Monsanto Petition (19-316-01p) for the Determination of Nonregulated Status for Dicamba, Glufosinate, Quizalofop and 2,4-Dichlorophenoxyacetic Acid Tolerant MON 87429 Maize with Tissue-Specific Glyphosate Tolerance Facilitating the Production of Hybrid Maize Seed

OECD Unique Identifier:
MON-87429-9

Draft 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 of Department Agriculture (USDA) seeking a determination of nonregulated status for herbicide resistant[1] maize (*Zea mays*) event MON 87429 (OECD Unique Identifier MON 87429-9, hereafter referred to as MON 87429 maize) developed using genetic engineering (modified maize) that is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived and, therefore, should no longer be a regulated organism under the APHIS' 7 Code of Federal Regulations (CFR) part 340. This petition was assigned the number 19-316-01p, and is hereafter referenced as Monsanto 2019. 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. 7702 *et seq.*)[2]. This plant pest risk assessment was conducted to determine if MON 87429 maize is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived.

The petition for non-regulated status described in this PPRA is being evaluated under the version of the regulations effective at the time that it was received. The Animal and Plant Health Inspection Service (APHIS) issued a final rule, published in the Federal Register on May 18, 2020 (85 FR 29790-29838, Docket No. APHIS-2018-0034)[2], revising 7 CFR part 340; however, the final rule is being implemented in phases. The new Regulatory Status Review (RSR) process, which replaces the petition for determination of nonregulated status process, became effective on April 5, 2021 for corn, soybean, cotton, potato, tomato, and alfalfa. The RSR process is effective for all crops as of October 1, 2021. However, for corn petitions received prior to April 5, 2021 APHIS will review petitions for determination of non-regulated status in accordance with the legacy regulations at 7 CFR § 340.6. (85 FR 29815). This petition for a determination of non-regulated status is being evaluated in accordance with the regulations at 7 CFR § 340.6 (2020) as it was received by APHIS on 6/27/2019. MON 87429 maize was produced by the *Agrobacterium tumefaciens*-mediated transformation method (Monsanto 2019), and three of the introduced genetic sequences come from plant pest organisms, including a promoter from the 35S RNA of cauliflower mosaic virus (CaMV) and the 25 base pairs of the right- and left-border T-DNA repeats from *A. tumefaciens*. Therefore, the MON 87429 maize is considered a regulated organism under APHIS regulations at 7 CFR part 340. Monsanto has conducted introductions of MON 87429 maize as a regulated organism under APHIS-authorized permit/notification since 2014 (Appendix A, Table A-1 in Monsanto 2019), in part, to gather information to support that MON 87429 maize is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived.

Potential impacts in this Plant Pest Risk Assessment are those that pertain to plant pest risk associated with MON 87429 maize and its progeny and their use in the absence of

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[1] WSSA (1998) ""Herbicide Resistance" and "Herbicide Tolerance" defined. (Technology Note)." *Weed Technology.* 12 (4): p 789. Last Accessed: 02/21/2020. http://www.jstor.org/stable/3989101

[2] To view the final rule, go to www.regulations.gov and enter APHIS-2018-0034 in the Search Field.

confinement relative to the unmodified recipient and/or other appropriate comparators. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if MON 87429 maize is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived. 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 87429 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 organism on non-target organisms; weediness of the regulated organism; 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 for a new or previously registered pesticide, EPA must determine through testing that the pesticide does not cause unreasonable adverse effects on humans, the environment, and non-target species when used in accordance with label instructions. EPA must also approve the language used on the pesticide label in accordance with 40 CFR part 158. Other applicable EPA regulations include 40 CFR part 152 - Pesticide Registration and Classification Procedures, part 174 - Procedures and Requirements for Plant Incorporated Protectants (PIPs), and part 172 - Experimental Use Permits. In support of Monsanto's third generation herbicide resistant MON 87419 maize, deregulated by USDA in 2016, Monsanto requested that EPA allows the 0.5 lb a.e./ac postemergence application window for dicamba to be extended from V5 to V8 growth stage or 36-inch height of maize, whichever occurs first, without reducing the application rate of dicamba (US-EPA 2019). Pending approval of the expanded label at EPA, the dicamba use pattern for MON 87429 would be no different than that of MON 87419. Use of glufosinate, quizalofop and 2,4-D over the top of MON 87429 will follow the current labeled use patterns of these individual herbicides[3,4,5]. The introduction of

[3] Federal Register, 77 FR 59106-59113, Glufosinate Ammonium, Pesticide Tolerances
[4] Federal Register, 82 FR 9523-9529, 2,4-D; Pesticide Tolerances
[5] Federal Register, 83 FR 7111-7115, Quizalofop ethyl; Pesticide Tolerances

MON 87429 stacked with a deregulated glyphosate resistance trait will follow the current labeled use patterns of glyphosate herbicide[6].

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 crops developed using genetic engineering 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 indicated that they are currently in consultation with the FDA following the policy, "Foods Derived from New Plant Varieties," on the food and feed safety of MON 87429 maize (Submitted on February 5, 2019).

B. Development of Dicamba, Glufosinate, Quizalofop and 2,4-Dichlorophenoxyacetic Acid Resistant MON 87429 Maize with Tissue-Specific Glyphosate Resistance Facilitating the Production of Hybrid Maize Seed

Maize (*Zea mays* L. ssp *mays*) belongs to genus *Zea*, which consists of five species including *Z. mays*, *Z. diploperennis*, *Z. luxurians*, Z. *nicaraguensis,* and *Z. perennis* (OECD 2003; OTGR 2008). Maize is the only cultivated subspecies, and all the other subspecies and species in genus *Zea* are wild grasses and referred to as tesosintes (OTGR 2008). The closest known relative of genus *Zea* is genus *Tripsacum*. Maize can easily cross with the species of its own genus under natural conditions, but can only be crossed experimentally with the genus *Tripsacum* (OECD 2003).

Maize is widely grown in the world from 58° North (e.g., Canada and Russia) to 40° South (e.g., Chile) (Farnham et al. 2003; OTGR 2008), and it is the largest grain crop in the world in total metric ton production as of 2017 (FAOSTAT 2019). The top five maize production countries in 2016/2017 include USA (385 million metric tons (MMT)), China (264 MMT), Brazil (99 MMT), the European Union (62 MMT), and Argentina (41 MMT) (USDA-FAS 2019). In the United States, maize is grown in almost all the states (Figure 1, colored areas) (USDA-NASS 2019a). As shown in Figure 2, there exists a significant year-to-year variability in planted acreages, ranging from 68 to 97 million acres in the past 20 years (USDA-NASS 2019b). Maize yields (bushels/acre) also differ from year to year but show an apparent increase over the years (Figure 3) (USDA-NASS 2019c-b).

Maize has been used as a basic food crop, but its primary use in industrialized countries shifts more towards animal feed in the form of grain, forage or silage (Farnham et al. 2003; OECD 2003). In developed countries, more than 85% of the maize is used to feed animals (Farnham et al. 2003). Maize can also be processed for a range of uses as

[6] Federal Register, 76 FR 27268-27271, Glyphosate; Pesticide Tolerances

ingredients in food or drinks, or for industrial purposes, e.g., alcohol including fuel ethanol (OECD 2003; OTGR 2008).

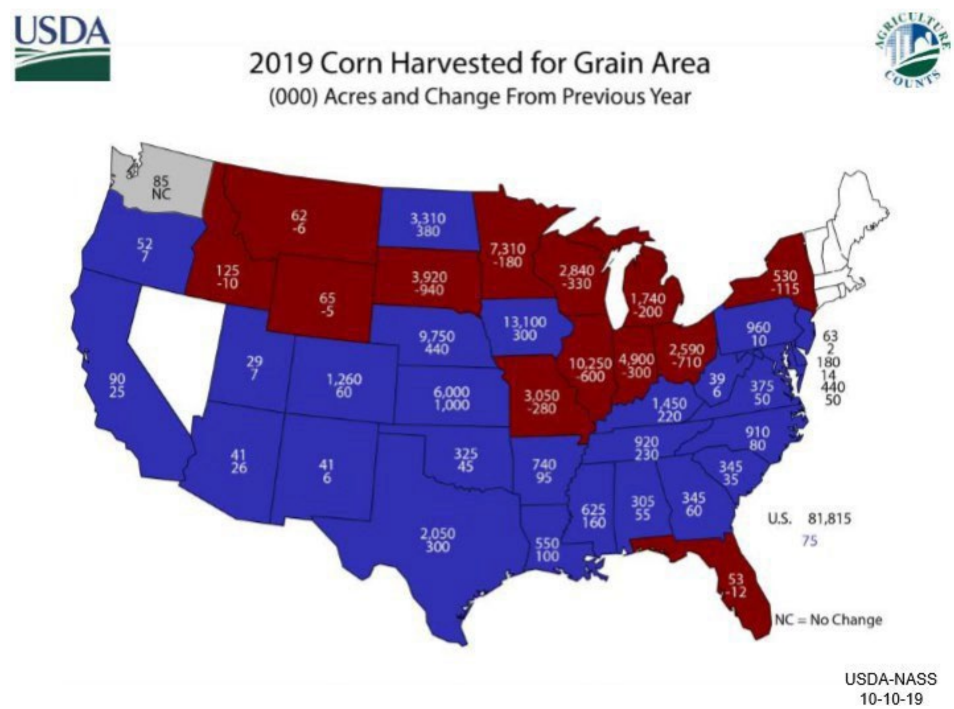


Figure 1. Maize production areas in the U.S. (USDA-NASS 2019a)

