OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

WASHINGTON, D.C. 20460

June 20, 2024

MEMORANDUM

SUBJECT: Product Characterization Review and Human Health Risk Assessment of the Insecticidal Plant-Incorporated Protectant Active Ingredient *Ophioglossum pendulum* IPD079Ea Protein and the Genetic Material Necessary (PHP83175) for its Production in DP-915635-4 maize and Establishment of a Permanent Tolerance Exemption. Data was provided in support of a FIFRA Section 3 Seed Increase Registration.

File Symbol:	29964-GU
PC Code:	017310
Petition No.:	2F9010
Pesticide:	Plant-Incorporated Protectant
Existing Tolerances:	Phosphinothricin acetyltransferase (PAT) 40 CFR § 174.522; Phosphomannose isomerase (PMI) 40 CFR § 174.527
MRID Nos.:	51426403-16, 51426418, 51426421, 51426434, 51426437, 51426441, 51426447-50, 51597101, 51597102, 51920301-06, 51920309, 51920316, 51920318, 52030401, 52321401, 52321402
Case Nos.:	00367241, 00367242

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I. Executive Summary

On June 23, 2022, Pioneer Hi-Bred International, Inc. (Pioneer) submitted a FIFRA Section 3 seed increase registration request (EPA File Symbol: 29964-GU) for event DP-915635-4 maize and an associated permanent tolerance exemption petition for residues of the new plant-incorporated protectant (PIP) active ingredient IPD079Ea protein (EPA Tolerance Petition: 2F9010). In addition to the new active ingredient, Event DP-915635-4 maize also expresses two herbicide tolerance genes, phosphinothricin acetyltransferase (PAT) and phosphomannose isomerase (PMI). Both PAT and PMI have been determined to be exempted from the requirement of a tolerance under the existing exemptions at 40 CFR § 174.522 and § 174.527, respectively.

The IPD079Ea protein was derived from *Ophioglossum pendulum* (common name: ribbon fern), a Southeast Asian epiphyte. The protein has activity against the corn pest *Diabrotica virgifera virgifera* (Western Corn Rootworm). Pioneer has provided to EPA several studies that describe the transformation of DP-915635-4 maize, characterize the inserted DNA sequence and expressed proteins, and otherwise support the human health risk assessment. The submitted label indicates that the product is proposed for breeding purposes, agronomic testing, increasing inbred seed, and producing hybrid seed corn up to a total of 100,000 acres per year.

Table 1. Plant-Incorporated Protectant Active Ingredient in DP-915635-4 Maize and Their Target Pests.

Common Name	Protein	PC Code	Target Pest
IPD079Ea	IPD079Ea	017310	*Diabrotica virgifera virgifera*

To support the human health risk assessment, Pioneer submitted one non-guideline acute oral toxicity study that showed that the protein is not toxic at a level well above maximum possible dietary exposures that are reasonably anticipated in the crop. A supplemental study determining the amino acid similarity of IPD079Ea to known toxins did not find significant homology. Scientific rationales were provided for all other routes of potential exposures (dermal, ocular, and inhalation) and were based on the absence of toxicity through the oral route of exposure, presence of the protein in the plant cells and the specificity of the mode of action to the target organism. Due to the proteinaceous composition of the active ingredient, IPD079Ea protein, the potential for food allergenicity was also considered by the Agency. A "weight of evidence" approach consistent with the Annex to the Codex Alimentarius "Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants" was used. Bioinformatics analysis of IPD079Ea protein's amino acid sequence homology to known and putative allergens was performed. *In vitro* studies were conducted to characterize the stability of IPD079Ea protein in simulated gastric and intestinal fluids. IPD079Ea protein was also evaluated to determine if the protein was inactivated by heat *in vitro*. Finally, the glycosylation status of IPD079Ea protein was also determined. The Agency's allergenicity assessment for the IPD079Ea protein determined the amino acid sequence of IPD079Ea protein did not have significant homology with known or putative allergens. IPD079Ea protein degraded in the presence of simulated gastric fluid, indicating the protein would not be intact after stomach digestion and is not expected to present a dietary exposure. Additionally, IPD079Ea protein was inactivated at a temperature of 50°C (122°F) and lacked glycosylation.

The information presented in support of this registration application is adequate to inform the product characterization and human health risk assessment for the permanent tolerance exemption under the standards of the Food Quality Protection Act (FQPA).

