Preliminary Extended Determination[1] of Nonregulated Status for Pioneer Hi-Bred International, Inc. Request (20-043-01.ext) for Extension of Determination of Nonregulated Status for MS44 Maintainer Line DP56113 for use in the Seed Production Technology for Africa (SPTA) Process

In response to a request from Pioneer Hi-Bred International, Inc. (hereinafter referred to as Pioneer) to extend a determination of nonregulated status to DP56113 maize which is engineered for maintenance and recovery of male-sterile maize breeding lines, the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has determined, based on similarity to its antecedent organism that DP56113 maize and progeny derived from it are not likely to pose a plant pest risk and are no longer to be considered regulated articles under APHIS' Biotechnology Regulations at Title 7 of the Code of Federal Regulations, part 340 (7 CFR part 340). This extension request is based upon APHIS' determination of nonregulated status of its antecedent organism: Pioneer's DP-32138-1 maize. DP-32138-1 maize was deregulated on June 28, 2011 (Petition No. 08-338-01p). APHIS approved permits or acknowledged notifications that were previously required for environmental release, interstate movement, or importation will no longer be required for DP56113 maize and its progeny. Importation of DP56113 maize seed, other propagative material, and bulk or table stock, will still be subject to APHIS foreign quarantine notices at 7 CFR part 319 and the Federal Seed Act regulations at 7 CFR parts 201 and 361.

DP56113 maize is similar to the antecedent DP-32138-1 maize. DP-32138 is also a breeding maintainer line (This line was referred to as 32138 SPT maintainer by Pioneer). It contains *zm-aa1* and *DsRed2* cassettes that are identical to the cassettes inserted into DP56113. DP-32138 also contains a functional *MS45* gene which restores fertility to *ms45* maize mutants by encoding a functional copy of the MS45 protein. This is considered the same mechanism of action as restoring fertility by gene silencing of *Ms44*. APHIS evaluated the plant pest risk of DP56113 maize by assessing its similarity to the deregulated DP-32138-1 maize.

APHIS previously conducted a Plant Pest Risk Assessment on the antecedent organism and concluded that it is unlikely to pose a plant pest risk. Based on the plant pest risk similarity assessment (see Appendix A) of DP56113 maize to the antecedent, APHIS concludes that DP56113 maize is unlikely to pose a plant pest risk and should no longer be regulated under 7 CFR part 340. From the similarity assessment, APHIS concludes the following with respect to DP56113 maize and its progeny:

(1) No plant pest risk was identified from the transformation process, the insertion and/or expression of new genetic material, or from metabolism changes in DP56113 maize.

(2) Disease and pest incidence and/or damage are not expected to be increased or atypical

[1] This extended determination is not effective until officially signed and published.

in DP56113 maize. No plant pest effects are expected on these or other agricultural products and no impacts are expected to APHIS pest control programs.

(3) Based on an evaluation of the gene products and their similarity to the antecedent, and on data submitted in the extension request, DP56113 maize is are unlikely to adversely impact nontarget organisms beneficial to agriculture.

(4) DP56113 maize is no more likely to become weedier or more difficult to control as a weed than the antecedents, which are not weedy.

(5) DP56113 maize is not likely 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 DP56113 maize to other sexually compatible relatives with which it can interbreed is not likely to occur.

(6) Significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of DP56113 maize is not expected.

(7) Horizontal gene transfer of the new genetic material inserted into the GE plant 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.

In addition to our findings that DP56113 maize is unlikely to pose a plant pest risk, APHIS prepared a Record of Categorical Exclusion Determination for this action based on an Environmental Assessment completed for the antecedent DP-32138-1 maize in 2011. DP56113 maize will have no significant impacts, individually or collectively, on the quality of the human environment and will have no effect on federally listed threatened or endangered species, species proposed for listing, or their designated or proposed critical habitats.

Based on my review and consideration of all of the scientific and environmental data, analyses, information, and previous conclusions regarding the plant pest risk assessment for the antecedent organism, the plant pest risk similarity assessment, and record of categorical exclusion determination, and my knowledge and experience as APHIS' Deputy Administrator for Biotechnology Regulatory Services, I have determined and decided that this determination of nonregulated status of DP56113 maize is the most scientifically sound and appropriate regulatory decision.

