



Marketing and  
Regulatory  
Programs

Animal Plant  
Health Inspection  
Service

Biotechnology  
Regulatory  
Services

5601 Sunnyside  
Ave, Beltsville,  
MD 20705

## **Extended Determination of Nonregulated Status for Pioneer Hi-Bred International, Inc. Request (25-171-01ext) for Extension of Determination of Nonregulated Status for Insect Resistant and Herbicide Tolerant DP51291 Maize (*Zea mays*)**

In response to a request from Pioneer Hi-Bred International, Inc. (hereinafter referred to as Pioneer) to extend a determination of nonregulated status to DP51291 maize which is engineered to express the *ipd072Aa* and *mo-pat* genes, which confer insect resistance and herbicide resistance, and the *pmi* gene as a selectable marker, the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has determined, based on similarity to DP23211 maize, its antecedent organism, that DP51291 maize is unlikely to pose a greater plant pest risk than the nonmodified maize from which it was derived and is no longer to be considered a regulated article 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 DP23211 maize. DP23211 maize was deregulated on 11/30/2023 (Petition No. 20-203-01p). APHIS-approved permits or acknowledged notifications that were previously required for environmental release, interstate movement, or importation will no longer be required for DP51291 maize and its progeny. Importation of DP51291 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.

APHIS evaluated the plant pest risk of DP51291 maize by assessing its similarity to the deregulated DP23211 maize. DP51291 maize contains a single insertion of *ipd072Aa*, *mo-pat*, and *pmi* cassettes that are identical to the cassettes inserted into the antecedent DP23211 maize. Relative to DP23211 maize, DP51291 maize contains four different noncoding recombination sites and lacks the *DvSSJ1* cassette. The three mechanisms of action in DP51291 maize are the same as the mechanisms of action in DP23211 maize, and the absence of the *DvSSJ1* cassette from DP23211 maize does not affect conclusions for the other mechanisms of action. DP51291 maize is therefore similar to the antecedent DP23211 maize.

APHIS previously conducted a Plant Pest Risk Assessment (PPRA) on the antecedent organism DP23211 maize and concluded that it is unlikely to pose a greater plant pest risk than the nonmodified maize from which it was derived. Based on our Plant Pest Risk Similarity Assessment (PPRSA, see Appendix A), APHIS did not identify any known or expected phenotypic differences resulting from the genetic modification between the antecedent organism and DP51291 maize, except for lack of insecticidal *DvSSJ1* dsRNA and increased expression of the insecticidal IPD072Aa protein, based on information in the extension and publicly available data. As discussed in the PPRSA, these phenotypic differences between DP51291 maize and the antecedent DP23211 maize did not result in

any increased plant pest risk. From the PPRSA, we conclude the following with respect to DP51291 maize and its progeny:

- No plant pest risk was identified from the transformation process, the insertion and/or expression of new genetic material, or from metabolic changes in DP51291 maize.
- DP51291 maize is unlikely to pose greater risk related to plant pest and disease impacts because DP51291 maize, like the antecedent DP23211 maize, is not expected to have altered pest or disease susceptibility, or any other phenotypic changes that could directly or indirectly affect pests and diseases.
- DP51291 maize is unlikely to adversely impact nontarget organisms beneficial to agriculture based on established understanding of the function of the genetic modification in the plant, data submitted with the antecedent organism, and EPA's environmental risk assessment.
- DP51291 maize is unlikely to have enhanced weediness that would lead to greater plant pest risk. Maize is not known to be a weed in the United States or its territories. Like the antecedent DP23211 maize, the phenotypic changes imparted by the modification are not expected to increase the weediness of DP51291 maize or the ability to manage the plant as a weed based on the function of the genetic modification.
- No natural hybridization between maize and other plants in the United States is known because maize has no sexually compatible relatives in the United States or its territories except for other cultivated varieties of *Zea mays*. Thus, DP51291 maize is not expected to increase the weed risk potential of other species.
- Adoption of DP51291 maize, like the antecedent DP23211 maize, is not expected to cause significant changes to agriculture or cultivation practices, including pesticide applications, tillage, irrigation, harvesting, or any other pest management practices.
- Horizontal gene transfer of the genetic material inserted in DP51291 maize, like the antecedent DP23211 maize, to other organisms is extremely 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.

Based on my full and complete review and consideration of the scientific data, analyses, information, previous conclusions of the Plant Pest Risk Assessment for the antecedent organism, input from the public involvement process, and the conclusions of the Plant Pest Risk Similarity Assessment, I am issuing this determination of nonregulated status.