II. Recommendation

The data and information submitted for Event DP-915635-4 maize expressing the new plant-incorporated active ingredient IPD079Ea protein are considered acceptable and sufficient to support its registration. The human health effects data, including the acute oral toxicity study, bioinformatics analysis, and *in vitro* studies, support the finding that there is reasonable certainty that no harm will result to the U.S. population, including infants and children, to the aggregate exposure of IPD079Ea protein. Therefore, the submitted data were deemed sufficient to support the exemption from a requirement for a tolerance for residues of IPD079Ea protein in or on food and feed commodities of corn: corn, field; corn, sweet; and corn, pop. In addition, occupational exposure is determined to be negligible.

III. Data Review Record

A. Background

Pioneer has submitted a FIFRA Section 3 seed increase registration for DP-915635-4 maize expressing the new plant-incorporate protectant (PIP) active ingredient IPD079Ea protein and herbicide tolerance proteins phosphinothricin acetyltransferase (PAT) and phosphomannose isomerase (PMI). The IPD079Ea protein is derived from the ribbon fern (*Ophioglossum pendulum*), a Southeast Asian epiphyte. The naturally occurring amino acid sequence of IPD079Ea protein has not been altered during the development of Event DP-915635-4 maize. Similar to the *Bacillus thuringiensis* crystal (Cry) toxins, the IPD079Ea protein oligomerizes to form pores, disrupting the midgut of the target insect (MRID 51920316; Wei et al., 2023). IPD079Ea protein is active against Western corn rootworm (*Diabrotica virgifera virgifera*). In DP-915635-4 maize, *ipd079Ea* expression is under the control of a root-preferred promoter, reducing the presence of the protein in tissues other than the roots of the corn plant.

B. Product Characterization

1. The Transformation System

An adequate description of the transformation process of DP-915635-4 maize was provided. A detailed summary and evaluation of that process is captured in the corresponding Data Evaluation Record in the Confidential Appendix.

2. Characterization of the DNA Inserted in the Plant

The inserted DNA sequence was characterized with several different studies. Genomic DNA was isolated from leaf tissue of control plants and Event DP-915635-4 maize to conduct the studies. To determine the sequence of the insert and to identify the number of genetic inserts, a southern-by-sequencing (SBS) study was conducted followed by bioinformatics analysis (Zastrow-Hayes et al, 2015). For this study biotinylated probes for the sequences of each plasmid were generated, a library was constructed, and next-generation sequencing was conducted. The results of the SBS study indicated that a single insert of the single copy was detected in Event DP-915635-4 maize. The insert consisted of the intended DNA sequence with a single nucleotide change in a non-coding region. No unintended inserts of any plasmid sequence in other parts of the plant genome were detected. Three new open reading frames (ORFs) were present adjacent to the DP-915635-4 event, two in the 5' flanking region and one in the 3' flanking region, but no fusion proteins were created and their expression was deemed unlikely given the absence of proximity to available promoters. To determine the stability and inheritance pattern of the insert, a Southern blot was performed using dioxygenin (DIG)-labeled probes for the IPD079Ea, PAT, and PMI genes on five generations of Event DP-915635-4 maize. The Southern blot data confirmed the DP-915635-4 DNA insert was stable over five generations. Additionally, the T-DNA insert was shown to follow the expected Mendelian inheritance pattern using both genotypic (RT-PCR) and phenotypic (herbicide tolerance) testing over five generations.

3. Protein Characterization and Expression

To characterize the IPD079Ea protein as expressed in event DP-915635-4, extractions of lyophilized plant tissues were analyzed with multiple methods. To determine the molecular weight of IPD079Ea protein, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis were conducted. In a separate experiment, SDS-PAGE and glycosylation staining were performed to demonstrate IPD079Ea protein is not glycosylated. Mass spectrometry was performed to determine the amino acid sequence of IPD079Ea protein in event DP-915635-4. A protein detection method using Enzyme-Linked Immunosorbent Assay (ELISA) was also submitted and found to be acceptable.