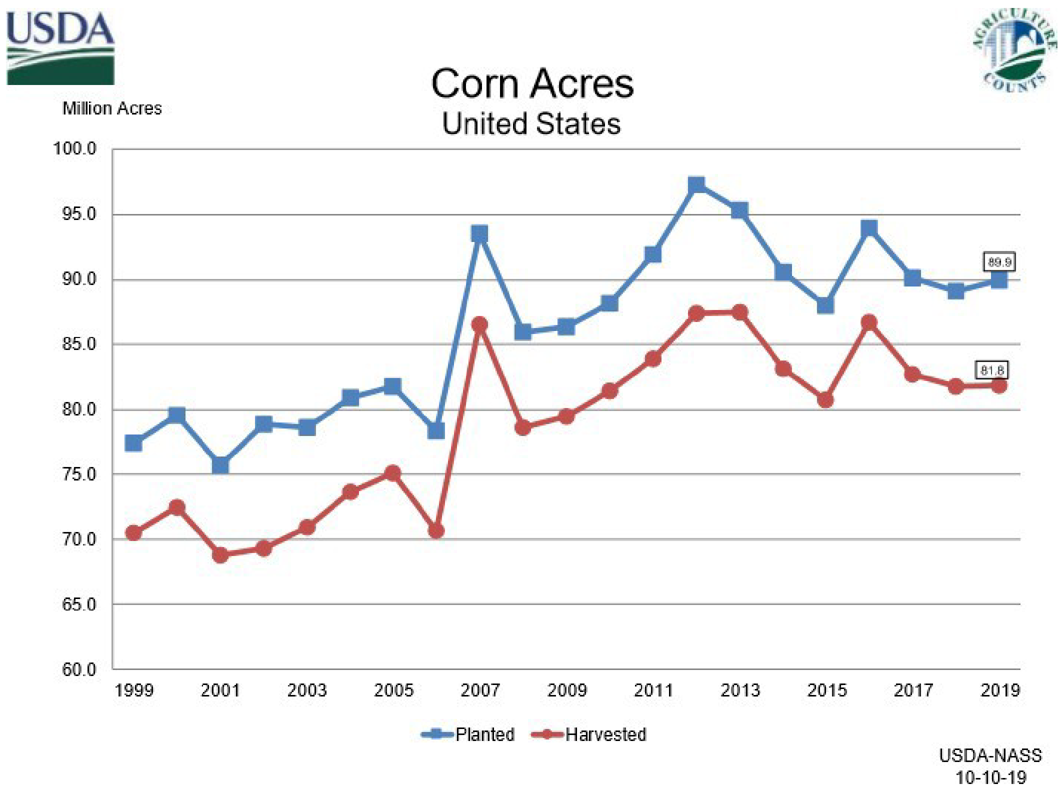


Figure 2. Maize acreage by year in the U.S. (USDA-NASS 2019b)

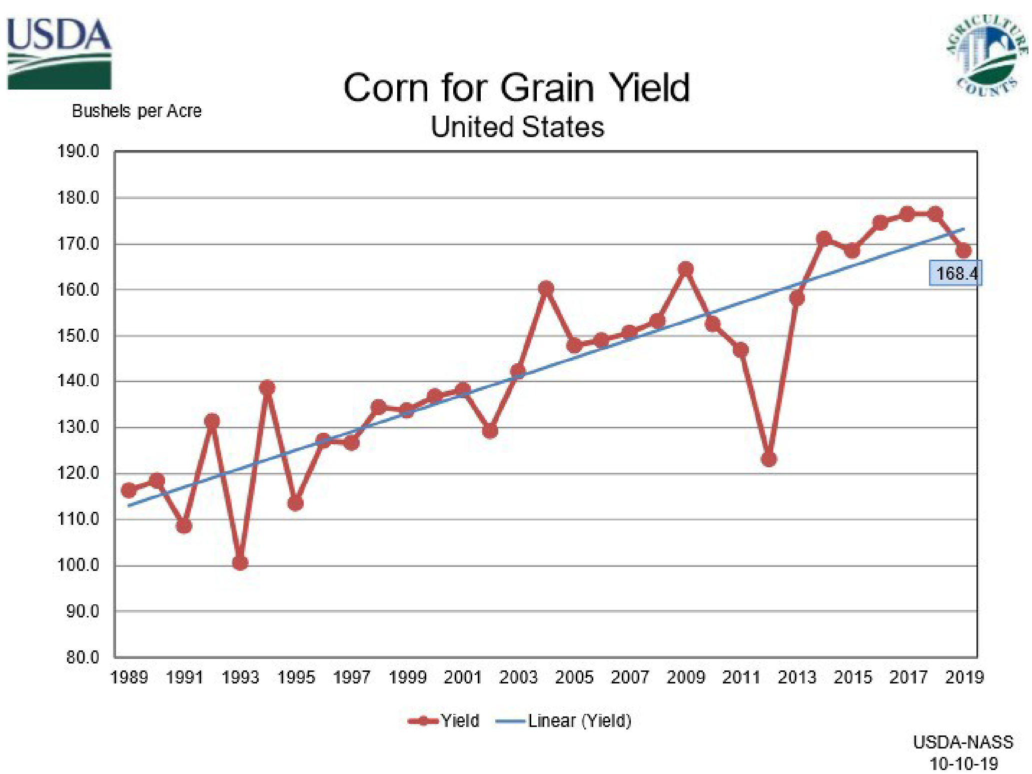


Figure 3. Maize yield by year in the U.S. (USDA-NASS 2019c-b)

Monsanto by *Agrobacterium* mediated-transformation of immature embryos of the inbred maize line LH244 developed the herbicide resistant MON 87429 maize that is resistant to the herbicides dicamba, glufosinate, aryloxyphenoxypropionate (AOPP) acetyl coenzyme A carboxylase (ACCase) inhibitors (so called "FOPs" herbicides such as quizalofop) and 2,4-dichlorophenoxyacetic acid (2,4-D). In addition, it provides tissue-specific glyphosate resistance to facilitate the production of hybrid maize seeds. The genetic engineering and breeding steps for the development of MON 87429 maize are described in the petition (Monsanto 2019).

The conventional control materials include the original transformation inbred line (LH244) and the F_1 hybrid from the cross between LH244 and another conventional inbred line HCL617. LH244 was used as the control in molecular characterization studies. The F_1 hybrid (LH244 × HCL617) was used as the control in compositional analysis studies and in phenotypic, agronomic and environmental interactions assessments. Where appropriate, commercial hybrid maize materials (reference hybrids) were also used to establish a range of variability or responses representative of commercial maize in the United States.

Based on maize biology (OECD 2003; OTGR 2008) and the data presented by Monsanto, APHIS concludes that MON 87429 maize was developed in a manner common to other modified maize and modified crops using *Agrobacterium*-mediated transformation (USDA-APHIS 2019a). APHIS believes the use of the non-GE control and other reference varieties as comparators is sufficient to determine that MON 87429 maize does not differ from the other maize varieties currently used in commercial production.

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 modified 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 the MON 87429 maize relative to the conventional controls. The assessment encompasses a consideration of the expressed dicamba mono-oxygenase (DMO) protein, phosphinothricin-N-acetyltransferase (PAT) protein, FOPs and 2,4-D dioxygenase (FT_T) protein and 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4 (CP4 EPSPS) as well as any observed or anticipated effects on plant metabolism including, e.g., any relevant changes in levels of metabolites, antinutrients, or nutrients in harvested seed/forage etc. derived from MON 87429 maize compared to those in the conventional control and 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 greater plant pest risks in the MON 87429 maize; or for expression of inserted DNA, new proteins or enzymes, or changes in metabolism to affect plant pest or diseases, non-target 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 87429 maize was developed through *Agrobacterium*-mediated transformation of the immature embryo of maize inbred line LH244 using the disarmed PV-ZMHT519224 binary vector (Monsanto 2019). The disarmed binary vector does not have the native T-DNA region from tumor-inducing (Ti) plasmids normally responsible for the incitation of crown gall tumors upon *A. tumefaciens* infection (Gelvin 2003).

Binary Plasmid Vector PV-ZMHT519224

The disarmed PV-ZMHT519224 binary vector is approximately 17.8 kb. It contains four gene expression cassettes for the expression of four herbicide resistance genes, which are delineated by a right border (RB) and left border (LB) sequences of T-DNA as well as backbone vector sequences outside of the two T-DNA border sequences. Transgene elements within the T-DNA regions are shown in Figure 4 and Table 1 below (Monsanto 2019).

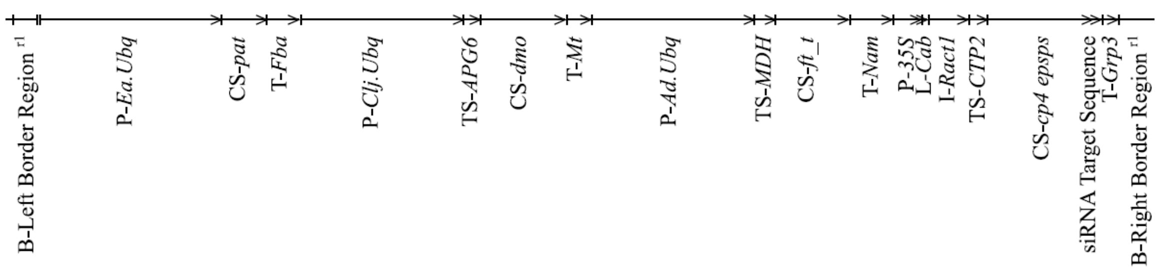


Figure 4. Schematic Representation of transgene elements within the T-DNA regions in MON 87429 maize (Figure IV-3, Monsanto 2019).

Table 1. The genetic elements and functions of the tandemly arrayed four gene expression cassettes in MON 87429 maize for the expression of genes *pat*, *dmo, ft_t*, and *cp4 epsps*.