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Bernadette Juarez
APHIS Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture

Date

Appendix A

Pioneer Hi-Bred International, Inc. Request (20-043-01.ext) for Extension of Determination of Nonregulated Status for MS44 Maintainer Line DP56113 for use in the Seed Production Technology for Africa (SPTA) Process

OECD Unique Identifier: DP-056113-9

Plant Pest Risk Similarity Assessment

November 2020

Agency Contact
Cindy Eck
Biotechnology Regulatory Services
4700 River Road
USDA, APHIS
Riverdale, MD 20737
Fax: (301) 734-8669

TABLE OF CONTENTS

A. Introduction

The Animal and Plant Health Inspection Service (APHIS) of the United States Department Agriculture (USDA) has received an extension request (20-043-01.ext) from Pioneer Hi-Bred International, Inc. (hereafter referred to as Pioneer). In accordance with §340.6(e)(2), Pioneer requests that APHIS extend the nonregulated status for the antecedent DP-32138-1 maize line, which is engineered for the recovery and maintenance of male-sterile lines during the maize breeding process (hereafter referred to as a maintainer line) to the genetically engineered (GE) DP56113 maize line, which is engineered for the same purpose, and any progeny derived from crosses of DP56113 maize with conventional maize or with other GE maize events that have received a determination of nonregulated status, or are not considered regulated articles under regulations at 7 CFR Part 340. USDA announced its determination of nonregulated status for DP-32138-1 maize on June 28, 2011.

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 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. DP56113 maize was produced by *Agrobacterium tumefaciens* mediated transformation of immature maize embryos (Pioneer Hi-Bred International 2020), and some of the introduced border sequences and regulatory elements in the insert come from plant pest organisms listed in 7 CFR part 340.2 (Pioneer Hi-Bred International 2020). Therefore, the DP56113 maize is considered a regulated article under APHIS regulations at 7 CFR part 340.

Potential impacts in this Plant Pest Risk Similarity Assessment are those that pertain to plant pest risk associated with the DP56113 maize and its progeny and their use in the absence of confinement relative to the antecedent DP-32138-1 maize. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if the DP56113 maize is any more likely than DP-32138-1 maize to pose a plant pest risk. APHIS specifies in 7 CFR part 340.6(e) that an extension request for nonregulated status shall include information to establish the similarity of the antecedent organism to the regulated article in question.

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; 80 FR 60414 2017). Under the Coordinated Framework, the oversight of biotechnology-derived plants rests with USDA-APHIS, the Food and Drug Administration (FDA), and the Office of Pesticide Programs of the U.S.

Environmental Protection Agency (EPA). Depending on the characteristics, certain biotechnology-derived products are subjected to review by one or more of these agencies.

B. Development of DP56113 maize

As described in the extension request (Pioneer Hi-Bred International 2020), Pioneer genetically engineered maize to produce DP56113 maize. DP56113 is a maize breeding maintainer line that contains a gene silencer (*zm-Ms44* artificial micro RNA) which will produce fragments of double-stranded RNA which match the sequence of the *Ms44* gene from maize, an α-amylase (*zm-aa1*) gene under a pollen-specific promoter, and a gene encoding a DsRed red fluorescent protein (*DsRed2*) under a seed-specific promoter. *Ms44* is a dominant male sterile mutant gene which produces male sterility in maize and inactivation of the gene was found to restore fertility (Fox et al. 2017). DP56113 is homozygous for *Ms44* and heterozygous for the inserted construct. The gene silencer inserted into DP56113 will impair the function of *Ms44*, such that the plant is fertile. The inserted α-amylase is active during pollen production, and renders pollen that contains the inserted gene non-functional. The DsRed gene is activated in seeds, giving seeds containing the transgene a red fluorescent pigment. The result of the activity of the two inserted genes and the gene silencer in the homozygous *Ms44* background is that DP56113 will produce 50% fertile pollen that lack the inserted material and 50% sterile pollen that contain the inserted material.

