Pioneer Petition (19-101-01p) for Determination of Nonregulated Status for Enhanced Grain Yield Potential and Glufosinate-ammonium Resistant DP202216 Maize.

OECD Unique Identifier:
DP-2Ø2216-6

Plant Pest Risk Assessment

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A. Introduction

Pioneer Hi-Bred International, Inc. (hereafter referred to as Pioneer) has petitioned the Animal and Plant Health Inspection Service (APHIS) of the United States Department Agriculture (USDA) for a determination that the genetically engineered (GE**)** Enhanced Grain Yield Potential and Glufosinate-ammonium Resistant Maize event DP-2Ø2216-6 (hereafter referred to as DP202216 maize) is unlikely to pose a plant pest risk and, therefore, should no longer be a regulated article under the APHIS' 7 Code of Federal Regulations (CFR) part 340. This petition was assigned the number 19-101-01p, and is hereafter referenced as Pioneer (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. 7701 *et seq*.)[1]. This plant pest risk assessment was conducted to determine if DP202216 maize is unlikely to pose a plant pest risk.

APHIS regulations in 7 CFR part 340 regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is no longer subject to the plant pest provisions of the PPA or to the regulatory requirements of Part 340 when APHIS determines that it is unlikely to pose a plant pest risk. A GE organism is considered a regulated article under Part 340 if the donor organism, recipient organism, or vector, or vector agent used in engineering the organism belongs to any genera or taxa designated in 7 CFR 340.2 and meets the definition of plant pest, or is an unclassified organism and/or an organism whose classification is unknown, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the Administrator determines is a plant pest or has reason to believe is a plant pest[2]. DP202216 maize was produced through the use of *Agrobacterium tumefaciens* as a vector to transfer specific genetic sequences from plasmid PHP40099 (Section III-A, p. 23 in Pioneer 2019). *A. tumefaciens* is a plant pest listed in 7 CFR 340.2. Additionally, portions of the introduced genetic sequences in the T-DNA of plasmid PHP40099 come from plant pest organisms listed in 7 CFR 340.2 (Table 3, pp. 32-33 Pioneer 2019). Therefore, DP202216 maize is considered a regulated article under APHIS regulations at 7 CFR part 340. Pioneer has conducted introductions of DP202216 maize as a regulated article under APHIS authorizations since 2009 (Appendix 1, pp. 114-116, Pioneer 2019), in part, to gather information to support that DP202216 maize is unlikely to pose a plant pest risk.

Potential impacts in this plant pest risk assessment are those that pertain to plant pest risk associated with DP202216 maize and its progeny and their use in the absence of confinement relative to the unmodified recipient and/or other appropriate comparators.

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

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

APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if DP202216 maize is unlikely to pose a plant pest risk. APHIS regulations in 7 CFR 340.6(c) specify the information needed for consideration in a petition for nonregulated status. APHIS will assess information submitted by the applicant about DP202216 maize related: to plant pest risk characteristics; expression of the gene product, new enzymes, or changes to plant metabolism; disease and pest susceptibilities and indirect plant pest effects on other agricultural products; effects of the regulated article on non-target organisms; weediness of the regulated article; impact on the weediness of any other plant with which it can interbreed; changes to agricultural or cultivation practices that may impact diseases and pests of plants; and transfer of genetic information to organisms with which it cannot interbreed.

APHIS may also consider information relevant to reviews conducted by other agencies that are part of the 'Coordinated Framework for the Regulation of Biotechnology'(51 FR 23302 1986; 57 FR 22984 1992). Under the Coordinated Framework, the oversight of biotechnology-derived plants rests with APHIS, the Food and Drug Administration (FDA), and the Office of Pesticide Programs of the U.S. Environmental Protection Agency (EPA). Depending on its characteristics, certain biotechnology-derived products are subjected to review by one or more of these agencies.

EPA regulates 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, under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 USC 136 *et seq.*). EPA also sets tolerance limits for residues of pesticides on and in food and animal feed, or establishes an exemption from the requirement for a tolerance, under the Federal Food, Drug and Cosmetic Act (FFDCA) (21 USC 301 *et seq*.). Prior to registration for a new use for a new or previously registered pesticide, EPA must determine through testing that the pesticide does not cause unreasonable adverse effects on humans, the environment, and 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 Pesticide Registration and Classification Procedures (40 CFR part 152) and Experimental Use Permits (40 CFR part 172). At present, no pending EPA reviews are relevant to DP202216 maize.

The FDA is responsible for ensuring the safety and proper labeling of all plant-derived food and feed, including those developed through modern biotechnology, under the FFDCA. To help sponsors of foods and feeds derived from genetically engineered crops comply with their obligations, the FDA encourages them to participate in its voluntary early food safety evaluation for new non-pesticidal proteins produced by new plant varieties intended to be used as food (US-FDA 2006) and a more comprehensive voluntary consultation process prior to commercial distribution of food or feed (57 FR 22984). Pioneer (2019) submitted a voluntary safety and nutritional assessment for food and feed derived from DP202216 maize to FDA in 2018. As of August 31st, 2020, FDA has not completed its consultation on DP202216 maize (FDA 2020).

B. Development of DP202216 maize

Maize (*Zea mays* ssp. *mays*), commonly referred to as corn in English-speaking countries, is the most widely cultivated grain crop in the United States and the United States is the world's largest producer (FAOSTAT 2015; USDA-ERS 2019). Maize is primarily grown for animal feed grain in the United States, accounting for more than 95% of 2018 total feed grain production, when over 14 billion bushels were produced on approximately 82 million acres (USDA-ERS 2019; USDA-NASS 2019b, a). The average maize yield within the United States was an estimated 176 bushels per acre in 2018 (USDA-NASS 2019a).

To optimize yield and economic return, growers select maize lines adapted to local environmental and climatic conditions, growing them as annual row crops using appropriate cultivation practices (e.g., seedbed preparation, planting timing and density, and integrated pest management to handle weed and disease pressure; see Hoeft et al. 2000; OECD 2003).

Maize productivity is impacted by losses due to abiotic factors (light, water, temperature and nutrients) and biotic factors (weeds, pests and pathogens). Plant pests can have a considerable influence on yield and productivity of crops; for example, total losses in maize due to biotic factors was estimated to be 31.2 - 38.3% between 1964 and 2003 (Oerke 2006). In particular, the presence of weeds in maize fields may cause greater production losses than other biotic factors (Aref and Pike 1998; Gibson et al. 2005; Oerke 2006).

Losses in maize productivity due to biotic factors have been reduced through practices that include the increased use of herbicides, pesticides, and hybrids resistant to pests and diseases (Russell 1991; Duvick 2005; USDA-ERS 2017). Global grain production has doubled since the 1960s and increases in maize grain yield is generally considered to be the result of the interaction between improved agronomic practices and improvements in maize genetics (Tollenaar and Lee 2004; Oerke 2006).

Selective breeding programs have altered maize genetics by selecting for desired plant phenotypes, resulting in incremental improvements in maize grain yield over time. Thus, maize grain yield has been positively affected by selective maize breeding programs. Maize breeding programs generally selected for yield-associated phenotypic characteristics, including decreased tassel size, leaf angle changes, increased kernel number and weight, delayed senescence, and a long period of grain fill (Rajcan and Tollenaar 1999; Duvick 2005; Echarte et al. 2013). These selected characteristics often promote efficiencies in growth, development, and resource partitioning in production agricultural systems (Duvick 2005). Prior to the broad adoption of selective maize breeding programs, average maize yield in the United States changed little from 1866 to 1930; however, yields increased steadily from 1930 to 2000 following the advent of the hybrid era (Tollenaar and Lee 2004; Egli 2008).

Modern biotechnology allows for targeted changes to maize genetics. Using biotechnology tools to alter the expression of specific maize genes known to play a role in certain phenotypic characteristics correlated with positive grain yield represents an approach that complements the selection of genes through selective breeding programs.

