Proposed Registration Decision for the New Active Ingredients:

DvSSJ1 dsRNA complementary to the DvSSJ1 gene sequence from *Diabrotica virgifera virgifera* and the genetic material (PHP74643 T-DNA) necessary for its production in DP23211 Maize (OECD Unique Identifier DP-Ø23211-2)

***Pseudomonas chlororaphis* IPD072Aa protein and the genetic material (PHP74643 T-DNA) necessary for its production in DP23211 Maize (OECD Unique Identifier DP-Ø23211-2)**

Approved by:

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1. Summary

This document announces that the U.S. Environmental Protection Agency (EPA) completed its initial evaluation of the proposed Plant-Incorporated Protectant active ingredients *Pseudomonas chlororaphis* IPD072Aa protein and the genetic material (PHP74643 T-DNA) necessary for its production in DP23211 Maize (OECD Unique Identifier DP-Ø23211-2) and DvSSJ1 dsRNA complementary to the DvSSJ1 gene sequence from *Diabrotica virgifera virgifera* and the genetic material (PHP74643 T-DNA) necessary for its production in DP23211 Maize (OECD Unique Identifier DP-Ø23211-2) and concluded that they meet the regulatory and safety standards under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the Federal Food, Drug, and Cosmetic Act (FFDCA), and the Endangered Species Act (ESA). EPA is seeking public comment on its proposed registration decision.

DP23211 maize expresses the new plant-incorporated protectant (PIP) active ingredients IPD072Aa protein and DvSSJ1 double-stranded RNA (dsRNA). The gene for the insecticidal protein IPD072Aa was derived from *Pseudomonas chlororaphis* and is intended to provide protection from certain coleopteran pests through disruption of the midgut epithelium. The DvSSJ1 dsRNA transcript targets *Diabrotica virgifera virgifera* (Western Corn Rootworm, WCR) and functions by weakening smooth septate junctions in the insect gut via RNA interference (RNAi). DP23211 maize also contains the inert ingredients phosphinothricin acetyltransferase protein and phosphomannose isomerase protein.

Available data demonstrated that, with regard to humans, IPD072Aa protein and DvSSJ1 dsRNA are not toxic or allergenic via any route of exposure. The Agency used a "weight of evidence" approach and determined that IPD072Aa protein represents a negligible risk to humans or livestock that consume DP23211 maize. The most likely exposure to the IPD072Aa protein is dietary through consumption of food products made from corn containing the protein. Although there may be dietary exposure to residues of IPD072Aa protein, such exposure presents no concern for adverse effects. Oral exposure from ingestion of drinking water is unlikely because the IPD072Aa protein is present at very low levels within the plant cells and the amounts likely to enter the water column from leaves, pollen or plant detritus are low. Additionally, proteases and nucleases found in water and the environment would likely degrade the biological material containing the active ingredients and treatment process for municipal water plants are likely to remove IPD072Aa residues. Although there may be dietary exposure to residues of IPD07Aa protein, such exposure presents no concern for adverse effects. Submitted data show that the IPD072Aa protein is not toxic via the oral route of exposure and bioinformatics analysis did not indicate a toxigenic potential in silico.

Likewise, the potential for allergenicity is low because: (1) The bacterium source of IPD07Aa protein, *Pseudomonas chlororaphis*, is not considered to be a source of allergenic proteins; (2) bioinformatic analysis indicates no similarity between IPD072Aa protein and known allergens; (3) IPD072Aa protein degrades rapidly when exposed to simulated gastric fluid and completely digested in simulated intestinal fluid or exposed to heat via food cooking; and (4) IPD072Aa protein is not glycosylated, which further reduces its allergenicity potential. Glycosylation is an enzymatic post-translational process in which carbohydrates (glycans) link to proteins, creating structures which could lead to an immune response in humans. Inhalation exposure to IPD072Aa protein is not likely due to corn pollen being too large to be respirable. Inhalation from sources other than pollen is unlikely as IPD072Aa proteins are contained within plant cells.

A "weight of evidence" approach also determined that, DvSSJ1 dsRNA represents a negligible risk to human or livestock that consume DP23211 maize. EPA has previously established an exemption from the requirement of a tolerance for residues of nucleic acids that are part of a plant-incorporated protectant (40 CFR § 174.507). EPA's analysis confirms that this established exemption is applicable to the DvSSJ1 dsRNA expressed in DP23211 maize. Further, the specific nature of the RNAi mode of action to WCR lowers the likelihood that DvSSJ1 dsRNA would pose a hazard to humans or livestock that consume DP23211 maize. Additionally,

humans and other mammals possess physiological barriers (i.e., nucleases in saliva and the gastrointestinal tract, acidic conditions in the stomach) that impede the uptake of plant RNA in mammalian cells.

After reviewing the submitted and publicly available data and information for IPD072Aa protein and DvSSJ1 dsRNA, EPA concluded that there is a reasonable certainty of no harm from residues of IPD072Aa protein or DvSSJ1 dsRNA, and their use will not cause unreasonable adverse effects to human health or the environment. Under FIFRA section 3(c)(5), EPA is proposing to register one new end-use product (EP), DP23211 Maize, containing the new active ingredients IPD072Aa protein and DvSSJ1 double stranded RNA and the genetic materials necessary for their production in corn. The proposed registration will be restricted to breeding and seed increase uses only; commercial plantings will not be permitted.

Furthermore, EPA is establishing a tolerance exemption for residues of IPD072Aa in or on corn, and EPA has already established a tolerance exemption for residues of nucleic acids (DvSSJ1 dsRNA) that are part of a plant-incorporated protectant (40 CFR § 174.507) when used in as a plant-incorporated protectant in corn.

