Request for USDA-APHIS-BRS Regulatory Status Review Step 1 of Insect and Herbicide Resistant DP915635 Maize

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Executive Summary

Pioneer Hi-Bred International, Inc. (Pioneer, member of Corteva Agriscience group of companies) is submitting a request for a regulatory status review (RSR) of a genetically engineered (GE) plant, maize event DP-915635-4, hereafter referred to as DP915635 maize. Pioneer requests, based on the information contained herein, that the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) conduct an initial review to determine whether there is a plausible pathway by which DP915635 maize would pose an increased plant pest risk relative to conventional maize and, if none are identified, that DP915635 maize is not subject to the regulations in 7 CFR part 340.

DP915635 maize was genetically modified to express the IPD079Ea protein for control of susceptible corn rootworm (CRW) pests, the phosphinothricin acetyltransferase (PAT) protein for tolerance to glufosinate-ammonium herbicides, and the phosphomannose isomerase (PMI) protein used as a selectable marker.

The IPD079Ea protein, encoded by the *ipd079Ea* gene from *Ophioglossum pendulum* (fern), confers control of susceptible CRW pests when expressed in plants by causing disruption of the midgut epithelium. The IPD079Ea protein does not have a mechanism of action on the plant itself; it is not intended to interact with any biochemical pathways or change the metabolism or physiology of the plant. It is produced by the plant solely to protect against CRW feeding and provides a new mode of action (MOA) that is separate and distinct from the currently available *Bt* protein-based MOAs for CRW control.

The PAT protein, encoded by a maize-optimized version of the phosphinothricin acetyltransferase (*mo-pat*) gene from *Streptomyces viridochromogenes*, confers tolerance to the herbicidal active ingredient glufosinate-ammonium at current labeled rates by acetylating phosphinothricin, the active component of glufosinate-ammonium herbicides, to an inactive form. The PAT protein present in DP915635 maize is identical to the corresponding protein found in a number of approved events across several different crops that are currently in commercial use (CERA - ILSI Research Foundation, 2011; CERA - ILSI Research Foundation, 2016; Hérouet et al., 2005). APHIS has previously determined maize expressing PAT does not pose an increased plant pest risk and PAT in maize is a plant-trait-MOA combination that has been determined by APHIS not to require regulation under 7 CFR part 340 (https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/regulatory-processes/confirmations/moa/moa-table).

The phosphomannose isomerase (PMI) protein, encoded by the *pmi* gene from *Escherichia coli*, serves as a selectable marker in plant tissue during transformation which allows for tissue growth using mannose as the carbon source. The PMI protein present in DP915635 maize is found in several approved events that are currently in commercial use (ISAAA, 2022). APHIS has previously determined maize expressing PMI does not pose an increased plant pest risk and PMI

in maize is a plant-trait-MOA combination that has been determined by APHIS not to require regulation under 7 CFR part 340 (https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/regulatory-processes/confirmations/moa/moa-table).

Based on the information contained herein and publicly available information, and APHIS' knowledge and experience with the plant, trait, and MOA, Pioneer requests APHIS conduct an initial review to determine whether there is a plausible pathway by which DP915635 maize would pose an increased plant pest risk relative to conventional maize and, if none are identified, that DP915635 maize is not subject to the regulations in 7 CFR part 340.

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List of Abbreviations

A

APHIS ..... Animal and Plant Health Inspection Service, Animal and Plant Health Inspection Service

B

Bt ..... Bacillus thuringiensis

C

CRW ..... Corn Rootworm
Cry ..... Crystalline

D

DNA ..... Deoxyribonucleic Acid
DP915635 ..... Maize event DP-915635-4

E

ELISA ..... Enzyme-Linked Immunosorbent Assay

F

FOIA ..... Freedom of Information Act

G

GE ..... Genetically engineered

I

ipd079Ea ..... ipd079Ea gene
IPD079Ea ..... IPD079Ea protein

L

L-PPT ..... L-phosphinothricin

M

MACPF ..... Membrane attack complex, perforin like
MOA ..... Mode of action
mo-pat ..... Maize-optimized version of the phosphinothricin acetyltransferase gene

N

non-GE ..... Non-Genetically engineered

P

PAT ..... Phosphinothricin acetyltransferase protein
pmi ..... Phosphomannose isomerase gene

PMI..... Phosphomannose isomerase protein

R

RSR..... Regulatory Status Review

S

SA ..... Salicylic acid

U

USDA..... United States Department of Agriculture, United States Department of Agriculture

W

WCR ..... Western corn rootworm

I. Requestor Information

I.A. Requestor

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II. The Information on the Comparator Plant

The comparator plant for DP915635 maize is conventional maize, *Zea Mays* L.

Biology documents on the non-Genetically Engineered (non-GE) (also referred to as "conventional") plant species, maize, have been published by the Canadian Food Inspection Agency (CFIA) (CFIA, 1994) and by the Organization for Economic Co-operation and Development (OCED) (OECD, 2003).

III. Genotype of DP915635 Maize

III.A. Description of the Genetic Modification of DP915635 Maize

DP915635 maize was genetically modified to produce the IPD079Ea protein for control of susceptible corn rootworm (CRW) pests, the phosphinothricin acetyltransferase (PAT) protein for tolerance to the herbicidal active ingredient glufosinate-ammonium, and the phosphomannose isomerase (PMI) protein that was used as a selectable marker. Table 1 provides a detailed description of the inserted genetic material, including accession numbers (when available).