DP202216 maize was developed by Pioneer to exhibit enhanced grain yield potential and glufosinate-ammonium resistance. DP202216 maize was created by the insertion of *zmm28* and a maize-optimized phosphinothricin acetyltransferase (*mo-pat*) gene cassettes into a proprietary Pioneer maize line through *Agrobacterium*-mediated transformation. Expression of the *zmm28* gene cassette results in extended and increased activity of the ZMM28 protein, a transcriptional regulator derived from *Z. mays* that confers enhanced grain yield potential. Additionally, expression of the *mo-pat* gene cassette results in accumulation of phosphinothricin acetyltransferase (PAT), a protein derived from *Streptomyces viridochromogenes* that confers resistance to glufosinate-ammonium, the active ingredient in phosphinothricin herbicides (Section III-A, p. 23, Pioneer 2019).

The proprietary Pioneer maize line that forms the genetic background of DP202216 maize was chosen as a recipient for transformation because it is both an elite line used for commercial products and is amenable to transformation (Section II-B, p. 22, Pioneer 2019). Several proprietary non-GE Pioneer maize hybrid and inbred lines were used as comparators in field and safety assessments of DP202216 maize; these maize hybrid and inbred lines were chosen because they represent the genetics of the maize lines used to produce DP202216 maize. Additionally, several non-GE Pioneer maize hybrid lines were used to obtain tolerance intervals for use in field and safety assessments of DP202216 maize. These non-GE Pioneer maize hybrid lines represent the normal range of variation of commercial maize that may be planted in the United States and allow further comparability of DP202216 maize to maize lines currently used in commercial production (Section III-B, p. 27, Pioneer 2019). Collectively, the near-isogenic and commercial maize lines represent conventional controls that DP202216 maize can be compared to in field and safety assessments.

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 in DP202216 maize, APHIS assessed data and information presented in the petition related to: the transformation process; the source of the inserted genetic material and its function in both the donor organism and the GE crop event; and the integrity, stability and mode of inheritance of the inserted genetic material through sexual 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 composition of DP202216 maize relative to conventional controls. The assessment encompasses a consideration of the expressed ZM228 and MO-PAT proteins and any observed or

anticipated effects on composition of DP202216 maize, including any relevant changes in levels of metabolites, anti-nutrients, or nutrients in grain and forage derived from DP202216 maize compared to those in the conventional controls.

This information is used later to inform whether there is any potential for plant pest vectors or sequences to cause disease or greater plant pest risks in DP202216 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

DP202216 maize was developed using *A. tumefaciens* as a vector. *Agrobacterium*-mediated transformation of a proprietary Pioneer maize line was facilitated through the co-cultivation of immature maize embryos and *A. tumefaciens* strain JTLBA4404 containing the plasmid PHP40099. Following 3-6 days of co-cultivation, the immature maize embryos were transferred to selection media (containing glufosinate) to identify positive transformants; additionally, this selection media also contained carbenicillin to eliminate residual *A. tumefaciens*. Following glufosinate selection and carbenicillin treatment, positive transformants developed as calli; transformed calli were then transferred to regeneration media to facilitate somatic embryogenesis and growth. Healthy regenerated plants were then selected for further analysis (Section III-A, pp. 23-24, Pioneer 2019).

PHP40099 is approximately 50 kb in length and contains backbone sequences necessary for maintenance/selection of the plasmid in bacteria and to facilitate transfer of DNA to a recipient plant, but which are not expected to be transferred into maize. The PHP40099 plasmid backbone sequences are identified and fully described in Figure 3 and Table 2 of the petition (pp. 29 and 30, Pioneer 2019).

Additionally, PHP4009 contains a single Transfer DNA (T-DNA) that is approximately 7.5 kb in length (Figure 4, p. 31, Pioneer 2019). This T-DNA was inserted into the proprietary Pioneer maize line and contains the following genetic elements[3] (Table 3, pp. 32-33, Pioneer 2019):

- Right Border: T-DNA Right Border (RB) sequence from the *A. tumefaciens* Ti plasmid (Komari et al. 1996).

[3] Various short intervening sequences are present in the T-DNA of PHP40099 to facilitate cloning (Table 3, pp. 32-33, Pioneer 2019); however these short intervening sequences are not included in the description of the T-DNA within the text. Additionally, several recombination sites to facilitate cloning are also present in the T-DNA of PHP40099 (Table 3, pp. 32-33, Pioneer 2019). These recombination sites include two flippase (Flp) sites, FRT1/RT87; two loxP sites; and four attB sites, attB1/attB2 and attB3/attB4. The presence of these sites does not cause recombination in the absence of a suitable recombinase enzyme; these recombinases are not naturally present in plants (Cox, 1988; Dale and Ow, 1990; Thorpe and Smith, 1998). These recombination sites are also not included in the description of the T-DNA within the text.

- Ti Plasmid Region: Sequence from the *A. tumefaciens* Ti plasmid (Komari et al. 1996).
- *Zm-gos2* Promoter: Promoter region from the *Z. mays* translation initiation factor *gos2* gene (de Pater et al. 1992).
- *ubiZM1* Intron: Intron region from the *Z. mays* ubiquitin gene 1 (*ubiZM1*) (Christensen et al. 1992).
- *zmm28* 5' UTR: 5' untranslated region (UTR) from the *Z. mays zmm28* gene.
- *zmm28*: Transcriptional regulator from *Z. mays*; the coding sequence for *zmm28* gene is 756 bp in length.
- *zmm28* 3' UTR: 3' untranslated region from the *Z. mays zmm28* gene.
- *pinII* Terminator: Terminator region from the *Solanum tuberosum* (potato) proteinase inhibitor II gene (*pinII*) (Keil et al. 1986; An et al. 1989).
- *ubiZM1* Promoter: Promoter region from the *Z. mays* ubiquitin gene 1 (*ubiZM1*) (Christensen et al. 1992).
- *ubiZM1* 5' UTR: 5' untranslated region from the *Z. mays* ubiquitin gene 1 (*ubiZM1*) (Christensen et al. 1992).
- *ubiZM1* Intron: Intron region from the *Z. mays* ubiquitin gene 1 (*ubiZM1*) (Christensen et al. 1992).
- *mo-pat*: Maize-optimized phosphinothricin acetyltransferase (*mo-pat*) gene from *S. viridochromogenes* strain Tü494 (Wohlleben et al. 1988).
- *pinII* Terminator: Terminator region from the *S. tuberosum* proteinase inhibitor II gene (*pinII*) (Keil et al. 1986; An et al. 1989).
- Ti Plasmid Region: Sequence from the *A. tumefaciens* Ti plasmid (Komari et al. 1996).
- Left Border: T-DNA Left Border (LB) from the *A. tumefaciens* Ti plasmid (Komari et al. 1996).

Pioneer confirmed the insertion and stability of the genetic elements listed above by conducting a detailed molecular characterization of the inserted T-DNA in DP202216 maize. An initial Polymerase Chain Reaction (PCR) screen in tandem with a Southern-by-Sequencing analysis (SbSTM technology, hereafter referred to as SbS; see Zastrow-Hayes et al. 2015) was utilized to determine the copy number and complexity of the T-DNA insertion, and the absence/presence of PHP4009 plasmid backbone sequences in DP202216 maize (Figure 5 and Appendix 2, p. 36 and 117 - 128, Pioneer 2019). Additionally, Southern blot analysis and PCR/herbicide screening was utilized to determine the genetic stability of the T-DNA insertion in DP202216 maize (Appendix 3 and 4, pp. 129 - 133, Pioneer 2019). Methods and data from these molecular characterization techniques, provided in Section V and Appendices 2, 3, and 4 of the petition (Pioneer 2019) and reviewed by APHIS, demonstrated that:

- A single, intact T-DNA from PHP40099 was inserted into the genome of DP202216 maize. Results from the SbS analysis identified the presence of two unique junction sites in a representative DP202216 maize individual that was initially identified as PCR positive for the PHP40099 T-DNA insert (Figure 6 and 7, pp. 40 - 41, Pioneer 2019). The presence of these two junction sites was identical across multiple individuals of DP2022116 maize that tested positive for

the PHP40099 T-DNA insert (Table 4 and Appendix 2, pp. 39 and 117-128, Pioneer 2019). Additionally, SbS data from negative and positive control samples reinforced the integrity of the DP202216 maize SbS analysis. Negative control samples demonstrated the absence of exogenous genetic elements derived from PHP40099 in the proprietary Pioneer maize line (Figure 8, p.42, Pioneer 2019), while positive control samples demonstrated appropriate alignment and read signal with maize genomic DNA spiked with PHP40099 plasmid DNA at a level corresponding to one copy per maize genome and the intact PHP4009 plasmid itself (Figure 9, p.43, Pioneer 2019). No junction sites were observed in the negative control sample (Figure 8, p.42, Pioneer 2019), a result that is expected given the nature of the negative control sample.

