassette was then segregated away by conventional breeding. R_0 plants with the *cp4 epsps* expression and T-DNA cassettes were self-pollinated to produce R_1 seed and plants, and molecular analysis was used to identify plants containing only the intended T-DNA and not the *cp4 epsps* (Huang *et al.*, 2004). R_1 plants homozygous for the T-DNA

and negative for the *cp4 epsps* expression cassette were identified by polymerase chain reaction (PCR) and selected for further development, with line MON 874Ø3 eventually selected as the lead line for commercialization (Monsanto, 2014, p. 31-32).

MON 874Ø3 was characterized using a combination of sequencing, PCR and bioinformatics methods. Data from molecular characterization of MON 874Ø3 using Next Generation Sequencing and Junction Sequence Analyses (NGS/JSA), directed DNA sequence analysis and bioinformatics analysis, provided and reviewed by APHIS, demonstrated that:

- There is a single copy of the T-DNA containing the *ATHB17* expression cassette insert in MON 874Ø3. Additionally, no sequences from the vector backbone or other unintended plasmid sequences are present in MON 874Ø3 (Monsanto, 2014, Section IV pp. 35, 39, Section IV.A, Tables IV-1 and IV-2, Figure IV-4, pp 43-51).
- The insert is 3,132 bp, no re-arrangements were observed and the sequence and organization of the insert are identical to the corresponding T-DNA of PV-ZMAP5741, except for small terminal deletions in both border regions which are otherwise identical in sequence in PV-ZMAP5741 (Monsanto, 2014, Section IV.B, Figure IV-7, pp. 52-53).
- Sequence analysis of over 1.2 kb flanking each end of the insertion site showed that the 5' and 3' genomic DNA sequence flanking the insert in MON 874Ø3 is identical to the corresponding region of the conventional control maize, with no major rearrangements except for a 149 base pair deletion of maize genomic DNA at the insertion site in MON 874Ø3 (Monsanto, 2014, Section IV.B Figure IV-7, Section IV.C, Figure IV-8, pp. 54-55). In the petition Monsanto states that this deletion is not expected to affect food or feed safety (Monsanto, 2014, Section IV.F, p. 61). Such deletions can occur during plant transformation and are presumed to be due to plant double-stranded DNA break repair mechanisms during the Agrobacterium-mediated transformation process (Salomon and Puchta, 1998).
- The generational stability of the introduced *ATHB17* gene was determined by NGS/JSA analyses for several generations of MON 874Ø3 (Monsanto, 2014, Section IV.D, Table IV-3, p. 56-57). Analysis of phenotypic and genotypic data of MON 874Ø3 maize segregating progeny indicated that the MON 874Ø3 T-DNA resides at a single locus within the maize genome and is inherited according to Mendelian ratios as confirmed by Chi-square analysis (Monsanto, 2014, Sections IV.D and IV.E. Tables IV-3 and IV-4, figure IV-9, pp.56-60)

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

MON 874Ø3 maize was genetically modified to contain the coding region of the full-length *ATHB17* gene from the plant species *Arabidopsis thaliana*. ATHB17 is a protein of the class II subfamily of the homeodomain-leucine zipper (HD-Zip) transcription factors. HD-Zip proteins are found in many plant species and are unique to the plant kingdom; they regulate gene expression and are important in the modulation of plant growth and development. Proteins in this family are classified into four subfamilies or classes: I, II, III and IV (Ariel *et al.*, 2007), and can form homodimers or heterodimers

with other HD-Zip proteins of the same class. Many have been shown to function as repressors of gene expression and to down regulate HD-Zip family gene transcription (Monsanto, 2014, Appendix B., p. 157). Class II HD-zip proteins have functions in response to light conditions, shade avoidance and response to auxins (Ariel *et al.*, 2007), and some have been reported to be involved in regulation of reproductive growth and development (Meijer *et al.*, 1997). Up to 55 non-redundant HD-Zip genes have been identified in the maize genome (Zhao *et al.*, 2011), and systematic bioinformatics analysis has identified 18 HD-Zip II genes in maize (Zhao *et al.*, 2011). Evaluations of the expression patterns of HD-Zip II genes in two different maize hybrids were performed to study the involvement of the HD-Zip II proteins in the regulation of ear growth. The results showed that HD-Zip II genes were expressed in all sampled maize tissues and across developmental stages and eight genes were predominantly expressed in the ear tissue. This suggested that they might be involved in regulation of plant reproductive and ear growth (Monsanto, 2014, Section B.9, p.160; Rice *et al.*, 2014).

HD-Zip proteins have a putative repression domain adjacent to a homeodomain (responsible for DNA binding), a leucine zipper (acts as a dimerization motif) and a carboxy terminal domain. High sequence variability in other gene regions has been reported (Zhao *et al*, 2011). Additionally to those four domains ATHB17 also has a unique N-terminus that is involved in the regulation of its cellular localization, and was shown to function as a transcriptional repressor (Monsanto, 2014, p. 157; Rice *et al.*, 2014).