---

Donna A. Lalli, Ph.D.  
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. Petition for an Extension of Determination of Nonregulated Status for Insect Resistant and Herbicide Tolerant DP51291 Maize (*Zea mays*)**

## **Plant Pest Risk Similarity Assessment**

**September 2025**

#### **Agency Contact**

Joseph Tangredi  
Biotechnology Regulatory Services  
Animal and Plant Health Inspection Service  
United States Department of Agriculture

5601 Sunnyside Ave, AP100  
Beltsville, MD 20705

Mention of companies or commercial products in this report does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned. USDA neither guarantees nor warrants the standard of any product mentioned. Product names are mentioned solely to report factually on available data and to provide specific information.

---

This publication reports research involving pesticides. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

## TABLE OF CONTENTS

|    |                                                                                                                                  |    |
|----|----------------------------------------------------------------------------------------------------------------------------------|----|
| 1  | Introduction .....                                                                                                               | 1  |
| 2  | Background .....                                                                                                                 | 3  |
|    | Purpose of the modification .....                                                                                                | 3  |
|    | Biology of the comparator plant .....                                                                                            | 3  |
| 3  | Description of Inserted Genetic Material, Expression Profile, Gene Products, and Changes to Plant Metabolism and Phenotype ..... | 3  |
|    | Description of the genetic modification and inheritance of inserted DNA .....                                                    | 3  |
|    | Expression profile of inserted DNA, and changes in gene expression, new proteins, or metabolism .....                            | 4  |
| 4  | Applicability of Previous Review and Assessment of Unique Pathways to Increased Plant Pest Risk .....                            | 4  |
| 5  | Potential Plant Pest and Disease Impacts.....                                                                                    | 5  |
| 6  | Potential Impacts on Nontarget Organisms Beneficial to Agriculture .....                                                         | 5  |
| 7  | Potential for Increased Weediness of the Modified Plant.....                                                                     | 5  |
| 8  | Potential Impacts on the Weediness of Other Plants with which the Modified Plant Can Interbreed .....                            | 6  |
| 9  | Potential Changes to Agricultural or Cultivation Practices .....                                                                 | 6  |
| 10 | Potential Impacts from Transfer of Genetic Information to Organisms with which the Modified Plant Cannot Interbreed .....        | 6  |
| 11 | Conclusions .....                                                                                                                | 7  |
| 12 | Similarity Table.....                                                                                                            | 8  |
| 13 | References .....                                                                                                                 | 11 |

## 1 INTRODUCTION

In June 2025, Pioneer Hi-Bred International, Inc. (Pioneer) submitted a petition to the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) requesting an extension of the determination of nonregulated status for genetically engineered insect-resistant and herbicide-resistant maize<sup>1</sup>, *Zea mays*, (OECD Unique Identifier DP-051291-2, referred to as DP51291 maize) and any progeny derived from it under Title 7 of the Code of Federal Regulations part 340 (7 CFR part 340). APHIS assigned number 25-171-01ext to the extension. 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.*). Pioneer engineered DP51291 maize to express the *ipd072Aa* and *mo-pat* genes, which confer insect resistance and herbicide resistance, and the *pmi* gene as a selectable marker. APHIS conducted this Plant Pest Risk Similarity Assessment (PPRSA) to determine whether DP51291 maize is similar to its antecedent organism DP23211 maize, reviewed in Petition No. 20-203-01p, and is therefore unlikely to pose a greater plant pest risk than nonmodified maize (7 U.S.C. 7701 *et seq.*).

APHIS regulations in part 340 govern the introduction (importation, interstate movement, or release into the environment) of certain organisms and products altered or produced through genetic engineering. A genetically engineered organism is considered a “regulated article” under § 340.1 if the donor organism, recipient organism, or vector (or vector agent) belongs to any genera or taxa designated in § 340.2 and meets the definition of a 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 as defined in § 340.1. A genetically engineered plant (modified plant) that meets the definition of a regulated article is no longer subject to the regulatory requirements of part 340 when APHIS determines that it is unlikely to pose a greater plant pest risk than the nonmodified plant from which it was derived. According to § 340.6(e), if a modified plant is similar to a previously deregulated plant(s), a request to extend the previous determination of nonregulated status to the new event may be filed.

Pioneer developed DP51291 maize by site-specific integration (SSI) to insert specific genetic sequences using *Agrobacterium*-mediated and microprojectile bombardment transformations (Pioneer 2025). DP51291 maize contains sequences derived from plant pests such as a banana streak virus promoter and a cauliflower mosaic virus 35S terminator. In addition,

---

<sup>1</sup> Pioneer described the phenotype of DP51291 maize as “herbicide tolerant,” and historically, APHIS has referred to GE plants with reduced herbicide sensitivity as herbicide tolerant. However, the phenotype would fall under the Weed Science Society of America’s (WSSA) definition of “herbicide resistance” because DP51291 maize has an “inherited ability to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type.” By the WSSA definition, “resistance [to an herbicide] may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis.” Herbicide tolerance, by the WSSA definition, only applies to plant species with an “inherent ability to survive and reproduce after herbicide treatment,” which implies there was no genetic manipulation to make the plant tolerant (WSSA 1998). Therefore, APHIS refers to this trait as “herbicide resistant.”