DP56113 is designed as a breeding maintainer line for maintenance and recovery of male-sterile maize lines for hybrid breeding. When DP56113 is selfed, all the progeny will be homozygous for *Ms44*, but only half of the progeny will contain the gene silencer, α-amylase gene, and DsRed gene. Seeds containing the gene silencer, α-amlyase gene, and DsRed gene will be marked with the DsRed fluorescent protein, and can be visually sorted from the seeds that do not contain the inserted construct. When DP56113 maize is crossed to a male-sterile line homozygous for *Ms44*, none of the progeny will contain the gene silencer, α-amylase gene, or DsRed gene. These male-sterile progeny may then be used as the female parent in hybrid seed production, without the need for interventions such as mechanical detasseling of the female parent or cytoplasmic incompatibility systems, which can be expensive, reduce seed production, or limit the choices of parent lines.

DP56113 maize is similar to the antecedent DP-32138-1. DP-32138 is also a breeding maintainer line. It contains *zm-aa1* and *DsRed2* cassettes that are identical to the cassettes inserted into DP56113. DP-32138 also contains a functional *MS45* gene which restores fertility to *ms45* maize mutants by encoding a functional copy of the MS45 protein. This is considered by the applicant as the same mechanism of action as restoring fertility by gene silencing of *Ms44*.

APHIS completed a detailed plant pest risk assessment (PPRA) and environmental assessment (EA) for the antecedent DP-32138 maize (APHIS 2011a, b). The EA fully addressed all resource areas of potential concern. In the antecedent petition, 08-338-01p, APHIS concluded on the basis of the EA that the impacts would not be significant. The

agency issued Findings of No Significant Impacts (FONSI) and made a determination of nonregulated status for the antecedent event.

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 extension request related to the similarity of the DP56113 maize line to the antecedent DP-32138-1 maize line, including the transformation process; the source of the inserted genetic material and its function in both the donor organism and the GE crop event; and the integrity, stability and mode of inheritance of the inserted genetic material through sexual or asexual reproduction and the number of loci inserted.

APHIS also assessed data presented in the extension request on whether the genetic modification results in expression of new genes, proteins, or enzymes, suppression of existing genes and their products, or changes in plant metabolism or composition in the DP56113 maize line. The assessment encompasses a consideration of the expressed double stranded RNA (dsRNA), the expressed α-amylase and DsRed proteins, and any observed or anticipated effects on plant metabolism including, e.g. any relevant changes in levels of metabolites, anti-nutrients, or nutrients in harvested maize from the DP56113 maize line compared to the antecedent DP-32138-1 maize line or those in the conventional counterparts and other comparators.

Description of the genetic modification and inheritance of inserted DNA

Pioneer used transformation plasmid PHP70533 to produce DP56113 maize by disarmed *Agrobacterium tumefaciens*-mediated transformation as described in the extension request (Pioneer Hi-Bred International 2020). The inserted material contained a pollen infertility cassette with *zm-aa1* and a color sorting cassette with *DsRed2* that are identical to the pollen infertility and color sorting cassettes used in the antecedent DP-32138-1 maize line. In addition, the insertion into DP56113 maize contains a cassette containing *zm-Ms44* artificial micro RNA, which is functionally equivalent to the sterility restoration cassette containing *ms45* in the antecedent DP-32138-1 maize line.

Pollen infertility cassette *zm-aa1*:

- *Pg47* promoter from *Zea mays* which confers pollen-specific expression
- *zm-bt1* Transit peptide from *Zea mays* which targets expressed products to the amyloplast
- *zm-aa1* gene from *Zea mays*, a truncated version of the *Zea mays* α-amylase gene
- *ln2-1* terminator from *Zea mays* which terminates gene transcription

Color sorting cassette *DsRed2*:

- CaMV 35S enhancer from cauliflower mosaic virus which increases gene expression

- *Ltp2* promoter from *Hordeum vulgare* which confers expression in the aleurone
- *DsRed2* gene from *Discosoma sp.* modified with an internal *BstE II* restriction site removed, which encodes a red fluorescent protein
- *pinII* terminator from *Solanum tuberosum*, which terminates gene transcription

Fertility restoration cassette containing *zm-Ms44* amiRNA

- *zm-Ms44* promoter from *Zea mays*, which confers expression in the same tissues and developmental stages as the endongenous *Ms44* gene
- *zm*-miRNA 5' precursor sequence, which precedes the artificial micro RNA sequence
- *zm*-Ms44 amiRNA sequence, a sequence encoding artificial micro RNA complementary to the *Ms44* gene from *Zea mays*
- *zm*-miRNA precursor 396h, a precursor sequence of the mciroRNA backbone 396h from *Zea mays*
- *zm*-Ms44 Star sequence, and artificial star sequence complementary to the *zm-Ms44* artificial micro RNA sequence except for one mismatched nucleotide
- *zm*-MiRNA 3' precursor 396h, a precursor sequence of the microRNA backbone 396h from *Zea mays*
- *zm-Ms44 terminator* from *Zea mays*, which terminates gene transcription.