According to information in the petition (Monsanto, 2014, p. 157) and Rice *et al.* (2014), maize-specific splicing of the *ATHB17* transcript in MON 874Ø3 results in the expression of a truncated protein, ATHB17Δ113, that lacks the first 113 N-terminal amino acids (part of the repression domain) and has a molecular weight of approximately 22 kilodaltons (kDa) instead of the predicted 32 kDa for the full length ATHB17 protein. The truncated protein does not function as a transcriptional repressor, but retains the ability to form homo and heterodimers with endogenous maize HD-Zip II proteins and bind to target DNA (Monsanto, 2014, Appendix B., pp. 157-158, Figure B-4, p.158). The likely action of ATHB17Δ113 is to attenuate the repressor activity of endogenous HD-Zip II proteins through a dominant-negative mechanism. This can occur through the formation of non-functional homodimers or of less active heterodimers that compete for DNA binding sites (Monsanto, 2014, Section B.8 and B.9, pp. 158-160, figure B-5 p.159). Experimental evidence described in the petition suggests that ATHB17Δ113 can function as a regulator of endogenous HD-Zip proteins that are transcriptional repressors. ATHB17Δ113 likely modulates HD-Zip II regulated pathways in the ear, which leads to increased biomass partitioning to the ear and increased ear biomass at an early reproductive phase (R1) in MON 874Ø3, compared to the maize plants used as controls, according to the data provided in the petition (Monsanto, 2014, p. 162; Rice *et al.*, 2014).

Monsanto measured biomass of the top-most ear (including the husk, shank, cob, silk and ovules) and remaining stover biomass at the R1 growth stage in MON 874Ø3 and in conventional control plants with the same genetic background grown at 13 field locations within maize production regions of the United States in 2012 (Monsanto, 2014, Table I-1,

pp. 25-26 and Section B.10 pp. 160-162, Table B-1 p. 161). In the combined site analysis there was a statistically significant increase (11.7%) in R1 ear biomass in MON 874Ø3 compared to the conventional controls. Biomass partitioning to the ear, calculated as the ratio of ear biomass to total biomass (ear plus stover) was also increased significantly (10%), however, no statistically significant differences were observed in R1 stover and total biomass between MON 874Ø3 and the control (Monsanto, 2014, Table B-1 p.161). A study conducted in the greenhouse compared biomass in MON 874Ø3 to conventional controls at several growth stages and statistically significant increase in ear biomass was observed in MON 874Ø3. According to information in the petition this increased ear growth is associated with increased partitioning of dry matter (photosynthate) from the source (vegetative) tissue to the sink (ear) tissue in MON 874Ø3 compared to control plants (Monsanto, 2014, Section B.10, pp. 161-162).

MON 874Ø3 expresses the ATHB17Δ113 protein, which consists of a single polypeptide chain of 162 amino acids (Monsanto, 2014, Section V.A.2., p. 64). Bioinformatics analyses of the amino acid sequence of ATHB17Δ113, identified homologous sequences from several food plants, and sequence identity ranged from approximately 58 to 83%. The data also indicated that ATHB17Δ113 does not have structural similarity to known allergens, gliadins, glutenins or protein toxins that may have adverse effects on human or animal health (Monsanto, 2014, pp. 69-70).

To determine compositional equivalence between MON 874Ø3 and conventional maize, Monsanto conducted analysis of the composition of mature harvested grain and forage (collected at early dent, R5 stage) from MON 874Ø3, MPA640B (a conventional control with the same genetic background) and 17 conventional commercial reference maize hybrids (Monsanto, 2014, Sections VI. pp. 73-92, IX.C. pp. 124 and Appendix F, pp. 196-211). The samples were collected from plants grown during 2012 in the United States in eight replicated geographically varied field sites under agronomic conditions typical for maize production. Compositional analyzes were performed for 78 components, (nine in forage and 69 in grain) encompassing key nutrients, antinutrients and secondary metabolites. For 16 of the 78 components, more than 50% of observations were below the assay limit of quantitation and were therefore excluded from statistical analysis. Sixty components were statistically assessed from the combined site analysis, and no significant differences were found between MON 874Ø3 and the conventional control, and the mean component values for MON 874Ø3 were within the calculated 99% tolerance intervals for the reference maize hybrids combined across all field sites (Monsanto, 2014, Tables VI-1-VI-7, pp.76-89). The mean component values for MON 874Ø3 were also within the maize compositional values in the literature, and/or the International Life Sciences Institute Crop Composition Database values (ILSI, 2010; Monsanto, 2014, Table VI-8, pp. 90-91)The data presented suggests that grain and forage from MON 874Ø3 maize has compositional equivalence to the conventional control and other conventional reference 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 874Ø3 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 874Ø3 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 United States of America; and supports trade and exports of U.S. agricultural products. PPQ responds to many new introductions of plant pests to eradicate, suppress, or contain them through various programs in cooperation with state departments of agriculture and other government agencies. These may be emergency or longer term domestic programs that target a specific pest.

Currently, PPQ has several active pest management programs that target insect pests and a noxious weed that can affect maize because maize is listed as one of the host plants. These include programs for the grasshopper and Mormon cricket on rangelands, the Light Brown Apple Moth (*Epiphyas postvittana*) in California, and of more relevance, the Japanese beetle (*Popilla japonica*), the Old World Bollworm (*Helicoverpa armigera*), and witchweed (*Striga asiatica*) (for more information on each of these 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 (USDA-APHIS, 2015)

Witchweed (*Striga asiatica*) is a parasitic plant listed as a Federal Noxious Weed that affects several crop plants including maize. Infested areas are found in North and South Carolina, and APHIS and state collaborators aim to stop the spread from infested areas and eradicate the pest (USDA-NRCS, 2015a).