*Agrobacterium*, used in the transformation of DP51291 maize, is a known plant pest. Therefore, unless and until APHIS makes a determination to extend the previous determination of nonregulated status, DP51291 maize is considered a regulated article under APHIS regulations in part 340.

APHIS utilized data and information submitted by the petitioner, in addition to current literature, to evaluate the similarity between DP51291 maize and the antecedent DP23211 maize. For those aspects of plant pest risk where the prior review of the antecedent applies equally to the new event, no further review is conducted. If there are pathways to increased plant pest risk for DP51291 maize that the previous review did not fully cover, then those pathways are analyzed during review of the extension.

APHIS specifies in § 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. The three transgenes in DP51291 maize, *ipd072Aa*, *mo-pat*, and *pmi* genes, encode proteins that are identical to those present in the previously deregulated DP23211 maize (Petition No. 20-203-01p). DP23211 maize additionally contained the *DvSSJ1* gene encoding an insecticidal double-stranded ribonucleic acid (dsRNA) (Pioneer 2020). There is no interaction between the product of this gene and the products of the other genes in DP23211 maize, and its absence in DP51291 maize would therefore not affect the risk conclusions for the other mechanisms of action. Therefore, Pioneer DP23211 maize is a suitable antecedent organism for extension.

APHIS considered information submitted by the applicant on DP51292 maize, information available on the antecedent DP23211 maize, and publicly available information related to the genetic and phenotypic differences between DP51291 maize and the antecedent organism. This PPRSA outlines the potential impacts of DP51291 maize and its progeny that are relevant to plant pest risk compared to the antecedent DP23211 maize, considering expression of the gene product, new enzymes, or changes to plant metabolism; plant pest risk characteristics; potential impacts on nontarget organisms beneficial to agriculture; the potential for increased weediness of the modified plant; increased weediness of sexually compatible relatives; changes to agricultural and cultivation practices; and impacts from the transfer of genetic information to organisms with which the modified plant cannot interbreed; and any other information which the Administrator believes to be relevant to a determination.

DP51291 maize has undergone regulatory review by other U.S. agencies. Under the Coordinated Framework, the oversight of biotechnology-derived plants rests with the USDA, the U.S. Food and Drug Administration (FDA), and the U.S. Environmental Protection Agency (EPA) (51 FR 23302 1986; 57 FR 6753 1992; Biotechnology Working Group 2017). Biotechnology-derived plants are subject to review by one or more of these agencies depending on their characteristics and intended use. FDA has primary responsibility for ensuring the safety of human food and animal feed (Biotechnology Working Group 2017). EPA regulates pesticides, including plants with plant-incorporated protectants (pesticides intended to be produced and used in a living plant), to ensure public safety (Biotechnology Working Group 2017). FDA (2024)

and EPA (2025) completed their reviews of DP51291 maize as well as the antecedent DP23211 maize (FDA 2022; EPA 2024). Biotechnology-derived plants are subject to review by one or more of these agencies depending on their characteristics and intended use. FDA has primary responsibility for ensuring the safety of human food and animal feed (Biotechnology Working Group 2017). EPA regulates pesticides, including plants with plant-incorporated protectants (pesticides intended to be produced and used in a living plant), to ensure public safety (Biotechnology Working Group 2017). FDA (2024) and EPA (2025) completed their reviews of DP51291 maize as well as the antecedent DP23211 maize (FDA 2022; EPA 2024).

## **2 BACKGROUND**

### **Purpose of the modification**

According to the extension request, DP51291 maize is intended for insect resistance to control certain corn rootworm (CRW) pests using the *ipd072Aa* gene which codes for the IPD072Aa protein. DP51291 maize also contains two other genes (Pioneer 2025). The *mo-pat* gene codes for the phosphinothricin acetyltransferase (PAT) protein, which provides herbicide resistance to glufosinate-ammonium, and the *pmi* gene codes for the phosphomannose isomerase (PMI) protein, which allows for identification of transgenic plants during development.

### **Biology of the comparator plant**

Both DP51291 maize and the antecedent DP23211 maize are genetically engineered maize. Therefore, maize is the comparator plant for both events.

## **3 DESCRIPTION OF INSERTED GENETIC MATERIAL, EXPRESSION PROFILE, GENE PRODUCTS, AND CHANGES TO PLANT METABOLISM AND PHENOTYPE**

APHIS assessed genetic, metabolic, and phenotypic changes to DP51291 maize that are known or plausibly expected to occur due to the modification, which are used later in this PPRSA to evaluate the potential to pose an increased plant pest risk. To determine whether the review of the antecedent DP23211 maize applies to DP51291 maize, APHIS compared the two plants.