In addition to the above genetic elements, the inserted T-DNA contains short non-coding intervening DNA sequences. These intervening sequences contain restriction enzyme recognition sites used for cloning purposes. The T-DNA also contains a Ti plasmid region and border sequences from the *Agrobacterium tumefaciens* Ti plasmid.

APHIS reviewed the information provided by Pioneer in the extension request and determined the following:

- The T-DNA inserted into the maize genome is present at a single locus and contains a single copy of the transgene.
- The T-DNA is stably inherited from generation to generation.
- DP56113 maize does not contain any back bone sequence of extraneous DNA fragments from the transformation plasmid PHP70533.
- During the transformation process, portions of the left border sequence and right border sequence of the T-DNA (originally 25 bp each) were truncated. These sequences are outside of the functional DNA elements and are not expected to impact expression of the transgenes.

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

The endogenous maize *zm-aa1* gene has been predominantly reported to be expressed in seed tissues of maize (Akazawa and Hara-Nishimura 1985; Oliveira et al. 2015). In DP56113 maize and the antecedent DP-32138-1, α-amylase was detected in leaf, whole plant, pollen, forage, and seed tissue assayed at several timepoints during vegetative growth and reproductive life stages of maize, although always at much lower expression levels than in the pollen (Pioneer Hi-Bred International 2008, 2020). The concentration

of α-amylase was apparently higher in DP56113 than the antecedent for all tissues and life stages tested. However, the expressed α-amylase is equivalent to endogenous maize α-amylase. α-amylase proteins are widely present in plants and commonly consumed by humans and animals in sprouted maize seed and other plant tissues. The apparently higher α-amylase expression in DP56113 relative to the antecedent is not expected to be associated with a change in plant pest risk in DP56113 maize relative to the antecedent DP-32138-1 maize.

DsRed was expressed in leaf, whole plant, forage, and seed samples in both DP56113 maize and the DP-32138-1 antecedent (Pioneer Hi-Bred International 2008, 2020) at several vegetative growth and reproductive life stages. DsRed concentrations were apparently slightly higher for DP56113 relative to the DP-32138-1 antecedent for at least one life stage in leaf, whole plant, pollen, and seed tissues. However, these differences were smaller than the apparent differences in α-amylase concentrations. In all cases, DsRed concentrations remained several orders of magnitude below levels that could be of toxicological concern. The apparently slightly higher DsRed expression in DP56113 relative to the antecedent DP-32138-1 maize is not expected to be associated with a change in plant pest risk in DP56113 relative to the antecedent.

The *zm-Ms44* amiRNA cassette is not expected to result in a change in protein expression. This cassette is expected to produce fragments of micro RNA that match maize *Ms44* (Fox et al. 2017). Micro RNAs are involved in post-transcriptional gene silencing in plants through mechanisms such as transcript cleavage or translational repression (Borges and Martienssen 2015). Thus, the artificial micro RNA targeting *Ms44* is expected to prevent this gene from being expressed at the tissues and life stages where it would normally occur. Micro RNAs are found in plant tissues throughout development (Borges and Martienssen 2015) and are expected to be rapidly broken down in the environment or when ingested.

Compositional analyses indicate that the levels of the majority of nutritional components did not differ between DP56113 and nontransgenic control maize, and that those levels that did differ fell within ranges considered to be normal for conventional maize (Pioneer Hi-Bred International 2020).

D. Potential Plant Pest and Disease Impacts

APHIS assessed data and information presented in the extension request related to the similarity of DP56113 maize to the antecedent DP-32138-1 maize to determine 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 DP56113 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 DP56113 maize is more likely to have significantly increased disease and pest susceptibility as compared to antecedent DP-32138-1 maize. Impacts or changes in similarity to the antecedent DP-32138-1 maize

to the DP56113 maize were assessed to determine if they would (1) affect 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.