Table 1. Description of Inserted Genetic Material in DP915635 Maize

Event Sequence	Genetic Element	Size	Description
1 - 134	Intervening Sequence	134	Synthetic DNA sequence used for cloning
135 - 168	*lox*P	34	Bacteriophage P1 recombination site recognized by Cre recombinase (Dale and Ow, 1990)
169 - 180	Intervening Sequence	12	Synthetic DNA sequence used for cloning
181 - 1080	*ubi*ZM1 Promoter	900	Promoter region from the *Zea mays* (maize) ubiquitin gene 1 (Christensen *et al.*, 1992; GenBank accession S94464.1)
1081 - 1163	*ubi*ZM1 5' UTR	83	5' untranslated region from the *Zea mays* (maize) ubiquitin gene 1 (Christensen *et al.*, 1992; GenBank accession S94464.1)
1164 - 2176	*ubi*ZM1 Intron	1013	Intron region from the *Zea mays* (maize) ubiquitin gene 1 (Christensen *et al.*, 1992; GenBank accession S94464.1)
2177 - 2198	Intervening Sequence	22	Synthetic DNA sequence used for cloning
2199 - 2246	FRT1	48	Flippase recombination target site from *Saccharomyces cerevisiae* (Proteau et al., 1986; GenBank accession NC_001398.1)
2247 - 2264	Intervening Sequence	18	Synthetic DNA sequence used for cloning
2265 - 2268	*pmi* 5' UTR	4	5' untranslated region from the Phosphomannose isomerase gene from *Escherichia coli* (Negrotto *et al.*, 2000; GenBank accession ARE47603.1)
2269 - 3444	*pmi*	1176	Phosphomannose isomerase gene from *Escherichia coli* (Negrotto *et al.*, 2000); GenBank accession ARE47603.1
3445 - 3480	*pmi* 3' UTR	36	3' untranslated region from the Phosphomannose isomerase gene from *Escherichia coli* (Negrotto *et al.*, 2000; GenBank accession ARE47603.1)
3481 - 3490	Intervening Sequence	10	Synthetic DNA sequence used for cloning
3491 - 3801	*pin*II Terminator	311	Terminator region from the *Solanum tuberosum* (potato) proteinase inhibitor II gene (An *et al.*, 1989; Keil *et al.*, 1986; GenBank accession X04118.1)
3802 - 3811	Intervening Sequence	10	Synthetic DNA sequence used for cloning
3812 - 4553	Z19 Terminator	742	Terminator region from the *Zea mays* (maize) 19-kDa zein gene (GenBank accession KX247647; Dong et al., 2016)
4554 - 4756	Intervening Sequence	203	Synthetic DNA sequence used for cloning

Table 1. Description of Inserted Genetic Material in DP915635 Maize (continued)

Event Sequence	Genetic Element	Size	Description
4757 - 6438	*os*-actin Promoter	1682	Promoter region from the *Oryza sativa* (rice) actin gene (GenBank accession CP018159; GenBank accession EU155408.1)
6439 - 6907	*os*-actin Intron	469	Intron region from the *Oryza sativa* (rice) actin gene (GenBank accession CP018159; GenBank accession EU155408.1)
6908 - 6922	Intervening Sequence	15	Synthetic DNA sequence used for cloning
6923 - 7474	*mo-pat*	552	Maize-optimized phosphinothricin acetyltransferase gene from *Streptomyces viridochromogenes* (Wohlleben *et al.*, 1988; GenBank accession M22827.1)
7475 - 7492	Intervening Sequence	18	Synthetic DNA sequence used for cloning
7493 - 7686	CaMV 35S Terminator	194	35S terminator region from the cauliflower mosaic virus genome (Franck *et al.*, 1980; Guilley *et al.*, 1982; GenBank accession NC_001497.2)
7687 - 7707	Intervening Sequence	21	Synthetic DNA sequence used for cloning
7708 - 7741	*lox*P	34	Bacteriophage P1 recombination site recognized by Cre recombinase (Dale and Ow, 1990)
7742 - 7837	Intervening Sequence	96	Synthetic DNA sequence used for cloning
7838 - 8421	*sb-ubi* Terminator	584	Terminator region from the *Sorghum bicolor* (sorghum) ubiquitin gene (Phytozome gene ID Sobic.004G049900.1; US Patent 9725731 (Abbitt, 2017))
8422 - 8462	Intervening Sequence	41	Synthetic DNA sequence used for cloning
8463 - 8926	*sb-gkaf* Terminator	464	Terminator region from the *Sorghum bicolor* (sorghum) γ-kafarin gene (de Freitas et al., 1994; GenBank accession X62480.1)
8927 - 8959	Intervening Sequence	33	Synthetic DNA sequence used for cloning
8960 - 8983	*att*B1	24	Bacteriophage lambda integrase recombination site from the Invitrogen Gateway® cloning system (Hartley *et al.*, 2000; Katzen, 2007)
8984 - 9021	Intervening Sequence	38	Synthetic DNA sequence used for cloning
9022 - 10602	*sb*-RCc3 Enhancer	1581	Enhancer region, showing root-specific activity, from the *Sorghum bicolor* (sorghum) root cortical RCc3 (*sb*-RCc3) gene (WO Patent 2012/112411 (Diehn and Peterson-Burch, 2012); GenBank accession JB072168.1)
10603 - 10608	Intervening Sequence	6	Synthetic DNA sequence used for cloning

Table 1. Description of Inserted Genetic Material in DP915635 Maize (continued)