- The transformation event in DP202216 maize represents a non-complex T-DNA insertion. Results from the SbS analysis demonstrated an absence of unique junction sites within the inserted PHP40099 T-DNA (Figure 6 and 7, pp. 40 - 41, Pioneer 2019); this indicates an absence of molecular rearrangement within the inserted T-DNA. Additionally, the SbS analysis demonstrated minor truncations within the RB and LB elements of the PHP40099 T-DNA at the two junction sites in DP202216 maize (Section V-B, p. 37, Pioneer 2019); however, minor truncations within the RB and LB sequences following *Agrobacterium*-mediated transformation of plants is common and expected (Kim et al. 2007). This nature of the PHP40099 T-DNA insertion was observed across multiple individuals of DP2022116 maize that tested positive for the PHP40099 T-DNA insert (Figure A2-1, A2-2, A2-3, A2-4, A2-5, A2-6, and A2-7, pp. 122-128, Pioneer 2019).
- Backbone sequences from the PHP40099 plasmid are not present in DP202216 maize. SbS data demonstrated an absence of unique junction sites beyond the RB and LB elements of the PHP40099 T-DNA when sequencing reads were compared between DP202216 maize and the intact PHP40099 plasmid (Figure 6, p. 40, Pioneer 2019). The absence of PHP4009 plasmid backbone sequences was consistently observed across multiple individuals of DP202216 maize that tested positive for the PHP40099 T-DNA insert (Figure A2-1, A2-2, A2-3, A2-4, A2-5, A2-6, and A2-7, pp. 122-128, Pioneer 2019). Accordingly, no junction sites were observed in the negative control sample (Figure 8, p.42, Pioneer 2019), a result that is expected given the nature of the negative control sample.
- The PHP40099 T-DNA insert is stably integrated into DP202216 maize and its progeny. Southern blot analysis utilizing probes corresponding to full-length sequences of *zmm28* and *mo-pat* genes (Table 5 and Figure 11, pp. 46 and 48, Pioneer 2019) demonstrated the consistent presence of *zmm28* and *mo-pat* restriction fragments across 5 breeding generations of DP202216 maize (Figure 13 and 14, pp. 50-51, Pioneer 2019). The observed restriction fragments for *zmm28* and *mo-pat* (~10 kb and ~7 kb for fragments hybridizing with the zmm28 and mo-pat probes, respectively; see Table 6, p. 46, Pioneer 2019) corresponded to predicted fragment sizes following digestion with the restriction enzyme, *NcoI* (>3.5 kb and >3.9 kb for fragments hybridizing with the zmm28 and mo-pat probes, respectively; see Table 6, p. 46, Pioneer 2019). Furthermore, negative and positive controls subject to the same Southern blot analysis reinforced the integrity of the study by confirming the absence of hybridizing bands derived

from the PHP40099 T-DNA in proprietary Pioneer maize lines (negative control, lanes 3 and 11) and the presence of predicted hybridizing bands from proprietary Pioneer maize lines spiked with the PHP40099 plasmid (positive control, lanes 2 and 12) (Table 6, Figure 13 and 14, pp. 46, 50-51, Pioneer 2019).

- The PHP40099 T-DNA insert is functional and segregates according to Mendelian rules of inheritance in DP202216 maize and its progeny. In conjunction with the Southern blot results described above, PCR and herbicide screening of five DP202216 maize breeding generations demonstrated sufficient overlap between expected and observed segregation ratios for each breeding generation of DP202216 maize examined (Table 7, p. 53, Pioneer 2019).

In summary, methods and results provided in Section V and Appendices 2, 3, and 4 of the petition (Pioneer 2019), and reviewed by APHIS, demonstrated that DP202216 maize contains a single, intact PHP40099 T-DNA within its genome. The insertion in DP202216 maize represents a non-complex T-DNA integration event; the data demonstrated no rearrangement of genetic elements in the PHP40099 T-DNA and only minor truncations in the RB/LB elements following integration. Additionally, the PHP40099 T-DNA is stably integrated into the plant genome across multiple breeding generations of DP20216 maize and its progeny.

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

DP202216 maize was developed by Pioneer to exhibit enhanced grain yield potential and glufosinate-ammonium resistance. These two traits are derived from the activity of two gene cassettes within the PHP40099 T-DNA that is integrated into the genome of DP202216 maize. The *zmm28* gene cassette confers enhanced grain yield potential and the *mo-pat* gene cassette confers resistance to glufosinate-ammonium in DP202216 maize.

zm228

The *zmm28* gene is a plant transcriptional regulator endogenous to *Z. mays* that is 251 amino acids in length and has a molecular weight of approximately 28 kDa (Table 3 and Figure 15, pp. 32-33 and 56, Pioneer 2019). *Zmm28* represents a family of plant transcriptional regulators containing conserved domains; the presence of these domains allow the ZMM28 protein to bind specific sequences of genomic DNA as homo-, hetero-, or multi-mers to regulate gene expression (Section VI-A.1A, p. 56, Pioneer 2019).

The *zmm28* gene contained within the PHP40099 T-DNA is regulated by the *zm-gos2* promoter, *ubiZM1* intron, and *pinII* terminator (Table 3, p. 32-33, Pioneer 2019). The *zm-gos2* promoter was cloned from the *Z. mays* translation initiation factor *gos2* gene and the the *ubiZM1* intron is a non-coding sequence from the *Z. mays* ubiquitin gene 1 (Christensen et al. 1992; de Pater et al. 1992); both genetic elements function to transcribe the introduced *zmm28* gene in DP202216 maize. Additionally, the *pinII* terminator from the *S. tuberosom* proteinase inhibitor II gene (Keil et al. 1986; An et al. 1989) functions to end transcription of the introduced *zmm28* gene in DP202216 maize. The presence of these regulatory elements within the *zm228* gene cassette of the

PHP40099 T-DNA leads to the constitutive expression of the introduced *zmm28* gene, which in turn facilitates its anticipated increased and extended expression relative to the endogenous *zmm28* gene in DP202216 maize.

Based on a bioinformatics analysis, the *in silico* translation of the introduced *zmm28* cDNA produced a protein that is identical to the endogenous ZMM28 protein already present in *Z. mays* (Figure 15, p. 56, Pioneer 2019). Western blot analysis utilizing a ZMM28 protein monoclonal antibody reinforced the results of the bioinformatics analysis (Figure 17, p. 58, Pioneer 2019), with both data sets demonstrating that the introduced ZMM28 and endogenous ZM228 proteins are equivalent in sequence and size.

As previously discussed, the constitutive expression pattern of the introduced *zmm28* gene facilitates its increased and extended expression relative to the endogenous *zmm28* gene in DP202216 maize. Data from Pioneer (2019) demonstrated that the ZMM28 protein accumulates to higher levels in grain and vegetative tissue compared to its non-GE comparator (Figure 17, p. 58, Pioneer 2019). Furthermore, a more detailed analysis of ZMM28 protein accumulation using an enzyme-linked immunosorbent assay (ELISA) or Western blot analysis over the course of a growing season in multiple in DP202216 maize tissues (Appendix 5 and 6, Pioneer 2019) demonstrated a similar pattern of results consistent with the Western blot analysis described earlier. Specifically, ZMM28 protein levels (the sum of introduced and endogenous ZMM28 protein levels) in DP202216 maize were generally higher than its non-GE, near isogenic control (Table 8, p. 67, Pioneer 2019). This included[4]:

- Whole plant samples from various maize developmental stages, including V9 (0.23 ng/mg dw versus 0.20 ng/mg dw); and R1 (0.18 ng/mg dw versus 0.14 ng/mg dw).
- Leaf samples from various maize developmental stages, including V6 (0.087 ng/mg dw versus 0.062 ng/mg dw); V9 (0.28 ng/mg dw versus 0.21 ng/mg dw); R1 (0.32 ng/mg dw versus 0.22 ng/mg dw); R4 (0.12 ng/mg dw versus 0.079 ng/mg dw); and R6 (<0.054 ng/mg dw versus ND[5]).
- Root samples from various maize developmental stages, including V9 (0.031 ng/mg dw versus 0.019 ng/mg dw); R4 (0.019 ng/mg dw versus ND); and R6 (0.015 ng/mg dw versus 0.014 ng/mg).
- Forage samples from the R4 maize developmental stage (0.049 ng/mg dw versus 0.029 ng/mg dw).
- Pollen sample from the R1 maize developmental stage (0.015 ng/mg dw versus ND).
- Grain samples from the R6 maize developmental stage (0.012 ng/mg dw versus ND ng/mg dw).