2. Background

On July 16, 2019, EPA received an application from Pioneer Hi-Bred International, Inc. (Pioneer) that proposed to register the plant-incorporated protectant (PIP) DP23211 Maize, containing the active ingredients DvSSJ1 double stranded RNA and IPD072Aa protein, for a FIFRA Section 3 seed increase registration. Pioneer provided data and other information (e.g., scientific rationales and published literature) to support the registration action. In addition, Pioneer submitted a petition to establish a tolerance exemption for residues of IPD072Aa protein in or on corn.

In the Federal Register of March 27, 2020 (85 FR 17328), EPA published a Notice of Receipt (NOR) that announced receipt of an application for registration of a product containing the new active ingredients DvSSJ1 dsRNA and IPD072Aa protein. Adhering to the current practices at the time, EPA had established a docket for each active ingredient on Regulations.gov (EPA-HQ-OPP-2019-0697 for IPD072Aa protein and EPA-HQ-OPP-2020-0027 for DvSSJ1 dsRNA). Current practice is to establish a single docket per decision (both active ingredients) to simplify publishing of proposed decisions, completed risk assessments, and to adequately collect comments. To improve ease of access EPA will only be using docket EPA-HQ-OPP-2019-0697 for the registration application.

In the Federal Register of April 15, 2020 (85 FR 20910), EPA published a Notice of Filing (NOF) for the petition requesting the exemption from the requirement of a tolerance for residues of *Pseudomonas chlororaphis* IPD072Aa protein in or on maize.

No comments were received for the Notice of Filing and two comments were received for the Notice of Receipt. These are addressed in Section 4 of this Proposed Decision.

3. Evaluation

In evaluating a pesticide registration application, EPA assesses a variety of studies to determine the likelihood of adverse effects (i.e., risk) from exposures associated with the use of the product. Risk assessments are developed to evaluate how the active ingredient might affect a range of nontarget organisms, including humans and terrestrial and aquatic wildlife (plants and animals). Based on these assessments, EPA evaluates and approves uses and terms of registration to mitigate any potential risk.

The conclusions conveyed in the assessments described below were developed in full compliance with EPA Scientific Integrity Policy for Transparent and Objective Science, and EPA Scientific Integrity Program's Approaches for Expressing and Resolving Differing Scientific Opinions. The full text of EPA Scientific Integrity Policy for Transparent and Objective Science, as updated and approved by the Scientific Integrity Committee and EPA Science Advisor can be found here: https://www.epa.gov/sites/default/files/2014-

02/documents/scientific_integrity_policy_2012.pdf. The full text of the EPA Scientific Integrity Program's Approaches for Expressing and Resolving Differing Scientific Opinions can be found here: https://www.epa.gov/scientificintegrity/approaches-expressing-and-resolving-differing-scientific-opinions.

3.1 Assessment of Human Health Exposure and Risk

To assess risk to human health, EPA requires information on the PIP's toxicity and allergenicity. If the PIP produces a protein, analyses include an acute oral toxicity test at maximum hazard dose, amino acid sequence homology and comparisons to known allergens and toxins, heat stability testing, and an in vitro digestion assay in a simulated gastric environment. In cases where it is necessary to produce the test substance (the PIP) in a yeast or a bacterium to obtain sufficient quantities to conduct acute oral toxicity tests, EPA also requires the applicant to demonstrate that the microbially-produced and plant-produced substances have similar biochemical characteristics and bioactivity.

3.1.1. Product Characterization

DP23211 maize is produced using site-specific integration using two sequential transformation steps; microprojectile bombardment to insert an integration site sequence at a specific location of the genome, then *Agrobacterium*-mediated transformation with a plasmid containing the target T-DNA in addition to several genetic elements. Integration of the T-DNA region from the plasmid into the landing pad occurred via flippase-mediated recombination.

The IPD072Aa protein produced in DP23211 maize is an insecticidal protein whose gene was originally isolated from *Pseudomonas chlororaphis*, a ubiquitous plant and soil bacterium that is more commonly found in the root environment of several plants and is well known for its biocontrol abilities. The first product using *P. chlororaphis* as an active ingredient was registered with the EPA in September 2001 (EPA Reg. No.: 75801-2) and this bacterium has not demonstrated toxicity or pathogenicity to humans, wildlife, or the environment. Under field conditions, the IPD072Aa protein protects against feeding damage from certain coleopterans by way of midgut epithelium disruption.

The DvSSJ1 dsRNA transcript targets *Diabrotica virgifera virgifera* (Western Corn Rootworm, WCR) and functions by weakening smooth septate junctions in the insect gut via RNA interference (RNAi). The DvSSJ1 dsRNA in DP23211 maize suppresses the target mRNA transcript in WCR through RNA interference (RNAi), a mechanism which regulates gene expression and defense against transposable elements and RNA-based viruses in almost all eukaryotic organisms. Suppression of the DvSSJ1 transcript through RNAi results in injury to the WCR midgut epithelium and is WCR-specific.

The data and information submitted to address the product analysis data requirements for the plant-incorporated protectant DP23211 containing IPD072Aa protein and DvSSJ1 dsRNA have been classified as acceptable.

3.1.2 Toxicological and Allergenicity Data and Information

In support of the application, Pioneer submitted data and information to evaluate toxicity and allergenicity which EPA examined via a "weight of evidence" approach.

EPA's assessment of the toxicity for the IPD072Aa protein concluded the following: (1) acute oral toxicity studies showed no toxicity to mice after exposure to a single dose of 2000 mg/kg bw; (2) the oral LD_{50} was determined to be >2000 mg/kg bw for mice (EPA Toxicity Category III); (3) the mode of action has a narrow spectrum of activity which is specific to the target organism, which lowers the likelihood that IPD072Aa would pose a hazard to humans or livestock and; (4) bioinformatic searches revealed no sequence homology between IPD072Aa protein and any known toxins that target mammals. Finally, *P. chlororaphis* as an active ingredient has been registered and in use for over 20 years and this bacterium has not demonstrated toxicity

or pathogenicity to humans, wildlife, or the environment. Based on this analysis, IPD072Aa protein represents a negligible toxicity risk to humans or livestock who consume DP23211 Maize.