The Federal Select Agent Program lists two pathogens of maize as USDA Plant Protection and Quarantine Select Agents: *Peronosclerospora philippinensis* (*Peronosclerospora sacchari*), the causal agent of Philippine downy mildew of maize found in parts of Africa and Asia; and *Sclerophthora rayssiae* var. *zeae*. which causes brown stripe downy mildew of maize and has also been reported in parts of Asia. Neither one of these pathogens has been reported in the United States (Magill, 2013; FSAP, 2014).

Maize itself is not considered a plant pest in the United States (7 CFR part 340.2) . The use of plant pest vectors or sequences does not increase the plant pest risk of MON 874Ø3. *Agrobacterium tumefaciens*, a designated plant pest, was used in the transformation, however the T-DNA was disarmed of sequences known to be required for formation of crown gall disease and the initial transformants were treated with antibiotic to kill the *Agrobacterium*. The use of genetic elements from *A. tumefaciens* and the duplicated enhancer region from the *Cauliflower mosaic virus* (CaMV) 35S RNA promoter did not impart plant pest characteristics to MON 874Ø3 (Monsanto, 2014, Section III. D and E. p. 35). MON 874Ø3 contains an expression cassette for the full-length *ATHB17* gene from *Arabidopsis thaliana*. Maize-specific splicing of the *ATHB17* transcript results in the expression of a truncated protein, ATHB17Δ113 that can alter the activity of endogenous maize HD-Zip II proteins, predominantly expressed in ear tissue. A statistically significant increase in ear weight at the R1 stage (silking stage) was observed in MON 874Ø3 compared to the maize plants used as controls (Monsanto, 2014, Table I-1, pp. 25-26).

Monsanto assessed phenotypic, agronomic and environmental interaction characteristics for MON 874Ø3, the conventional control (MPA640B) and 21 reference hybrids grown under similar agronomic conditions (Monsanto, 2014, Table VII-1, pp. 95-97, Table H-1, pp. 220-221) during 2012. Field trial sites were established at 13 locations that provided a range of environmental and agronomic conditions representative of U.S. maize growing regions (Monsanto, 2014, VII-3, p. 106, Table H-2, p.222). The change in ear biomass does not appear to affect pest potential based on agronomic, phenotypic and environmental interaction characteristics assessed for MON 874Ø3.

Maize yields can be reduced by diseases that affect the crop; and disease incidence and severity vary depending on factors that include weather conditions, crop production practices, hybrid selection and susceptibility of the plant to disease. In 2012 dry conditions across many states in the United States affected the prevalence and severity of corn diseases during the growing season. The greatest estimated losses in millions of bushels overall in 22 states were caused by Fusarium stalk rot, Aspergillus ear rot, Pythium damping off, Fusarium ear rot and gray leaf spot. Other major losses were caused by common smut, nematodes and charcoal rot, several other diseases not listed here also caused losses in maize in 2012 (Mueller, 2014). Pathogens associated with the diseases listed above are: Fusarium stalk rot, *Fusarium moniliforme*, *F. proliferatum* and *F. subglutinans*; Aspergillus ear rot, *Aspergillus flavus* and *A. parasiticus*; Pythium damping off, *Pythium spp*; Fusarium ear rot, *Fusarium moniliforme*; gray leaf spot, *Cercospora zeae-maydis*; common smut, *Ustilago maydis*; charcoal rot, *Macrophomina*

phaseolina. Some nematodes that affect maize are: root knot nematodes, *Meloydogyne* spp.; lesion nematodes, *Pratylenchus* spp; sting nematodes, *Belonolaimus spp*. (White, 1999).

Several viruses also cause disease in maize with varying degrees of damage depending on susceptibility of the host, presence of the vector and of weed hosts that can act as reservoirs for the virus. Maize viruses are mostly transmitted by arthropod vectors that can also cause direct damage to the plant, and a few viruses of maize are soilborne. More than 15 species of aphids can transmit *Maize dwarf mosaic potyvirus*. *Maize chlorotic dwarf waikairus* is transmitted by the leafhopper *Graminella nigrifons. Maize chlorotic mottle machlomovirus* (MCMV) can cause significant damage to maize reducing crop yields by 10 to 15%. However, co-infections of MCMV with *Wheat streak mosaic potyvirus, Sugarcane mosaic potyvirus* or *Maize dwarf mosaic potyvirus,* induce Maize Lethal Necrosis Disease (or Corn Lethal Necrosis Disease), a synergistic disease that can reduce crop yields by up to 90% (White, 1999; Cabanas *et al.*, 2013). MCMV is vectored by Chrysomelid beetles including the western, northern and southern corn rootworms and by thrips *Frankliniella occidentalis* and *Frankliniella willliamsi* (Cabanas *et al.*, 2013; Zhao *et al.*, 2014).

MON 874Ø3 and the conventional control were evaluated for response to diseases during crop development and at harvest in several locations, and the severity of response to disease was evaluated. Assessments of response to disease were performed for the following diseases and pathogens: anthracnose, bacterial leaf spot, ear rot, eyespot, *Fusarium sp*., Goss' bacterial wilt, gray leaf spot, leaf blight, *Maize rough dwarf potyvirus*, northern leaf spot, *Pythium sp*., *Rhizoctonia sp*., rust, seedling blight, smut, stalk rot and Stewart's bacterial wilt (Monsanto, 2014, Tables VII-5 and H-6, pp. 110, 239). Although plant viruses were not specifically assessed, abundance of important maize virus vectors were assessed.