Genetic Element	**Function**
B-Left Border Region	DNA region from *A. tumefaciens* containing the left border sequence used for transfer of the T–DNA

P2-*Ea.Ubq*	Promoter, 5' UTR, and intron sequences for a ubiquitin gene (*Ubq*) from *Erianthus ravennae* (plume grass) that directs transcription in plant cells
CS-*pat*	Coding sequence for the PAT protein of *Streptomyces viridochromogenes* that confers resistance to glufosinate
T-*Fba*	3' UTR sequence of the *fructose-bisphosphate aldolase* (*Fba*) gene from *Setaria italica* (foxtail millet) that directs polyadenylation of mRNA
P-*Clj.Ubq*	Promoter, 5' UTR, and intron sequences for a ubiquitin gene (*Ubq*) from *Coix lacryma-jobi* (adlay millet) that directs transcription in plant cells
TS-*APG6*	Codon optimized targeting sequence of the Albino and pale green 6 (Apg6) gene from *Arabidopsis thaliana* encoding a chloroplast-targeted Hsp101 homologue transit peptide region that directs the protein to the chloroplast
CS-*dmo*	Codon optimized coding sequence for the dicamba mono–oxygenase (DMO) protein of *Stenotrophomonas maltophilia* that confers dicamba resistance
T-*Mt*	3' UTR sequence of the OsMt gene from *O. sativa* encoding metallothionein-like protein that directs polyadenylation of mRNA
P-*Ad.Ubq*	Promoter, 5' UTR, and intron sequences for a ubiquitin gene (*Ubq*) from *Arundo donax* (giant reed) that directs transcription in plant cells
TS-*MDH*	Targeting sequence from *A. thaliana* Mdh gene encoding the malate dehydrogenase transit peptide region that directs the protein to the chloroplast
CS-*ft_t*	Coding sequence of modified version of R-2,4-dichlorophenoxypropionate dioxygenase (Rdpa) gene from *Sphingobium herbicidovorans* that expresses a FOPs and 2,4-D dioxygenase protein (FT_T) that confers tolerance to quizalofop and 2,4-D herbicides
T-*Nam*	3' UTR sequence from the gene coding for a no apical meristem (Nam) protein domain containing protein from *O. sativa*
P-*35S*	Promoter and leader from the 35S RNA of cauliflower mosaic virus (CaMV) that directs transcription in plant cells
L6-*Cab*	5' UTR leader sequence from the gene coding for chlorophyll a/b-binding (CAB) protein of *Triticum aestivum* (wheat) that is involved in regulating gene expression
I7-*Ract1*	Intron and flanking UTR sequence of the act1 gene from *O. sativa* encoding rice Actin 1 protein that is involved in regulating gene expression.
TS-CTP2	Targeting sequence of the ShkG gene from *A. thaliana* encoding the EPSPS transit peptide region that directs transport of the protein to the chloroplast
CS-*cp4 epsps*	Codon optimized coding sequence of the 5-enolpyruvylshikimate-3-phosphate synthetase (aroA) gene from the Agrobacterium sp. strain CP4 encoding the CP4 EPSPS protein that provides glyphosate tolerance
siRNA Target Sequence	Modified partial 3' UTR sequence of *Zea mays* cDNA that contains male tissue specific siRNA target sequence
T-Grp3	3' UTR sequence of the glycine-rich RNA binding- protein (Grp3) gene from *O. sativa* encoding the GRP3 protein that directs polyadenylation of mRNA

B-Right Border Region	DNA region from *A. tumefaciens* containing the right border sequence used for transfer of the T–DNA

T-DNA in transformation vector PV-ZMHT519224 contains the following four gene expression cassettes.

- The *pat* gene expression cassette contains a ubiquitin gene (*Ubq*) promoter from *Erianthus ravennae* (plume grass), the phosphinothricin-N-acetyltransferase gene (*pat*) from *Streptomyces viridochromogenes* that expresses the PAT protein to confer resistance to glufosinate herbicide, and a 3' UTR sequence of the *fructose-bisphosphate aldolase* (*Fba*) gene from *Setaria italica* (foxtail millet).

- The *dmo* gene expression cassette contains a ubiquitin gene (*Ubq*) promoter from *Coix lacryma-jobi* (adlay millet), a targeting sequence of the *Albino and pale green 6* (*Apg6)* gene from *Arabidopsis thaliana* encoding a chloroplast-targeted Hsp101 homologue transit peptide region that directs the protein to the chloroplast, a demethylase gene (*dmo*) from *Stenotrophomonas maltophilia* that expresses a dicamba mono-oxygenase (DMO) protein to confer tolerance to dicamba herbicide, and a 3' UTR sequence of the *OsMt* gene from *O. sativa* encoding metallothionein-like protein.

- The *ft_t* gene expression cassette contains a ubiquitin gene (*Ubq*) promoter from *Arundo donax* (giant reed), a, targeting sequence from *A. thaliana Mdh* gene encoding the malate dehydrogenase transit peptide region that directs the protein to the chloroplast, a R-2,4-dichlorophenoxypropionate dioxygenase (*Rdpa)* gene from *Sphingobium herbicidovorans* that expresses a FOPs and 2,4-D dioxygenase protein (FT_T) that confers tolerance to quizalofop and 2,4-D herbicides, and a 3' UTR sequence from the gene coding for a no apical meristem (*Nam*) protein domain containing protein from *O. sativa.*

- The *cp4 epsps* gene expression cassette contains the promoter and leader from the 35S RNA of cauliflower mosaic virus (CaMV), an intron and flanking UTR sequence of the *act1* gene from *O. sativa* (rice) encoding rice Actin 1 protein, the 5-enolpyruvylshikimate-3-phosphate synthetase gene from the *Agrobacterium* sp. strain CP4 (*cp4 epsps*) encoding the CP4 EPSPS protein that provides glyphosate resistance, the modified partial 3' UTR sequence of *Zea mays* cDNA that contains male tissue specific siRNA target sequence, and the 3' UTR sequence of the glycine-rich RNA binding- protein (*Grp3*) gene from *O. sativa*. Tissue-specific expression of CP4 EPSPS protein in MON 87429, allowing for glyphosate induced non-viable pollen phenotype, is the second generation of Monsanto's Roundup® Hybridization System (RHS) for hybrid seed production.

Among the above transgene elements inserted into MON 87429 maize, three elements, including the T-DNA right- and left-border sequences from *A. tumefaciens* and a promoter from the 35S RNA of cauliflower mosaic virus (CaMV) are associated with plant pests that are listed in 7 CFR 340.2. However, none of them is known to cause plant diseases.

<u>Characteristics, Stability, and Inheritance of the Introduced DNA</u>

Monsanto has provided data to characterize the inserted DNAs in MON 87429 maize with a combination of techniques including sequencing, PCR and bioinformatics (Monsanto 2019). The data demonstrate that MON 87429 maize contains a single copy of the T-DNA inserted into a single locus but does not contain DNA fragments from the vector backbone of plasmid PV-ZMHT519224.

A sequence comparison between the sequence generated from the 5' and 3' flanking sequences of MON 87429 and the sequence from the conventional control indicates that 54 bases of maize genomic DNA were deleted during integration of the T-DNA. There also was a 29-base insertion in the MON 87429 5′ flanking sequence and a 31 base insertion in the MON 87429 3' flanking sequence (Monsanto, 2019). Such changes are common during plant transformation (Anderson et al. 2016).

To assess the inheritance and stability of the MON 87429 T-DNA, phenotypic and genotypic segregation data were recorded during development of MON 87429 and analyzed using Chi square ($\chi2$) analysis over several generations. The $\chi2$ analysis results indicate that the MON 87429 T-DNA resides at a single locus within the maize genome and is stably inherited according to Mendelian principles.

Next Generation Sequencing (NGS) reads from five breeding generations of MON 87429 demonstrate the genetic stability of the T-DNA present in MON 87429 through multiple breeding generations. This comprehensive NGS and bioinformatic analysis of NGS data from multiple generations also supports the conclusion that MON 87429 contains a single, stable, inserted T-DNA.

Monsanto (2019) also demonstrates that MON 87429 maize showed the expected reduction of CP4 EPSPS protein expression in pollen with no impact on endogenous plant gene expression through using an endogenous maize regulatory element to target CP4 EPSPS mRNA for degradation in tassel tissues.

In summary, these results demonstrate that MON 87429 contains a single copy of the intended T-DNA containing the *pat, dmo, ft_t,* and *cp4 epsps* expression cassettes that is stably integrated at a single locus with no plasmid backbone sequences and is inherited according to Mendelian principles over multiple generations.

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

As described above, MON 87429 maize contains *dmo, pat, cp4 epsps* and *ft_t* expression cassettes for the expression of the DMO, PAT, CP4 EPSPS and FT_T proteins, respectively (Figure 4). DMO, PAT, CP4 EPSPS and FT_T protein levels in forage, root, leaf and grain of MON 87429 were determined using a multiplexed immunoassay. To further demonstrate the MON 87429 RHS trait mode-of-action (MOA), CP4 EPSPS expression in pollen tissue was determined to illustrate the differential expression between vegetative and pollen tissue.

DMO Protein

The *dmo* expression cassette expresses two forms of mature DMO protein with only one amino acid (cysteine) difference at the N-terminus due to alternative processing of the chloroplast transit peptide (CTP). One form consists of 341 amino acids while the other form of the DMO protein consists of 340 amino acids. The expression of the DMO protein confers resistance to dicamba herbicide. The mean DMO protein level in MON 87429 across all sites was highest in leaf at 35 µg/g dw and lowest in root at 2.3 µg/g dw. The mean DMO protein level in MON 87429 grain was 2.4 µg/g dw (Monsanto, 2019).

PAT Protein

Expression of the *pat* gene in MON 87429 results in a single polypeptide of 182 amino acids that are identical to the wild type PAT protein encoded by *S. viridochromogenes* and to the PAT proteins produced in several commercially available glufosinate resistant crops except for the first methionine that is removed due to co-translational processing in MON 87429 (Monsanto 2019). PAT protein levels were determined in forage, leaf, root and grain tissues. The mean PAT protein level in MON 87429 across all sites was highest in leaf at 5.8 µg/g dw and lowest in grain at 0.84 µg/g dw.