### **Description of the genetic modification and inheritance of inserted DNA**

DP51291 maize was genetically modified via *Agrobacterium*-mediated and microprojectile bombardment transformations. There is a single insertion site into the maize genome that contains three genes of interest (*ipd072Aa*, *mo-pat*, and *pmi*) and other regulatory components. Promoters and terminators for the genes of interest are identical between DP51291 maize and the antecedent DP23211 maize. The only elements present in DP51291 maize but not in the antecedent DP23211 maize are four noncoding recombination sites (*loxP*, *attB1*, *attB2*, and *attB3*). These elements are used in the transformation process to guide the insertion of the cassettes to a location in the genome and do not alter the final plant phenotype, as these sites are otherwise inert and designed to minimize disruption of the genome. All genes were stably inherited across five generations, as confirmed by Southern blot

followed by Next Generation sequencing analysis (Pioneer 2025). Compared to the antecedent DP23211 maize, DP51291 maize lacks the entire *DvSSJ1* gene cassette, which encodes an insecticidal dsRNA intended to suppress translation of the DvSSJ1 protein via RNA interference (RNAi), and four noncoding components (*KpnI*, *zm-SEQ9*, *KpnI*, *zm-SEQ8*) (Pioneer 2020). A comparison of the genetic elements contained within the extension and antecedent organism is found in Table 1.

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

Insect-protected DP51291 maize produces an insecticidal protein, IPD072Aa, coded for by the *ipd072Aa* gene from *Pseudomonas chlororaphis*, which protects against feeding damage caused by corn rootworms (*Diabrotica spp.*). While the IPD072Aa protein from DP51291 maize is structurally and functionally equivalent to the IPD072Aa protein from the antecedent DP23211 maize, DP51291 maize expresses IPD072Aa protein at least two times higher than the antecedent DP23211 maize in all tissues examined (Pioneer 2020, 2025). Pioneer observed the largest expression difference in the roots, where there was over an 8.5-fold higher expression level in DP51291 maize than the antecedent organism (Pioneer 2025 Table 13). Because higher protein expression levels in certain plant tissues may pose a greater risk to nontarget organisms (NTOs), APHIS discusses this issue in more detail below.

DP51291 maize also contains two additional exogenous genes, *mo-pat* and *pmi*, also found in DP23211 maize. The *mo-pat* gene encodes the PAT protein that allows plants to survive in the presence of glufosinate-ammonium herbicide. The *pmi* gene encodes the PMI protein that allows plants to survive on media with an alternative carbon source, D-mannose, during the screening process. Plants producing the PMI protein metabolize D-mannose, which can lead to a visible color change of some media, allowing the identification of transgenic plants. Both genes have been well-characterized and do not cause any known additional changes to plant metabolism or phenotype (Herouet et al. 2005; Stoykova and Stoeva-Popova 2011; Das et al. 2023; Mmbando 2023). In addition, both the *mo-pat* and *pmi* genes were previously reviewed and deregulated in multiple petitions for maize and other plants, e.g., 00-136-01p, 03-353-01p, 20-203-01p (the full list of petitions may be found on the petition table at USDA's website) (USDA 2001, 2005, 2023, 2025).

## **4 APPLICABILITY OF PREVIOUS REVIEW AND ASSESSMENT OF UNIQUE PATHWAYS TO INCREASED PLANT PEST RISK**

APHIS did not identify any known or expected genetic, metabolic, or phenotypic differences resulting from the genetic modification between the antecedent organism and DP51291 maize, except for lack of insecticidal *DvSSJ1* dsRNA and increased expression of the insecticidal IPD072Aa protein, based on information in the extension, information about the antecedent organism, and publicly available data. Because there is no interaction between the product of *DvSSJ1* and the products of the other genes in DP23211 maize, the absence of insecticidal *DvSSJ1* dsRNA in DP51291 maize does not affect the risk conclusions for the other mechanisms

of action. Therefore, the conclusions of the previous PPRA were applied to each category of plant pest risk, except potential impacts on NTOs resulting from increased expression of the IPD072Aa protein, as described below.

## **5 POTENTIAL PLANT PEST AND DISEASE IMPACTS PEST RISK**

APHIS did not identify any differences between the antecedent organism and DP51291 maize which could alter plant pest and disease impacts. The evaluation of the antecedent organism, DP23211 maize, found no increased risk of plant pest and disease impacts due to the genetic modifications of that plant (USDA 2023). Based on APHIS' review of the extension, the antecedent petition, and antecedent PPRA, the previous findings of the antecedent PPRA with respect to changes to pest resistance, pest susceptibility, plant defense, the ability to host a disease or pest, or to affect plant pests or diseases apply to DP51291 maize. Therefore, APHIS concludes that DP51291 maize is unlikely to alter potential plant pest and disease impacts or lead to greater plant pest risk than the antecedent organism.