Several arthropod and gastropod pests can infest maize and reduce yields in the U.S. and there are regional differences in the importance of these pests. Among the most damaging are: European corn borer (*Ostrinia nubilalis*); western, northern, and southern corn rootworms (*Diabrotica virgifera, D. barberi*, and *D. undecimpunctata,* respectively); southwestern corn borer (*Diatrea grandiosella*), corn earworm (*Helicoverpa zea*), western bean cutworm (*Striacosta albicosta*); Japanese beetle (*Popillia japonica*) and black cutworms (*Agrostis ipsilion*) (Flanders *et al.*, 2013).

Evaluations of damage caused by 18 arthropod pests and one gastropod (slug) pest of maize were conducted in 2012 U.S. field trials for MON 874Ø3. The arthropods included: aphids (Aphididae), armyworms and cutworms (Noctuidae), bean leaf beetle (*Ceratoma trifurcata*), billbugs (*Sphenophorus parvulus*), corn earworm, corn flea beetle (*Chaetocnema pulicaria*), corn rootworm beetles (*Diabrotica* spp.), European corn borer, grasshoppers (*Melanoplus* spp.), Japanese beetles, June beetles (Scarabaeidae), sap beetles (Nitidulidae), southwestern corn borer, spider mites (*Tetranychus* sp.), stink bugs (Pentatomidae), western bean cutworm (*Richia albicosta*) and click beetles (Elateridae) (Monsanto, 2014, Table H-7, p. 240).

Observations of 13 field trial sites during 2012 encompassing 18 arthropod and one gastropod pest and 17 diseases, showed no biologically significant differences in the range of responses observed between MON 874Ø3 and the conventional control LH244 for 150 comparisons for plant damage caused by arthropods and gastropods (Monsanto, 2014, Tables VII-5 and H-7, pp. 110, 240) or for the 176 comparisons for plant damage caused by diseases, (Monsanto, 2014, Tables VII-5 and H-6, pp. 110, 239). Specific arthropod (corn earworm and European corn borer) damage and arthropod abundance were assessed quantitatively from observations and collections performed at four field sites during the 2012 growing season at different development stages, comparing MON 874Ø3 to the conventional control and 11 conventional commercial reference maize hybrids (Monsanto, 2014, VII.C.2.2.2, pp 111 -114, including Tables VII-6 and VII-7, pp. 112-114). No differences were detected that were considered biologically meaningful in terms of increased pest potential of MON 874Ø3 compared to the conventional control.

Additionally, abundance of arthropods (including pests and beneficial species) was assessed using sticky traps and visual counts five times during the growing season at four sites, comparing MON 874Ø3 and the conventional control. Sticky trap collections were performed for: aphids, billbugs, corn flea beetles, corn rootworm beetles, delphacid planthoppers, grasshoppers, lacewings, ladybird beetles, leafhoppers, macro-parasitic hymenoptera, micro-parasitic hymenoptera, minute pirate bugs, damsel bugs, sap beetles, seedcorn beetles, spiders, syrphid flies, tachinid flies, tarnished plant bugs, thrips and click beetles (Monsanto, 2014, Table H-9, pp. 242-249). No statistically significant differences between MON 874Ø3 and the conventional control were detected for 130 out of 144 comparisons, (Monsanto, 2014, p.228). The only pest arthropod that had some mean counts higher than the conventional control and above the range of reference varieties in the sticky traps in more than one observation was Thrips, but it was not consistently observed to be higher across all sites or collections, and not considered to be biologically significant.

Sixty six statistical comparisons for arthropod abundance by visual counts were made for antlike flower beetles, corn flea beetles, Japanese beetles, lacewing adults, lacewing larvae, ladybird beetle adults, ladybird beetle larvae, minute pirate bugs, corn rootworm beetles, sap beetles, shining flower beetles, spiders, stink bugs and click beetles (Monsanto, 2014, Table H-10, pp. 250-255). No statistical differences were detected for 61 of the 66 comparisons. Differences were not consistently observed across all sites and collections and were not considered meaningful in terms of increased pest potential of MON 874Ø3 (Monsanto, 2014, pp. 228-230).

The data from phenotypic and agronomic studies and environmental interactions such as plant disease interactions, arthropod damage and arthropod abundance collected for MON 874Ø3 and conventional controls did not indicate adverse impacts to non-target arthropod populations or changes to plant-disease interactions (Monsanto, 2014, Sections IX.D. and IX.E, pp. 125-126).

In summary, the introduced genetic elements did not significantly alter the observed insect pest infestation and disease occurrence or resulting damage on MON 874Ø3 over the control line and other comparators. As discussed earlier there were no significant changes in MON 874Ø3 grain or forage composition compared to the conventional control with a similar genetic background or to reference commercial varieties, so no changes in composition are anticipated that would render MON 874Ø3 more susceptible to pests and diseases. The observed agronomic traits also did not reveal any significant changes that would indirectly indicate that MON 874Ø3 is or could be relatively more susceptible to pests and diseases over the control or reference maize varieties. Thus, MON 874Ø3 is unlikely to be more susceptible to plant pathogens and insect pests than conventional maize and existing commercial varieties, 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. MON 874Ø3 is not expected to have an adverse impact on APHIS PPQ pest management programs for maize.