CP4 EPSPS Protein

MON 87429 contains a 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) gene from *Agrobacterium* sp. strain CP4 (*cp4 epsps*) that expresses an EPSPS protein with a single polypeptide chain of 455 amino acids (CP4 EPSPS). The mean CP4 EPSPS protein level in MON 87429 across all sites was the highest in leaf at 54 µg/g dw and lowest in grain at 0.63 µg/g dw. As intended the mean CP4 EPSPS protein level in MON 87429 pollen across all sites was below the limit of quantitation (0.11 µg/g dw).

FT_T Protein

The *ft_t* gene in MON 87429 is a modified version of the R-2,4-dichlorophenoxypropionate dioxygenase (*Rdp*a) gene from the soil bacteria *Sphingobium herbicidovorans*. The mean FT_T protein level in MON 87429 across all sites was the highest in leaf at 440 µg/g dw and lowest in root at 41 µg/g dw. The mean FT_T protein level in MON 87429 grain was 47 µg/g dw.

Potential new ORFs

Directed sequencing (locus-specific PCR, DNA sequencing and analyses) performed on MON 87429 was used to determine the complete sequence of the single DNA insert from

PV-ZMHT519224, the adjacent flanking genomic DNA, and the 5' and 3' insert-to-flank junctions. This analysis demonstrated that the sequence and organization of the DNA is identical to the corresponding region in the PV-ZMHT519224 T-DNA. Furthermore, the analysis of the genomic organization at the insertion site in MON 87429 showed that 54 bases were deleted upon T-DNA integration. There also was a 29 base insertion in the MON 87429 5′ flanking sequence and a 31 base insertion in the MON 87429 3′ flanking sequence. Such changes are common during plant transformation (Anderson et al. 2016) and these changes presumably resulted from DNA repair mechanisms in the plant during *Agrobacterium*-mediated transformation processes (Salomon and H. 1998). No major DNA rearrangement occurred at the insertion site in MON 87429 upon DNA integration and the flanking sequences are not functional elements (Monsanto 2019).

Metabolism composition Analysis

To assess any potential metabolite alteration as a result of the expression of the above inserted genes, Monsanto analyzed the metabolism composition of MON 87429 maize grain and forage samples collected from five field sites that were representative of U.S. maize growing regions (Monsanto 2019). The production of materials for compositional analyses used a sufficient variety of field trial sites, reflecting a range of environmental conditions under which MON 87429 is expected to be grown and robust field designs (randomized complete block design with four replicates). MON 87429 plots were treated with dicamba, glufosinate, quizalofop and 2,4-D to generate samples under conditions of the intended use of the product. Samples were subjected to sensitive analytical methods that allow quantitative and accurate measurements of key components.

For MON 87429 maize, the introduced proteins, DMO, PAT, CP4 EPSPS and FT_T, confer herbicide resistance and lack catalytic activity that is intended to or expected to affect the plant's metabolism. Given the nature of these introduced traits and the overall lack of meaningful unintended compositional characteristics observed for biotechnology-derived products characterized to date (Herman and Price 2013; Venkatesh et al. 2015), compositional changes that would affect the levels of components in MON 87429 maize were not expected.

Composition data for 25 metabolic components including major nutrients in grain (protein, amino acids, total fat, linoleic acid, carbohydrates, acid detergent fiber, neutral detergent fiber and ash), major nutrients in forage (protein, total fat, carbohydrates, acid detergent fiber, neutral detergent fiber and ash) and anti-nutrients in grain (phytic acid and raffinose) were analyzed. The statistical comparison of MON 87429 maize and the conventional control was based on compositional data combined across all five field sites. It is noteworthy that a statistically significant difference between MON 87429 and the conventional control does not necessarily imply biological relevance from a food and feed safety perspective. A mean difference less than the variability seen due to natural environmental variation within the single, closely related germplasm is typically not a food or feed safety concern (Venkatesh et al. 2015). Only if the impact of MON 87429 on levels of metabolic components is large relative to natural variation inherent to conventional maize would the difference in composition be potentially meaningful from a food and feed safety and nutritional perspective. Therefore, the difference between MON

87429 and the conventional control was evaluated in the context of variation within the conventional control germplasm grown across multiple sites (i.e., variation due to environmental influence) by determining the range of replicate values for the conventional control.

There were no statistically significant differences for 23 of the 25 metabolic components analyzed from MON 87429 grain and forage (Table VI-1–Table VI-4 in Monsanto 2019). Two components (total fat and linoleic acid in grain) showed a statistically significant difference between MON 87429 and the conventional control (Table VI-1 in Monsanto 2019). For total fat, the mean values for MON 87429 maize and the conventional control were 3.76 % dw and 3.88 % dw, respectively with a difference of -0.15 % dw. For linoleic acid, the mean values for MON 87429 and the conventional control were 55.53 % Total Fatty Acid (FA) and 55.21 % Total FA, respectively, with a difference of 0.32 % Total FA. For these components, however, the difference between MON 87429 and the conventional control was less than the conventional control range values. Furthermore, the MON 87429 mean component values were within the range of values observed in the literature and/or the ILSI-CCDB values (Table VI-5 in Monsanto 2019). Thus, these data indicated that the statistically significant differences observed in total fat and linoleic acid in grain did not imply biological relevance from a food and feed safety perspective.

In summary, the expression of the inserted DNAs and the resulting phenotype in MON 87429 maize are consistent with the inheritance of the introduced genetic material. The sequence analysis showed no evidence supporting any potential creation of new ORFs or any unintended effects resulting from the insertion of the genetic materials (Monsanto 2019). The compositional analyses demonstrated that introduction of the PV-ZMHT519224 T-DNA in MON 87429 maize achieved the intended expression of DMO, PAT, CP4 EPSPS and FT_T for herbicide resistance while maintaining the equivalent metabolism composition of gain and forage tissues in comparison to the control and other conventional maize varieties.

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 of plant pests, or from any other expression products, new enzymes, proteins or changes in plant metabolism or composition in MON 87429 maize that are known or anticipated to cause disease symptoms, or to affect plant pests or diseases or plant defense responses. APHIS also assessed whether MON 87429 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 modified 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. A variety of insect, plant disease, mollusk, nematode or weed programs exist including the programs for grasshoppers (Order Orthoptera) on rangelands, light brown apple moth (*Epiphyas postvittana*) in California, and of more relevance, Japanese beetle (*Popillia japonica*), Old World bollworm (*Helicoverpa armigera*), and witchweed (*Striga asiatica*) that can affect maize (USDA-APHIS 2019b).

The grasshoppers are normally natural components of the rangeland ecosystem (Figure 5), but they can invade adjacent cropland and cause serious economic losses when their populations reach outbreak levels, especially when accompanied by a drought condition.

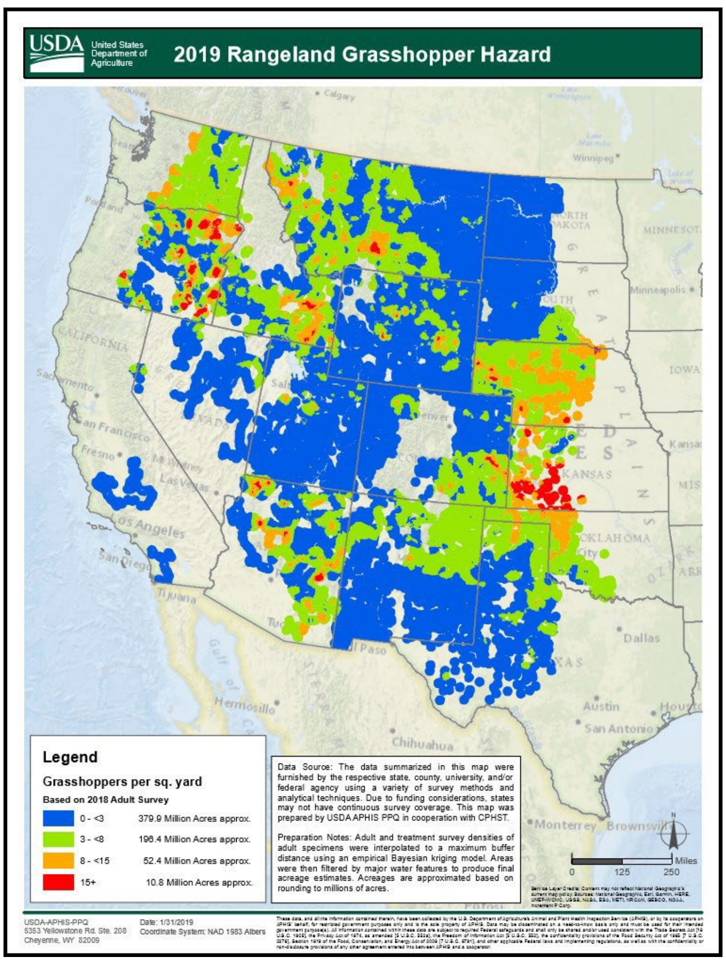
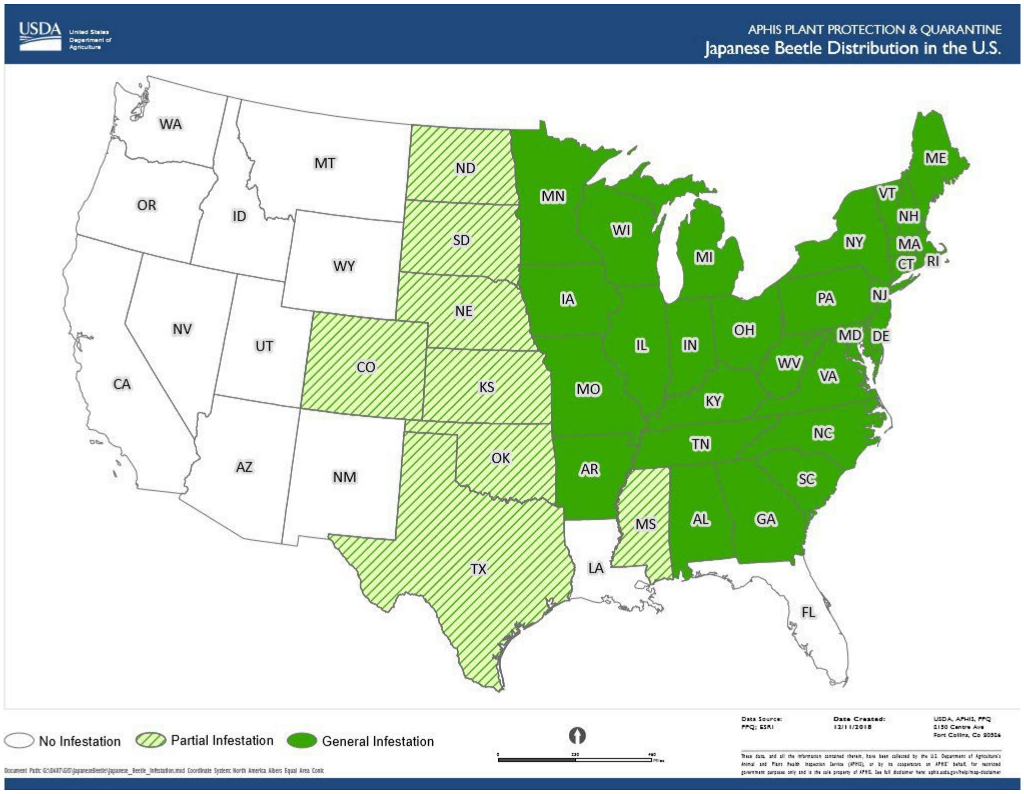