## **6 POTENTIAL IMPACTS ON NONTARGET ORGANISMS BENEFICIAL TO AGRICULTURE**

For insecticidal proteins such as IPD072Aa, risk to nontarget organisms (NTOs) depends on both the toxicity of the protein and the amount of the protein that the NTOs will be exposed to over their lifetimes. Because IPD072Aa protein expression in all DP51291 maize tissues is at least two times greater than in the antecedent DP23211 maize, APHIS undertook additional review. APHIS considers the potential effect of genetic modifications on organisms beneficial to agriculture, such as predators and parasitoids, pollinators, and organisms that influence soil health. EPA conducted an environmental risk assessment of DP51291 maize that considered risk to NTOs, such as red-lipped green lacewings (predator), pink ladybeetles (predator), parasitic wasps (predator), honeybees (pollinator), and collembola (soil organism). EPA's environmental risk assessment is sufficient to address the nontarget evaluation that APHIS normally conducts for PPRAs or PPRSAs because both assessments assess risk to NTOs. As part of its environmental risk assessment, EPA re-evaluated toxicity data provided by the petitioner from feeding studies of IPD072Aa protein, particularly for those species with higher sensitivity to the protein, in the context of the higher expression levels found in DP51291 maize. EPA determined that DP51291 maize is unlikely to adversely affect NTOs (EPA 2025). Based on EPA's ecological risk assessment for DP51291 maize, APHIS concludes that DP51291 maize is unlikely to alter impacts on NTOs beneficial to agriculture that would lead to greater plant pest risk than the antecedent organism.

## **7 POTENTIAL FOR INCREASED WEEDINESS OF THE MODIFIED PLANT**

APHIS did not identify any differences between the antecedent organism and DP51291 maize which could alter potential for increased weediness of the modified plant or the ability to manage the plant as a weed based on the function of the genetic modification. Maize is not known to be a weed in the United States or its territories. The evaluation of the antecedent organism, DP23211 maize, found no increased potential for increased weediness due to the

genetic modifications of that plant (USDA 2023). Based on APHIS' review of the extension, the antecedent petition, and antecedent PPRA, the previous findings of the antecedent PPRA with respect to characteristics such as establishment, competitiveness, reproduction, survival, persistence, and/or spread that could influence weediness or the ability to manage the plant as a weed apply to DP51291 maize. Therefore, APHIS concludes that DP51291 maize is unlikely to have increased weediness that would lead to greater plant pest risk than the antecedent organism.

## **8 POTENTIAL IMPACTS ON THE WEEDINESS OF OTHER PLANTS WITH WHICH THE MODIFIED PLANT CAN INTERBREED**

APHIS did not identify any differences between the antecedent organism and DP51291 maize which could alter potential for increased weediness of other plants with which the modified plant can interbreed. Maize has no sexually compatible relatives in the United States or its territories (Beadle 1980; Sánchez González et al. 2018), except for other cultivated varieties of *Zea mays*. Therefore, APHIS concludes that DP51291 maize is unlikely to cause increased weediness with other plants with which the modified plant can interbreed.

## **9 POTENTIAL CHANGES TO AGRICULTURAL OR CULTIVATION PRACTICES**

APHIS did not identify any differences between the antecedent organism and DP51291 maize which could lead to significant changes to agricultural or cultivation practices of the modified plant. The evaluation of the antecedent organism, DP23211 maize, found no potential changes to agriculture or cultivation practices due to the genetic modifications of that plant (USDA 2023). Based on APHIS' review of the extension, the antecedent petition, and antecedent PPRA, the previous findings of the antecedent PPRA with respect to pesticide applications, tillage, irrigation, harvesting, or any other pest management practice apply to DP51291 maize. Therefore, APHIS concludes that DP51291 maize is unlikely to alter agricultural or cultivation practices that would lead to greater plant pest risk than the antecedent organism.