EPA's assessment of the allergenicity for the IPD072Aa protein concluded the following: (1) the source of the trait, *P. chlororaphis*, is a ubiquitous soil bacterium with a long history of pesticidal use that has not demonstrated any toxicity or pathogenicity and is therefore not considered a source of allergenic proteins; (2) bioinformatics searches indicated no alignments to known allergens; (3) complete degradation of IPD072Aa protein in a simulated gastric environment indicated that the intact protein will not pass from the stomach into the intestinal lumen; (4) characterization studies conducted on both the DP23211 maize-derived and microbially-expressed IPD072Aa protein demonstrated that the IPD072Aa protein is not glycosylated; finally (4) heat treatment testing found that the IPD072Aa protein is not stable beyond 95 ℃, and corn products are typically cooked beyond 100 ℃ or higher, it is expected that the IPD072Aa protein would become denatured during these processes. Based on this analysis, IPD072Aa protein represents a negligible allergenicity risk to humans or livestock who consume DP23211 Maize

EPA's hazard assessment for the DvSSJ1 dsRNA concluded the following: (1) the mode of action for DvSSJ1 dsRNA is highly specific, naturally occurring gene regulation mechanism, that is specific to the target organism, which lowers the likelihood that DvSSJ1 dsRNA would pose a hazard to humans or livestock; (2) the bioinformatics study did not identify any exact matches between DvSSJ1 21-mers and human transcripts and indicates that the potential for off-target effects of the DvSSJ1 fragment in humans in silico is negligible; (3) the low expression values in DP23211 maize, specificity of the dsRNA, and history of safe exposure to RNA, suggest that potential off-target effects in mammals as a result of consuming DP23211 maize are unlikely and; (4) physiological and biochemical barriers exist in mammals that may play a role in preventing the uptake of plant RNAs by mammalian cells. Based on this analysis, there is a reasonable expectation that DvSSJ1 poses no hazard to humans.

EPA has established an exemption from the requirement of a tolerance for residues of nucleic acids that are part of a plant-incorporated protectant (40 CFR § 174.507). EPA has determined that the DvSSJ1 dsRNA meets the definition of a nucleic acid residue in a PIP and confirms that this established exemption is applicable to the DvSSJ1 dsRNA expressed in DP23211 maize.

3.1.3 Aggregate Exposure and Risk Characterization of the IPD072Aa Protein

No adverse effects of concern were observed in toxicological tests IPD072Aa protein and DvSSJ1 dsRNA as described previously; therefore, the EPA did not conduct a quantitative exposure assessment.

Dietary Exposure and Risk Characterization:

The proposed exemption from the requirement of a tolerance for residues of the IPD072Aa protein applies to the food and feed commodities of corn: corn, field; corn, sweet; and corn, pop. Grain serves as a basis for many commodities consumed by humans, thus dietary exposure to IPD072Aa protein is expected. The highest mean concentration of IPD072Aa protein was found in roots and was measured to be 42 ng/mg dry weight. The acute oral toxicity study found the LD_{50} of IPD072Aa to be >2000 mg/kg body weight in male and female mice. These values indicate that food exposure would be unlikely to surpass levels of IPD072Aa protein which were tested in the acute oral toxicity study and not toxic at that level. Further, IPD072Aa protein is rapidly digested in SGF and completely digested in SIF. Bioinformatics analyses did not indicate a toxigenic or allergenic potential in silico. Therefore, IPD072Aa protein does not exhibit any mammalian toxicity via the oral route of consumption, and it also presents a minimal risk of being an allergen.

Drinking Water Exposure and Risk Characterization:

A quantitative drinking water exposure and risk assessment has not been conducted because drinking water

exposure to residues of the active ingredient are expected to be negligible. A soil dissipation study found that the estimated dissipation time of IPD072Aa protein in various soils (loam, sandy clay loam, and silt loam) was within 7 days, it is therefore expected that biological processes will reduce run-off and potential exposure of drinking water to negligible levels.

Proteases and nucleases found in water and the environment would likely degrade the biological material containing the active ingredients, as they have been demonstrated to be susceptible to degradation through these means. Further, given the treatment process for municipal water plants (chemical addition, coagulation and flocculation, sedimentation and clarification, filtration, and disinfection) it is likely that IPD072Aa protein residues would be removed during the water treatment process (https://www.cdc.gov/healthywater/drinking/public/water_treatment.html). If exposure should occur, the Agency concludes that such exposure would not be expected to present any risk due to the lack of toxicity observed for IPD072Aa protein.

Non-occupational, Residential Exposure and Risk Characterization:

Non-dietary residential exposure via inhalation is not likely. Corn is pollinated by wind, so it is possible that air in residential areas will carry transgenic corn pollen; however, corn pollen is not respirable, as it consists of spherical particles ranging in size from 80 to 125 µm, in contrast with respirable particles that are less than 10 µm. Inhalation exposure from sources other than pollen is not likely. IPD072Aa protein is contained within plant cells, which essentially eliminates non-occupational and residential inhalation exposure route or reduces it to negligible levels.

Non-dietary exposure via the skin is somewhat more likely via the contact with corn products which might have been processed in a way that disrupts cellular structure. The most likely way the proteins can have an effect via dermal exposure is by eliciting an allergic reaction. The weight-of-evidence arguments in the section 3.1.2 support the lack of allergenicity for the IPD072Aa protein. Additionally, there exists a large number of proteases on the surface of the human skin. These proteases will contribute to degradation of any proteins coming in contact with the skin. The EPA concludes that there will be no risks associated with dermal exposure to IPD072Aa protein.

3.1.4 Cumulative Effects

Section 408(b)(2)(D)(v) of FFDCA requires that, when considering whether to establish, modify, or revoke a tolerance, the Agency consider "available information" concerning the cumulative effects of a particular pesticide's residues and "other substances that have a common mechanism of toxicity." No risk of cumulative toxicity/effects from the IPD072Aa protein have been identified as no toxicity has been shown in the submitted studies. Therefore, EPA has not assumed that the IPD072Aa protein has a common mechanism of toxicity with other substances.