Event Sequence	Genetic Element	Size	Description
10609 - 12189	*sb*-RCc3 Enhancer	1581	Enhancer region, showing root-specific activity, from the *Sorghum bicolor* (sorghum) root cortical RCc3 (*sb*-RCc3) gene (WO Patent 2012/112411 (Diehn and Peterson-Burch, 2012); GenBank accession JB072168.1)
12190 - 12202	Intervening Sequence	13	Synthetic DNA sequence used for cloning
12203 - 13786	*sb*-RCc3 Enhancer	1584	Enhancer region, showing root-specific activity, from the *Sorghum bicolor* (sorghum) root cortical RCc3 (*sb*-RCc3) gene (WO Patent 2012/112411 (Diehn and Peterson-Burch, 2012)); GenBank accession JB072168.1)
13787 - 14697	*zm*-PCOa Promoter	911	Promoter region upstream of a *Zea mays* (maize) PCO118362 mRNA sequence identified as having root-specific activity (Seq ID No: 4 found in WO Patent 2017/222821(Crow et al., 2017))
14698 - 14715	Intervening Sequence	18	Synthetic DNA sequence used for cloning
14716 - 15571	*zm*-HPLV9 Intron	856	Intron region from the *Zea mays* (maize) ortholog of an *Oryza sativa* (rice) hypothetical protein (*zm*-HPLV9) gene, a predicted *Zea mays* (maize) calmodulin 5 gene (Phytozome gene ID Zm00008a029682, WO Patent 2016109157 (Abbitt and Shen, 2016)); Genbank accession LQ422921.1)
15572 - 15580	Intervening Sequence	9	Synthetic DNA sequence used for cloning
15581 - 17020	*ipd079Ea*	1440	Insecticidal protein gene from *Ophioglossum pendulum* (fern) (Seq ID No: 55 found in WO Patent 2017/023486 (Allen et al., 2017))
17021 - 17037	Intervening Sequence	17	Synthetic DNA sequence used for cloning
17038 - 17990	*sb-SCI-1B* Terminator	953	Terminator region of the *Sorghum bicolor* (sorghum) subtilisin-chymotrypsin inhibitor *1B* gene (WO Patent 2018/102131 (Abbitt et al., 2018); GenBank accession LQ750838.1)
17991 - 18036	Intervening Sequence	46	Synthetic DNA sequence used for cloning
18037 - 18496	Z27G Terminator	460	Terminator region from the *Zea mays* (maize) W64 line 27-kDa gamma zein gene (Das et al., 1991; Liu et al., 2016; GenBank accession GX289463.1)
18497 - 18502	Intervening Sequence	6	Synthetic DNA sequence used for cloning
18503 - 19404	*UBQ14* Terminator	902	Terminator region from the *Arabidopsis thaliana* (Arabidopsis) ubiquitin 14 gene (Callis et al., 1995; GenBank accession NM_001125450.2)
19405 - 19410	Intervening Sequence	6	Synthetic DNA sequence used for cloning
19411 - 19904	*In2-1* Terminator	494	Terminator region from the *Zea mays* (maize) *In2-1* gene (Hershey and Stoner, 1991; GenBank accession AR014639)

Table 1. Description of Inserted Genetic Material in DP915635 Maize (continued)

Event Sequence	Genetic Element	Size	Description
19905 - 19961	Intervening Sequence	57	Synthetic DNA sequence used for cloning
19962 - 19985	*att*B2	24	Bacteriophage lambda integrase recombination site from the Invitrogen Gateway® cloning system (Hartley *et al.*, 2000; Katzen, 2007)
19986 - 20132	Intervening Sequence	147	Synthetic DNA sequence used for cloning
20133 - 20153	*att*B3	21	Bacteriophage lambda integrase recombination site (Cheo *et al.*, 2004)
20154 - 20388	Intervening Sequence	235	Synthetic DNA sequence used for cloning
20389 - 20436	FRT6	48	Modified flippase recombination target site from *Saccharomyces cerevisiae* (Proteau et al., 1986; GenBank accession NC_001398.1)
20437 - 20564	Intervening Sequence	128	Synthetic DNA sequence used for cloning

III.B. Sequence of the Insert

Sequence characterization analysis was performed to determine the DNA sequence of the DP915635 insert. It should be noted that while DNA sequencing provides certain molecular information, the exact nucleotide sequence should not be viewed as static. Spontaneous mutations are a very common phenomenon in plants, presenting a biological mechanism of adaptation to constantly changing environment (Weber et al., 2012). Spontaneous mutations can occur in any part of the plant genome and in both non-GE and GE plants (Waigmann et al., 2013). In GE plants, there is no scientific basis to expect that the frequency of spontaneous mutations in transgenic insert or flanking genomic regions would be greater than in the rest of the plant genome, or that they would have a differential impact on safety (La Paz et al., 2010; Waigmann et al., 2013).

Sanger sequence is provided in section VII. APPENDIX-Sequence of the Inserted Genetic Material

IV. Description of the Traits Expressed in DP915635 Maize

IV.A. Purpose and Intended Phenotype of DP915635 Maize

Maize has multiple downstream uses for feed, fuel, and food that are significant for the global supply of this crop commodity. The introduction of insect-resistant and herbicide-tolerant DP915635 maize is intended to help growers keep pace with increasing maize demand globally by mitigating the effects of certain plant pests (*Diabrotica spp.*) and enabling effective management of agriculturally important weeds. The United States is one of the world's largest maize producers and a leading exporter of maize, with approximately 88.6 million acres of maize planted in 2022 (USDA-NASS, 2022). One of the most serious pests of maize in the United States is western corn rootworm (WCR; *Diabrotica virgifera virgifera),* with economic losses of greater than $1 billion annually from both management costs and yield loss (Metcalf, 1986; PHI, 2010; Shrestha et al., 2018).

WCR damage has historically been managed with crop rotation, broad-spectrum soil insecticides, and transgenic crops expressing crystalline (Cry) proteins, such as Cry3 and Cry34/35 classes of protein, developed from *Bacillus thuringiensis* (*Bt*). Certain *Bt* maize events expressing these classes of Cry proteins have repeatedly demonstrated efficacy against susceptible corn rootworm pests, while limiting impacts to populations of non-target organisms, including those beneficial to agriculture and provide plant-pest mitigation (predators and parasitoids of plant pests) (Devos et al., 2012; Svobodová et al., 2015; Svobodová et al., 2017). As adoption of *Bt* maize has increased, the selection pressure on target insects to develop resistance has become greater (Cullen et al., 2013). Insect resistance to transgenic traits can pose a threat to the long-term durability of *Bt* crops. As reduced performance of Cry3 and Cry34/35 proteins in maize has been reported in the scientific literature (Gassmann et al., 2016; Jakka et al., 2016), new mechanisms of action (MOA) are important for maintaining sustainable and durable CRW management (Gassmann et al., 2016; Niu et al., 2017).