E. Potential Impacts on Nontarget Organisms Beneficial to Agriculture

MON 874Ø3 is not engineered for pest resistance, thus there are no 'target' species, or 'nontarget' species. APHIS assessed whether exposure or consumption of MON 874Ø3 would have a direct or indirect adverse impact on species beneficial to agriculture**.** Organisms considered were representative of the species associated with production of the regulated crop in the agricultural environment. The assessment includes analyses of data and information on MON 874Ø3 **c**ompared to the conventional control and other comparators used as a reference range for any biologically relevant changes in the phenotype or proteins produced which may be novel or expressed at significantly altered amounts that are associated with impacts on organisms beneficial to agriculture, and/or any observations of beneficial organisms associated with the plants.

Beneficial organisms include arthropods that can be pollinators, or predators or parasites of arthropod pests. Other beneficial organisms include earthworms, termites, ants, beetles, and millipedes present in the soil macrofauna that have major roles in the breakdown of dead plant material (Ruiz *et al.*, 2008). Arthropod damage and abundance were assessed quantitatively from observations and collections at four field trial sites using visual counts and sticky traps and included enumeration of pest and beneficial arthropods at four field sites. MON 874Ø3 was compared to the conventional control and to a reference range of eleven conventional maize cultivars and no biologically meaningful differences were found. (Monsanto, 2014, Section H.8, pp. 224-225, Table H-9 pp. 242-249 and Table H-10, pp. 250-255).

Although there are many genetic elements in MON 874Ø3, there is only one protein coding sequence and one new protein that is expressed (Monsanto, 2014, Table IV-1, pp. 45-46). As summarized previously, expression of *ATHB17* results in the production of a truncated protein ATHB17Δ113 due to RNA splicing in maize. ATHB17 belongs to the HD-ZIP II subfamily of proteins, transcription factors that are common in plants. ATHB17Δ113 shares sequence homology with proteins present in food crops consumed by humans and animals without noted allergenicity or toxicity. The potential for

allergenicity, toxicity and biological activity of ATHB17Δ113 was analyzed using simulated gastric and intestinal fluid digestion assays, bioinformatics, a mouse gavage study and consideration of the potential exposure to the protein (Monsanto, 2014, Sections V.D., V.E. and IX.B.1, pp. 68-71, 124). The data and information presented demonstrate that it is rapidly digested and does not share amino acid sequence similarity with known allergens, gliadins, glutenins, or protein toxins which could have adverse effects to human or animal health. The mouse gavage acute oral toxicity study demonstrated a "No Observable Adverse Effects Level (NOAEL) of 1,335 mg/kg body weight" (the highest dose tested). This supports the conclusion of safety for consumption by vertebrate organisms, especially given the low exposure estimates determined for the ATHB17Δ113 protein in grain (no more than 0.001%) (Monsanto, 2014, pp. 9, 124, 126-127).

Harvested grain and forage from MON 874Ø3, the conventional control MPA640B and 17 commercial reference maize hybrids (Monsanto, 2014, Table F-1, p.196) were compositionally assessed. The analysis of key nutrients, anti-nutrients and secondary metabolites of MON 874Ø3 demonstrated that MON 874Ø3 grain and forage are compositionally equivalent to the conventional control (Monsanto, 2014, Section IX.C, p. 125).

MON 874Ø3 does not have pesticidal activity, and results from extensive phenotypic and agronomic studies and observational data on environmental interactions such as plant-disease interactions, arthropod damage and arthropod abundance (summarized in Section D above) support the conclusion of no adverse impacts to non-target arthropod populations and no changes to plant-disease interactions.

Therefore, based on the above analysis of the peer-reviewed literature and the information provided in the petition on the safety and expression level of the expressed ATHB17Δ113 protein, the compositional analysis and phenotypic and agronomic studies, APHIS concludes that exposure to and/or consumption of MON 874Ø3 maize are unlikely to have any adverse impacts to organisms beneficial to agriculture.

F. Potential for Enhanced Weediness of MON 874Ø3 Maize

APHIS assessed whether MON 874Ø3 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 non-transgenic conventional control comparator or other varieties of maize currently under cultivation. The assessment considers the basic biology of maize, the situations in which maize volunteers are considered weeds, and an evaluation of the characteristics of MON874Ø3 maize that could influence weediness. Monsanto conducted evaluations in both laboratory experiments and field trials on phenotypic, agronomic, and environmental interactions of MON 874Ø3 maize, compared to the conventional control and up to 21 commercial maize hybrid (Monsanto, 2014, Appendices G, H, I and Tables G-1,G-2, H-1,H-4, H-5 and I-1, pp. 212-260) The data collected and analyzed indicate that MON 874Ø3 maize performs similarly to the convention control MPA640B and the tested conventional corn hybrids.

MON 874Ø3 has no herbicide tolerance or insect resistance traits. Data presented in the petition discussed earlier indicate that the combined site analysis over 13 field test sites in 2012 showed that MON 87403 had statistically significant increases in R1 ear biomass and biomass partitioning to the ear, compared to the conventional control, however, no statistically significant differences were observed in R1 stover and total biomass. Data for 13 other phenotypic characteristics were collected from 13 field sites (Monsanto, 2014, Table VII-3, p. 106) representative of U.S. commercial maize production areas. In combined site analysis no statistically significant differences were observed in these characteristics between MON 874Ø3 and the conventional control MPA640B except for increased ear height in MON 874Ø3; however, the mean ear height value was within the maize reference range (Monsanto, 2014, Table VII-4. p. 107). Ear height is not a reported weediness characteristic, and a small change would not be expected to change agronomic practices.