An ELISA was used to determine IPD079Ea expression in various tissues (leaf, pollen, root, whole plant/forage, and grain tissues) of plants grown in 6 locations across the United States and Canada. IPD079Ea protein is overall expressed highest in roots, which is to be expected given that it is under the control of a root-preferred promoter. The single highest value was determined to be in maturing roots (V6; 16 ng/mg tissue dry weight). Lowest expression of IPD079Ea was found in the growing leaf tissues with levels at or below the limit of detection (<0.14 ng/mg tissue dry weight). Expression in grain (R6) was comparable to that value. Pollen (R1) expression was above the limit of detection but about 16-times lower than in the highest expressing root stage (V6). Herbicide treatment of the plants did not meaningfully affect the expression of the protein.

Two herbicide tolerance proteins are expressed by DP-915635-4 maize, PAT and PMI. Mass spectrometry was used to deduce the PAT and PMI amino acid sequences expressed by DP-915635-4 maize. The review compared the amino acid sequences for PAT and PMI in DP-

915635-4 maize using a Basic Local Alignment Search Tool (BLAST) to the sequences previously exempted from a tolerance under 40 CFR §174.522 and 40 CFR § 174.527, respectively. The review concluded the existing tolerance exemptions apply to the PAT and PMI proteins as expressed in DP-915635-4 maize.

4. Mode of action

Molecular evidence was provided showing that IPD079Ea toxicity in WCR is the result of receptor binding in the insect gut, followed by oligomerization and pore formation (MRID 51920316). This mode of action is similar to insecticidal proteins derived from the soil bacterium *Bacillus thuringiensis* (Wei et al., 2023). Pore formation of IPD079Ea was demonstrated using transmission electron microscopy and protein binding to the insect midgut was shown using IPD079Ea specific antibody and histopathology. At 6-hours, IPD079Ea was detected in the lumen of the WCR midgut with interaction with the apical microvilli of the enterocytes of the epithelium. After 24-hours of feeding, the treated insects were stunted in size and the cross-sections revealed the lumen of the gut was collapsed and contained cellular debris. Specificity of IPD079Ea to the target organism WCR was molecularly determined by comparing the affinity of the protein to the brush border membrane vesicles (BBMV; the receptor to which the protein binds) of WCR and the related Lepidopteran species corn earworm, European corn borer, and fall armyworm. Binding to BBMVs was only detected in WCR, but not any of the other tested Lepidopteran species.

5. Supporting Data

The submitted product characterization studies to support the registrant's application for the FIFRA Section 3 seed increase registration for IPD079Ea protein as expressed in the Event DP-915635-4 maize product are summarized with their classifications in Table 2. The Agency individually reviewed the submitted studies in Data Evaluation Records (DERs).

The information provided is sufficient to support the product characterization for the FIFRA section 3 seed increase registration of this PIP.

Table 2. Summary of Product Identity, Manufacturing Process, and Analytical Methods Data Submitted in Support of the Section 3 Registration of DP-91365-4 maize Containing IPD079Ea Protein.

STUDY TYPE	RESULT	MRID NO.
Characterization of Event DP-915635-4 maize	An adequate description of the transformation of DP-915635-4 maize was provided. Southern-by-sequencing confirmed the identity and integrity of the singular PIP cassette, its presence as a single copy, and the absence of plasmid backbone sequences. The PIP cassette was determined to be stable over five generations via Southern blot. Additionally, the T-DNA insert was shown to follow a Mendelian inheritance pattern using both genotypic (RT-PCR) and phenotypic (herbicide tolerance) testing. Three new open reading frames (ORFs) were present in the DP915635 event at	51426406 51426407 51426408 51426409 51426410 51426441 51597101 51920301 51920302 51920303 51920318