Overall, the ELISA and Western blot data provided by Pioneer (2019) and reviewed by APHIS indicates that the introduced *zmm28* gene cassette results in the production of a

[4] All values reported as dry weight (dw) in ng/mg for DP202216 maize and then for control maize.
[5] Not detected.

protein that is identical to the endogenous *Z. mays* ZMM28 protein. Additionally, based on the regulatory elements in the *zmm28* gene cassette within the PHP40099 T-DNA, constitutive expression of the introduced *zmm28* gene results in increased and extended accumulation of the introduced ZMM28 protein relative to the non-GE, isogenic control maize. These two results are expected, given that the introduced *zmm28* gene was originally cloned from *Z. mays* and that the insertion of the PHP40099 T-DNA results in a constitutively expressed, additional copy of the *zmm28* gene to contribute to ZM228 protein production in DP202216 maize.

mo-pat

The introduced *mo-pat* gene and its corresponding MO-PAT protein in DP202216 maize is identical to the trait within several crop plants that were previously reviewed by USDA as part of the petition process and are currently in U.S. commercial production (USDA-APHIS 2001, 2005, 2013a). Thus, the *mo-pat* gene and its corresponding MO-PAT protein in DP202216 maize is already well studied.

The *mo-pat* gene is a maize-optimized version of the phosphinothricin acetyltransferase (*pat*) gene originally cloned from *S. viridochromogenes* (Table 3, p. 32-33, Pioneer 2019). The translated MO-PAT protein is 183 amino acids in length and has a molecular weight of approximately 21 kDa (Figure 18, p. 60, Pioneer 2019). MO-PAT acetylates phosphinothricin and disrupts its ability to function as a competitive inhibitor of glutamine synthetase, an important enzyme in amino acid metabolism (Hérouet et al. 2005; CERA - ILSI Research Foundation 2016).

The *mo-pat* gene contained within the PHP40099 T-DNA is regulated by the *ubiZM1* promoter, *ubiZM1* 5' untranslated region (UTR), *ubiZM1* intron, and *pinII* terminator (Table 3, pp. 32-33, Pioneer 2019). Both the *ubiZM1* promoter, 5' UTR, and intron were cloned from the regions flanking or within the *Z. mays* ubiquitin gene 1 (Christensen et al. 1992); collectively, these three genetic elements function to initiate and maintain transcription of the introduced *mo-pat* gene in DP202216 maize. Additionally, the *pinII* terminator from the *S. tuberosom* proteinase inhibitor II gene (Keil et al. 1986; An et al. 1989) functions to end transcription of the introduced *mo-pat* gene in DP202216 maize. The presence of these regulatory elements within the *mo-pat* gene cassette of the PHP40099 T-DNA leads to the constitutive expression of the introduced *mo-pat* gene, resulting in accumulation of the MO-PAT protein and facilitating resistance to phosphinothricin and phosphinothricin-based herbicides.

Based on a bioinformatics analysis, the *in silico* translation of the introduced *mo-pat* cDNA produced a protein that is identical to the translated PAT protein native to *S. viridochromogenes*; both proteins are 183 amino acids in length and have a molecular weight of approximately 21 kDa (Figure 18, p. 60, Pioneer 2019). Western blot analysis reinforced the results of the bioinformatics analysis (Figure 19, p. 62, Pioneer 2019), with both data sets demonstrating that the introduced MO-PAT protein is equivalent in size to the microbially-derived PAT protein.

Pioneer (2019) also demonstrated, through Western Blot analysis, that the MO-PAT protein accumulates in DP202216 maize vegetative and reproductive tissues (Table 9, p. 68, Pioneer 2019). In general, MO-PAT accumulated to a higher level in DP202216 maize above-ground (e.g., leaf, forage, whole plant) and pollen tissues than root and grain tissues (Table 9, p. 68, Pioneer 2019). Expected functionality of the introduced MO-PAT protein was demonstrated through the herbicide-resistant phenotype screening of the DP202216 segregation analysis (Section V-E, p. 52, Pioneer 2019).

Overall, the Western blot data presented by Pioneer (2019) and reviewed by APHIS indicates that the introduced *mo-pat* gene cassette results in the production of a protein that is identical to the PAT protein that is produced microbially. Additionally, based on the regulatory elements in the *mo-pat* gene cassette within the PHP40099 T-DNA and the absence of *mo-pat* in control maize, constitutive expression of the introduced *mo-pat* gene results in accumulation of the introduced MO-PAT protein in maize vegetative and reproductive tissues.

Compositional analysis of DP202216 maize

As previously discussed, the presence of *zmm28* and *mo-pat* gene cassettes in DP202216 maize results in the accumulation of ZMM28 and MO-PAT proteins, respectively. To determine if these introduced gene cassettes affected maize metabolism, Pioneer (2019) examined the nutritional composition of DP202216 maize. This comparative assessment is based on compositional considerations for new varieties of maize, including GE varieties of maize (see OECD 2002; Codex Alimentarius Commission 2008) and was undertaken in tissues intended for use in forage and grain. Material for this compositional analysis was grown and collected across eight different sites in maize-growing regions of the United States in 2017 (i.e., Iowa, Illinois, Indiana, Missouri, Nebraska, Pennsylvania, and Texas; see Appendix 9, Pioneer 2019).

DP202216 maize forage at the R4 stage of development was collected and analyzed for important nutritional components, including proximates, fibers, and minerals. No statistically-significant differences were observed between DP202216 maize forage and its non-GE, near isogenic control for maize proximates, fibers, or minerals (Table 10, p. 75, Pioneer 2019). Additionally, DP202216 maize grain at the R6 stage of development was collected and analyzed for important nutritional components; this included maize proximates (i.e., crude protein, crude fat, crude fiber, acid detergent fiber (ADF), neutral detergent fiber (NDF), ash, and carbohydrates); fatty acids; total amino acids; minerals and vitamins; and key secondary metabolites and anti-nutrients. No statistically-significant differences were observed between DP202216 maize and its non-GE, near isogenic control for maize proximates (Table 11, p. 77, Pioneer 2019), fatty acids (Tables 12 and 13, pp. 79-80, Pioneer 2019), minerals (Table 15 and 16, pp. 85 - 86, Pioneer 2019), or key secondary metabolites and anti-nutrients (Table 19 and 20, p. 91, Pioneer 2019). Statistically-significant differences were observed between DP202216 maize and its non-GE, near isogenic control for three amino acids (glycine, methionine, and serine) and two vitamins (vitamin B1 (thiamine) and vitamin B3 (niacin)); however, the observed values for these three amino acids and two vitamins from DP202216 maize

were within the tolerance intervals identified from other commercially-available maize varieties that were concurrently grown and collected (Table 14 and 17, pp. 82 - 83 and 88 - 89, Pioneer 2019). Accordingly, these three amino acid and two vitamin differences are observed to be within the range of variation typically found in maize. Examination of other amino acids and vitamins between DP202216 maize and its non-GE, near isogenic control did not result in any observed statistically significant differences (Table 14 and 17, pp. 82 - 83 and 88 - 89, Pioneer 2019).

In summary, the majority of compositional metrics measured between DP202216 maize and its non-GE, near isogenic control did not result in any significant differences in forage or grain. While significant differences were observed in DP202216 maize grain for three amino acids (glycine, methionine, and serine) and two vitamins (vitamin B1 (thiamine) and vitamin B3 (niacin)), the observed values of these components were within the range of variation observed in other commercially-available maize varieties. Based on these compositional analyses, grain and forage are compositionally similar between DP202216 maize and its controls.