Based on the results of the acute oral toxicity study for the IPD072Aa protein, there is no indication of mammalian toxicity resulting from the plant-incorporated protectant IPD072Aa as found in DP23211 maize. In the absence of such effects, we conclude that there are no identifiable cumulative effects for the IPD072Aa proteins.

3.1.5 Determination of Safety for U.S. Population, Infants, and Children

U.S. Population: Based upon its evaluation described above and in the Human Health Risk Assessment, EPA concludes that there is a reasonable certainty that no harm will result to the U.S. population, including infants and children, from aggregate exposure to residues of IPD072Aa protein. This includes all anticipated dietary exposures and all other exposures for which there is reliable information.

Infants and Children: Although FFDCA section 408(b)(2)(C) provides for an additional tenfold margin of safety

for infants and children in the case of threshold effects, EPA has determined that there are no such effects due to the lack of toxicity of IPD072Aa proteins. As a result, an additional margin of safety for the protection of infants and children is unnecessary.

3.1.6 Occupational Exposure and Risk Characterization

Dermal or pulmonary exposure to IPD072Aa is not likely as the PIP is contained within plant cells, which reduces these exposure routes to negligible levels. Worker exposure to the IPD072Aa protein via seed dust is also expected to be negligible due to the low amount of protein expressed in transformed plants. If such exposure should occur, the Agency concludes that such exposure would not be expected to present any risk due to the lack of toxicity.

3.1.7 Analytical Method

Although an analytical method is typically not required when the Agency establishes an exemption from the requirement of a tolerance for an active ingredient, Pioneer submitted a protocol for a lateral flow test strip kit to be used for the detection of IPD072Aa protein in maize grain samples. The protocol adequately describes the methodology Pioneer is currently in the process of having the test kit certified by USDA's Grain Inspectors, Packers and Stockyards Administration (GIPSA) and has requested that the availability of a validated lateral flow test kit be a condition of registration.

3.1.8 Human Health Conclusions

IPD02Aa Protein: Based on the molecular characterization, protein expression, bioactivity, acute oral toxicity, lack of glycosylation, complete digestion in both SGF and SIF, and bioinformatics, the weight of evidence suggests that the IPD072Aa protein, and the genetic material necessary for its production in DP23211 maize, is unlikely to cause an adverse effect on humans when exposed via the oral route. EPA has determined that there is reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to the IPD072Aa protein and the genetic material necessary for its production. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. The Agency has arrived at this conclusion because, as previously discussed, no toxicity to mammals has been observed, nor any indication of allergenicity potential for this plant-incorporated protectant.

DvSSJ1 dsRNA: Based on the bioactivity data, bioinformatics studies, and human health waivers, the weight of evidence suggests that DvSSJ1, and the genetic material necessary for its production in DP23211 maize, is unlikely to cause an adverse effect on humans. Further, the existing tolerance exemption for residues of nucleic acids that are part of a plant-incorporated protectant (40 CFR § 174.507) is applicable to the DvSSJ1 dsRNA expressed in DP23211 maize, indicating a negligible risk to human or livestock that consume maize products. The database of studies required to support the assessment of risk to human health is complete. For more information on the human health risk assessment of IPD072Aa protein and DvSSJ1 dsRNA, please see the supporting documentation provided in the associated regulatory docket (search for "EPA-HQ-OPP-2020-0697" at www.regulations.gov).

3.2 Assessment of Ecological Exposure and Risk

EPA's current ecological risk assessment approach for PIPs was developed from previous experience with Bt-derived Cry and Vip proteins targeting lepidopteran and coleopteran pests. These proteins are generally understood to be specific to their target pests and related insects within the same taxonomic order, and with nearly two decades of history indicating safe use, EPA considers the current approach sufficient for determining ecological risk of Bt-derived protein PIPs. Accordingly, to assess risk to the environment from PIPs, EPA requires toxicity data/information on nontarget organisms including avian and mammalian wildlife, aquatic animals, non-target insects (including honeybees), and non-target plants. Potential effects to federally

listed threatened and endangered species are evaluated, as are gene flow, invasiveness, horizontal gene transfer and fate in the environment.

The database of studies and information required to support the assessment of risk to the environment is adequate for making a safety determination for seed increase registration of DP23211 Maize containing *Pseudomonas chlororaphis* IPD072Aa protein and DvSSJ1 double stranded RNA. Pioneer submitted guideline studies and scientific rationales supported by the open literature to address the data requirements. Based on the analysis described below, EPA has determined that there is a reasonable expectation of no discernible effects to occur to any non-coleopteran, non-target species exposed to both IPD072Aa protein or DvSSJ1 dsRNA as expressed within DP23211 Maize as a result of the proposed Section 3 seed increase registration. Further, most listed coleopteran species are habitat specialists and do not use corn or corn fields as habitat. Thus, exposure to such species is not anticipated to occur. Additionally, even where exposure to listed coleopteran species may occur, EPA does not reasonably expect such exposure to result in discernible effects to listed coleopteran species because such species do not consume corn or corn tissue. Therefore, because EPA has determined there is a reasonable expectation of no discernible effects to occur to any non-coleopteran nontarget organisms exposed to IPD072Aa protein and DvSSJ1 dsRNA as expressed within DP23211 Maize and EPA has concluded that negligible to no exposure to IPD072Aa protein or DvSSJ1 dsRNA is expected for listed coleopteran species, effects to listed species and their designated critical habitats are not expected and EPA is making a "No Effect" determination under the Endangered Species Act (ESA).

A summary of the data and information reviewed for IPD072Aa protein and DvSSJ1 dsRNA is provided below. The Agency's full environmental risk assessment (U.S. EPA 2024b) can be found in the associated regulatory docket (search for "EPA-HQ-OPP-2019-0697" at regulations.gov).