Figure 5. 2019 U.S. rangeland grasshopper hazard

The light brown apple moth (LBAM) can damage a wide range of crops and other plants. The LBAM was found in California in 2007, and some areas have been designated as quarantined areas (Figure 6).

Figure 6. 2018 Quarantined areas for light brown apple moth in California

The Japanese beetle is a highly destructive plant pest that can be very difficult and expensive to control. Japanese beetle adults attack the foliage, flowers, or fruits of more than 300 different ornamental and agricultural plants. Japanese beetles have spread throughout many states of the U.S. (Figure 7). APHIS maintains the Japanese Beetle Quarantine and Regulations that can be found in 7 CFR 301.48 with the objective to protect the agriculture of the Western United States and prevent the human-assisted spread of the beetle from the Eastern U.S.



The Old-World bollworm can affect 180 species of plants, with maize listed as one of its preferred hosts. It is closely related to the corn earworm (*Helicoverpa zeae*). Old World bollworm was first detected in western Puerto Rico in September 2014, and APHIS is conducting a variety of activities to protect the continental U.S from this pest (USDA-APHIS 2019b).

Witchweed (*Striga asiatica*) is a parasitic plant listed as a Federal Noxious Weed that affects maize and several other crops. 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 2019j).

Maize itself is not considered a plant pest in the United States (7 CFR 340.2). The Agrobacterium strain *A. tumefaciens* used in the generation of MON 87429 maize was disarmed and also was already removed with antibiotics during the transformation process. The inserted DNA elements derived from plant pests do not result in the production of infectious agents or disease symptoms in plants. The genetic modifications of MON 87429 maize including genetic elements, expression of the gene products and their functions have been summarized above and are not expected to impart any new plant pest or disease risk than comparator maize lines.

Monsanto evaluated the differences between MON 87429 maize and conventional control plants of the damages from the 14 assessed arthropods (aphids, armyworms, billbugs, corn earworms, corn flea beetles, corn rootworm beetles, cutworms, European corn borers, grasshoppers, Japanese beetles, sap beetles, slugs, spider mites, and stink bugs) and 10 assessed diseases (anthracnose, corn stunt, eyespot, Goss's bacterial wilt, gray leaf spot, leaf blight, northern leaf spot, rust, smut (head and ear), and Stewart's wilt) (Monsanto 2019). The differences of MON 87429 maize, conventional control, and commercial references in their responses to arthropod pests and diseases were evaluated at natural levels (no artificial infestation) during the growing season at the following growth stages: V5 – V8, V12 – R1, R1 – R3, and R4 – R5 at each of eight field sites. No differences were observed between MON 87429 and the conventional control for both arthropod and disease damages.

These data demonstrate that the integration of the T-DNA of plasmid PV-ZMHT519224 and the expression of DMO, PAT, CP4 EPSPS and FT_T for herbicide resistance in MON 87429 maize did not significantly alter the insect predation and disease occurrence or render MON 87429 maize more susceptible to pests and diseases over its control or reference maize varieties. Also, as discussed earlier, there were no observed or anticipated unintended metabolic composition changes in MON 87429 maize that could impart any new plant pest or disease risk compared to conventional maize (Monsanto 2019). Thus, MON 87429 maize is unlikely to be more susceptible to plant pathogens and insect pests than conventional maize. For this reason, MON 87429 maize 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 Non-target Organisms Beneficial to Agriculture

The MON 87429 maize is not engineered for pest resistance other than for the expression of DMO, PAT, CP4 EPSPS and FT_T for herbicide resistance. Thus, Mon 87429 maize does not possess pesticidal activity, nor has 'target' species. APHIS assessed whether exposure or consumption of the MON 87429 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 87429 maize compared to the conventional maize counterpart for any biologically relevant changes in the phenotype or substances (e.g., proteins, nutrients, or anti-nutrients) 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.

As described above in Section C, the inserted T-DNA in MON 87429 maize encodes four proteins novel to maize, DMO, PAT, CP4 EPSPS and FT_T that confer herbicide resistance. These proteins have been assessed in multiple products by USDA-APHIS and U.S. FDA in the past years. The modes-of-action and experimental evidence reviewed in the current and/or previously submitted Petitions supports that these four proteins are safe; and no safety concerns have occurred during the history of environmental exposure to DMO, PAT, CP4 EPSPS and the alpha-ketoglutarate-dependent dioxygenase family of proteins to which FT_T belongs.

DMO Protein

MON 87429 maize contains a demethylase gene (*dmo)* from *Stenotrophomonas maltophilia* that expresses a dicamba mono-oxygenase (DMO) protein. The DMO protein enzymatically catalyzes the demethylation of the broadleaf herbicide dicamba to the non-herbicidal compound 3,6-dichlorosalicylic acid (DCSA) and formaldehyde, thus conferring dicamba resistance (Chakraborty et al. 2005). DMO protein is part of a large oxygenase family of enzymes that incorporate one or two oxygen atoms into substrates and are widely distributed in many universal metabolic pathways (Harayama et al. 1992). The DCSA product of the reaction catalyzed by the DMO protein is a known metabolite of dicamba in conventional cotton, soybean, soil, and livestock, and its safety has been evaluated by the FAO-WHO (FAO-WHO 2010).

The DMO protein in MON 87429 maize possesses a high level of sequence identity and structural and functional similarity with DMO proteins expressed in modified crops that were deregulated by USDA-APHIS (USDA-APHIS 2019a). Furthermore, these modified crops expressing DMO proteins completed consultation with U.S. FDA, where it was demonstrated that food and feed derived from these events are not materially different than the respective conventional crops. Thus, prior safety assessments of the DMO protein expressed in other modified crops demonstrating the lack of impact on plant pest potential, the lack of homology to known protein toxins or allergens, digestibility in *in vitro* digestion assays and lack of acute oral toxicity are directly applicable to the DMO protein expressed in MON 87429 maize.

PAT Protein

The phosphinothricin N-acetyltransferase (PAT) acetylates herbicide glufosinate in the presence of acetyl CoA to form non-herbicidal N-acetyl glufosinate, thus conferring glufosinate resistance to the crop. The PAT proteins have been isolated from two separate species of *Streptomyces*, *S. hygroscopicus* (Thompson et al. 1987) and *S. viridochromogenes* (Wohllehen et al. 1988). The PAT protein isolated from *S. hygroscopicus* is encoded by the *bar* gene, and the PAT protein isolated from *S. viridochromogenes* is encoded by the *pat* gene. MON 87429 maize contains the *pat* gene from *S. viridochromogenes*. These two PAT proteins are made up of 183 amino acids with 85% identity to each other at the amino acid level (Wohllehen et al. 1988), and are considered to be equivalent with regard to function and safety (OECD 1999).

The PAT proteins have a robust history of safe consumption and safe use in agriculture that is supported by the lack of any documented reports of adverse human or animal affects since the introduction of modified crops expressing PAT proteins in 1995 (Duke 2005). Numerous glufosinate-resistant crops including maize, canola, soybean, sugar beet, rice and cotton have been deregulated by USDA-APHIS (USDA-APHIS 2019a), where it was demonstrated that food and feed derived from these crops are not materially different than the respective conventional crops. The safety of PAT proteins has been confirmed following extensive reviews by regulatory agencies in many other countries for more than 30 modified crop events in several different crop species (e.g., maize, soybean, cotton, canola and sugar beet). Furthermore, the EPA has issued a tolerance exemption for PAT protein (US-EPA 1999). Given that the PAT protein expressed in MON 87429 is almost identical to PAT proteins produced in other commercially available glufosinate resistant crops, prior safety assessments of the PAT proteins expressed in these other modified crops demonstrating lack of impact on plant pest potential, lack of homology to known protein toxins or allergens, digestibility in *in vitro* digestion assays and lack of acute oral toxicity are directly applicable to the PAT protein expressed in MON 87429 maize.

CP4 EPSPS Protein

5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) is a key enzyme involved in aromatic amino acid biosynthesis and it catalyzes an enzymatic reaction to form 5-enolpyruvylshikimate-3-phosphate (EPSP) and inorganic phosphate, leading to the biosynthesis of aromatic amino acids (phenylalanine, tryptophan and tyrosine) and other aromatic molecules that are necessary for plant growth (Alibhai and Stallings 2001). Glyphosate inhibits the endogenous plant EPSPS enzyme, thereby depriving plants of essential amino acids (Steinrucken and Amrhein 1980).