STUDY TYPE	RESULT	MRID NO.
	the transjunction sites, two in the 5' flanking region and one in the 3' flanking region, but none of them created a fusion protein. **CLASSIFICATION: ACCEPTABLE**	52030401 52321401 52321402
Characterization of the IPD079Ea protein expressed by DP-915635-4 maize	Multiple studies have been submitted to characterize IPD079Ea protein expressed in DP-915635-4 maize and to determine its expression level within the various plant tissues. The identity of the protein was confirmed in lyophilized plant tissues via protein size determination (SDS-PAGE), immunoreactivity (Western blot), and amino acid identity (N-terminal and LC-MS sequencing). Glycosylation analysis demonstrated that IPD079Ea protein expressed in DP-915635-4 maize is not glycosylated. A study detecting IPD079Ea protein in DP915635-4 maize grown in 6 locations across the United States and Canada quantified the expression levels of the protein as well as the two inert proteins PAT and PMI in plant tissues. IPD079Ea protein is overall expressed highest in roots, which is to be expected given that it is under a root-preferred promoter. The single highest value was determined to be in maturing roots (V6; 16 ng/mg tissue dry weight). Lowest expression of IPD079Ea was found in the growing leaf tissues with levels at or below the limit of detection (<0.14 ng/mg tissue dry weight). Expression in grain (R6) was comparable to that value. Pollen (R1) expression was above the limit of detection but about 16-times lower than in the highest expressing root stage (V6). **CLASSIFICATION: ACCEPTABLE**	51426411 51426421 51920304 51920309
Characterization of the PAT and PMI proteins expressed by DP-915635-4 maize	Multiple studies have been submitted to characterize and detect the herbicide resistance proteins PAT and PMI expressed in DP915635-4 maize. The identity of the proteins was confirmed in lyophilized plant tissues via protein size determination (SDS-PAGE), immunoreactivity (Western blot), and amino acid identity (N-terminal and LC-MS sequencing). Glycosylation analysis demonstrated that neither PAT nor PMI, as expressed in DP915635, is glycosylated. **CLASSIFICATION: ACCEPTABLE**	51426434 51426437
Characterization and equivalency of the microbially-derived IPD079Ea protein test substance	Two studies were submitted which characterized the IPD079Ea protein from two separate microbially-derived batches. Each of the studies analyzed the amino acid concentration, amino acid sequence, and determined the mass of the proteins. The proteins were also subject to SDS-PAGE, western blot, and protein glycosylation analysis. Both of the microbially-derived proteins were identical in sequence, were immuno-reactive, and lacked glycosylation. To demonstrate the biological equivalency of IPD079Ea protein test substances, three batches of microbially-derived test substance and one batch of DP915635 maize-derived IPD079Ea protein were tested in bioactivity assays. *Diabrotica virgifera virgifera* neonates were dispensed into agar-diet wells coated in IPD079Ea protein dosing solution (0, 1.56, 3.13, 6.25, 12.5, and 25.0 µg/cm^2) and assessed for mortality after seven days. The LC_{50} for the maize-derived batch was 6.93 µg/cm^2 and the microbially-derived batches were determined to be overall higher (more potent), albeit not to a biologically meaningful degree. Based on the data provided, the microbially-derived test substances are a suitable surrogate for the maize-produced IPD079Ea.	51426415 51426416 51920305
Validation of the IPD079Ea protein ELISA Method	Extracted protein from leaf, pollen, root, whole plant, and grain samples of a maize event expressing IPD079Ea were analyzed for IPD079Ea protein using a sandwich Enzyme-Linked Immunosorbent Assay (ELISA) method. Extraction efficiency of the protein from plant tissues was	51426418 51920306

STUDY TYPE	RESULT	MRID NO.
	determined. A standard curve was established. The method was validated by analyzing for matrix effects, specificity, accuracy, and repeatability. A microbially produced IPD079Ea was used as an analytical reference standard. **CLASSIFICATION: ACCEPTABLE**	

C. Mammalian Toxicology Assessment

1. Hazard Analysis

IPD079Ea is derived from the fern *O. pendulum*. There is some indication that the family of *Ophioglossum* contains several members that are consumed as vegetables, either processed or unprocessed (Carlson et al, 2022). Some of the *Ophioglossum* family members have been noted to have medicinal properties including anti-microbial, anti-inflammatory properties, and wound healing (Baskaran 2018; Clericuzio 2012; Clericuzio 2014). There are also some reports of medicinal properties of *O. pendulum* including uses as a cough remedy and a hair treatment (Horner 1958; Mannan 2008; Nugraha 2020). However, no food use of *O. pendulum* was identified in a literature search, and therefore no direct history of safe exposure could be established through use in food. History of safe exposure can be useful in establishing a (low) hazard profile of a novel PIP but, as with IPD079Ea, such history is not always available or, indeed required. Because the hazard assessment is based on a weight-of-evidence approach other information elements support the hazard assessment in those cases.