IV.A.1. Insect Resistance

DP915635 maize produces the IPD079Ea protein that has demonstrated to be efficacious against certain susceptible CRW pests, including WCR (Boeckman et al., 2022). DP915635 maize is anticipated to diversify the currently available *Bt* protein-based MOAs for CRW control through the use of a new protein MOA. DP915635 maize is intended to help mitigate against CRW plant pest risk by providing farmers with an additional option and an alternative to soil-applied or foliar-applied insecticides for controlling susceptible CRW pests to protect maize grain yield.

IV.A.2. Herbicide Tolerance

GE herbicide-resistant maize lines are widely cultivated because they provide flexible weed management options for growers. Herbicide-resistant maize has a significant impact on growers' earnings and sustainable agricultural practices as they enable specific herbicides to be used on an as-needed basis and can help growers to adopt reduced or no tillage practices which can reduce soil erosion, improve soil health, and reduce carbon footprint, among other benefits (Fawcett and Towery, 2003; Fernandez-Cornejo et al., 2012).

The PAT protein confers tolerance to the herbicidal active ingredient glufosinate-ammonium (CERA - ILSI Research Foundation, 2016). DP915635 maize provides farmers with an additional control option for herbicide management practices.

IV.A.3. Selectable Marker

The PMI protein was incorporated into DP915635 maize to enable selection of plants containing the desired constructs during the event development process (Negrotto et al., 2000). The PMI present in DP915635 maize is found in several approved events that are currently in commercial use (ISAAA, 2022).

IV.B. Characterization of the IPD079Ea Protein Expressed in DP915635 Maize

IV.B.1. MACPF Protein Background

The IPD079Ea protein, encoded by the *ipd079Ea* gene from *Ophioglossum pendulum*, has a sequence motif that place it among a family of pore forming proteins known as membrane attack complex, perforin like (MACPF).

Certain MACPF proteins (like the IPD079Ea protein) are known for multistep transmembrane pore formation. This begins with recognition and binding to a membrane receptor, using the C terminal domain as an anchor and specific binding recognition site. The bound protein then assembles into a ring-shaped complex (referred to as oligomerization), followed by pore formation via protein conformational changes (in α-helices and β-strands of the MACPF domain) to begin membrane insertion. Toxicity is then conferred by either the transport of toxins across the membrane, or by flux leading to osmotic imbalance causing cell lysis (Reboul *et al.*, 2016, Rosado *et al.*, 2008; Tilley and Saibil, 2006)).

MACPF proteins are found in bacterial species, proteins of the immune system, and have been identified in plant species. MACPF proteins have been investigated in *Arabidopsis thaliana* immune and salicylic acid (SA) mediated defense signaling pathways (Morita-Yamamuro et al., 2005; Yu et al., 2020), and more recently in six species of the Poaceae clade and one species of green algae (Yu *et al.*, 2020).

Poaceae family (including *Zea mays*) MACPF gene expression has been shown to be induced as a defense response to environmental (biotic and abiotic) stresses and that gene expression is preferential to vegetative tissues (Yu *et al.*, 2020). Stress response of MACPF proteins in the Poaceae family has shown that promoters upstream of *MACPF* genes in maize are activated in response to external stimuli (biotic and abiotic stressors) (Yu *et al*., 2020), but the function of MACPF proteins and their role in development and response to environmental stress in maize is largely unknown.

IV.B.2. Mechanism of Action of the IPD079Ea protein

Similar to the mode of action of 3-domain Cry toxins from *Bacillus thuringiensis*, the IPD079Ea protein is a pore-forming protein which localizes in the target insect midgut. Once ingested by target insects, the IPD079Ea protein binds to a specific midgut receptor and forms a pore structure. Mortality in target insects is then accomplished by the pore's disruption of midgut membranes via osmotic imbalance and cell lysis. The crystal structure of the IPD079Ea protein has been determined and shows an N-terminal MACPF domain (used for pore formation), and a C terminal β-tripod domain which is likely used for receptor recognition. Similar β-tripod domains have been identified in other MACPF proteins and the C-terminal domain of that protein demonstrated specificity in receptor recognition in insect cells, conferring activity against WCR (Zaitseva *et al.*, 2019).

Insect midgut binding of the IPD079Ea protein requires the presence of an appropriate receptor to mediate the protein's toxicity. Although the specific receptor is presently unknown, Pioneer laboratory assays show that the IPD079Ea protein exhibits preferred binding in the midgut membranes of WCR, does not bind to European corn borer midgut membranes, and does not utilize receptors previously identified for Cry proteins.

Unlike MACPF proteins which are driven by promoters that initiate expression in response to external stimuli, the *ipd079Ea* gene in DP915635 maize is driven by a maize root specific promoter (*zm-pcoa*; Table 1) (WO Patent 2017/222821; Crow et al., 2017) and was designed to be expressed in DP915635 maize roots at vegetative stages when CRW pests are active. The expression data presented in Table 2 below demonstrate that the IPD079Ea protein is preferentially expressed in DP915635 maize roots during vegetative stages of plant development.

The IPD079Ea protein is produced by DP915635 maize solely to confer protection against certain coleopteran insects. As the IPD079Ea protein is receptor-mediated, specific to WCR, and designed to express when the target pest is active, Pioneer does not expect the IPD079Ea protein to affect DP915635 plant response to biotic or abiotic stressors, or developmental differences when compared to a non-GE, near isoline control maize.