MON 87429 contains an EPSPS gene from *Agrobacterium* sp. strain CP4 (*cp4 epsps*) that expresses the CP4 EPSPS protein. Although the CP4 EPSPS protein expressed in MON 87429 is structurally similar and functionally identical to endogenous plant EPSPS enzymes, it has a much-reduced affinity for glyphosate and thus confers resistance to glyphosate (Sikorski and Gruys 1997). Additionally, MON 87429 maize utilizes an endogenous maize regulatory element to target CP4 EPSPS mRNA for degradation specifically in tassel tissues, resulting in reduced CP4 EPSPS protein expression in pollen. Appropriately timed glyphosate applications result in a sterile pollen phenotype and thus, allow for desirable cross pollinations to be made in maize without using traditional methods to control self-pollination in female inbred parents.

The safety and mode-of-action of CP4 EPSPS protein is studied extensively and well documented (ILSI 2010). The CP4 EPSPS protein has a robust history of safe consumption and safe use in agriculture as supported by the lack of any documented reports of adverse human or animal effects since the introduction of modified crops expressing CP4 EPSPS protein. Numerous glyphosate-resistant, commercially available CP4 EPSPS containing crops have been deregulated by USDA-APHIS (USDA-APHIS 2019a) and have completed consultations with the FDA, where it was demonstrated that food and feed derived from these crops are not materially different than the respective conventional crops. The safety of the CP4 EPSPS protein has also been reviewed by regulatory agencies around the world (OECD 1999, 2002). Furthermore, the U.S. EPA in 1996 established an exemption from the requirement of a tolerance for residues of the plant pesticide inert ingredient CP4 EPSPS and the genetic material necessary for its production in all plants (40 CFR § 174.523, re-designated from § 180.1174, effective April 25, 2007). Prior safety assessments of the CP4 EPSPS protein expressed in these other modified crops are directly applicable to the MON 87429 CP4 EPSPS protein because the MON 87429 CP4 EPSPS protein is identical to the CP4 EPSPS proteins in these modified crops.

FT_T Protein

The *ft_t* gene in MON 87429 is a modified version of the R-2,4-dichlorophenoxypropionate dioxygenase (*Rdp*a) gene from the soil bacteria *Sphingobium herbicidovorans*. The amino acid sequence of the FT_T protein shares ~ 89% sequence identity with wild type RdpA (Monsanto 2019). The FT_T protein is an alpha-ketoglutarate-dependent non-heme iron dioxygenase that catalyzes a dioxygenase reaction in the presence of alpha-ketoglutarate (αKG) and oxygen by incorporating oxygen into quizalofop, thus degrading it into the herbicidally-inactive quizalofop phenol and pyruvate. The FT_T protein also catalyzes the dioxygenase reaction that degrades auxin herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) into herbicidally-inactive 2,4-dichlorophenol (2,4-DCP) and glyoxylic acid in the presence of alpha-ketoglutarate and oxygen. Thus, the FT_T protein in MON 87429 confers resistance to aryloxyalkanoate herbicides including the aryloxyphenoxypropionate acetyl coenzyme A carboxylase (ACCase) inhibitors (so called “FOPs” herbicides such as quizalofop) and some synthetic auxins, such as 2,4-D.

Monsanto demonstrated that the FT_T protein lacks structural similarity to allergens, toxins or other proteins known to produce adverse effects in mammals. The FT_T protein is rapidly digested by proteases found in the human gastrointestinal tract (pepsin and pancreatin) and demonstrated no acute oral toxicity in mice at the high dose tested. In addition, the ubiquitous presence of *Sphingobium* species in the environment has resulted in widespread human and animal exposure and is not commonly known for allergenicity and human or animal pathogenicity. Furthermore, the FT-T protein as an α-ketoglutarate-dependent non-heme iron dioxygenase belongs to a diverse superfamily of Fe(II)/α-ketoglutarate dependent hydroxylases, and this protein superfamily is broadly distributed across the plant, animal and bacterial kingdoms, which have been extensively consumed by both humans and animals (Hausinger 2004) without any reports of adverse effects. Therefore, it is unlikely that exposure to the FT_T protein in food and feed products derived from MON 87429 pose a risk to human and animal health.

Additionally, molecular characterization revealed no potential for unintended effects and no unintended changes to plant metabolism, as supported by the substantially equivalent compositional characteristics between MON 87429 and the conventional control. The germination and dormancy, phenotypic and agronomic, and pollen characteristics were not indicative of increased weediness or plant pest risk for MON 87429 compared to conventional maize. Furthermore, the arthropod and disease damages data showed a lack of differences in plant responses to a subset of non-target organisms (specific diseases and arthropod pests) between MON 87429 and conventional control maize. Similarly, there has been no reported adverse impacts to non-target organisms due to the exposure to DMO, PAT, or CP4 EPSPS proteins from either extensive testing and/or wide scale commercial cultivation of a number of different modified herbicide-resistant crops. The FT_T protein is unlikely to have adverse impacts to non-target organisms because of its non-toxic mode-of-action (MOA), lack of acute oral toxicity, and lack of impact on plant pest potential.

Corn possesses few of characteristics of weeds and has a long history of cultivation around the globe without any report that it is a serious weed or that it forms persistent feral populations. In the United States, the regions where maize is grown have no plants listed as threatened or endangered, or that are proposed for listing, that are sexually compatible with maize. Also, there are only a limited number of threatened or endangered species that may be found in U.S. maize fields, and there is an even more limited number of species that might feed on maize plants or maize grain. Furthermore, none of the listed species in states where maize is grown require maize as a host plant. The safety of the MON 87429 DMO, PAT, CP4 EPSPS and FT_T proteins, and the compositional, agronomic, and phenotypic equivalence of MON 87429 to conventional maize, support a conclusion that the planting of MON 87429 is not expected to affect listed threatened or endangered plant species or designated critical habitat for listed plant or animal species.

Therefore, based on the data including the mode of action, the low expression levels, and the molecular characterization and safety assessment of DMO, PAT, CP4 EPSPS and FT_T proteins; the demonstration of compositional, agronomic and phenotypic equivalence to conventional maize; and the environmental interactions assessment, APHIS concludes that exposure to and/or consumption of MON 87429 maize is unlikely to have any adverse impacts to non-target organisms, nor does it pose an additional risk to organisms beneficial to agriculture or threatened and endangered species compared to conventional maize.

F. Potential for Enhanced Weediness of MON 87429 maize

APHIS assessed whether the MON 87429 maize is likely to become weedier (i.e. more prevalent, competitive, damaging or difficult-to-control in situations where it is not wanted) than the unmodified progenitor from which it was derived, or other varieties of the crop currently under cultivation. The assessment considers the basic biology of maize, the situations in which maize volunteers or feral populations are considered weeds, and an evaluation of MON 87429 maize compared to conventional maize control and other reference maize hybrids 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 seed dormancy and germination, agronomic and phenotypic traits, disease and pest susceptibility, and environmental interactions. The assessment also considers whether the stacked herbicide resistance 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 United States, maize is not listed as a weed in the major weed references (Crockett 1977) and it is not designated as a noxious weed by the federal government (USDA-APHIS 2019b). Furthermore, maize does not possess weedy characteristics such as high level of seed dormancy, ease of seed shattering, and strong growth competitiveness (OECD 2003; OTGR 2008). Maize seeds do not exhibit dormancy and are well retained on the cob and covered by multiple layers of husk leaves. Also, maize is sensitive to low temperatures, and the germinating seedlings and plants do not survive freezing winter conditions (OECD 2003; OTGR 2008) (Andersson and de Vicente 2010). Although maize seed does not shatter, harvest process or foraging wildlife can result in seed disperse, and some may overwinter and germinate when conditions are ideal and develop into volunteer plants the following year. However, maize has not been reported to be able to establish self-sustaining populations outside of cultivation (OECD 2003; OTGR 2008). This is further supported by data from controlled experiments where maize plants were left unharvested but no feral plants were discovered within a year or two after planting (Raybould et al. 2012; Sammons et al. 2014). Similar to conventional maize volunteers, MON 87429 maize volunteers can be managed by employing 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).

In comparative studies between MON 87429 and a conventional control, germination and dormancy, phenotypic and agronomic, environmental interaction (plant responses to abiotic stressors, diseases, and arthropod pests), and pollen characteristics were evaluated for changes that would impact the plant pest potential, and in particular, plant weediness potential. In each of these assessments, MON 87429 was compared to an appropriate conventional control that had a genetic background similar to MON 87429 but did not possess the inserted traits. In addition, multiple commercial maize hybrids developed through conventional breeding and selection were included to provide a range of comparative values for each characteristic that are representative of the variability in existing commercial maize hybrids.

To assess the seed germination and dormancy potential of MON 87429 maize seed, Monsanto conducted a comparative assessment of seed germination and dormancy characteristics for MON 87429 and the conventional control. MON 87429 was compared to the conventional control for percentages of germinated, dead, viable firm-swollen, and viable hard seed using two temperature regimes: optimum (alternating 20°C and 30°C) and suboptimum (constant 10°C for seven days followed by 25°C for four days). No statistically significant differences were detected between MON 87429 and the conventional control in either the optimum or suboptimum temperature regimes for any of the evaluated characteristics. These results demonstrate that the introduction of the dicamba-, glufosinate-, quizalofop-, and 2,4-D-tolerance and RHS traits does not result in different seed germination characteristics and dormancy potential of MON 87429 maize compared to conventional maize.