To that end, the applicant submitted acute oral toxicity data demonstrating the lack of mammalian toxicity at high levels of exposure to pure IPD079Ea protein. This data demonstrates the safety of the product at a level well above maximum possible exposure levels that are reasonably anticipated in the crop. Specifically, an acute oral toxicity study in mice (MRID 51597102) indicated that IPD079Ea is non-toxic to mammals. Microbially produced and purified IPD079Ea protein was dosed by oral gavage to 6 female and 6 male Crl:CD1 (ICR) mice in a repeat dose study totaling 5,000 mg/kg bw of the test substance. Individuals in the control group, which also consisted of 6 female and 6 male mice, were dosed with an equivalent concentration of bovine serum albumin (5,000 mg/kg bw). Under these conditions, exposure to IPD079Ea did not result in any mortality or other evidence of acute oral toxicity. Thus, the LD_{50} of IPD079Ea was determined to be greater than 5,000 mg/kg. In general, when proteins are toxic, they are known to act via acute mechanisms and at very low dose levels (Sjoblad et al., 1992). Therefore, since no acute effects were shown to be caused by IPD079Ea, even at relatively high dose levels, the IPD079Ea protein is not considered toxic. Further, although there were some limitations in the bioinformatics analysis to identify homologous toxins (as described in Table 3), the amino acid sequence comparisons determined no similarities between the IPD079Ea and known toxic proteins in protein databases that would raise a safety concern.

Since IPD079Ea is a protein, allergenic potential was also considered. Currently, no definitive tests for determining the allergenic potential of novel proteins exist. Therefore, EPA uses a weight-of-evidence approach where the following factors are considered: source of the trait;

amino acid sequence comparison with known allergens; and biochemical properties of the protein, including in-vitro digestibility in simulated gastric fluid (SGF) and glycosylation. This approach is consistent with the approach outlined in the Annex to the Codex Alimentarius "Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants." The allergenicity assessment for IPD079Ea follows:

1. Source of the trait: There is no information in the literature indicating that *Ophioglossum pendulum* is a known source of allergenic proteins.
2. Amino acid sequence: A comparison of the amino acid sequence of IPD079Ea with known allergens showed no overall sequence similarity or identity at the level of >35% over 80 amino acid residues.
3. Digestibility: The IPD079Ea protein was digested within 30 seconds in simulated gastric fluid containing pepsin.
4. Heat inactivation: IPD079Ea is inactivated at temperatures of 50 °C for 30-35 minutes.
5. Glycosylation: IPD079Ea expressed in corn was shown not to be glycosylated.
6. Conclusion: Considering all of the available information, EPA has concluded that the potential for IPD079Ea to be a food allergen is minimal.

Due to the low toxicity of the IPD079Ea protein in the acute oral toxicity study coupled with the results of the bioinformatics analyses and the minimal potential for the protein to be an allergen, there is the reasonable expectation that IPD079Ea in DP-915635-4 poses no hazard to humans.

Note that because obtaining sufficient quantities of purified protein for the various *in vivo* and *in vitro* tests from the transformed plant is challenging, the company produced IPD079Ea in a heterologous microbial system. Bioequivalence between the plant-produced and microbially produced proteins was demonstrated by comparing the amino acid concentration, amino acid sequence, and the mass of the proteins. The proteins were also subject to SDS-PAGE, western blot, and protein glycosylation analyses. Like the plant-produced protein, the microbially-derived protein was identical in sequence, immuno-reactive to an IPD079Ea-specific antibody, and lacked glycosylation. To further demonstrate the biological equivalency of IPD079Ea protein test substances, three batches of microbially-derived test substance and one batch of DP915635 maize-derived IPD079Ea protein were tested in bioactivity assays. WCR neonates were dispensed into agar-diet wells coated in IPD079Ea protein dosing solution (0, 1.56, 3.13, 6.25, 12.5, and 25.0 $\mu g/cm^2$) and assessed for mortality after seven days. The LC_{50} for the plant-derived batch was 6.93 $\mu g/cm^2$ and the microbially-derived batches were determined to be overall higher (more potent), albeit not to a biologically meaningful degree.

2. Supporting Data

The submitted mammalian toxicity studies to support the registrant's application for the FIFRA Section 3 registration for a PIP expressing IPD079Ea protein in Event DP-915635-4 maize are summarized with their classifications in Table 3. An acute oral toxicity study was submitted to address potential risks associated with oral exposure to IPD079Ea protein. Requests to waive the acute dermal toxicity, acute inhalation toxicity, and acute dermal irritation studies were submitted and considered acceptable by the Agency. Information from the scientific rationales

and studies is included in the section below, and Data Evaluation Records (DERs) of the scientific rationales and studies are attached.