Plant Protection and Quarantine (PPQ) is an APHIS program that safeguards agriculture and natural resources from the entry, establishment, and spread of animal and plant pests and noxious weeds into the United States; and supports trade and exports of U.S. agricultural products. PPQ responds to new introductions of plant pests to eradicate, suppress, or contain them through various programs in cooperation with state departments of agriculture and other government agencies. These may be an emergency or longer term domestic programs that target a specific pest. A variety of insect, plant disease, mollusk, nematode or weed programs exist (APHIS 2020), however, none specifically target pests of DP56113 maize.

Because the genetic makeup of two of the three cassettes involved in the the GE traits of DP56113 maize is identical, and the function of the *Ms44*-silencer cassette is equivalent to the previously deregulated antecedent DP-32138-1 maize, no significant changes in composition are expected from the expression of the inserted genes in the DP56113 maize. Compositional analysis of DP56113 maize showed that this GE maize line did not differ from non-transgenic control maize lines (Pioneer Hi-Bred International 2020). Similarly, DP56113 maize is not expected to differ from the antecedent DP-32138-1 maize in its ability to harbor or transmit plant pathogens or pests and cause indirect plant pest effects on other agricultural products. Data presented for DP56113 maize did not indicate that DP56113 is different from non-transgenic maize in terms of its ability to harbor or transmit plant pathogens and pests (Pioneer Hi-Bred International 2020).

E. Potential Impacts on Non-target Organisms Beneficial to Agriculture

APHIS has previously evaluated the potential impacts on non-target organisms beneficial to agriculture that could result from the deregulation of the antecedent DP-32138-1 maize. The DP-32138-1 maize was determined by APHIS to be unlikely to have an adverse effect on non-target organisms in the environment. DP56113 maize is engineered to produce micro RNA for deactivation of a dominant male sterility gene through gene silencing. DP56113 maize and the antecedent DP-32138-1 maize are both engineered to produce an endogenous maize α-amylase protein and the DsRed red fluorescent protein.

The same pollen infertility cassette (*zm-aa1*) and color sorting cassette (*DsRed*) 2 were inserted into DP56113 maize and the antecedent DP-32138-1 maize. The fertility restoration cassettes in DP56113 maize and the DP-32138-1 antecedent have equivalent function. Therefore, based on the high similarity of DP56113 maize to the antecedent DP-32138-1 maize; the compositional similarity of DP56113 maize to non-transgenic maize; the unlikely impacts of non-target effects due to gene silencing, α-amylase production, or DsRed production; and on the finding that the antecedent DP-32138-1 maize was unlikely to harm non-target organisms, APHIS concludes that it is unlikely

that DP56113 maize will have an adverse effect on non-target organisms, including those beneficial to agriculture.

F. Potential for Enhanced Weediness of DP56113 Maize

The biology of maize is well studied and understood (OECD 2003). As documented in the Plant Pest Risk Assessment of the antecedent DP-32138-1 maize, maize is not known to be weedy or persistent; it is are incapable of survival outside of cultivation (Gould 1968; Holm et al. 1979; Muenscher 1980; OECD 2003).

In addition to considerations of the known biology of maize, APHIS analyzed information on a suite of agronomic characteristics and plant-disease and plant-insect interactions submitted in the petition on the antecedent organism and in the extension request (Pioneer Hi-Bred International 2008, 2020). This agronomic data showed that the antecedent DP-32138-1 maize and DP56113 maize are not different than non-transgenic comparators. Based on this data, both DP56113 and the antecedent DP-32138-1 are unlikely to become weeds. In addition, APHIS has also assessed the potential weediness of many other GE maize events representing a variety of traits. Therefore, because of the similarity of the DP56113 maize to the antecedent DP-32138-1 maize and the data presented for DP56113 maize, APHIS has determined that DP56113 maize is not likely to be a weed.