3.2.1 Terrestrial Exposure and Risk Characterization

A. Birds and Mammals

Corn grain is the plant tissue that birds and mammals are most likely to directly consume, although it is possible that insectivorous birds and mammals that inhabit corn agroecosystems are exposed via other sources (e.g., insect prey). A "worst case" scenario that reduces uncertainty is based on the highest concentration expressed in the PIP plant based on expression studies submitted to the EPA.

The Agency risk assessment for avian and mammalian species for IPD072Aa protein and DVssJ1 dsRNA as expressed in DP23211 Maize are based on acute toxicity tests and waiver rationales. The worst-case EECs for terrestrial vertebrate species in the relevant tissues likely to be consumed (i.e., maximum-above ground expression) is 45 ng/mg for IPD072Aa protein and 0.113 ng/mg for DvSSJ1 dsRNA.

In an acute toxicity study conducted with a representative avian species, the NOEC for IPD072Aa protein was 2000 mg/kg, which far exceeds the worst-case EEC. In a 14-day mouse acute oral study for IPD072Aa protein, no mortality or other evidence of acute oral toxicity was observed, based on evaluation of body weight, clinical signs, and gross pathology.

In an acute toxicity study conducted with a representative avian species, the NOEC for DvSSJ1 dsRNA was 105 mg/kg (the highest concentration tested), which exceeds the worst-case EEC by several orders of magnitude. No sublethal effects were observed at any dose level.

Waiver rationales for avian inhalation toxicity studies were accepted because associated acute toxicity studies indicated no effect to birds and because EPA does not anticipate significant exposure of birds to IPD072Aa protein and DvSSJ1 dsRNA via inhalation. This is due to the nature of PIPs being embedded in the cellular matrix of the plant materials and thus being unavailable for direct exposure via the inhalation route.

The spectrum of activity for IPD072Aa protein is limited to beetles as no hazard was identified for IPD072Aa protein consumption outside of the coleopteran order in any hazard testing. Furthermore, there is a history of safe use of insecticidal proteins from *P. chlororaphis* in commercialized oversprays as well as seed treatments and no toxicity incidents have been reported in the use of proteins from this bacterial species in commercial sprays.

In addition to lack of hazard to birds and mammals due to the specificity of DvSSJ1 dsRNA to the *Diabrotica* genus, there also exist physiological barriers that minimize the exposure of mammals/birds to dsRNA: (1) without a known transporter, the gut and vascular system provide a barrier to uptake of these molecules in mammalian and avian species; (2) low pH in the gut and nucleases in saliva as well as the lumen of the gastrointestinal tract degrade free-standing RNAs; (3) RNA that would potentially enter systemic circulation via the pulmonary route would encounter nucleases in the blood, resulting in degradation, and be degraded by filtration in kidneys; (4) if dsRNA did circulate, it would be unlikely to cross the hydrophobic cellular membrane to penetrate tissues due to the hydrophilic nature of the macromolecule; and (5) were the dsRNA to pervade the cell, it would be unlikely to escape endosomal capture, lysosomal degradation, or accumulate to levels that could impact gene regulation. Further, bioinformatics analysis indicates that it is unlikely there is a molecular target for DvSSJ1 dsRNA to interact with in either mammalian or avian species.

In conclusion, due a lack of toxicity, the specificity of IPD072Aa protein and DvSSJ1 dsRNA, and the physiological barriers to exposure in vertebrates of RNA, no discernible effects to birds or mammals are expected from IPD072Aa protein or DvSSJ1 dsRNA as expressed in DP23211 Maize.

B. Honeybees and Nontarget Insects

For both IPD072Aa protein and DvSSJ1 dsRNA a series of representative non-target insects were assayed against the two AIs separately and a spectrum of analysis test was initiated to determine the host range of each pesticide active ingredient.

The representative non-target insects tested included pollinators (honeybees), predators and parasitoids (green lacewings, pink spotted lady beetle, convergent lady beetle, parasitic wasp), and detritivores (springtails). Selection criteria for the relevant spectrum of activity test species included phylogenetic relation to the target insects, ecological function, presence in the agroecosystem, and practical considerations regarding laboratory settings. Testing for the spectrum of analysis of both pesticide active ingredients was conducted with 11 species across the coleopteran and lepidopteran orders. For full details on Nontarget insect testing, please see the full Ecological Effects Review (U.S. EPA, 2024b).

i. *Honeybees*

The potential exposure of honeybees to DvSSJ1 dsRNA and the IPD072Aa protein in DP23211 Maize pollen was assessed due to the species importance as beneficial organisms in agriculture and agroecosystems. Studies with both larval and adult honeybees were submitted to support the registration of DP23211 Maize.

No adverse effects on survival, behavior, or appearance were observed when IPD072Aa protein was assayed against larval or adult honeybees in oral toxicity tests. For the assays, EPA considered the assumption that honeybee larvae consume 2.0 mg of pollen and 4.3 mg of pollen as adults. Based on these assumptions the worst-case EEC for IPD072Aa protein is 3.0 ng/larvae and 6.45 ng/adult bee, and the worst-case EEC for DvSSJ1 dsRNA is 4.04×10^{-3} ng/larvae and 8.69×10^{-3} ng/adult bee. For the IPD072Aa protein assay, the maximum protein concentration that the larvae were exposed to was 0.20 µg protein/larva, while adults were exposed to a mean daily dose of 1.3 µg protein per adult bee per day. No effects were observed at any dose tested, and the corresponding margins of exposure (MOE) are 67 times the worst-case EEC for honeybee larvae and 201 times the worst-case EEC for adult bees. For the DvSSJ1 dsRNA assay, the larvae were exposed to 0.0040 µg dsRNA/larva, while adults were exposed to 0.026 µg dsRNA per adult bee per day. No effects

were observed at any dose tested, and the corresponding margins of exposure (MOE) are 990 times the worst-case EEC for honeybee larvae and 2,993 times the worst-case EEC for adult bees. Therefore, EPA has determined there is a reasonable expectation of no discernable effects to honeybees from either IPD072Aa protein or DvSSJ1 dsRNA.