## **10 POTENTIAL IMPACTS FROM TRANSFER OF GENETIC INFORMATION TO ORGANISMS WITH WHICH THE MODIFIED PLANT CANNOT INTERBREED**

APHIS did not identify any differences between the antecedent organism and DP51291 maize which could alter the potential for the genetic material inserted into DP51291 maize to be horizontally transferred without sexual reproduction to other organisms. The evaluation of the antecedent organism, DP23211 maize, found no greater risk of horizontal gene transfer to organisms with which the modified plant cannot interbreed due to the genetic modifications of that plant (USDA 2023). Based on APHIS' review of the extension, the antecedent petition, and antecedent PPRA, the previous findings of the antecedent PPRA with respect to disease, damage, injury, or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants via horizontal gene transfer apply to DP51291 maize. Therefore, APHIS concludes that horizontal gene transfer of the genetic material inserted into DP51291 maize to organisms with which DP51291 maize cannot interbreed is highly unlikely and is not

expected to lead directly or indirectly to disease, damage, injury, or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

## **11 CONCLUSIONS**

APHIS reviewed the information from the extension, supporting documents, public literature, and other relevant sources to assess the potential for increased plant pest risk of the DP51291 maize compared to the antecedent DP23211 maize. APHIS compared the similarity between antecedent DP23211 maize and the DP51291 maize and found that the assessment of DP23211 maize also applies to DP51291 maize, including impacts on plant pest and disease, increased weediness of the modified plant, increased weediness of sexually compatible relatives, changes to agricultural and cultivation practices, and impacts from transfer of genetic information to organisms with which the modified plant cannot interbreed. APHIS also reviewed EPA's ecological risk assessment for DP51291 maize (EPA 2025) and concluded that DP51291 maize is unlikely to pose a greater plant pest risk than DP23211 maize regarding harm to nontarget organisms beneficial to agriculture. Based on this Plant Pest Risk Similarity Assessment, APHIS concludes that DP51291 maize is unlikely to pose a greater plant pest risk than the antecedent organism, DP23211 maize.

## 12 SIMILARITY TABLE

Table 1. Similarity Table of Extension Request Compared to Antecedent Petition.

| Similarity Table   |                                                                                                                                                                           |                                                                                                                                                                           |                                                                                               |
|--------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| Description        | Extension Request DP51291 maize<br>Extension 25-171-01ext                                                                                                                 | Antecedent DP23211 maize<br>Petition 20-203-01p                                                                                                                           | Comments                                                                                      |
| Organism           | Maize                                                                                                                                                                     | Maize                                                                                                                                                                     | Same                                                                                          |
| Phenotype Category | Insect Resistance<br>Herbicide-resistance<br>Selective Marker                                                                                                             | Insect Resistance<br>Herbicide-resistance<br>Selective Marker                                                                                                             | Same phenotype categories                                                                     |
| Genetic Components | <i>loxP</i>                                                                                                                                                               | <i>KpnI</i><br><i>zm-SEQ9</i>                                                                                                                                             | Noncoding sequences used during the transformation process that do not affect plant phenotype |
|                    | <i>ubiZM1</i> Promoter<br><i>ubiZM1</i> 5' UTR<br><i>ubiZM1</i> Intron<br><i>FRT1</i><br><i>Pmi</i><br><i>pinII</i> Terminator<br><i>Z19</i> Terminator                   | <i>ubiZM1</i> Promoter<br><i>ubiZM1</i> 5' UTR<br><i>ubiZM1</i> Intron<br><i>FRT1</i><br><i>Pmi</i><br><i>pinII</i> Terminator<br><i>Z19</i> Terminator                   | Same <i>pmi</i> cassette                                                                      |
|                    | <i>os-actin</i> Promoter<br><i>os-actin</i> Intron<br><i>mo-pat</i><br><i>CaMV</i> 35S Terminator<br><i>loxP</i><br><i>sb-ubi</i> Terminator<br><i>sb-gkaf</i> Terminator | <i>os-actin</i> Promoter<br><i>os-actin</i> Intron<br><i>mo-pat</i><br><i>CaMV</i> 35S Terminator<br><i>loxP</i><br><i>sb-ubi</i> Terminator<br><i>sb-gkaf</i> Terminator | Same <i>mo-pat</i> cassette                                                                   |
|                    | <i>attB1</i><br><i>attB2</i>                                                                                                                                              | <i>KpnI</i>                                                                                                                                                               | Noncoding sequences used during the                                                           |
|                    |                                                                                                                                                                           |                                                                                                                                                                           |                                                                                               |