In the phenotypic, agronomic, and environmental interactions assessment of MON 874Ø3, data were collected to evaluate altered plant pest potential. The plant characterization of MON 874Ø3 encompassed: seed germination, dormancy, and emergence; vegetative growth; reproductive development (including pollen characteristics); lodging; seed yield; dropped ears; plant response to abiotic stress and interactions with diseases and arthropods; and intended phenotype (increased ear biomass) (Monsanto, 2014, Table VII-1 pp. 95-97and Figure VII-1 p. 99). Results comparing MON 874Ø3 and the conventional control demonstrated that MON 874Ø3 does not possess: increased weediness characteristics; increased susceptibility or tolerance to specific abiotic stress, to diseases, arthropods or slug; or characteristics that would confer a plant pest risk compared to conventional maize (Monsanto, 2014, Section VII.A, pp. 93-97, Section VII.C.2 pp. 104-107).

Maize has not been listed as a weed in the United States (Crockett, 1977; Muenscher, 1980) and it is not present on the Federal Noxious Weed List (7 CFR part 360). Although the Plants Database lists maize as a plant that can be a weed according to the Southern Weed Science Society (USDA-NRCS, 2015i) maize is grown throughout the world and has not been reported to be a serious weed outside of agriculture or to form persistent feral populations (Gould, 1968). Maize has not been found to establish self- sustaining populations outside agriculture. The seeds are retained on the cob and are poorly dispersed, lack dormancy and are susceptible to low temperatures, and furthermore, maize is a week competitor in native environments and is outcompeted by native perennial species (Andersson and de Vicente, 2010; Raybould *et al.*, 2012). Plants genetically engineered for resistance to weeds and insects and non GE plants were unable to form self-sustaining feral populations in agricultural land (Raybould *et al.*, 2012). Although seeds of modern maize cultivars have no dormancy characteristics, some seeds may overwinter and germinate when weather conditions allow (OGTR, 2008). Chemical or mechanical methods are often applied to remove volunteers, but the plants that are not removed do not typically result in feral populations in following years because maize is incapable of sustained reproduction outside of cultivation and it is non-invasive in natural habitats (Andersson and de Vicente, 2010). Maize possesses few of the characteristics of those plants that are notably successful as weeds (Baker, 1965; Keeler, 1989; Andersson

and de Vicente, 2010). MON874Ø3 maize has not been engineered for herbicide resistance, and there are many options for control of MON874Ø3 maize occurring as volunteers in fields.

Seed dormancy is an important characteristic often associated with plants considered to be weeds, the presence of a hard seed coat is a characteristics that contributes to dormancy (Anderson, 1996). Laboratory studies indicated that MON 874Ø3 seeds have dormancy and germination characteristics similar to seeds of the conventional control which were produced from the same field locations; in both cases seeds exhibited high rates of seed germination under optimal conditions and no hard viable seeds, and were within the range for nine commercially available reference hybrids (Monsanto, 2014, Section VII.C.1 pp. 101-103, Table VII-2, p. 103, Appendix G, pp. 212-217, Table G-1, p. 214, Table G-2. p. 215-216).

Pollen viability and morphology of MON 874Ø3 was compared to the conventional control and four commercial references grown at the same field site in assessments of the potential for gene flow and introgression of the trait into sexually compatible plants. No statistically significant differences (α=0.05) were detected between MON 874Ø3 and the conventional control for pollen viability and diameter, and no visual differences in general pollen morphology were observed (Monsanto, 2014, Section VII.C.3. p. 115-116, Table VII-8, p. 116 and Appendix I, pp 257-260, Figure I-1 p. 259).

In assessments of abiotic stress, no differences in the range of responses observed between MON 874Ø3 and the conventional control were observed for 143 comparisons of plant responses to abiotic stressors. Similarly no biologically significant differences were observed for 176 comparisons for disease responses to 17 diseases. And no biologically meaningful differences in the range of responses to arthropod damage or in arthropod abundance were observed between MON 874Ø3 and the conventional control. The results for the above observations were within the reference ranges observed for reference commercial hybrids (Monsanto, 2014, Section VII.C.2.2.1, p. 239; Table H-5, Abiotic Stressors p. 238; Table H-6, Disease Damage pp. 239; Tables H-7 and H-8 for Arthropod Damage Evaluations, pp. 340-241; Tables H-9 and H-10, pp. 242-255 for Arthropod Abundance).

Based on the agronomic field data presented in the petition (Monsanto, 2014) and literature survey concerning weediness potential of the crop, MON 874Ø3 is unlikely to persist as a troublesome weed or to have an impact on current weed management practices. These data suggest that MON 874Ø3 is no more likely to become a weed than conventional varieties of the crop.