D. Potential Plant Pest and Disease Impacts

APHIS assessed whether potential plant pest or disease impacts are likely to result from the transformation process, from DNA sequences from plant pests, or from any other expression products, new enzymes, proteins or changes in plant metabolism or composition in DP202216 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 DP202216 maize is likely to have significantly increased disease and pest susceptibility based on data and observations from field trials on specific pest and disease damage or incidence and any agronomic data that might relate to such damage. Impacts or changes are assessed to determine if they would (1) affect the new GE crop and/or result in significant introduction or spread of a damaging pest or disease to other plants; (2) result in the introduction, spread, and/or creation of a new disease; and/or (3) result in a significant exacerbation of a pest or disease for which APHIS has a control program. Any increase in pest or disease susceptibility is evaluated with respect to the context of currently cultivated varieties, the ability to manage the pest or disease, and the potential impact on agriculture.

Plant Protection and Quarantine (PPQ) is an APHIS program that safeguards agriculture and natural resources from the entry, establishment, and spread of animal and plant pests and noxious weeds into the United States of America. PPQ also supports trade and exports of U.S. agricultural products. PPQ responds to many new introductions of plant pests to eradicate, suppress, or contain them through various programs in cooperation with state departments of agriculture and other government agencies. These may be emergency or longer-term domestic programs that target a specific pest. A variety of insect, plant disease, mollusk, nematode or weed programs exist (USDA-APHIS 2019a).

Currently, PPQ has several active pest management programs that target insect pests and a noxious weed that can affect maize. These include programs for grasshoppers (Order

Orthoptera) on rangelands, 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*) (for more information on each of these programs, see USDA-APHIS 2019a).

The Japanese beetle can cause significant damage feeding on many plant species; when adults feed on maize silk, it affects pollination and kernel formation. A recently established program targets the Old World bollworm. This pest can affect 180 species of plants, with maize listed as one of its preferred hosts. It is closely related to the corn earworm (*H. zeae*). Old World bollworm was first detected in western Puerto Rico in September 2014; while three adults were detected the following year in FL, this was considered a transient event and Old World bollworm is not considered present in the continental United States at this time (USDA-APHIS 2019a).

Witchweed (*S. 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 2019).

Maize itself is not considered a plant pest in the United States (7 CFR 340.2). The plant pest vector used to insert the DNA do not pose a plant pest risk to DP202216 maize. The PHP40099 T-DNA is disarmed; the T-DNA inserted into DP202216 maize lacks sequences from tumor-inducing (Ti) plasmids normally responsible for the formation of crown gall tumors following *A. tumefaciens* infection (Hoekema et al. 1983; Hellens et al. 2000). The transformation event contained only the intended sequences in addition to common insertion site mutations (See Section C. Description of Inserted Genetic Material, Its Inheritance and Expression, Gene Products, and Changes to Plant Metabolism). Furthermore, following transformation, plant tissue was treated with the antibiotic carbenicillin to eliminate *A. tumefaciens* (Section III-A, p. 24, Pioneer 2019).

DP202216 maize was grown within confined field trials in the United States from 2009 through 2017 in at least 131 locations across 16 states and territories covering a diverse range of areas where maize is currently cultivated and where DP202216 is expected to be grown (Appendix 1, pp. 114 - 116, Pioneer 2019). In these confined field trials, a general survey of insects and diseases were undertaken for DP202216 maize and compared to its near isogenic control and other reference maize hybrids every four weeks. In addition to the observational data from these confined field trials that was annually reported to APHIS (i.e., data on unusual pest and/or disease incidence), Pioneer also specifically assessed the biotic interactions of DP202216 maize, its near isogenic control, and 4 reference hybrids grown under similar agronomic conditions during the 2017 growing season (Appendix 13, pp. 176 - 213, Pioneer 2019). For this specific study, field trials were established at 12 sites representative of U.S. maize growing regions (Figure 20, p. 97, Pioneer 2019) in order to determine if DP202216 maize responds differently to naturally-occurring insects and disease than its near isogenic control or other conventional maize lines.

Between 2009 and 2017, common maize pests were observed during the confined field trials of DP202216 maize. A full list of observed maize pests is available in Appendix 13 of Pioneer (2019). Common arthropod pests observed included Chinese rose beetle (*Adoretus sinicus*), corn earworm (*H. zea*), Lacewings (*Neuroptera spp*.), spider mites (*Tetranychidae spp*.), flea beetle (*Chaetocnema pulicaria*), fall armyworm (*Spodoptera frugiperda*), aphids (*Aphididae spp*.), corn planthoppers (*Delphacidae spp*.), corn sap beetle (*Carpophilus spp*.), thrips (*Frankliniella spp.*), and cornsilk fly (*Eucesta stigmatias*) (Table A13-13, pp. 190-203, Pioneer 2019). Common microbial pests observed included common rust (*Puccinia sorghi*), maize chlorotic mottle machlovirus, maize mosaic rhabdovirus, northern corn leaf blight (*Exserohilum turicum*), common smut (*Ustilago maydis*), and gray leaf spot (*Cercospora zeae-maydis*) (Table A13-14, pp. 204 - 213, Pioneer 2019). For the observed arthropod and microbial pests, no unexpected differences in overall severity were observed between DP202216 maize, its near isogenic control, and other reference maize hybrids (Table A13-13 and A13-14, pp. 190 - 213, Pioneer 2019).

During a 2017 study, biotic interactions were specifically assessed in DP202216 maize, its near isogenic control, and 4 reference hybrids. Across the 12 sites utilized in this study, DP202216 maize did not respond differently to arthropod or microbial pests when compared to its near isogenic control and reference hybrids (Table A13-1 through A13-12, pp. 177 - 188, Pioneer 2019).

The data presented in Appendix 13 of Pioneer (2019) suggests that integration of the PHP40099 T-DNA and accumulation of the ZMM28 and MO-PAT proteins did not significantly alter the insect pest infestation, disease occurrence, or resulting damage on DP202216 maize over its near isogenic control or reference maize varieties. As discussed earlier, there were no significant changes in DP202216 maize composition relative to its near isogenic control or reference maize varieties (see Section C. Description of Inserted Genetic Material, Its Inheritance and Expression, Gene Products, and Changes to Plant Metabolism). Taken collectively, these data demonstrate that DP202216 maize is unlikely to be more susceptible to plant pathogens and insect pests than conventional maize. For this reason, DP202216 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 Nontarget Organisms Beneficial to Agriculture

DP202216 maize is not engineered for pest resistance. Thus, there are no ‘target’ or ‘nontarget’ species. As a result, APHIS assessed whether exposure or consumption of DP202216 maize would have a direct or indirect adverse impact on species beneficial to agriculture. Organisms considered were representatives of species associated with production of the regulated crop in the agricultural environment. Additionally, the assessment includes an analysis of data and information on DP202216 maize compared to the non-GE counterpart (or other comparators) for any biologically relevant changes in the phenotype or substances (e.g., proteins, nutrients, anti-nutrients, metabolites, etc.)

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.

Although many genetic elements were introduced into DP202216 maize, there are only two protein coding sequences; of these two protein coding sequences, only one is not endogenous to maize (Table 3, pp. 32 - 33, Pioneer 2019). In DP202216 maize, the activity of the *zmm28* and *mo-pat* gene cassettes results in the production of the ZMM28 and MO-PAT proteins, respectively (Figure 4 and Table 3, pp. 31 - 33, Pioneer 2019). As described in Section C, ZMM28 is identical to the endogenous maize protein and MO-PAT has been reviewed previously and multiple times by APHIS as part of its petition process. Thus, each introduced protein possesses a history of safe use. This history of safe use is further supported by *in silico*, *in vitro*, and experimental data provided by Pioneer (2019) and reviewed by APHIS.