IV.B.3. IPD079Ea Protein Trait Expression Assessment in DP915635 Maize

The expression levels of the IPD079Ea protein were evaluated in DP915635 maize.

Tissue samples were collected during the 2019 growing season at six sites in commercial maize-growing regions of the United States and Canada. A randomized complete block design with four blocks was utilized at each site. The following tissue samples were collected: root (V6, V9, and R4 growth stages), leaf (V9, R1, and R4 growth stages), pollen (R1 growth stage), forage (R4 growth stage), and grain (R6 growth stage). The concentrations of the IPD079Ea protein were determined using a quantitative enzyme-linked immunosorbent assay (ELISA).

The maximum concentration of IPD079Ea found in several growth stages and tissues are summarized across sites in Table 2.

Table 2. Across-Sites Summary of Maximum IPD079Ea Protein Concentrations in DP915635 Maize

Tissue (Growth Stage)	Maximum ng IPD079Ea/mg Tissue Dry Weight
Root (V6)	26
Root (V9)	30
Root (R4)	2.7
Leaf (V9)	1.6
Leaf (R1)	0.29
Leaf (R4)	<0.14
Pollen (R1)	1.3
Forage (R4)	0.46
Grain (R6)	0.36

Note: Growth stages (Abendroth et al., 2011).

IV.C. Characterization of the PAT Protein Expressed in DP915635 Maize

IV.C.1. Mechanism of Action of the PAT Protein

The mode of action of the PAT protein has been previously characterized and described (CERA - ILSI Research Foundation, 2011; Hérouet et al., 2005). The United States EPA has granted an exemption from the requirement of a tolerance for the PAT protein as an inert ingredient in plants (US-EPA, 1996). The PAT protein confers tolerance to the herbicidal active ingredient glufosinate-ammonium, the active ingredient in phosphinothricin herbicides. Glufosinate chemically resembles the amino acid glutamate and acts to inhibit an enzyme called glutamine synthetase, which is involved in the synthesis of glutamine. Glutamine synthetase is also involved in ammonia detoxification. Due to its similarity to glutamate, glufosinate blocks the activity of glutamine synthetase, resulting in reduced glutamine levels and a corresponding increase in concentrations of ammonia in plant tissues, leading to cell membrane disruption and cessation of photosynthesis resulting in plant death. The PAT protein confers tolerance to glufosinate-ammonium herbicides by acetylating phosphinothricin, an isomer of glufosinate-ammonium, thus detoxifying the herbicide (CERA - ILSI Research Foundation, 2011; Hérouet et al., 2005).

IV.D. Characterization of the PMI Protein Expressed in DP915635 Maize

IV.D.1. Mechanism of Action of the PMI Protein

The mode of action of PMI has been previously characterized and described (Negrotto et al., 2000; Privalle, 2002; Reed et al., 2001; Weisser et al., 1996). PMI is widely present in nature and is expressed in fungi, insects, plants, and mammals (Slein, 1950; US-EPA, 2004). The United States EPA has granted an exemption from the requirement of a tolerance for the PMI protein as an inert ingredient in plants (US-EPA, 2004). The PMI protein catalyzes the reversible interconversion between mannose-6-phosphate and fructose-6-phosphate. Mannose is phosphorylated by hexokinase to mannose-6-phosphate and in the presence of PMI enters the glycolytic pathway after isomerization to fructose 6-phosphate. In the absence of PMI, mannose-6-phosphate accumulates in the plant cells and inhibits glycolysis; additionally, high levels of mannose can lead to other impacts on photosynthesis and adenosine triphosphate (ATP) production (Negrotto et al., 2000; Privalle, 2002). However, in the presence of PMI, plant cells may survive on media containing mannose as a carbon source, thus allowing PMI to be utilized as a selectable marker (Negrotto et al., 2000; Reed et al., 2001).

IV.E. Expected Changes in the Metabolism, Physiology, or Development of DP915635 Maize

No changes in metabolism, physiology or development are expected due to the traits/genetic modifications in DP915635 maize relative to its comparator plant, non-GM maize.

V. Additional Information

The IPD079Ea protein is not intended nor expected to interact with any biochemical pathways or change the metabolism or physiology of maize. The IPD079Ea protein does not have a mechanism of action on the plant itself. It is expressed in DP915635 maize solely to confer protection against certain susceptible coleopteran pests.

The mode of action of the IPD079Ea insecticidal protein is through specific binding to WCR midgut binding sites. Therefore, non-target organisms, including plant-pest parasitoids and predators,

are unlikely to be adversely affected by the IPD079Ea protein in DP915635 maize (see Table 2 in O'Neill et al., 2024).

Overall, studies to date have shown that the effects of IR (insect resistant) crops on non-target populations are minimal to negligible in comparison to the effects of broad-spectrum chemical insecticides (NAS, 2016; Romeis et al., 2019). Naranjo (2009) found that the use of Bt crops has the potential to enhance, rather than reduce, the role of biological control in integrated pest management systems. A recent more extensive review article examining evidence from 25 years of research into GM crops confirmed that the new analysis is "largely consistent with previous analysis" (Meissle et al. 2022). Although the trait/MOA analyzed in these reviews are mostly Bt proteins, it is unlikely that the effects of IDP079Ea protein in DP915635 maize on plant pest populations would be different than those of Bt and other GM crops. The conclusions of these publications are applicable to DP915635 maize, as the IPD079Ea protein has a similar narrow spectrum of activity and the same routes of exposure (expressed *in planta*) and ecological interactions (in maize).