G. Potential Impacts on the Weediness of Any Other Plants with which DP56113 Maize Can Interbreed

APHIS evaluated the potential for gene introgression to occur from the antecedent DP-32138-1 maize to sexually compatible wild relatives and considered whether such introgression would result in increased weediness (Pioneer Hi-Bred International 2008). APHIS has also evaluated previously evaluated the potential for many previously deregulated maize events to impact the weediness of other plants with which they can interbreed. Those assessments found that while first generation hybrids can be formed with maize's closest relative, teosinte, the hybrids are weak and do not contribute to gene flow in subsequent generations. Also, the geographic distribution of teosinte is highly limited in the United States to fairly rare, sparsely dispersed feral populations in Florida. *Tripsacum* is not as closely related to maize as teosinte, but can be successfully hand crossed with maize to form hybrids. However, the many biological and geographic constraints such as distribution, genetic incompatibility, sterility of hybrids and temporal separation of flowering time make gene flow nearly impossible. Thus introgression from cultivated maize to either of these wild relatives is highly unlikely.

These sexually compatible relatives of maize are not considered to be weeds in the United States (Holm et al. 1979) and the PPRAs of the antecedents conclude that in the highly unlikely event that they acquire the new traits through gene flow; the traits would not be expected to transform them into weeds. Based on the high similarity of DP56113 maize to the antecedent, and on the finding that the antecedent organisms were unlikely

to cause wild relatives to become weeds, APHIS concludes that it is unlikely that DP56113 maize will cause the wild relatives of maize to become weeds.

H. Potential Changes to Agriculture or Cultivation Practices

APHIS assessed whether significant changes to agricultural or cultivation practices from the antecedent DP-32138-1 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. APHIS also considered differences in the anticipated breeding systems where DP56113 maize and the antecedent DP-32138-1 will be used.

APHIS did not identify any significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, rotations, management of volunteers, etc.) from the antecedent DP-32138-1 maize and concluded that no impact on plant diseases or pests or their management is likely to occur. DP-32138-1 maize is used as a maintainer line that pairs with a male-sterile female line with the male-sterile trait based on a homozygous recessive mutation. DP56113 maize is used as a maintainer line that pairs with a male-sterile female line with the male-sterile trait based on a homozygous dominant mutation. As a consequence, the hybrid progeny of the Ms44 female breeding line used in the DP56113 system will be male-sterile, and seeds of the hybrid progeny would need to be grown together with a male-fertile line for production to continue past the first hybrid generation (Fox et al. 2017). This change is plant breeding system is not expected to result in a change to agricultural practices with implication for for plant pest risk. Based on the similarity of DP56113 maize to the antecedent DP-32138-1 maize, APHIS concludes that it is unlikely that any significant changes to agriculture or cultivation practices would be associated with DP56113 maize and therefore no impact on plant diseases or pests of their management is likely to occur.

I. Potential Impacts from Transfer of Genetic Information to Organisms with which DP56113 Maize Cannot Interbreed

APHIS has previously examined the potential for the antecedent DP-32138-1 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 (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 HGT from GE organisms to another organism without reproduction or human intervention has been reviewed (Keese 2008). Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake and recombination and rearrangement, most notably through viruses and mobile genetic elements. APHIS has previously reviewed the potential for HGT from GE maize to bacteria, fungi, invertebrates, viruses, and parasitic plants (APHIS 2011a).

APHIS previously concluded that HGT of the inserted genetic material from the antecedent DP-32138-1 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. Therefore, APHIS concludes that HGT from DP56113 maize to other organisms is also highly unlikely.

J. Conclusion

APHIS has reviewed the information submitted in the extension request, supporting documents, and other relevant information to assess the similarity of plant pest risk of DP56113 maize compared to the antecedent DP-32138-1 maize. APHIS concludes that the DP56113 maize is unlikely to pose a greater plant pest risk than the previously deregulated antecedent DP-32138-1 maize.