|                        |                                                                                          |                                                                                                                                                                                                                                                                                                                   |                                                                                                                  |
|------------------------|------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|
|                        |                                                                                          |                                                                                                                                                                                                                                                                                                                   | transformation process that do not affect plant phenotype                                                        |
|                        |                                                                                          | <i>ubiZM1</i> Promoter<br><i>ubiZM1</i> 5' UTR<br><i>ubiZM1</i> Intron<br>All-stop Codon Sequence<br><i>DvSSJ1</i> Fragment<br>Mini-stop Codon Sequence<br><i>zm-Adh1</i> Intron Connector<br><i>DvSSJ1</i> Fragment<br>All-stop Codon Sequence<br>Z27G Terminator<br>UBQ14 Terminator<br><i>In2-1</i> Terminator | Removal of <i>DvSSJ1</i> cassette compared to the antecedent petition. Not expected to increase plant pest risk. |
|                        | BSV(AY) Promoter<br><i>zm-HPLV9</i> Intron<br><i>ipd072Aa</i><br><i>at-T9</i> Terminator | BSV(AY) Promoter<br><i>zm-HPLV9</i> Intron<br><i>ipd072Aa</i><br><i>at-T9</i> Terminator                                                                                                                                                                                                                          | Same <i>ipd072Aa</i> cassette                                                                                    |
|                        | <i>attB3</i><br><i>FRT87</i>                                                             | <i>FRT87</i><br><i>zm-SEQ8</i>                                                                                                                                                                                                                                                                                    | Noncoding sequences used during the transformation process that do not affect plant phenotype                    |
| Transformation Method  | <i>Agrobacterium</i> -mediated and microprojectile bombardment                           | <i>Agrobacterium</i> -mediated and microprojectile bombardment                                                                                                                                                                                                                                                    | Same                                                                                                             |
| Insert and Copy Number | Single intact insertion                                                                  | Single intact insertion                                                                                                                                                                                                                                                                                           | Same                                                                                                             |
| Backbone Absent        | Yes                                                                                      | Yes                                                                                                                                                                                                                                                                                                               | Same                                                                                                             |

|                                                                                              |                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                      |                                                                                                                                                      |
|----------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|
| Gene(s) of Interest                                                                          | <i>pmi</i> : Allows growth on D-mannose media<br><i>mo-pat</i> : Herbicide resistant to glufosinate-ammonium<br><i>ipd072Aa</i> : Insect resistance against susceptible corn rootworm (CRW) pests | <i>pmi</i> : Allows growth on D-mannose media<br><i>mo-pat</i> : Herbicide resistant to glufosinate-ammonium<br><i>DvSSI1</i> : Insect resistance against susceptible corn rootworm (CRW) pests<br><i>ipd072Aa</i> : Insect resistance against susceptible corn rootworm (CRW) pests | Removal of one insect resistance gene of interest compared to the antecedent petition.                                                               |
| Plant Pest and Disease Impacts                                                               | None                                                                                                                                                                                              | None                                                                                                                                                                                                                                                                                 | Same                                                                                                                                                 |
| Impacts on Nontarget Organisms                                                               | None                                                                                                                                                                                              | None                                                                                                                                                                                                                                                                                 | Although DP51291 maize has 2 – 8x higher expression than the antecedent depending on tissue type, no increased risk to nontarget organisms was found |
| Potential for Increased Weediness                                                            | None                                                                                                                                                                                              | None                                                                                                                                                                                                                                                                                 | Same                                                                                                                                                 |
| Potential for Increased Weediness of Sexually Compatible Relatives                           | None                                                                                                                                                                                              | None                                                                                                                                                                                                                                                                                 | Same                                                                                                                                                 |
| Changes to Agricultural or Cultivation Practices with Potential to Affect Plant Pest Risk    | None                                                                                                                                                                                              | None                                                                                                                                                                                                                                                                                 | Same                                                                                                                                                 |
| Transfer of genetic information to organisms with which the modified plant cannot interbreed | None                                                                                                                                                                                              | None                                                                                                                                                                                                                                                                                 | Same                                                                                                                                                 |