ii. *Non-target Insects*

a. *Predators and Parasitoids*

Hazard testing was conducted for four representative predators and parasitoids exposed to both pesticide active ingredients in isolation including green lacewing, pink spotted lady beetle, convergent lady beetle, and a parasitic wasp. Although predators or parasitoids do not feed directly on maize tissue, they may be exposed to IPD072Aa protein and DvSSJ1 dsRNA via the consumption of prey that has previously consumed tissue from DP23211 Maize. Accordingly, predators and parasitoids were assumed to be exposed to the highest expression levels in aboveground tissues in DP23211 Maize, which are 0.113 ng/mg DvSSJ1 dsRNA and 45 ng/mg IPD072Aa protein. The worst-case EEC assumes that: 1) 100% of the pesticide active ingredient from the PIP transfers to the predator/parasitoid through the prey, and that, 2) predators/parasitoids are exposed to the maximum pesticide active ingredient concentration expressed in the above ground tissue of the plant.

For IPD072Aa protein, no adverse effects were seen at the highest test concentrations for green lacewing (500 ng/mg) or the parasitic wasp (1000 ng/mg). Due to the lack of effects seen in the aforementioned studies and the specificity of IPD072Aa to beetles, no discernible effects are expected for non-coleopteran predator/parasitoid species, such as green lacewing or parasitic wasp, from exposure to IPD072Aa protein as expressed in DP23211 Maize. Effects were observed in the representative coleopteran species. For pink spotted ladybird beetle, the IPD072Aa protein MOE associated with mortality, mean weight, and mean days to adult emergence was 2 times the worst-case EEC (45 ng/mg). For convergent lady beetle, concentrations at which effects from IPD072Aa protein were observed for mean weight and mean days to emergence were 2 times (100 ng/mg) and for mortality were 11 times (500 ng/mg) the worst-case EEC of 45ng/mg. The LD_{50} values for both pink spotted ladybird beetle and convergent lady beetle were >22 times and 22 times the worst-case EEC, respectively. Lady beetles (e.g., pink spotted ladybird beetle and convergent lady beetle) are not known to eat corn leaf tissue, with pollen being the more likely source of direct consumption of plant tissue. Using the concentration of IPD072Aa protein in pollen as the expected environmental concentration would result in MOEs of 66 times. For the potential of prey species containing equivalent levels of IPD072Aa protein as the highest concentrations found in plants, lady beetles commonly prey on aphids and aphids have been shown to contain very low to no detectable levels of other transgenic proteins when collected from transgenic corn, making this exposure route unlikely to be significant.

For DvSSJ1 dsRNA, green lacewing, pink spotted ladybird beetle, convergent lady beetle, and the parasitic wasp were exposed to 1 ng DvSSJ1 dsRNA/mg diet, and no adverse effects were reported. The diagnostic dose used, 1 ng DvSSJ1 dsRNA/mg diet, is nine times above the 0.113 ng/mg worst-case EEC. Nontarget guideline toxicity testing along with bioinformatics and bioassay activity spectrum testing demonstrate that pesticidal activity of DvSSJ1 dsRNA is limited to the target pest genus.

Based on these results, EPA has concluded that there is a reasonable expectation of no discernable effects to predator and parasitoid species from exposure to IPD072Aa protein and DvSSJ1 dsRNA.

b. Soil-dwelling Organisms and Detritivores

IPD072Aa protein and DvSSJ1 dsRNA in DP23211 Maize may enter the soil through root exudates, root sloughing, pollen deposition, and post-harvest plant tissue decomposition. Soil-dwelling organisms may be exposed to IPD072Aa protein DvSSJ1 dsRNA via ingestion of DP23211 senescent maize tissues. Soil-dwelling decomposers and detritivores are most likely to consume senescent maize tissues that are incorporated into

the soil post-harvest. Multiple soil degradation and soil bioactivity studies were conducted for IPD072Aa protein and DvSSJ1 dsRNA.

Results from soil degradation studies indicate that IPD072Aa dissipates within two to seven days in western blots depending on the soil type assayed (i.e., loam, silt loam, sandy clay loam). When compared to other proteinaceous PIPs, the soil degradation timeline of IPD072Aa is similar to Bt maize hybrids. Bt PIPs have had terrestrial soil DT50 estimates between 1.6 to 22 days typically, with estimates at high concentration levels estimating the DT50 at up to 46 days for some Bt toxins.

Results from soil degradation studies indicate that most DvSSJ1 is dissipated by 80% within 24 hours via QuantiGene analysis in all representative soil types. However, the bioactivity of DvSSJ1 in bioassays against the target pest using dsRNA spiked soil were substantially longer than the limit of detection of DvSSJ1 evidenced by the QuantiGene analysis.

The worst-case EEC for soil-dwelling organisms that consume senescent plant material can be calculated based on the maximum concentration of DvSSJ1 dsRNA and the IPD072Aa protein in senescent whole plant tissue. The representative non-target detritivore selected for hazard testing with both pesticide active ingredients was a springtail (F. candida).

For IPD072Aa protein, *F. candida* was exposed to 500 ng IPD072Aa protein/mg diet. No effects were observed at this upper limit dose. The maximum concentration of IPD072Aa protein measured in senescent (R6) whole plant tissue was 33 ng IPD072Aa protein/mg. Thus, the worst-case EEC for the representative detritivore was 15 times for IPD072Aa protein.

For DvSSJ1 dsRNA, *F. candida* was tested as a representative non-target detritivore and exposed to 1 ng DvSSJ1 dsRNA/mg diet. No effects were observed at this upper limit dose. The maximum concentration of DvSSJ1 dsRNA protein measured in senescent whole plant tissue was 2.99×10^{-2}ng/mg. Thus, the worst-case EEC for the representative detritivore was 33 times for DvSSJ1 dsRNA.