Table 3. Summary of Mammalian Toxicity Data Submitted in Support of the Section 3 Registration of DP-915635-4 maize Containing IPD079Ea Protein.

STUDY TYPE	RESULT	MRID NO.
Acute Oral Toxicity Study in Rats	LC_{50} > 5000 mg/kg bw **CLASSIFICATION: ACCEPTABLE/Non-Guideline** **EPA Toxicity Category IV**	51426414 51597102
Waiver Requests for Acute Dermal Toxicity, Acute Inhalation Toxicity, Acute Dermal Irritation, and Acute Eye Irritation	Scientific rationales were provided to address the potential risk from the IPD079Ea protein through the ocular, inhalation, and dermal routes of exposure. Rationales were found to be acceptable based on the expected negligible exposure through these routes given that the protein is expressed within plant cells. The rationale was further supported by the demonstrated low oral toxicity of the protein in mice (EPA Tox Category IV) and its mode of action in the insect gut. **CLASSIFICATION: ACCEPTABLE**	51426447 51426448 51426449 51426450
Description of the Mode of Action of IPD079Ea Protein	The mode-of-action for IPD079Ea protein was determined using X-ray crystallography, competitive binding assays, transmission electron microscopy, and a larval feeding study followed by histopathology. Based on the presented studies, IPD079Ea protein shares structural homology with other proteins in the Membrane Attack Complex and Perforin (MACPF) family. IPD079Ea protein binds specifically to the brush border membrane vesicles (BBMVs) of Western corn rootworm but does not bind to the BBMVs of other Lepidopteran corn pests. IPD079Ea protein forms pores and ruptures the midgut of Western corn rootworm within 24-hours. The study was classified as SUPPLEMENTAL. Data would have been strengthened by providing BBMV positive controls in the specificity assay and a description of the pH conditions in the binding competition assay with various Cry proteins. **CLASSIFICATION: SUPPLEMENTAL**	51920316
In vitro Heat inactivation of IPD079Ea protein	Samples of IPD079Ea protein were heated to temperatures of 25°C, 50°C, 75°C, and 95°C for 30-35 minutes, mixed with stonefly diet and fed to *D. virgifera virgifera* neonates for 7 days. IPD079Ea protein no longer had bioactivity after heating the protein to ≥50°C. **CLASSIFICATION: ACCEPTABLE/Non-Guideline**	51426403
In vitro gastrointestinal tract stability of IPD079Ea protein	Stability in simulated gastric fluid (SGF): IPD079Ea protein was fully digested in SGF after 30 seconds and was no longer detectable by western blot. Stability in SGF and subsequent simulated intestinal fluid (SIF) digestion: Low molecular weight IPD079Ea protein fragments were detected after SGF digestion; the IPD079Ea protein fragments were fully digested in SIF after 30 seconds and was no longer detectable by Western blot. **CLASSIFICATION: ACCEPTABLE/Non-Guideline**	51426412 51426413

STUDY TYPE	RESULT	MRID NO.
Bioinformatics Assessment of Allergenicity	COMPARE database search: No alignments to known or putative allergens were obtained over a length of 80 amino acids or greater with a sequence identity of ≥ 35% over the entire length of the protein. FuzzPro search using the COMPARE database: No contiguous 8-residue matches between the IPD079Ea protein sequence and the protein sequence of the known and putative allergens were identified in the second search. **CLASSIFICATION: ACCEPTABLE/Non-Guideline**	51426405
Bioinformatics Assessment of Toxin Homology	An internal toxin database was assembled by filtering the database of annotated proteins in UniProtKB/Swiss-Prot for keywords which may indicate toxicity such as toxin, hemagglutinin, and vasoactive. The IPD09Ea amino acid sequence was used to conduct a BLASTP (version 2.9.0) search against the amino acid sequences of the proteins in the internal database. The data cut-off used for the study was an E-value of 10^{-4}. Based on the conducted BLASTP search, no significant hits indicating homology of IPD079Ea to toxic proteins were obtained. The bioinformatics assessment of the toxicity of IPD079Ea protein was considered Supplemental for the following reasons: 1) the full list of keywords used to filter for toxic proteins to compose the database was not provided and thus the relevance of the search terms could not be fully evaluated; 2) a list of the best hits of IPD079Ea to the sequences present in the database would have allowed for an evaluation of the degree of protein identity and thus strengthened the finding that no significant homologies were found. **CLASSIFICATION: SUPPLEMENTAL:/Non-Guideline**	51426404