Monsanto also evaluated the phenotypic, agronomic, and environmental interactions characteristics of MON 87429 maize by comparing with the conventional control and four commercial reference hybrids at eight field sites representing major U.S. maize growing regions in 2017 (Monsanto, 2019). A combined-site analysis for nine phenotypic and agronomic characteristics (early stand count, days to flowering, plant height, days to maturity, lodging, final stand count, moisture, seed weight, and yield) showed no statistically significant differences between MON 87429 and the conventional control for any of the analyzed characteristics (Table VII-4, Monsanto 2019). Also, in an assessment of plant responses to abiotic stressors (cold temperatures, drought, waterlogging, hail, high temperatures, high winds, nutrient deficiency, soil compaction, and sun scald), diseases (anthracnose, corn stunt, eyespot, Goss's bacterial wilt, gray leaf spot, leaf blight, northern leaf spot, rust, smut, and Stewart's wilt), and arthropod pests (aphids, armyworms, billbugs, corn earworms, corn flea beetles, corn rootworm beetles, cutworms, European corn borers, grasshoppers, Japanese beetles, sap beetles, slugs, spider mites, and stink bugs), no differences were observed between MON 87429 and the conventional control for any of the analyzed characteristics.

APHIS also assesses the potential for increased weedy or invasive characteristics of the sexually compatible plants and wild relatives through the potential gene flow and introgression from MON 87429 maize. The viability and morphology of MON 87429 maize were compared to that of the conventional control and four commercial references grown under similar agronomic conditions. No statistically significant differences were detected between MON 87429 and the conventional control for percentage viable pollen, pollen diameter and general pollen morphology.

These results support the conclusion that MON 87429 does not possess: 1) increased weediness characteristics; 2) increased susceptibility or tolerance to specific abiotic stressors, diseases, or arthropod pests; or 3) characteristics that would confer a plant pest risk compared to conventional maize. Therefore, based on the results of multiple assessments discussed above and the weight of evidence supports the overall conclusion that the introduction of the dicamba-, glufosinate-, quizalofop-, and 2,4-D-tolerance and RHS traits does not result in increased weediness or plant pest risk of MON 87429 compared to conventional maize and is unlikely to pose a plant pest risk. These results, in conjunction with the cultivation of existing various herbicide-resistant maize varieties in the United States, supports that MON 87429 maize is unlikely to be any more difficult to control as a volunteer in subsequent seasons after its planting. Thus, the existing numerous methods to effectively manage volunteer maize in agricultural fields (Ogg and Parker 2000; USDA-APHIS 2014) are also likely to be effective in controlling MON87429 maize volunteers.

Based on the agronomic field data and literature survey concerning weediness potential of the crop, MON 87429 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 field trial plots planted with MON 87429 maize under USDA-APHIS authorizations did not reveal any differences in survivability or persistence relative to other varieties of the same crop currently being grown. These data suggest that MON 87429 maize is no more likely to become a weed than conventional varieties of the crop. MON 87429 maize volunteers and feral populations 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 87429 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; Hegde et al. 2006), and even in the existing floras, the occurrence of hybridization or introgression is reported to be widespread (Stace 1987; Rieseberg and Wendel 1993; Peterson et al. 2002). It has been a common practice by plant breeders to artificially introgress traits from wild relatives into crop plants to develop new cultivars (Khoury et al. 2013). Hybridization and introgression are natural biological processes and do not constitute inherent environmental risks. 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)). This topic is covered in two sections: 1) the potential for gene flow, hybridization and introgression from the MON 87429 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 based on the phenotypic changes that have been observed in MON 87429 maize.

Potential for gene flow, hybridization, and gene introgression

Maize is a wind pollinated species with plant morphology and reproductive biology that facilitates cross pollination, leading to relatively high levels of pollen-mediated gene flow occurrence in this species. However, for pollen-mediated gene flow to occur between maize and its allied species and subspecies, certain conditions must be satisfied such as sexual compatibility, flowering synchrony and sufficient proximity to each other.

Hybridization with Teosinte

As described in Section B, the genus *Zea* consists of five species: 1) *Z. diploperennis*, perennial diploid (2n=20); 2) *Z. luxurians*, annual diploid (2n=20); 3) *Z. nicaraguensis*, annual diploid (2n=20); 4) *Z. perennis*, perennial tetraploid (2n=40); and the *Z. mays*, annual diploid (2n=20). The latter encompasses four annual diploid (2n=20) subspecies: ssp. *mays*, ssp. *huehuetenangensis,* ssp. *mexicana* and ssp. *parviglumis* (Hufford et al. 2012). Within genus *Zea*, *Z. mays* ssp. *mays* is the only domesticated maize and all the other species and subspecies are the wild relatives of the maize and are collectively named as teosintes (OTGR 2008; Andersson and de Vicente 2010).

All species in teosintes except the tetraploid perennial *Z. perennis* can cross with cultivated maize to produce fertile hybrids, but typically occur at very low rate (Doebley 1990; Baltazar et al. 2005; OTGR 2008). It is reported that hybridization between maize and *Z. mays* ssp. *mexicana* occurs sporadically and at very low rates (Baltazar et al. 2005; Ellstrand et al. 2007) but maize can hybridize with *Z. mays* ssp. *parviglumis* readily at higher rates (Ellstrand et al. 2007). However, while these hybridizations occurred in the direction of maize as female and teosinte as male, hybridizations in the opposite direction rarely occurred (Ellstrand et al. 2007; Mauricio et al. 2013). Gene flow from maize to teosinte most probably results from crosses where teosinte first pollinates maize (Baltazar et al. 2005). This limited and asymmetric gene flow, favoring teosinte introgression into maize may be attributed to the genetic barrier between teosinte and maize that is controlled by a gene called the *'Teosinte crossing barrier*' (*Tcb*) (Evans and Kermicle 2001). Gene flow and introgression between maize and teosintes is also limited by their geographical distribution, flowering synchrony and proximity.

Teosinte is not native to the United States. However, the annual teosinte (*Z. mays* ssp. *mexicana*) is reported to have feral populations in Florida, Alabama, and Maryland (USDA-NRCS 2019a) and *Z. perennis* is listed in Texas and South Carolina (USDA-NRCS 2019b). For *Z. diploperennis* and *Z. luxurians,* there are no reported information about their location and status in the United States (USDA-NRCS 2019c, 2019d). 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 in the United States (USDA-APHIS 2013).

Taken together, the genetic barrier, differences in developmental and morphological factors, potential flowering asynchrony and insufficient proximity between maize and teosinte as well as the limited geographical distribution of teosinte make natural crosses and gene introgression from MON 87429 maize into teosinte unlikely in the United States.

Hybridization with *Tripsacum*

Tripsacum is the genus that is the closet known relative of *Zea* and it consists of 16 recognized species (OECD 2003). *Tripsacum* species has a base chromosome number of x=18 compared to the base chromosome of maize (x=10) and can be represented by diploid (2n=36), triploid (2n=54), tetraploid (2n=72), pentaploid (2n=90) and hexaploid (2n=108). There are five species of *Tripsacum* that are present in the United States, including three species native to the U.S.: *T. floridanum* (Florida gamagrass), *T. lanceolatum* (Mexican gamagrass), and *T. dactyloides* (Eastern gamagrass); two species introduced in Puerto Rico: *T. latifolium* (wideleaf gamagrass) and *T. fasciculatum* (Guatemalan gamagrass) (USDA-NRCS 2019e, 2019f, 2019g, 2019h, 2019i).

Unlike teosinte that its member species can hybridize with maize under natural conditions, out-crossing between maize and *Tripsacum* species is not known to occur in the wild and can only be made experimentally with extreme difficulty (OECD 2003). *Tripsacum* species (*T. dactyloides*, *T. floridanum*, *T. lanceolatum*, and *T. pilosum*) have been crossed with maize under experimental conditions, however, the resultant hybrids have a high degree of sterility and are genetically unstable (Galinat 1988; OTGR 2008; Andersson and de Vicente 2010). Thus, *Tripsacum* species are unlikely to form viable hybrid progeny with maize under natural conditions.

The introduced genes encoding DMO, PAT, CP4 EPSPS and FT_T proteins in MON 87429 maize are not expected to change the ability of the plant to interbreed with other plant species. Indeed, the agronomic and phenotypic data of MON 87429 maize provided by Monsanto indicated no unintended changes likely to affect the potential gene flow from MON 87429 maize to sexually compatible species.

Based on all the above information, the genetic modification in MON 87429 maize is not expected to increase the potential for gene flow, hybridization and/or introgression to sexually-compatible taxa compared to the other maize varieties. Gene flow, hybridization and/or introgression of genes from MON 87429 maize to other sexually-compatible relatives with which it can interbreed is not likely to occur in the United States and its territories.

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

Based on the data presented in the petition, MON 87429 maize does not exhibit characteristics that may cause it to be any weedier than other cultivated maize (Monsanto 2019). Moreover, its potential impact due to the extremely limited potential for gene introgression into teosinte and *Tripsacum* species is not expected to be any different than that of other cultivated maize varieties. Additionally, none of the sexually compatible-relatives of maize in the United States are considered to be weeds in the United States (Holm et al. 1979). Therefore, even in the extremely unlikely event of successful hybrids and/or introgression between MON 87429 maize and its wild relatives, the inserted transgenes of MON 87429 maize are unlikely to transform its wild relatives into more weedy species. If the maize wild relatives were introgressed with the herbicide resistance traits from MON 87429 maize, they can be managed using alternative herbicides and/or other method of weed control. Based on the above considerations, MON 87429 maize is unlikely to adversely impact sexually-compatible wild relatives or their weediness characters.

Based on the information presented in the petition and in relevant literature, APHIS has reached the following conclusions. The genetic modification in MON 87429 maize is not expected to increase the potential for gene flow, hybridization, and/or introgression to occur to sexually-compatible taxa compared to the non-modified recipient or other varieties of maize that are commonly grown. Gene flow, hybridization, and/or introgression of genes from MON 87429 maize to other sexually-compatible relatives, including wild, weedy, feral or cultivated species in the United States and its territories is not likely to occur. Furthermore, both the maize and its sexually compatible relative species are not considered weedy or invasive. The modified phenotype is not expected to affect the current ability to control these species in situations where they are considered weedy or invasive; the following measures are still available for their control: herbicides, tillage and other methods. Therefore, MON 87429 maize is not expected to increase the weed risk potential of other species with which it can interbreed in the United States and its territories.