## 13 REFERENCES

- 7 CFR part 340. 2025. *Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There Is Reason to Believe Are Plant Pests*. Retrieved from <https://www.ecfr.gov/current/title-7/subtitle-B/chapter-III/part-340> Last accessed July 23, 2025.
- 7 U.S.C. 7701 *et seq.* 2000. *Plant Protection Act*. Retrieved from <https://uscode.house.gov/view.xhtml?path=/prelim@title7/chapter104&edition=prelim> Last accessed March 12, 2025.
- 51 FR 23302. 1986. *Coordinated Framework for Regulation of Biotechnology*. Retrieved from <https://www.federalregister.gov/citation/51-FR-23302>
- 57 FR 6753. 1992. *Exercise of Federal Oversight Within Scope of Statutory Authority: Planned Introductions of Biotechnology Products Into the Environment*. Retrieved from <https://www.federalregister.gov/citation/57-FR-6753>
- Beadle G. 1980. *The Ancestry of Corn*. Scientific American 242, pp. 112–119.
- Biotechnology Working Group. 2017. *Modernizing the Regulatory System for Biotechnology Products: Final Version of the 2017 Update to the Coordinated Framework for the Regulation of Biotechnology*. Biotechnology Working Group, under the auspices of the Emerging Technologies Interagency Policy Coordination Committee. Retrieved from <https://usbiotechnologyregulation.mrp.usda.gov/sites/default/files/2017-coordinated-framework-update.pdf> Last accessed March 12, 2025.
- Das S, Ray MK, Panday D, and Mishra PK. 2023. *Role of biotechnology in creating sustainable agriculture*. PLOS Sustainability and Transformation 2, pp. e0000069.
- EPA. 2024. *Human Health Risk Assessment and Review of Product Characterization of the Insecticidal Plant-Incorporated Protectants, Pseudomonas chlororaphis IPD072Aa protein and DvSSJ1 dsRNA Complementary to the DvSSJ1 Gene Sequence from Diabrotica virgifera virgifera, and the Genetic Material Necessary (vector PHP74643) for their Production in Event DP23211 Maize (OECD Unique ID DP-Ø23211-2) and Establishment of a Permanent Tolerance Exemption*. Data were provided in support of a FIFRA Section 3 Seed Increase Registration. Washington, DC. UNITED STATES ENVIRONMENTAL PROTECTION AGENCY.
- EPA. 2025. *Environmental Risk Assessment for the Plant-Incorporated Protectant Pseudomonas chlororaphis IPD072Aa Protein and the Genetic Material Necessary for its Production in Corn Event DP-Ø51291-2*. Washington, D.C. Environmental Protection Agency.
- FDA. 2022. *Biotechnology Notification File No. 000175 CFSAN Note to the File*. College Park, MD. Food and Drug Administration.
- FDA. 2024. *Biotechnology Notification File No. BNF 00o196*. College Park, MD. Food and Drug Administration.
- Herouet C, Esdaile DJ, Mallyon BA, Debruyne E, Schulz A, Currier T, Hendrickx K, van der Klis RJ, and Rouan D. 2005. *Safety evaluation of the phosphinothricin acetyltransferase proteins encoded by the pat and bar sequences that confer tolerance to glufosinate-ammonium herbicide in transgenic plants*. Regul Toxicol Pharmacol 41, pp. 134–149. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/15698537>

- Mmbando GS. 2023. *Recent Advances in Antibiotic-Free Markers; Novel Technologies to Enhance Safe Human Food Production in the World*. Mol Biotechnol 65, pp. 1011–1022. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/36443619>
- Pioneer Hi-Bred International. 2020. *Petition for the Determination of Nonregulated Status for Insect Resistant and Herbicide-Tolerant DP23211 Maize Volume 1- Petition*. Submitted by S.A. Catron, US Seeds Regulatory Affairs Leader. Pioneer Hi-Bred International. Johnston, IA.
- Pioneer Hi-Bred International, Inc. 2025. *Petition for an Extension of Determination of Nonregulated Status for Insect Resistant and Herbicide-Tolerant DP51291 Maize*. Submitted by P.T. Garcia, US Seeds Regulatory Affairs Leader. Pioneer Hi-Bred International, Inc. Johnston, IA
- Sánchez González JdJ, Ruiz Corral JA, García GM, Ojeda GR, Larios LDIC, Holland JB, Medrano RM, and García Romero GE. 2018. *Ecogeography of teosinte*. PLoS One 13, pp. e0192676.
- Stoykova P and Stoeva-Popova P. 2011. *PMI (manA) as a nonantibiotic selectable marker gene in plant biotechnology*. Plant Cell, Tissue, and Organ Culture 105, pp. 141–148.
- USDA. 2001. *Approval of Mycogen Seeds c/o Dow AgroSciences LLC and Pioneer Hi-Bred International, Inc., Request (00-136-01p) Seeking a Determination of Non-regulated Status for Bt Cry1F Insect Resistant, Glufosinate Tolerant Corn Line 1507: Environmental Assessment and Finding of No Significant Impact*. United States Department of Agriculture - Animal and Plant Health Inspection Service.
- USDA. 2005. *Approval of Mycogen Seeds/Dow AgroSciences LLC and Pioneer Hi-Bred International, Inc. Request 03-353-01p) Seeking a Determination of nonregulated Status for Bt Cry34Ab1/35Ab1 Insect Resistant, Glufosinate Tolerant Corn Line 59122-7. Environmental Assessment and Finding of No Significant Impact*. United States Department of Agriculture - Animal and Plant Health Inspection Service.
- USDA. 2023. *Plant Pest Risk Assessment on the Pioneer Petition (20-203-01p) for Determination of Non-regulated Status of DP23211 Maize Riverdale, MD*. United States Department of Agriculture - Animal and Plant Health Inspection Service.
- USDA. 2025. *Petitions for Determination of Nonregulated Status*. Retrieved from <https://www.aphis.usda.gov/biotechnology/legacy-petition-process/petitions> Last accessed 7/17/2025.
- WSSA. 1998. *"Herbicide resistance" and "Herbicide tolerance" defined*. Weed Technology 12, pp. 789–790. Retrieved from <http://www.jstor.org/stable/3989101> Last accessed 05/08/2010.