D. Exposure and Risk Characterization

1. Federal Food, Drug, and Cosmetic Act (FFDCA) Considerations

a. Aggregate Exposure and Risk Characterization, Not Including Occupational Exposure

In examining aggregate exposure, EPA considers available information concerning exposures from the pesticide residue in food and all other non-occupational exposures, including drinking water from ground water or surface water and exposure through pesticide use in gardens, lawns, or buildings (residential and other indoor uses).

The Agency has considered available information on the aggregate exposure levels of consumers (and major identifiable subgroups of consumers) to the pesticide chemical residue and to other related substances. These considerations include dietary exposure under the tolerance exemption and all other tolerances or exemptions in effect for the plant-incorporated protectant chemical residue, and exposure from non-occupational sources.

No adverse effects of concern were observed in toxicological tests conducted with IPD079Ea protein and all other available hazard information as described previously; therefore, the EPA did not conduct a quantitative exposure assessment.

i. Food Exposure

Corn products are made from grains and are consumed regularly by the American public and thus dietary exposure is deemed the most relevant route for assessing human risk of IPD079Ea. Corn grains are consumed in various products. Corn on the cob is primarily prepared by cooking but may be eaten raw. Many corn products such as canned corn and masa are cooked as part of the preparation of the product. Multiple corn products such as popcorn, corn meal, corn starch, and corn oil involve preparation of the kernels by drying. These products would then be heated as part of preparation of the product or as part of a dish. Exposure to IPD079Ea through consumption of DP-915635-4 is expected to be very low given its very low levels of expression in grain. Importantly, even if exposure were to occur, due to the low toxicity of the IPD079Ea protein and the minimal potential for the protein to be an allergen, there is the reasonable expectation that IPD079Ea in DP-915635-4 poses no hazard to humans.

ii. Drinking Water Exposure

Oral exposure to IPD079Ea protein via drinking water is considered unlikely because the protein is already expressed at very low levels in the whole corn plant. Leaching into the soil and groundwater, combined with environmental conditions and microbial activity is expected to further reduce its presence. In the unlikely event that IPD079Ea does enter drinking water, the protein has been shown to have no risk associated with dietary exposure.

iii. Non-Occupational and Residential Exposure

The current submission is noted to be for seed increase uses and residential and non-occupational exposures are expected to be minimal given that the active ingredient and the inert are a plant-incorporated protectant in corn. The only possible route of non-occupational exposure other than dietary is via handling of the plants and plant products. Exposures via the skin or inhalation are expected to be minimal given that the IPD079Ea protein is embedded in the matrix of the plant, which essentially eliminates or reduces dermal and inhalation routes of exposure to negligible levels. Furthermore, IPD079Ea protein expression in leaf tissue, forage, and pollen is very low. Together, there are no risks associated with these exposure routes because IPD079Ea is present in the plant at low levels and are not toxic or allergenic.

b. Cumulative Effects

Based on the results of the toxicity and allergenicity studies for IPD079Ea protein, there is no indication of mammalian toxicity resulting from the IPD079Ea protein. In the absence of such effects, we conclude that there are no identifiable cumulative effects for the IPD079Ea protein.

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

i. U.S. Population

For all of the reasons discussed previously, EPA concludes that there is reasonable certainty that no harm will result to the U.S. population, including infants and children, from aggregate exposure to residues of IPD079Ea protein. This includes all anticipated dietary exposures and all other exposures for which there is reliable information.

ii. Infants and Children

For all of the reasons discussed previously, EPA has concluded that IPD079Ea protein is not toxic or allergenic to mammals, including infants and children. Because there are no threshold levels of concern to infants, children, and adults, EPA concludes that no additional margin of safety is necessary to protect infants and children.

2. Occupational Exposure and Risk Characterization

Exposure via the skin or inhalation is not likely since the PIP active ingredient is contained within plant cells, which essentially eliminates these exposure routes or reduces these exposure routes to negligible levels. Corn pollen is not considered respirable, as it consists of spherical particles ranging in size from 90 to 100 μm (Goldstein et al., 2004), whereas respirable particles are typically considered less than 10 μm. In the case of agricultural dusts derived from activities such as planting, cultivation, and harvest, these particles also tend to be non-respirable sizes (Goldstein et al., 2004). Additionally, the very low expression of IPD079Ea in the grain of transformed plants further supports the expectation that exposure via seed dust would be negligible. If exposure should occur, the Agency concludes that such exposure would not be expected to present any risk due to the lack of toxicity.