The *in silico* analyses of DP202216 maize focused generally on Open Reading Frames (ORFs) within the PHP40099 T-DNA insertion and the specific amino acid sequence of MO-PAT. The introduced ZMM28 protein was not directly examined because it is identical to the endogenous maize ZMM28 protein in terms of amino acid sequence and molecular weight (Figure 15 and 17, p. 56, 58, Pioneer 2019) and shares homology with similar proteins in other fruits and vegetables (Anderson et al. 2019); as a result, the ZMM28 protein has been regularly consumed without any reports of allergenic or toxic effects, demonstrating a history of safe use. A comparison of ORFs within the PHP40099 T-DNA and the MO-PAT protein to amino acid sequences contained within various allergen and toxicity databases[6] resulted in no significant alignments (Section V-D, VI-A.2E, VI-A.2I, pp. 52, 63, 64, Pioneer 2019). These results provide evidence that neither the PHP40099 T-DNA insertion, nor the ZMM28 or MO-PAT proteins, results in the production of an allergen or toxin.

The *in vitro* analyses primarily focused on the introduced MO-PAT protein because the introduced ZMM28 protein is identical to the endogenous maize ZMM28 protein and has an extensive history of safe use. Specifically, MO-PAT was tested for stability when subject to heat and simulated gastric fluid; the results demonstrated that MO-PAT is inactivated by high temperatures and degraded in simulated gastric fluid (Hérouet et al. 2005). These results corroborate the heat inactivation results published by Wehrmann et al. (1996) and the heat and simulated gastric acid data previously reviewed by USDA-APHIS (2001, 2005, 2013a).

Experimental analyses quantified the amount of ZMM28 and MO-PAT proteins in edible tissues from DP202216 maize (Table 8 and 9, pp. 67 - 68, Pioneer 2019); this data was utilized to determine exposure levels of these proteins to humans and livestock under conservative total replacement values and to determine if DP202216 maize poses a substantial risk to human or livestock health following consumption (Appendix 10 and 11, pp. 158 - 166, Pioneer 2019). Exposure levels to ZMM28 and MO-PAT proteins

[6] i.e., Comprehensive Protein Allergen Resource (COMPARE) database and a proprietary Pioneer toxin database (a subset of the UniProtKB/Swiss-Prot database); see Sections V and VI in Pioneer (2019).

remain low and does not substantially raise the exposure of humans or animals to these proteins (Sections VI-B and VI-C, pp. 65 - 71, Pioneer 2019). As a result, the consumption of these two proteins by humans and livestock are unlikely to pose a substantial risk to human and livestock health. In particular, humans and livestock would have to consume a substantial amount of MO-PAT protein from DP202216 maize to equal the dose where no treatment-related adverse effects were observed in an acute toxicology study in mice (Appendix 10 and 11, pp. 158-166, Pioneer 2019).

This low level of risk to human and livestock health from consumption of the introduced ZM228 and MO-PAT proteins in DP202216 maize is further reinforced by the realities of maize commodity handling during processing and compositional data of the transformation event. In general, the exposure scenarios described in the previous paragraph assumed total replacement with DP202216 maize; however, when maize commodities are processed, this processing generally results in a high level of blending unless specific procedures are in place (e.g., in the case of some specialty maize products). As a result of this blending, exposure to the introduced ZMM28 and MO-PAT proteins is lower than what is assumed in the total replacement scenarios utilized in the exposure calculations described previously. Additionally, and as previously discussed in Section C, grain and forage from DP202216 maize is compositionally similar to its near-isogenic control and reference maize hybrids currently cultivated in the United States. Thus, the overall composition of DP202216 maize is not anticipated to pose a risk to humans or livestock, as it is compositionally similar to maize varieties that are currently consumed.

Therefore, based on the above analysis of the peer-reviewed literature; the information provided in the petition on the safety and expression level of the ZM228 and MO-PAT proteins; and the compositional analysis of DP202216 maize, APHIS concludes that exposure to and/or consumption of DP202216 maize is unlikely to have any adverse impacts to organisms beneficial to agriculture.

F. Potential for Enhanced Weediness of DP202216 maize

APHIS assessed whether DP202216 maize is likely to become more weedy (i.e., more prevalent, competitive, damaging or difficult-to-control in situations where it is not wanted) than the non-transgenic progenitor from which it was derived or other varieties of the crop currently under cultivation. This assessment considers the basic biology of maize, the situations in which maize volunteers are considered weeds, and an evaluation of DP202216 maize compared to its near isogenic control and other reference maize hybrids. Evaluations on DP202216 maize centered on characteristics related to establishment, competiveness, reproduction, survival, persistence and/or spread that could influence weediness and the ability to manage maize as a volunteer; these evaluations were undertaken in laboratory and field studies. Laboratory studies primarily focused on seed viability and germination/dormancy (Section VIII-A, pp. 93 - 96, Pioneer 2019), while field studies focused on maize characteristics, including early stand count, days to flowering, height, lodging, final stand count, days to maturity, pollen

viability, kernel rows per ear, kernels per ear, kernels per row, harvest grain moisture, yield, 100-kernel weight, and seed germination and dormancy (Section VIII-B and Appendix 12, pp. 96 - 104 and 167 - 175, Pioneer 2019). Additionally, responses to various abiotic stresses (e.g., above average rainfall, wind, hail, etc.) were observed and evaluated during the confined field testing of DP202216 maize (Appendix 13, pp. 176 - 188, Pioneer 2019).

In the United States, maize is not listed as a weed in the major weed references (Crockett 1977; Holm et al. 1979; Holm et al. 1997) and it is not designated as a noxious weed by the federal government (USDA-APHIS 2019a). Maize is unable to establish outside agriculture, as evidenced by the lack of reports of such behavior despite being one of the most widely cultivated grains in the world, and by data from controlled experiments where maize plantings left unharvested resulted in no feral plants within a year or two after planting (Raybould et al. 2012; Sammons et al. 2014). However, maize has been mentioned as an agricultural weed (i.e., volunteer plants) by the Southern Weed Science Society (USDA-NRCS 2016). Maize does not possess any of the attributes commonly associated with weeds (Baker 1965) such as long persistence of seed in the soil, the ability to disperse, invade, and become a dominant species in new or diverse landscapes, or the ability to compete well with native vegetation. Maize seeds are retained on the cob covered in a husk and are poorly dispersed, have no innate dormancy and are susceptible to low temperatures, although some seeds may overwinter and germinate when weather conditions allow; however germinating seedlings and plants are sensitive to cold and do not survive freezing winter conditions (Hoeft et al. 2000; OECD 2003; OGTR 2008; Andersson and de Vicente 2010). Although maize seed does not shatter, kernels are often scattered by harvest equipment or foraging wildlife, and some may survive to create volunteer plants the following year. Similar to conventional maize volunteers, herbicide-resistant maize volunteers, including DP202216 maize volunteers, can be managed by optimizing mechanical cultivation, crop rotation, and the careful selection of the modes of action for pre-emergent and post-emergent herbicides to balance competing herbicide sensitivities between volunteers and the rotational crop (Vencill et al. 2012).

In order to evaluate germination and dormancy in DP202216 maize, laboratory studies utilizing warm, cold, and diurnal conditions were performed (Table 21, p. 94, Pioneer 2019). Across these three conditions, DP202216 maize seeds demonstrated a germination rate no lower than 99%; this result was comparable to the germination rate of its near isogenic control and other reference maize hybrids under the same conditions (Table 23, 24, 25, pp. 95 - 96, Pioneer 2019). While these data demonstrate similar germination rates between DP202216 maize, its near isogenic control, and other reference maize hybrids, these data also suggest that seed dormancy rates between the DP202216 and its comparators are likely similar. This conclusion is reinforced by the absence of hard or fresh seed among the DP202216 maize seeds that did not germinate (Section VIII-A, p. 94, Pioneer 2019); the presence of these two characteristics is generally associated with seed dormancy germinate (Anderson 1996).