G. Potential Impacts on the Weediness of Any Other Plants with which MON 874Ø3 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 and

Soltis, 1993; Hegde *et al.*, 2006), and even in the existing floras, the occurrence of hybridization or introgression is reported to be widespread (Stace, 1987; Rieseberg and Wendel, 1993; Peterson *et al.*, 2002). It has been a common practice by plant breeders to artificially introgress traits from wild relatives into crop plants to develop new cultivar (Khoury *et al.*, 2013). However, gene flow from crops to wild relatives is also thought of as having a potential to enhance the weediness of wild relatives, as observed in rice, sorghum, sunflower and a few other crops (Ellstrand *et al.*, 1999). This topic is covered in two sections: 1) the potential for gene flow, hybridization and introgression from MON 874Ø3 to sexually compatible relatives, including wild, weedy, feral or cultivated species in the United States and its territories, and 2) if so, the risk potential with respect to weediness of those taxa based on the phenotypic changes that have been observed in the engineered plants.

APHIS evaluated the potential for gene introgression to occur from MON 874Ø3 maize to sexually compatible wild relatives and considered whether such introgression would result in increased weediness. Introgression is a process whereby gene(s) successfully incorporate into the genome of a recipient plant.

Cultivated maize (or corn), *Zea mays* subsp. *mays*, is a member of the grass family Poacae and 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, 2015j); *Zea perennis* is listed in Texas and

South Carolina (USDA-NRCS, 2015k). *Zea diploperennis* and *Zea luxurians* are also listed, but there is no information about their location and status (USDA-NRCS, 2015g; 2015h). 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-BRS, 2013), and introgression into teosinte is unlikely in the U.S.

The genus most closely related to *Zea* is *Tripsacum*, a genus with16 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; 2015d; 2015e) and *T. fasciculatum* and *T. latifolium* occur in Puerto Rico (USDA-NRCS, 2015c; 2015f). *Tripsacum* species (2n=18) can be represented by diploid, triploid, tetraploid and higher ploidy levels, and 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 (cross fertilizing), wind pollinated and 100% open pollinated, 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 crosses; Mexican maize landraces x Chalco teosinte) (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 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 and cross pollination between a donor field and receptor field can occur over a 7 day period and maize will cross-pollinate readily (Coe *et al.*, 1988; OGTR, 2008). However, adverse consequences of gene flow from MON 874Ø3 maize to wild or weedy related species in the U.S. are highly unlikely.

Based on the information presented in the petition and in relevant literature, APHIS has reached the following conclusions. The genetic modification in MON 874Ø3 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 varieties of the crop commonly grown. Gene flow, hybridization and/or introgression of genes from MON 874Ø3 to other sexually compatible relatives with which it can interbreed is not likely to occur in the United States and its territories. MON 874Ø3 does not exhibit characteristics that cause it to be any weedier or more difficult to control than other cultivated corn, and so is unlikely to transform corn wild relatives into more weedy species in the rare incidence of successful transgene introgression.

H. Potential Changes to Agriculture or Cultivation Practices

APHIS assessed whether significant changes to agricultural or cultivation practices from adoption of MON 874Ø3 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.

MON 874Ø3 maize has increased ear biomass at an early reproductive stage, and management practices currently employed for maize cultivation are not expected to change if MON 874Ø3 is determined to be no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act. Monsanto studies demonstrate that the cultivation practices needed for growing MON 874Ø3 are essentially indistinguishable from practices used to grow conventional maize (Monsanto, 2014, pp. 120-122). Additionally, no biologically significant differences in insect abundance, insect and disease damage were observed in field trials between MON 874Ø3 maize and the conventional control or comparators (Monsanto, 2014). APHIS does not foresee changes in either insects or disease damage or control measures employed due to agricultural or cultivation practices with MON 874Ø3.

APHIS could not identify any significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of MON 874Ø3; therefore, no impact on plant diseases or pests or their management is likely to occur.

I. Potential Impacts from Transfer of Genetic Information to Organisms with which MON 874Ø3 Maize Cannot Interbreed

APHIS examined the potential for the new genetic material inserted into MON 874Ø3 to be horizontally transferred without sexual reproduction to other organisms and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants. Horizontal gene transfer (HGT) 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 from genetically engineered organisms to another organism without reproduction or human intervention were reviewed by Keese (2008). Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake and recombination and rearrangement, most notably through viruses and mobile genetic elements. HGT has been a major contributor to the spread of antibiotic resistance amongst pathogenic bacteria; emergence of increased virulence in bacteria, eukaryotes and viruses; and, in the long run, to major transitions in evolution.

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

Horizontal gene transfer and expression of DNA from a plant species to bacterial, fungal or invertebrate species is unlikely to occur based on the following observations. Although there are many opportunities for plants to directly interact with fungi and bacteria (e.g. as commensals, symbionts, parasites, pathogens, decomposers, or in the guts of herbivores) and with invertebrates as plant pests, there are almost no evolutionary examples of HGT from eukaryotes to bacteria or from plants to fungi or invertebrates (Keese, 2008). Examples of HGT between eukaryotes and fungi primarily involve gene acquisition or transfer by fungi to or from other distantly related fungi or bacteria (Keeling and Palmer, 2008; Keese, 2008) and HGT between plants and fungi is extremely rare (Richards *et al.*, 2009). Examples of HGT between plants and invertebrates are also extremely rare, and most examples of HGT in insects involve acquisition of genes from their pathogens or endosymbionts (Keese, 2008; Zhu *et al.*, 2011; 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. Many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Wood et al., 2001; Kaneko et al., 2002*)*. There is no evidence that these organisms contain genes derived from plants. HGT from plants to bacteria is a very low frequency event, primarily because functional and selective barriers to HGT increase with genetic distance (Keese, 2008). 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). Additionally, transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression.