3. Residue Analytical Methods

A protocol for a lateral flow test kit was submitted for detection of IPD079Ea protein (MRID 51426440). The study is not currently commercially available and no validation data has been provided.

E. Human Health Risk Assessment Conclusion

Based on the molecular characterization data for corn event DP-915635-4, low expression of the IPD079Ea protein in the various plant parts, low demonstrated toxicity and minimal likelihood of allergenicity, EPA has determined that negligible risk to humans is expected from IPD079Ea and the genetic material necessary for its production in corn event DP-915635-4. Further, the existing tolerance exemption for phosphinothricin acetyltransferase (PAT) at 40 CFR § 174.522 and phosphomannose isomerase (PMI) at 40 CFR § 174.527 are applicable to the PAT and PMI proteins as expressed in DP-915635-4 corn.

IV. References

Baskaran X, Vigilia AG, Zhang S, Feng S, Liao W. A review of the use of pteridophytes for treating human ailments. *Journal of Zhejiang University Science B*. 2018; 19(2): 85-119. Doi: 10.1631/jzus.B1600344.

Carlson AB, Mathesius CA, Ballou S, Fallers MN, Gunderson TA, et al. Safety assessment of the insecticidal protein IPD079Ea from the fern, *Ophioglossum pendulum*. *Food and Chemical Toxicology*. 2022; 1666: 113187. Doi: 10.1016/j.fct.2022.113187.

Clericuzio M, Tinello S, Burlando B, Ranzato E, Martinotti S, et al. Flavonoid Oligoglycosides for *Ophioglossum vulgatum* L. Having Would Healing Properties. *Planta Medica*. 2012;78: 1639-1644. Doi: 10.105/s-0032-1315149.

Clericuzio M, Burlando B, Gandini G, Tinello S, Ranzato E, et al. Keratinocyte wound healing activity of galactoglycerolipids from the fern *Ophioglossum vulgatum L*. *Journal of Natural Medicines*. 2014; 68(1):31-7. Doi: 10.1007/s11418-013-0759-y.

Goldstein DA, Shelton PE, Cullen MR, Easterday PA, Eppard PJ, Cabanilla BR. Responding to the challenge of novel technology: An industrial hygiene and safety program for antibody production in maize. *Journal of Occupational and Environmental Medicine.* 2004; 46:784-790. Doi: 10.1097/01.jom.0000135691.69649.fb.

Horner E. An Unusual Hawaiian Population of *Ophioglossum pendulum*. *American Fern Journal*. 1958; 48(3):118-122. Doi: 10.2307/1545560.

Mannan MM, Maridass M, Victor B. A Review on the Potential Uses of Ferns. *Ethnobotanical Leaflets*. 2008; 12:281-285.

Nugraha AS, Triatmoko B, Wangchuk P, Keller PA. Vascular Epiphytic Medicinal Plants Sources of Therapeutic Agents: Their Ethnopharmacological Uses, Chemical Composition, and Biological Activities. *Biomolecules*. 2020; 10(2): 181. Doi: 10.3390/biom10020181.

Sjoblad RD, McClintock JT, Engler R. Toxicological considerations for protein components of biological pesticide products. *Regulatory Toxicology and Pharmacology*. 1992; 15: 3-9. Doi: 10.1016/0273-2300(92)90078-n.

Wei JZ, Lum A, Schepers E, Liu L, Weston RT, et al. Novel insecticidal proteins from ferns resemble insecticidal proteins from *Bacillus thuringiensis*. *Proceedings of the National Academy of Sciences*. 2023; 120(44): e2306177120. Doi: 10.1073/pnas.2306177120.

Zastrow-Hayes G, Lin H, Sigmund AL, Hoffman JL, Alarcon CM, et al. Southern-by-Sequencing: A Robust Screening Approach for Molecular Characterization of Genetically Modified Crops. *Plant Genome*. 2015; 8(1): eplantgenome2014.08.0037. Doi: 10.3835/plantgenome2014.08.0037.