Field evaluation of DP202216 maize was performed across 12 sites spanning regions of the United States where DP202216 maize is intended to be grown during the 2017

growing season (Appendix 12, pp. 167 - 175, Pioneer 2019). Each site was managed to maintain an environment that would produce a successful crop, including typical agronomic practices as appropriate. Agronomic data was collected from DP202216 maize and compared to its near isogenic control and other reference maize hybrids that were also cultivated at each site. The collected data demonstrated no substantial differences between DP202216 maize and its comparators for early stand count, days to flowering, height, lodging, days to maturity, pollen viability, kernel rows per ear, kernels per ear, kernels per row, harvest grain moisture, and 100-kernel weight (Table 28, pp. 101 - 102, Pioneer 2019). While a substantial difference was observed in final stand count between DP202216 maize and its near isogenic control, the values were also observed to be within the reference range of other corn hybrids, suggesting that variation is within the norm of commonly cultivated maize varieties (Table 28, p. 102, Pioneer 2019). These data provide evidence that DP202216 maize grows and develops in a similar manner as its conventional maize comparators.

Additionally, no substantial differences in abiotic stress responses between DP202216 maize and its near isogenic control and other reference maize hybrids across the 12 sites during the 2017 growing season (Table A13-1 through A13-12, pp. 177 - 188, Pioneer 2019). These data suggests that DP202216 maize responds to abiotic stressors in a similar manner as its conventional maize comparators, and is further reinforced by an absence of unexpected abiotic stress responses during the 9 years that DP202216 maize was field tested within the United States (Appendix 13, p. 189, Pioneer 2019). Also, as previously examined, DP202216 maize does not appear to respond differently to biotic stressors, including insect and disease pests, compared to its near isogenic control or reference maize varieties (Section D. Potential Plant Pest and Disease Impacts).

Interestingly, the 2017 field data suggests that DP202216 maize yields no more grain than its near isogenic control (193.7 versus 201.7 bushels/acre, respectively; see Table 28, p. 102, Pioneer 2019). While the observed yield values are within the reference range provided by the reference maize hybrids that were also grown at these sites (102-292 bushels/acre; see Table 28, p. 102, Pioneer 2019), the absence of significant yield differences between DP202216 maize and its near isogenic control may be an artifact of the design of the 2017 study (Section VIII-B.1, p. 99, Pioneer 2019). Utilizing a larger data set that spanned multiple years (2014 - 2016) and methods designed to detect small but significant differences in maize grain yield (Section VIII-B.2, p. 103, Pioneer 2019), DP202216 maize was observed to produce higher grain yield than its near isogenic controls across sites, years, and hybrids during 2014 – 2016 study (Table 29, p. 103, Pioneer 2019).

The data show that neither the enhanced yield potential trait nor the glufosinate resistant trait altered the weediness potential of DP202216 maize compared to the conventional control based on the assessed phenotypic and agronomic traits. This conclusion, in conjunction with the cultivation of existing glufosinate-resistant maize varieties in the United States, indicates that DP202216 maize is unlikely to be any more difficult to control as a volunteer in subsequent seasons after its planting. There are numerous methods to effectively manage volunteer maize in agricultural fields (Jeschke and Doerge

2010); given that absence of increased weediness potential in DP202216 maize, existing methods that are effective in control currently maize volunteers, including maize volunteers that are resistant to glufosinate, are also likely to be effective in controlling DP202216 maize volunteers.

In summary, DP202216 maize is unlikely to persist as a troublesome weed or to have an impact on current weed/volunteer management practices, based on the agronomic laboratory data, field data, and literature survey concerning weediness potential of the crop. These data suggest that DP202216 maize is no more likely to become a weed than conventional varieties of maize.

G. Potential Impacts on the Weediness of Any Other Plants with which DP202216 Maize Can Interbreed

Gene flow is a natural biological process with significant evolutionary importance. A number of angiosperm taxa are believed to be derived from hybridization or introgression between closely related taxa (Grant 1981; Rieseberg and Wendel 1993; Soltis and Soltis 1993; Hegde et al. 2006), and even in the existing floras, the occurrence of hybridization or introgression is reported to be widespread (Stace 1987; Rieseberg and Wendel 1993; Peterson et al. 2002). It has been a common practice by plant breeders to artificially introgress traits from wild relatives into crop plants to develop new cultivars (Khoury et al. 2013). However, gene flow from crops to wild relatives is also thought of as having a potential to enhance the weediness of wild relatives, as observed in rice, sorghum, sunflower, and a few other crops (see Table 1 in Ellstrand et al. 1999). This topic is covered in two sections: 1) the potential for gene flow, hybridization and introgression from the DP202216 maize to sexually compatible relatives, including wild, weedy, feral or cultivated species in the United States and its territories, and 2) if gene flow is likely, then risk potential with respect to weediness of those taxa based on the phenotypic changes that have been observed in DP202216 maize.

Potential for gene flow, hybridization, and gene introgression

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

Except for *Z. perennis*, teosintes can be crossed with cultivated maize to produce fertile hybrids (Doebley 1990; OGTR 2008). However, there are barriers that reduce or prevent gene flow between maize and teosinte in the environment. For example, temporal and

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

A search of the Plants Database yielded results showing that *Z. mexicana* (Syn. *Z. mays* subsp *mexicana*) is listed as present in Florida, Alabama and Maryland, having been introduced from Mexico (USDA-NRCS 2015b); *Z. perennis* is listed in Texas and South Carolina (USDA-NRCS 2015c). *Z. diploperennis* and *Z. luxurians* are also listed, but there is no information about their location and status (USDA-NRCS 2015a, i). 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 2013b). Therefore, introgression of DP202216 maize into teosinte is unlikely in the United States,

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

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

7 day period (Coe et al. 1988; OGTR 2008). However, adverse consequences of gene flow from DP202216 maize to wild or weedy related species in the United States are highly unlikely.

Gene flow potential of DP202216 maize was evaluated thoroughly. The introduced *zmm28* and *pat* genes in DP202216 maize are not expected to change the ability of the plant to interbreed with other plant species. Furthermore, the APHIS evaluation of data provided by Pioneer of agronomic and phenotypic properties of DP202216 maize, including those characteristics associated with reproductive biology such as seed germination and dormancy, early stand count, plant height, final stand count, grain moisture, test weight, yield and pollen morphology and viability indicated no unintended changes likely to affect the potential for gene flow from DP202216 maize to sexually compatible species (Table 28, pp. 101 - 102, Pioneer 2019). The potential for gene flow to occur specifically between herbicide-resistant crop varieties and their sexually compatible relatives has been previously addressed (Mallory-Smith and Sanchez Olguin 2010). Gene flow does not differ whether the herbicide resistance trait is introduced via genetic engineering or via conventional breeding techniques, and gene flow has been occurring between non-GE maize and GE maize hybrids. Therefore, the potential for gene flow and introgression of the glufosinate herbicide-resistant trait from DP202216 maize to other maize hybrids and its consequences are anticipated to be similar to those as for existing commercial maize hybrids.

Many conditions have been identified that are required for gene flow and introgression to occur between a crop and its wild relatives (Carpenter et al. 2002; Jenczewski et al. 2003; Stewart et al. 2003; Owen 2005), including flowering synchrony, abundance and method of pollen spread, distance of pollen movement, genetic compatibility, and environmental conditions pertinent to cross-pollination, but the foremost condition is the presence of wild relatives within pollen or seed dispersal range from the crop. In the United States, the lack of sexually compatible wild relatives of *Z. mays* ssp. *mays* precludes the opportunity for gene flow to occur between cultivated maize and its wild relatives. Based on the information presented in the petition and in relevant literature, APHIS has reached the following conclusions: The genetic modification in DP202216 maize is not expected to increase the potential for gene flow, hybridization and/or introgression to sexually-compatible taxa compared to the non-transgenic recipient or other hybrids of the crop commonly grown. Gene flow, hybridization and/or introgression of genes from DP202216 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

As described earlier, there is no indication that DP202216 maize possesses a selective advantage that would result in increased weediness. In the extremely unlikely event successful hybrids of cultivated maize and wild relatives were to occur in the United States, the herbicide-resistance trait would only provide selective advantage in situations in which DP202216 maize was in contact with the herbicide (i.e., in an agricultural or fallowed field or field edge). Any herbicide-resistant hybrid-derived populations are likely to be controlled using other available chemical or mechanical means. As discussed

in the Section F, many methods that are effective for control of DP202216 maize as volunteers would likely be effective for control of hybrids formed with other conventional maize or related species.