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L. Similarity Table

Description	Extension Request DP56113 Extension 20-043-01.ext	Antecedent DP-32138-1 Petition 08-338-01p	Comments
Organism	**Maize**	**Maize**	
Phenotype	Pollen Infertility Fluorescent Color Marker Fertility Restoration	Pollen Infertility Fluorescent Color Marker Fertility Restoration	Same phenotypes
Genotype	Pollen infertility cassette *zm-aa1:* *Pg47* promoter from *Zea mays* which promotes pollen-specific expression *zm-bt1* Transit peptide from *Zea mays* which targets expressed products to the amyloplast *zm-aa1* gene from *Zea mays*, a truncated version of the *Zea mays* α-amylase gene *ln2-1* terminator from *Zea mays* which terminates gene transcription	Pollen infertility cassette *zm-aa1:* *Pg47* promoter from *Zea mays* which promotes pollen-specific expression *zm-bt1* Transit peptide from *Zea mays* which targets expressed products to the amyloplast *zm-aa1* gene from *Zea mays*, a truncated version of the *Zea mays* α-amylase gene *ln2-1* terminator from *Zea mays* which terminates gene transcription	Same genes and regulatory elements

	Color sorting cassette *DsRed2*: CaMV 35S enhancer from cauliflower mosaic virus which increases gene expression *Ltp2* promoter from *Hordeum vulgare* which promotes expression in the aleurone *DsRed2* gene from *Discosoma sp.* modified with an internal *BstE II* restriction site removed, which encodes a red fluorescent protein *pinII* terminator from *Solanum tuberosum*, which terminates gene transcription Fertility restoration cassette *zm-Ms44* amiRNA: *zm-Ms44* promoter from *Zea mays*, which promotes expression in the same tissues and developmental	Color sorting cassette *DsRed2*: CaMV 35S enhancer from cauliflower mosaic virus which increases gene expression *Ltp2* promoter from *Hordeum vulgare* which promotes expression in the aleurone *DsRed2* gene from *Discosoma sp.* modified with an internal *BstE II* restriction site removed, which encodes a red fluorescent protein *pinII* terminator from *Solanum tuberosum*, which terminates gene transcription Fertility restoration cassette *zm-Ms45*: *5126* promoter from *Zea mays,* which promotes anther-preferred expression	Same genes and regulatory elements Same same mode of action (fertility restoration for male-sterile lines)

	stages than the endongenous *Ms44* gene is expressed *zm*-miRNA 5' precursor sequence, which precedes the artificial micro RNA sequence *zm*-Ms44 amiRNA sequence, a sequence encoding artificial micro RNA complementary to the *Ms44* gene from *Zea mays* *zm*-miRNA precursor 396h, a precursor sequence of the mciroRNA backbone 396h from *Zea mays* *zm*-Ms44 Star sequence, and artificial star sequence complementary to the *zm-Ms44* artificial micro RNA sequence except for one mismatched nucleotide *zm*-MiRNA 3' precursor 396h, a precursor sequence of the microRNA backbone 396h from *Zea mays*	*Ms45* gene from *Zea mays*, which encodes a functional copy of *Ms45*	

	zm-Ms44 terminator from *Zea mays*, which terminates gene transcription.	*Zm-MS45 terminator* from *Zea mays*, which terminates gene transcription	
Transformation Method	*Agrobacterium tumefaciens*–mediated	*Agrobacterium tumefaciens*–mediated	Same
Insert and Copy Number	Single intact insertion	Single intact insertion	Same
Compositional analysis	Compositionally equivalent to comparator	Compositionally equivalent to comparator	Same
Backbone Absent	Yes	Yes	Same
Mechanism of Action	*zm-aa1*: renders pollen infertile *DsRed2*: visual marker of seeds containing transgene *zm*-Ms44 amiRNA: Silences *Ms44* gene	*zm-aa1*: renders pollen infertile *DsRed2*: visual marker of seeds containing transgene *Ms45:* functional Ms45 gene	Same Same Same mode of action—Fertility Restoration

Date of antecedent EA/ EIS	N/A	May 2011	
Plant Pest Risk			
Disease and pest susceptibilities	Similar to antecedent	Unlikely to change disease and pest susceptibilities	
Impacts on beneficial non-targets	Similar to antecedent	Unlikely to impact beneficial non-target organisms	
Enhanced weediness	Similar to antecedent	Unlikely to enhance weediness	
Enhanced weediness of relatives	Similar to antecedent	Unlikely to enhance weediness of relatives	
Changes to agriculture or cultivation practices	Similar to antecedent	Unlikely to change agriculture or cultivation practices	
Horizontal Gene Transfer	Similar to antecedent	Unlikely to affect the probability of horizontal gene transfer	
Plant Pest Risk	Similar to antecedent	Unlikely to pose a plant pest risk	