Herbicide resistance to glufosinate by catalyzing the conversion of L-phosphinothricin (L-PPT) to a non-phytotoxic form (N-acetylphosphinothricin) (PAT protein) and the PMI marker which catalyzes the reversible conversion of mannose 6-phosphate and fructose 6-phosphate have previously been evaluated and deregulated in maize by USDA, are found in multiple commercial maize products grown in the environment (including in combination with other modifications that confer protection against certain coleopteran insects), and have not increased plant pest risk when compared to non-GE maize. Both PAT and PMI in maize are plant-trait-MOA combinations that have been determined by APHIS not to require regulation under 7 CFR part 340 (https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/regulatory-processes/confirmations/moa/moa-table).

Based on the information contained herein and publicly available information, and APHIS' knowledge and experience with the plant, trait, and MOA, Pioneer requests APHIS conduct an initial review to determine whether there is a plausible pathway by which DP915635 maize would pose an increased plant pest risk relative to conventional maize and, if none are identified, that DP915635 maize is not subject to the regulations in 7 CFR part 340.

VI. References

Abbitt SE, inventor, Aug. 8, 2017. SB-UBI terminator sequence for gene expression in plants. United States Patent, Patent No. US 9,725,731 B2

Abbitt SE, Klein K, Selinger D, inventors. 07 June 2018. Transcriptional Terminators for Gene Expression in Plants. World Intellectual Property Organization, Patent No. WO 2018/102131 A1

Abbitt SE, Shen B, inventors. 7 July 2016. Maize and sorghum s-adenosyl-homocysteine hydrolase promoters. World Intellectual Property Organization, Patent No. WO 2016/109157 A1

Abendroth LJ, Elmore RW, Boyer MJ, Marlay SK (2011) Corn Growth and Development. Iowa State University Extension, PMR 1009

Allen SM, Barry J, Crane V, English J, Fengler K, Schepers E, Udranszky I, inventors. 9 February 2017. Plant Derived Insecticidial Proteins and Methods for Their Use. World Intellectual Property Organization, Patent No. WO 2017/023486 A1

An G, Mitra A, Choi HK, Costa MA, An K, Thornburg RW, Ryan CA (1989) Functional Analysis of the 3' Control Region of the Potato Wound-Inducible Proteinase Inhibitor II Gene. The Plant Cell 1: 115-122

Boeckman CJ, Ballou S, Gunderson T, Huang E, Linderblood C, Olson T, Stolte B, LeRoy K, Walker C, Wang Y, Woods R, Zhang J. Characterization of the Spectrum of Activity of IPD079Ea: A Protein Derived From *Ophioglossum pendulum* (Ophioglossales: Ophioglossaceae) With Activity Against Western Corn Rootworm *[Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae)]. J Econ Entomol. 2022 Oct 12;115(5):1531-1538. doi: 10.1093/jee/toac079. PMID: 35640234; PMCID: PMC9554786.

Callis J, Carpenter T, Sun CW, Vierstra RD (1995) Structure and Evolution of Genes Encoding Polyubiquitin and Ubiquitin-Like Proteins in *Arabidopsis thaliana* Ecotype Columbia. Genetics 139: 921-939

CERA - ILSI Research Foundation (2011) A Review of the Environmental Safety of the PAT Protein. International Life Sciences Institute, Center for Environmental Risk Assessment

CERA - ILSI Research Foundation (2016) A Review of the Food and Feed Safety of the PAT Protein. International Life Sciences Institute, Center for Environmental Risk Assessment

CFIA (1994) The Biology of *Zea mays* (L.) (Maize). Canadian Food Inspection Agency, BIO1994-11

Cheo DL, Titus SA, Byrd DRN, Hartley JL, Temple GF, Brasch MA (2004) Concerted Assembly and Cloning of Multiple DNA Segments Using In Vitro Site-Specific Recombination: Functional Analysis of Multi-Segment Expression Clones. Genome Research 14: 2111-2120

Christensen AH, Sharrock RA, Quail PH (1992) Maize polyubiquitin genes: structure, thermal perturbation of expression and transcript splicing, and promoter activity following

transfer to protoplasts by electroporation. Plant Molecular Biology 18: 675-689

Crow A, Diehn S, Sims L, inventors. 28 December 2017. Plant regulatory elements and methods of use thereof. World Intellectual Property Organization, Patent No. WO 2017/222821 A2

Cullen EM, Gray ME, Gassmann AJ, Hibbard BE (2013) Resistance to Bt Corn by Western Corn Rootworm (Coleoptera: Chrysomelidae) in the U.S. Corn Belt. Journal of Integrated Pest Management 4: D1-D6

Dale EC, Ow DW (1990) Intra- and intramolecular site-specific recombination in plant cells mediated by bacteriophage P1 recombinase. Gene 91: 79-85

Das OP, Ward K, Ray S, Messing J (1991) Sequence Variation between Alleles Reveals Two Types of Copy Correction at the 27-kDa Zein Locus of Maize. Genomics 11: 849-856

de Freitas FA, Yunes JA, da Silva MJ, Arruda P, Leite A (1994) Structural characterization and promoter activity analysis of the γ-kafirin gene from sorghum. Molecular and General Genetics 245: 177-186

Devos Y, De Schrijver A, De Clercq P, Kiss J, Romeis J. Bt-maize event MON 88017 expressing Cry3Bb1 does not cause harm to non-target organisms. Transgenic Res. 2012 Dec;21(6):1191-214. doi: 10.1007/s11248-012-9617-z. Epub 2012 May 11. PMID: 22576225.