DP202216 maize does not exhibit characteristics that may cause it to be any weedier than other cultivated maize based on the data presented in the petition (see Section F. Potential for Enhanced Weediness of DP202216 maize). Furthermore, 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 those instances of accidental gene flow between DP202216 maize and its wild relatives, the transgenes of DP202216 maize are unlikely to transform its wild relatives into more weedy species. Moreover, its potential impact due to the extremely limited potential for gene introgression into teosinte is not expected to be any different than that of other cultivated maize varieties. Based on the above considerations, DP202216 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 DP202216 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-transgenic recipient or other varieties of maize that are commonly grown. Gene flow, hybridization, and/or introgression of genes from DP202216 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. It is highly unlikely that maize plants will be found outside of an agricultural setting. It is also highly unlikely that gene flow and introgression will occur between DP202216 maize plants and sexually-compatible relatives in a natural environment, since sexually compatible relatives do not occur in the United States. Herbicides and other methods are available to control volunteer glufosinate-resistant maize and other maize and *Zea* species with which it might cross. Therefore, DP202216 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 DP202216 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.

DP202216 maize exhibits enhanced yield potential and glufosinate resistance. Information contained within the Pioneer (2019) petition demonstrates that the cultivation practices needed for growing DP202216 maize are similar to practices used to grow conventional maize. Additionally, no biologically significant differences in insect abundance, insect and disease damage were observed in field trials or targeted studies of

DP202216 maize and its near isogenic control or reference maize hybrid comparators (see Section D. Potential Plant Pest and Disease Impacts). Furthermore, DP202216 maize exhibits growth and developmental characteristic that are similar to conventional maize (see Section F. Potential for Enhanced Weediness of DP202216 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 DP202216 maize. Additionally, GE maize varieties with similar traits (if not identical in the case of mo-pat; see USDA-APHIS 2019b) 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.

APHIS could not identify any significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of DP202216 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 DP202216 maize Cannot Interbreed

APHIS examined the potential for the new genetic material inserted into DP202216 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 have been reviewed (Keese 2008). Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake and recombination and rearrangement, most notably through viruses and mobile genetic elements. HGT has been a major contributor to the spread of antibiotic resistance amongst pathogenic bacteria; emergence of increased virulence in bacteria, eukaryotes and viruses; and, over extended time scales, to major transitions in evolution (Brown 2003; Keeling and Palmer 2008; Keese 2008).

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

DP202216 maize contains protein coding regions derived from *S. viridochromogenes* strain Tü494 (*mo-pat*); it also contains small, non-coding regions from *A. tumefaciens* (e.g., sequences related to the T-DNA), *Saccharomyces cerevisiae* (i.e., flippase recombination sites), and Bacteriophage lambda (i.e., loxP and AttB recombination sites; see Table 3, pp. 32 - 33, Pioneer 2019).

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).

HGT from and expression in bacteria of the foreign DNA inserted into the nuclear genome of the GE plant is unlikely to occur. First, many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Wood et al. 2001; Kaneko et al. 2002). There is no evidence that these organisms contain genes derived from plants. HGT from plants to bacteria is a very low frequency event, primarily because functional and selective barriers to HGT increase with genetic distance (Keese 2008). Second, in cases where review of sequence data implied that HGT 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 HGT occurred, proteins corresponding to the transgenes are not likely to be produced. Fourth, the European Food Safety Authority (2009) has evaluated HGT from the use of antibiotic resistance marker genes and concluded that the likelihood of transfer of antibiotic resistance genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is very rare or remote.

Potential for horizontal gene transfer to viruses

APHIS also considered whether horizontal transfer of DNA from the GE plant to plant viruses was likely to occur and would lead to the creation or selection of plant viruses that are more virulent or have a broader host range. DP202216 maize contains no sequences from plant viruses (Table 3, pp. 32 - 33, Pioneer 2019). Nevertheless, 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 DNA viruses (e.g., gemini viruses that replicate in the nucleus; see Frischmuth and Stanley 1998) and RNA viruses (which typically replicate in the cytoplasm); however most have been under conditions that favor recombination to restore a defective virus (Fuchs and Gonsalves 2007; Keese 2008; Thompson and Tepfer

2010). Populations of recombinants between virus transgenes expressed in transgenic plants infected with related viruses are similar to recombinants found in mixed infections of the same viruses in non-transgenic plants, indicating that there was no novel recombination mechanism in the transgenic plants and no increased risk is expected over what is expected from mixed infections (Keese 2008; Turturo et al. 2008). Non-homologous 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 in the 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 virus-resistant plum, squash, or papaya engineered with genes from viruses that have been deregulated in the United States (Fuchs and Gonsalves 2007).

Potential for horizontal gene transfer to parasitic plants

Evidence for HGT from plants to other plants is limited to two specific scenarios: (1) exchange of genes between a parasitic plant and its host; and (2) exchange of genes between cells of two plants living in close proximity, such as in a graft junction. In both cases, this type of HGT requires physical contacts between the two plants. Most cases of HGT in plants involve transfer of mitochondrial genomes, which are primarily maternally inherited in plants to other mitochondria genomes (Barr et al. 2005), 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, *Striga hermonthica* (purple witchweed) from its monocot host (Yoshida et al. 2010). According to this study, the incorporation of the specific genetic sequence (with an unknown function) occurred between sorghum and *S. hermonthica*. However, this HGT occurred before speciation of *S. hermonthica* and related *S. gesnerioides* (cowpea witchweed) from their common ancestor. Furthermore, *S. hermonthica* is not found in the United States and *S. asiatica*, another related parasite of cereal crops, is only present in North Carolina and South Carolina (USDA-NRCS 2015j). 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 DP202216 maize crop, the DNA sequences were inserted into the nuclear genome, not the mitochondrial genome (Pioneer 2019).

If DP202216 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 it. However, in both scenarios, this newly introduced DNA would likely reside in somatic cells; 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 DP202216 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 the DP202216 maize compared to the unmodified variety from which it was derived and other maize reference hybrids. APHIS concludes that the DP202216 maize is unlikely to pose a plant pest risk based on the following findings:

- No plant pest risk was identified from the transformation process or the presence of new genetic material in DP202216 maize because the *Agrobacterium tumefaciens* transformation vector was disarmed, the transformed material was treated with an antibiotic to kill the bacterium, and the plant pest sequences inserted do not cause disease or create an infectious agent.
- No increase in plant pest risk was identified from expression of the inserted genetic material, the introduced ZMM28 or MO-PAT proteins, or changes in metabolism or composition. The expressed ZMM28 and MO-PAT proteins do not raise any plant pest concerns, and the composition of DP202216 maize grain and forage were determined to be substantially equivalent to its near isogenic comparator and other maize reference hybrids cultivated in the United States.
- Disease and pest incidence and/or damage were not observed to be significantly increased or atypical in DP202216 maize compared to its near isogenic comparator and other maize reference hybrids during field trials and targeted studies conducted in growing regions representative of where DP202216 maize is expected to be grown. Observed agronomic traits also did not reveal any significant differences that would indirectly indicate DP202216 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 DP202216 maize is unlikely to have any adverse impacts on organisms beneficial to agriculture based on APHIS' analysis of studies on DP202216 maize food and feed safety and composition.
- DP202216 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 DP202216 maize as a volunteer. Glufosinate resistance is not a new trait in maize and volunteers of the glufosinate-resistant DP202216 maize can be managed using a variety of currently available methods and alternative herbicides.
- DP202216 maize is not expected to increase the weed risk potential of other species with which it can interbreed in the United States or its territories. Gene flow, hybridization, and/or introgression of inserted genes from DP202216 maize to other

sexually compatible relatives with which it can interbreed is not likely to occur. Any possible introgression into teosintes or *Tripsacum* species of the new phenotype conferred by genetic engineering is not likely to increase the weediness of these relatives or affect the current ability to control them in situations where they might be considered weedy or invasive.

- Significant changes to agricultural or cultivation practices (e.g., pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of DP202216 maize were not identified and are not likely to increase plant diseases or pests or compromise their management. In particular, the glufosinate-resistant trait in DP202216 maize is not new and is already presented in some GE maize varieties that 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.
- Horizontal gene transfer of the new genetic material inserted into DP202216 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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