Based on these results, EPA has concluded that there is a reasonable expectation of no discernable effects to soil-dwelling organisms and detritivore species from exposure to IPD072Aa protein and DvSSJ1 dsRNA.

C. Nontarget Plants – Outcrossing and Weediness (Gene Flow)

EPA has previously determined that there is no significant risk of gene flow from corn PIPs to wild or weedy relatives in the U.S., its possessions or territories, based on lack of sexually compatible relatives (US EPA 2010). As this determination is based on corn plant biology, and is not active ingredient specific, there is no information to indicate that this assumption would not apply to IPD072Aa protein or DvSSJ1 dsRNA. Thus, no risk of gene flow or weediness is anticipated for the IPD072Aa protein and DvSSJ1 dsRNA PIPs in DP23211 Maize.

3.2.2 Aquatic Animals and Plants

Movement of corn plant foliage beyond planted fields and into nearby aquatic habitats is expected to be limited prior to harvest. Pollen shed may deposit IPD072Aa protein and DvSSJ1 dsRNA into aquatic areas, though aquatic areas that are further than 10-15 meters from the edge of a corn field are expected to receive minimal amounts of pollen expressing either active ingredient. Therefore, pollen from DP23211 Maize is not a likely contributor of either active ingredient to aquatic exposure.

Like Cry proteins, aquatic exposure from the IDP072Aa protein is predicted to be lower than levels that would elicit adverse effects due to the two-week period (which includes colonization and decomposition of the tissue by microorganisms) for corn to degrade sufficiently for consumption by aquatic taxa during which time the

pesticide active ingredient is anticipated to have largely leached out of the tissue. Similarly, DvSSJ1 dsRNA will likely be degraded beyond biologically relevant levels when the corn tissue is available as a food source to invertebrates and therefore exposure in aquatic systems is expected to be negligible.

For IPD072Aa protein, the tested representative species for aquatic invertebrate species was *D. magna*. No effects were observed from IPD072Aa protein exposure at a test concentration of 15mg/L with a resulting estimated MOE >455 times the EEC. In a supplemental catfish feeding study, no effect was observed for a freshwater fish species.

For DvSSJ1 dsRNA, the tested representative species for aquatic invertebrate species was *D. magna*. No effects were observed from DvSSJ1 dsRNA exposure at a test concentration of 0.1 mg/L with a resulting MOE >33,444 times the EEC. In a supplemental catfish feeding study, no effect was observed for a freshwater fish species. The bioinformatics analysis and spectrum of activity study for DvSSJ1 dsRNA indicate specificity to the target pest genus, *Diabrotica*. Additionally, beetles in the genus *Diabrotica* are not known to be aquatic.

Scientific rationale to waive testing was submitted for freshwater fish toxicity. This rationale was based on non-target organism toxicity studies, as well spectrum of activity studies, demonstrating that the DvSSJ1 dsRNA is highly specific to the target genus (*Diabrotica)*, and IPD072Aa protein is specific to the coleopteran insect order. Due to this specificity, and the expectation of negligible exposure levels, toxicity is not expected in freshwater fish. Based on these results, EPA has concluded that there is a reasonable expectation of no discernable effects to freshwater fish or invertebrates from IPD072Aa protein and DvSSJ1 dsRNA as expressed in DP23211 Maize.

EPA has previously determined that exposure to PIPs in marine and estuarine environments is not significant and therefore adverse effects are not anticipated for fish or invertebrates from these environments. Given exposure in freshwater settings is anticipated to be negligible, exposure in oceans and rivers is likewise negligible. At this time, there is no information to indicate that this assumption would not apply to IPD072Aa protein or DvSSJ1 dsRNA. Therefore, EPA accepted a waiver rationale from the registrant for this toxicity testing with these taxa.

3.2.3 Endangered Species Conclusion

Because DvSSJ1 and IPD072Aa expressed in DP23211 maize are selective for coleopteran species and because coleopteran-eating organisms have access to many sources of food outside of corn fields, no discernible effects to non-coleopteran listed species are expected. Due to the lack of direct or indirect effects, there is a reasonable expectation of no discernible effects to listed non-coleopteran species. Therefore, the endangered species assessment for DP23211 maize is focused on potential direct effects to listed coleopteran species. Listed coleopteran species are named in Table 1 below.

Table 1. Threatened and Endangered Beetles in the United States.

Common Name	Scientific Name	ESA Listing Status
Coffin Cave mold beetle	*Texamaurops reddelli*	Endangered
Kretschmarr Cave mold beetle	*Rhadine persephone*	Endangered
Tooth Cave ground beetle	*Rhadine exilis*	Endangered
[no common name] Beetle	*Rhadine infernalis*	Endangered
[no common name] Beetle	*Batrisodes venyivi*	Endangered
Helotes mold beetle	*Dinacoma caseyi*	Endangered

Casey's June Beetle	*Elaphrus viridis*	Endangered
Delta green ground beetle	*Desmocerus californicus dimorphus*	Threatened
Valley elderberry longhorn beetle	*Polyphylla barbata*	Threatened
Mount Hermon June beetle	*Stygoparnus comalensis*	Endangered
Comal Springs dryopid beetle	*Heterelmis comalensis*	Endangered
Comal Springs riffle beetle	*Cicindela ohlone*	Endangered
Ohlone tiger beetle	*Cicindela nevadica lincolniana*	Endangered
Salt Creek Tiger beetle	*Cicindelidia floridana*	Endangered
Miami tiger beetle	*Brychius hungerfordi*	Endangered
Hungerford's crawling water Beetle	*Habroscelimorpha dorsalis dorsalis*	Endangered
Northeastern beach tiger beetle	*Ellipsoptera puritana*	Threatened
Puritan tiger beetle	*Texamaurops reddelli*	Threatened
American burying beetle	*Rhadine persephone*	Threatened