Diehn S, Peterson-Burch B, inventors. 23 August 2012. Root-Preferred Promoter and Methods of Use. World Intellectual Property Organization, Patent No. WO 2012/112411 A1

Dong J, Feng Y, Kumar D, Zhang W, Zhu T, Luo M-C, Messing J (2016) Analysis of tandem gene copies in maize chromosomal regions reconstructed from long sequence reads. Proceedings of the National Academy of Sciences 113: 7949-7956

Fawcett R, Towery D (2003) Conservation Tillage and Plant Biotechnology: How New Technologies Can Improve the Environment By Reducing the Need to Plow. Conservation Technology Information Center, http://www.ctic.org/media/pdf/Biotech2003.pdf

Fernandez-Cornejo J, Hallahan C, Nehring R, Wechsler S (2012) Conservation Tillage, Herbicide Use, and Genetically Engineered Crops in the United States: The Case of Soybeans. AgBioForum 15: 231-241

Franck A, Guilley H, Jonard G, Richards K, Hirth L (1980) Nucleotide sequence of cauliflower mosaic virus DNA. Cell 21: 285-294

Gassmann AJ, Shrestha RB, Jakka SR, Dunbar MW, Clifton EH, Paolino AR, Ingber DA, French BW, Masloski KE, Dounda JW, St Clair CR (2016) Evidence of Resistance to Cry34/35Ab1 Corn by Western Corn Rootworm (Coleoptera: Chrysomelidae): Root Injury in the Field and Larval Survival in Plant-Based Bioassays. Journal of Economic Entomology 109: 1872-1880

Guilley H, Dudley RK, Jonard G, Balàzs E, Richards KE (1982) Transcription of cauliflower mosaic virus DNA: detection of promoter sequences, and characterization of transcripts. Cell 30: 763-773

Hartley JL, Temple GF, Brasch MA (2000) DNA Cloning Using In Vitro Site-Specific Recombination. Genome Research 10: 1788-1795

Hérouet C, Esdaile DJ, Mallyon BA, Debruyne E, Schulz A, Currier T, Hendrickx K, van der Klis R-J, 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. Regulatory Toxicology and Pharmacology 41: 134-149

Hershey HP, Stoner TD (1991) Isolation and characterization of cDNA clones for RNA species induced by substituted benzenesulfonamides in corn. Plant Molecular Biology 17: 679-690

ISAAA (2022) Gene: pmi (events list). International Service for the Acquisition of Agribiotech Applications, https://www.isaaa.org/gmapprovaldatabase/gene/default.asp?GeneID=37&Gene=pmi

Jakka SRK, Shrestha RB, Gassmann AJ (2016) Broad-spectrum resistance to *Bacillus thuringiensis* toxins by western corn rootworm (*Diabrotica virgifera virgifera*). Scientific Reports 6: 27860

Katzen F (2007) Gateway® recombinational cloning: a biological operating system. Expert Opinion on Drug Discovery 2: 571-589

Keil M, Sanchez-Serrano J, Schell J, Willmitzer L (1986) Primary structure of a proteinase inhibitor II gene from potato (*Solanum tuberosum*). Nucleic Acids Research 14: 5641-5650

La Paz JL, Pla M, Papazova N, Puigdomenech P, Vicient CM (2010) Stability of the MON 810 transgene in maize. Plant Molecular Biology 74: 563-571

Liu H, Shi J, Sun C, Gong H, Fan X, Qiu F, Huang X, Feng Q, Zheng X, Yuan N, Li C, Zhang Z, Deng Y, Wang J, Pan G, Han B, Lai J, Wu Y (2016) Gene duplication confers enhanced expression of 27-kDa γ-zein for endosperm modification in quality protein maize. Proceedings of the National Academy of Sciences 113: 4964-4969

Meissle, M., Naranjo, S.E. & Romeis, J. Does the growing of Bt maize change abundance or ecological function of non-target animals compared to the growing of non-GM maize? A systematic review. *Environ Evid* **11**, 21 (2022). https://doi.org/10.1186/s13750-022-00272-0

Metcalf RL (1986) Foreward. In JL Krysan, TA Miller, eds, Methods for the Study of Pest *Diabrotica*. Springer-Verlag, New York, pp vii-xv

Morita-Yamamuro C, Tsutsui T, Sato M, Yoshioka H, Tamaoki M, Ogawa D, Matsuura H, Yoshihara T, Ikeda A, Uyeda I, Yamaguchi J (2005) The *Arabidopsis Gene CAD1* Controls Programmed

Cell Death in the Plant Immune System and Encodes a Protein Containing a MACPF Domain. Plant and Cell Physiology 46: 902-912

Naranjo SE (2009) Impacts of *Bt* crops on non-target invertebrates and insecticide use patterns. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 4: 1-23

NAS. 2016. *Genetically Engineered Crops: Experiences and Prospects*. National Academies of Sciences, Engineering, and Medicine: National Academies of Sciences, Engineering, and Medicine. Retrieved from http://www.nap.edu/catalog/23395/genetically-engineered-crops-experiences-and-prospects

Negrotto D, Jolley M, Beer S, Wenck AR, Hansen G (2000) The use of phosphomannose-isomerase as a selectable marker to recover transgenic maize plants (*Zea mays* L.) via *Agrobacterium* transformation. Plant Cell Reports 19: 798-803

Niu X, Kassa A, Hu X, Robeson J, McMahon M, Richtman NM, Steimel JP, Kernodle BM, Crane VC, Sandahl G, Ritland JL, Presnail JK, Lu AL, Wu G (2017) Control of Western Corn Rootworm (*Diabrotica virgifera virgifera*) Reproduction through Plant-Mediated RNA Interference. Scientific Reports 7: 12591

OECD (2003) Consensus Document on the Biology of *Zea mays* subsp. *mays* (Maize). Organisation for Economic Co-operation and Development, ENV/JM/MONO(2003)11

O'Neill BF, Boeckman C, LeRoy K, Linderblood C, Olson T, Woods R, Challender M (2024) An environmental risk assessment of IPD079Ea: a protein derived from *Ophioglossum pendulum* with activity against *Diabrotica* spp.In maize. GM Crops & Food 15: 15-31