The genetic elements in the inserted gene cassette in MON 874Ø3 are derived from DNA from plants and the part of a regulatory sequence from a plant virus that was used in the promoter is optimized for expression in maize. The *ATHB17* gene used expresses a protein belonging to a subfamily of the HD-Zip family of proteins, this family of transcription factors is unique to the plant kingdom (Ariel *et al.*, 2007). Thus even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced.

Potential for horizontal gene transfer to viruses

APHIS also considered whether horizontal transfer of DNA from MON 874Ø3 to plant viruses is likely to occur and would lead to the creation or selection of plant viruses that are more virulent or have a broader host range. The only virus sequences inserted into MON 874Ø3 are the duplicated enhancer region from the *Cauliflower mosaic virus* (CaMV) 35S RNA promoter. This issue has been considered before by other science review panels and government regulatory bodies (EPA-FIFRA-SAP, 2006; Keese, 2008). HGT is not unusual among plant viruses; however, this is generally limited to exchange between viruses present in the same host organism in mixed infections, and most commonly involves homologous recombination, relying on sequence similarity at the point of crossover (Keese, 2008). HGT of 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 including in the CaMV 35 promoter, and strategies implemented in design of transgenes to avoid recombination have been suggested. No recombinant or undesirable viruses with new properties have been detected for over at least 8-10 years in field tests or during commercial growth of deregulated virus resistant plum, squash, or papaya engineered with genes from viruses that have been deregulated in the United States (Fuchs and Gonsalves, 2007).

Potential for horizontal gene transfer to parasitic plants

Evidence for HGT from plants to other plants is limited to two specific scenarios: (1) exchange of genes between a parasitic plant and its host; and (2) exchange of genes between cells of two plants living in close proximity, such as in a graft junction. In both cases, this type of HGT requires physical contacts between the two plants. Most cases of HGT in plants involve transfer of mitochondrial genomes, which are primarily maternally inherited in plants (Barr *et al.*, 2005), to other mitochondrial 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 of unknown function occurred between sorghum and purple witchweed. However, this HGT occurred before speciation of purple witchweed and related cowpea witchweed (*S. gesnerioides*) from their common ancestor. Furthermore, *S. hermonthica* is not found in the U.S. and *S. asiatica*, another related parasite of cereal crops, is only present in North Carolina and South Carolina (USDA-NRCS, 2015a). More recent studies of genetic sequences demonstrated that in a few parasitic species of the Rafflesiaceae family, 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. Furthermore, in MON 874Ø3 the DNA sequences were inserted into the nuclear genome, not the mitochondrial genome.

If MON 874Ø3 maize 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. 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 horizontal gene transfer of the genetic material inserted into MON 874Ø3 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.

J. Conclusion

APHIS has reviewed the information submitted in the petition, supporting documents, and other relevant information to assess the plant pest risk of MON 874Ø3 maize compared to the unmodified variety from which it was derived, the recipient maize line LH244. APHIS concludes that MON 874Ø3 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 874Ø3 because *A. tumefaciens* was eliminated using antibiotics. The inserted genetic material which was derived from plant pests, ie., the DNA borders from *A. tumefaciens* and regulatory sequences from *Cauliflower mosaic caulimovirus*, do not result in the production of infectious agents or disease symptoms in plants.
- No increase in plant pest risk was identified in MON 874Ø3 from the expression of the inserted genetic material or changes in metabolism or composition because the expressed ATHB17Δ113 protein raises no plant pest issues and MON 874Ø3 maize can be considered compositionally or nutritionally equivalent to its nontransgenic counterpart control derived from the recipient line LH244.
- Disease and pest incidence and/or damage were not observed to be significantly increased or atypical in MON 874Ø3 compared to the nontransgenic counterparts or other comparators in field trials conducted in growing regions representative of where MON 874Ø3 is expected to be grown. Observed agronomic traits also did not reveal any significant differences that would indirectly indicate that MON 874Ø3 is more susceptible to pests or diseases. Therefore no plant pest effects are expected on this or other agricultural products and no impacts are expected to APHIS pest control programs.
- Exposure to and/or consumption of MON 874Ø3 maize are unlikely to have any adverse impacts on organisms beneficial to agriculture based on the analysis of the low expression level of the expressed ATHB17Δ113 protein and its potential allergenicity or toxicity, the compositional analysis, and phenotypic and agronomic studies.
- MON 874Ø3 is no more likely to become a weed or weedier than conventional varieties of maize based on its observed agronomic characteristics, weediness

potential and current management practices available to control MON 874Ø3 as a volunteer.

- MON 874Ø3 maize is not expected to increase the weed risk potential of other species with which it can interbreed in the United States or its territories. Gene flow, hybridization and/or introgression of inserted genes from MON 874Ø3 to other sexually compatible relatives with which it can interbreed is not likely to occur. Any possible introgression into teosintes or *Tripsacum* species of the new phenotype conferred by genetic engineering is not likely to increase the weediness of these relatives or affect the current ability to control them in situations where they might be considered weedy or invasive.
- Significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of MON 874Ø3 were not identified. No impacts on pests or pest management practices are anticipated.
- Horizontal gene transfer of the new genetic material inserted into MON 874Ø3 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.

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