Source: (US FWS 2023)

An analysis of environmental and food requirements of listed beetle species is provided in the Ecological Effects and Environmental Fate Assessment found in the associated regulatory docket (search for "EPA-HQ-OPP-2020-0697" at www.regulations.gov). Most listed coleopteran species are habitat specialists and do not use corn or corn fields as habitat. Thus, exposure to such species is not anticipated to occur. Additionally, even where exposure to listed coleopteran species may occur, EPA does not reasonably expect such exposure to result in discernible effects to listed coleopteran species because such species do not consume corn or corn tissue. Based on the analysis presented in Table 15, EPA has determined that negligible to no exposure is expected for federally listed coleopteran species from the cultivation of DP23211 due to habitat or dietary requirements of listed coleoptera. Therefore, because EPA has determined there is a reasonable expectation of no discernible effects to occur to any non-coleopteran nontarget organisms exposed to IPD072Aa and DvSSJ1 as expressed within DP23211 corn, and because EPA has concluded that negligible to no exposure to IPD072Aa or DvSSJ1 is expected for listed coleopteran species, EPA is making a 'No Effect' determination under the Endangered Species Act (ESA) for all listed species and their designated critical habitats resulting from the proposed uses of IPD072Aa and DvSSJ1 in event DP23211 maize, and has concluded that consultation with the U.S. Fish and Wildlife Service and the National Marine Fisheries Service under ESA § 7(a)(2) is not required.

4. Benefits and Public Comments

Biopesticides are pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. Plant-incorporated protectants (PIPs), a class of biopesticides, consist of pesticidal traits inserted into an organism to be protected as the active ingredient and may have the following benefits:

- Usually are inherently less harmful than conventional pesticides.
- Generally affect only the target pest and closely related organisms, in contrast to broad-spectrum conventional pesticides that may affect many different organisms (e.g., birds, insects, and mammals).
- Often effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides.
- Can greatly decrease the use of conventional pesticides while crop yields remain high, when used as a component of integrated pest management (IPM) programs.

- Can offer another tool for pest management in areas where pesticide resistance, niche markets, environmental concerns, and organic preference limit the use of conventional pesticides.

Like other PIPs, DP23211 Maize aligns with potential benefits above and could fill specific pest control needs in areas where corn is grown. Although the proposed registration is for seed increase and breeding purposes, if commercialized in the U.S., the active ingredients may be effective against target pests that have developed resistance to other toxins. In addition, as described in this document, IPD072Aa protein and DvSSJ1 dsRNA are not toxic or allergenic to humans and do not pose unreasonable risks to nontarget species.

EPA has provided the public two opportunities to comment on the IPD072Aa protein and DvSSJ1 dsRNA pesticide product and its associated IPD072Aa protein tolerance exemption petition through information presented in the Federal Register and/or on www.regulations.gov. No comments were received through Notice of Filing. Two comments were received through the Notice of Receipt. The first comment referenced a chlorine product which is not relevant to DP23211 Maize. The second comment expressed opposition to the application and concerns about pesticide toxicity and lack of safety testing. However, the commenter provided no specific objection to the DP23211 Maize application, nor a basis for EPA to deny registration of the product.

Because Pioneer's pesticide product contains IPD072Aa protein and DvSSJ1 dsRNA, new active ingredients, and involves the first agricultural use of these active ingredients, EPA is opening a 15-day public comment period on the proposed decision. EPA is taking this action in accordance with a policy, first implemented in October 2009, designed to provide a more meaningful opportunity for the public to participate in major registration actions.

5. Proposed Registration Decision

The IPD072Aa protein and DvSSJ1 dsRNA databases are comprised of studies and information that meet the data requirements and support the labeled uses. In considering the assessed risk to human health and the environment, EPA concludes that *Pseudomonas chlororaphis* IPD072Aa protein and the genetic material (PHP74643 T-DNA) necessary for its production in DP23211 Maize (OECD Unique Identifier DP-Ø23211-2) and DvSSJ1 dsRNA complementary to the DvSSJ1 gene sequence from *Diabrotica virgifera virgifera* and the genetic material (PHP74643 T-DNA) necessary for its production in DP23211 Maize (OECD Unique Identifier DP-Ø23211-2) meet the regulatory standard under FIFRA. Therefore, EPA is proposing to grant the seed increase registration of the IPD072Aa protein and DvSSJ1 dsRNA active ingredients in pesticide product DP23211 Maize under FIFRA section 3(c)(5).

EPA is proposing to register one end use product, DP23211 Maize, for use in corn against targeted coleopteran pests as a seed increase registration for breeding operations, including seed manufacturing, research, agronomic testing, small scale research trials, productions in breeding nurseries, increasing inbred seed stocks and producing hybrid seed.

As a term of registration, Pioneer must make the lateral flow test kit and technical support for the IPD072Aa protein available to the U.S. Food and Drug Administration, if requested, and must make the lateral flow test kit for the IPD072Aa protein broadly available upon completion of certification by USDA's Grain Inspectors, Packers and Stockyards Administration (GIPSA).

The risk assessments and label supporting this proposed decision can be found in the associated regulatory docket (search for "EPA-HQ-OPP-2020-0697" at www.regulations.gov).

6. References

Additional references are provided in the risk assessment documents available in the docket for this action (search for "EPA-HQ-OPP-2020-0697" at www.regulations.gov).

U.S. EPA, 2024a. 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. Memorandum from N. Ortiz, Ph.D. to M. Weiner. Dated March 25, 2024

U.S. EPA, 2024b. Ecological Effects and Environmental Fate Assessment for the Plant-Incorporated Protectants IPD072Aa and DvSSJ1 and the Genetic Material Necessary for their Production in DP23211 Maize. Data and Information Were Provided in Support of an Application for a FIFRA Section 3 Seed Increase Registration. Memorandum from K. Welch to M. Weiner. Dated January 19, 2024.