H. Potential Changes to Agriculture or Cultivation Practices

APHIS assessed whether significant changes to agricultural or cultivation practices from adoption of the MON 87429 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.

As described in Section B, maize is widely grown in the world (Farnham et al. 2003; OTGR 2008), and it is grown in almost all the states in United States (USDA-NASS 2019a).Weeds, annual and/or perennial, are considered to be the greatest pest problem in maize production because they compete with maize for water, nutrients, and light resulting in substantial yield losses when left uncontrolled (Aref and Pike 1998). Weed management practices include mechanical practices (e.g., tillage), cultural practices (e.g., crop rotation, variety selection, optimizing planting date, plant population and row spacing), and chemical practices (e.g., herbicide application). Herbicide-Resistant maize is currently grown on approximately 90% of U.S. maize acres, and approximately 98% of the maize acreage in the U.S. receives an herbicide application (Monsanto 2019). Numerous herbicides are available for preplant, preemergence, and postemergence control of annual and perennial weeds in maize (USDA-NASS 2019c-a). MON 87429 maize offers the combination of dicamba, glufosinate, quizalofop, 2,4-D, and glyphosate resistance, and this combination will facilitate the utilization of multiple herbicide sites-of-action in a grower's integrated weed management system to control a broad spectrum of grass and broadleaf weed species, including herbicide-resistant and tough to control weed species. Mon/N 87429 can help reduce the potential for further resistance development to glyphosate, dicamba, glufosinate, quizalofop and 2,4-D herbicides as well as other important maize herbicides.

It is noteworthy that the MON 87429 event confers glyphosate resistance in specific plant tissues (i.e., not in tassels) to facilitate the production of hybrid seed. The susceptibility of tassels in MON 87429 to glyphosate results in intended non-viable pollen phenotype when applying glyphosate during reproductive development. Glyphosate can also be used for weed control in MON 87429 inbred seed increases or hybrid seed production. However, other agricultural management practices for the production of hybrid maize seed and for the cultivation of commercial maize would also be no different for MON 87429 than for conventional maize hybrids. Furthermore, MON 87429 is not intended to be offered for commercial use as a stand-alone product, but will be combined, through traditional breeding methods, with other deregulated events that confer full-plant glyphosate resistance (e.g., NK603). Upon stacking of MON 87429 maize with deregulated full-plant glyphosate resistance traits, growers will have the ability to use the established current glyphosate practice for weed control.

Glufosinate, quizalofop and 2,4-D herbicides are currently labeled for preplant applications on conventional and herbicide-resistant maize hybrids and for in-crop postemergence applications on herbicide-resistant (HR) hybrids. The intended preplant and postemergence uses of these herbicides with MON 87429 maize would not be any different than current labeled uses in HR maize. In support of Monsanto's new herbicide resistant product MON 87419 maize that has been deregulated by USDA-APHIS, Monsanto requested that EPA allow the 0.5 lb a.e./ac postemergence application window for dicamba to be extended from V5 to V8 growth stage or 36-inch height of maize, whichever occurs first (U.S. EPA, 2019). Pending approval of the expanded label at EPA, the dicamba use pattern for MON 87429 would be no different than that of MON 87419.

Therefore, it is not anticipated that commercialization of MON 87429 maize in the U.S. would have a notable impact on current maize production practices, beyond the intended benefits of effective management of common, troublesome weeds, and/or herbicide-resistant weeds and additional options for growers to rotate and/or use combinations of herbicides with multiple sites-of-action for preplant and in-crop postemergence herbicide applications.

Herbicides can impact pests or pathogens directly or indirectly through effects on the control of the crop or weeds associated with the crop. The issue of whether glyphosate use in glyphosate resistant plants increases disease severity has been addressed in a recent review article (Duke et al. 2012). For crops where no-till or conservation tillage have not been widely used, the wide-spread adoption of post-emergent herbicides in the crop might increase use of conservation tillage practices which could impact diseases that build up in crop residue left on the soil (Duke et al. 2012). Data provided within the Monsanto petition shows that MON 87429 maize is phenotypically similar to conventional maize and is no more susceptible to diseases or pests than commercially cultivated maize. Thus, MON 87429 maize is unlikely to exacerbate plant pests or diseases associated with maize. As a result, APHIS does not foresee changes in either insects or disease damage or control measures employed due to agricultural or cultivation practices with MON 87429 maize.

Information contained within the Monsanto petition demonstrates that except for greater diversity in herbicide chemistry resistance that will provide greater flexibility in weed control options, there are no expected changes to the inputs needed for MON 87429 maize production, and no expected impacts to most of the agronomic practices employed for production of maize compared to the current practices. The cultivation practices needed for growing MON 87429 maize are similar to that used to grow conventional maize. Additionally, 38 modified maize varieties have been previously evaluated and 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, in part due to an absence of these introduced traits to substantially alter maize cultivation practices (USDA-APHIS 2019a).

APHIS could not identify any significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of MON 87429 maize; 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 87429 maize Cannot Interbreed

APHIS examined the potential for the new genetic material inserted into MON 87429 maize to be horizontally transferred without sexual reproduction to other organisms and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants. The horizontal gene transfer between unrelated organisms is one of the most intensively studied fields in the biosciences since 1940, and the issue gained extra attention with the release of modified plants into the environment (Dröge et al. 1998). Potential risks from stable horizontal gene transfer (HGT) from modified organisms to another organism without reproduction or human intervention were recently reviewed (Keese 2008). Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake, 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 87429 maize contains tandemly arrayed four gene expression cassettes for the expression of genes *pat*, *dmo, ft_t*, and *cp4 epsps* with in total 20 genetic elements derived from plants, bacteria and plant virus.

HGT 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 gene transfer from and expression in bacteria of the foreign DNA inserted into the nuclear genome of the modified plant is unlikely to occur. First, many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Wood et al. 2001; Kaneko et al. 2002). There is no evidence that these organisms contain genes derived from plants. HGT from plants to bacteria is a very low frequency event, primarily because functional and selective barriers to HGT increase with genetic distance (Keese 2008). Second, in cases where review of sequence data implied that horizontal gene transfer occurred, these events are inferred to occur on an evolutionary time scale on the order of millions of years (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 US-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 modified plant to plant viruses was likely to occur and would lead to the creation or selection of plant viruses that are more virulent or have a broader host range. This issue has been considered before by other science review panels and government regulatory bodies (EPA-FIFRA-SAP 2006; Keese 2008). HGT is not unusual among plant viruses; however, this is generally limited to exchange between viruses present in the same host organism in mixed infections, and most commonly involves homologous recombination, relying on sequence similarity at the point of crossover (Keese 2008). HGT from virus sequences engineered into plants has been demonstrated with infecting or challenge viruses, including both DNA viruses (e.g., geminiviruses which replicate in the nucleus) (Frischmuth and Stanley 1998) and RNA viruses (which typically replicate in the cytoplasm); however most have been under conditions that favor recombination to restore a defective virus (Fuchs and Gonsalves 2007; Keese 2008; Thompson and Tepfer 2010). Populations of recombinants between virus transgenes expressed in modified plants infected with related viruses are similar to recombinants found in mixed infections of the same viruses in non-modified plants, indicating that there was no novel recombination mechanism in the modified 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).

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. 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 2019j). 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 MON 87429 maize, the DNA sequences were inserted into the nuclear genome, not the mitochondrial genome (Monsanto 2019).

If MON 87429 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 MON 87429 maize. 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 87429 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, public comments in response to Federal Register notices concerning this petition, and other relevant information to assess the plant pest risk of MON 87429 maize compared to the unmodified variety from which it was derived. APHIS concludes that the MON 87429 maize is unlikely to pose a greater plant pest risk compared to the unmodified variety from which it was derived based on the following findings.

- No plant pest risk was identified from the transformation process or the insertion of new genetic material in MON 87429 maize because the *A. tumefaciens* transformation vector was disarmed, the transformed material was treated with an antibiotic to kill the bacterium, and the inserted plant pest sequences do not cause disease or create an infectious agent.
- No increase in plant pest risk was identified in MON 87429 maize from expression of the inserted genetic materials, or changes in metabolism or composition because there were no significant changes in agronomic, ecological and compositional characteristics that would render MON 87429 maize more susceptible to pests and diseases over its control or reference maize varieties.

- Disease and pest incidence and/or damage were not observed to be significantly increased or atypical in MON 87429 maize compared to the unmodified counterpart or other comparators in field trials conducted in growing regions representative of where MON 87429 maize is expected to be grown. Observed agronomic traits also did not reveal any significant differences that would indirectly indicate that MON 87429 maize 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 87429 maize is unlikely to have any adverse impacts on organisms beneficial to agriculture based on the analysis of studies on MON 87429 maize food and feed safety and composition.
- MON 87429 maize is no more likely to become a weed or become weedier than conventional varieties of the crop based on its observed agronomic characteristics, weediness potential of the crop and current management practices available to control MON 87429 maize as a weed. Volunteers and feral populations of MON 87429 maize can be managed using a variety of currently available methods and herbicides.
- MON 87429 maize is not expected to increase the weed risk potential of other species with which it can interbreed in the U.S. or its territories. Gene flow, hybridization and/or introgression of inserted genes from MON 87429 maize to other sexually compatible relatives with which it can interbreed is not likely to occur. The sexual compatible relatives of maize are not considered weedy or invasive, and the new phenotype conferred by genetic engineering is not likely to increase the weediness of these sexually compatible relatives or affect the current ability to control these relatives in situations where they are considered weedy or invasive.
- Significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of MON 87429 maize were not identified and are not likely to increase plant diseases or pests or compromise their management.
- Horizontal gene transfer of the new genetic material inserted into MON 87429 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.

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