PHI (2010) CropFocus: Corn Rootworm. Pioneer Hi-Bred International, Inc, https://www.pioneer.com/CMRoot/Pioneer/US/Non_Searchable/agronomy/cropfocus_pdf/corn_rootworm.pdf

Privalle LS (2002) Phosphomannose Isomerase, a Novel Plant Selection System. Annals of the New York Academy of Sciences 964: 129-138

Proteau G, Sidenberg D, Sadowski P (1986) The minimal duplex DNA sequence required for site-specific recombination promoted by the FLP protein of yeast *in vitro*. Nucleic Acids Research 14: 4787-4802

Reboul CF, Whisstock JC, Dunstone MA (2016) Giant MACPF/CDC pore forming toxins: A class of their own. Biochimica et Biophysica Acta 1858: 475-486

Reed J, Privalle L, Powell ML, Meghji M, Dawson J, Dunder E, Sutthe J, Wenck A, Launis K, Kramer C, Chang Y-F, Hansen G, Wright M (2001) Phosphomannose isomerase: An efficient selectable marker for plant transformation. In Vitro Cellular & Developmental Biology - Plant 37: 127-132

Romeis J, Naranjo SE, Meissle M, and Shelton AM. 2019. *Genetically engineered crops help support conservation biological control*. Biological Control,Vol. 130, pp. 136-154. Retrieved from http://www.sciencedirect.com/science/article/pii/S1049964418305103

Rosado CJ, Kondos S, Bull TE, Kuiper MJ, Law RHP, Buckle AM, Voskoboinik I, Bird PI, Trapani JA, Whisstock JC, Dunstone MA (2008) The MACPF/CDC family of pore-forming toxins. Cellular Microbiology 10: 1765-1774

Shrestha RB, Dunbar MW, French BW, Gassmann AJ (2018) Effects of field history on resistance to Bt maize by western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae). PLoS ONE 13: e0200156

Slein MW (1950) Phosphomannose Isomerase. Journal of Biological Chemistry 186: 753-761

Svobodová Z, Skoková Habuštová O, Hutchison WD, Hussein HM, Sehnal F. Risk Assessment of Genetically Engineered Maize Resistant to Diabrotica spp.: Influence on Above-Ground Arthropods in the Czech Republic. PLoS One. 2015 Jun 17;10(6):e0130656. doi: 10.1371/journal.pone.0130656. PMID: 26083254; PMCID: PMC4470634.

Svobodová Z, Shu Y, Skoková Habuštová O, Romeis J, Meissle M. Stacked Bt maize and arthropod predators: exposure to insecticidal Cry proteins and potential hazards. Proc Biol Sci. 2017 Jul 26;284(1859):20170440. doi: 10.1098/rspb.2017.0440. PMID: 28724730; PMCID: PMC5543214.

Tilley SJ, Saibil HR (2006) The mechanism of pore formation by bacterial toxins. Current Opinion in Structural Biology 16: 230-236

US-EPA (1996) Phosphinothricin Acetyltransferase (PAT) and the Genetic Material Necessary for Its Production (Plasmid Vector pZ01502) in Corn; Exemption from Requirement of a Tolerance. Federal Register 61: 40336-40338

US-EPA (2004) Phosphomannose Isomerase and the Genetic Material Necessary for Its Production in All Plants; Exemption from the Requirement of a Tolerance. Federal Register 69: 26770-26775

USDA-NASS (2022) Quick Stats: Corn Acres Planted. United States Department of Agriculture, National Agricultural Statistics Service, https://quickstats.nass.usda.gov/results/6100380F-8E61-3C32-9925-458A64591D46

Waigmann E, Gomes A, Lanzoni A, Perry JN (2013) Editorial: New Commission Implementing Regulation on Risk Assessment of GM plant applications: novel elements and challenges. The EFSA Journal 11: e11121

Weber N, Halpin C, Hannah LC, Jez JM, Kough J, Parrott W (2012) Editor's Choice: Crop Genome Plasticity and Its Relevance to Food and Feed Safety of Genetically Engineered Breeding Stacks. Plant Physiology 160: 1842-1853

Weisser P, Krämer R, Sprenger GA (1996) Expression of the *Escherichia coli pmi* gene, encoding

phosphomannose-isomerase in *Zymomonas mobilis*, leads to utilization of mannose as a novel growth substrate, which can be used as a selective marker. Applied and Environmental Microbiology 62: 4155-4161

Wohlleben W, Arnold W, Broer I, Hillemann D, Strauch E, Punier A (1988) Nucleotide sequence of the phosphinothricin *N*-acetyltransferase gene from *Streptomyces viridochromogenes* Tü494 and its expression in *Nicotiana tabacum*. Gene 70: 25-37

Yu L, Liu D, Chen S, Dai Y, Guo W, Zhang X, Wang L, Ma S, Xiao M, Qi H, Xiao S, Chen Q (2020) Evolution and Expression of the Membrane Attack Complex and Perforin Gene Family in the Poaceae. International Journal of Molecular Sciences 21: 5736

Zaitseva J, Vaknin D, Krebs C, Doroghazi J, Milam SL, Balasubramanian D, Duck NB, Freigang J (2019) Structure–function characterization of an insecticidal protein GNIP1Aa, a member of an MACPF and β-tripod families. Proceedings of the National Academy of Sciences 116: 2897-2906

VII. APPENDIX – Sequence of the Inserted Genetic Material

GGTACCTCACTGACTAGCTAATCGAGCTAGTTACCCTATGAGGTGACATGAAGCGCTCACGGTTACTATGACGGTT
AGCTTCACGACTGTTGGTGGCAGTAGCGTACGACTTAGCTATAGTTCCGGACTTACCGATAACTTCGTATAGCATA
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