Proposed Registration Decision for the New Active Ingredient:

***Bacillus thuringiensis* Cry1Da2 protein and the genetic material (PHP88492) necessary for its production in DAS1131 Maize (OECD Unique Identifier: DAS-Ø1131-3)**

Approved by:

Digitally signed by EDWARD MESSINA
Date: 2024.12.04 15:27:10 -05'00'

Ed Messina, Esq., Director
Office of Pesticide Programs
U.S. Environmental Protection Agency

1. Summary

This document announces that the U.S. Environmental Protection Agency (EPA) completed its initial evaluation of the proposed Plant-Incorporated Protectant active ingredient *Bacillus thuringiensis* Cry1Da2 protein and the genetic material (PHP88492) necessary for its production in DAS1131 Maize (OECD Unique Identifier: DAS-Ø1131-3) and concluded that it meets 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.

The gene for the insecticidal protein Cry1Da2 was derived from *Bacillus thuringiensis* (Bt) and is intended to provide protection from certain lepidopteran pests. Cry1Da2 is a chimeric protein consisting of sequences from Bt crystal toxins, Cry1Ab and Cry1D. Similar to other Bt-derived Cry proteins, Cry1Da2 disrupts the insect midgut epithelium upon ingestion though a pore-forming mechanism.

Available data demonstrated that, with regard to humans, Cry1Da2 protein is not toxic or allergenic via any route of exposure. The Agency used a "weight of evidence" approach and determined that, Cry1Da2 is not expected to pose any risk of toxicity to humans and the likelihood of the protein to be a food allergen is minimal. The most likely exposure to the Cry1Da2 protein is dietary through consumption of food products made from corn containing the protein. Oral exposure from ingestion of drinking water is unlikely because the Cry1Da2 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 ingredient and treatment process for municipal water plants are likely to remove Cry1Da2 residues.

Although there may be dietary exposure to residues of Cry1Da2 protein, such exposure presents no concern for adverse effects. Submitted data show that the Cry1Da2 protein is not toxic via the oral route of exposure and a bioinformatics analysis did not indicate any homology to known toxins. Likewise, the potential for allergenicity is low because: (1) The bacterial source of Cry1Da2 protein, *Bacillus thuringiensis*, has a long history of safe use and the Cry proteins produced by Bt have not been shown to exhibit either toxicity or allergenicity to humans; (2) bioinformatic analysis indicates no similarity between Cry1Da2 protein and known allergens; (3) Cry1Da2 protein degrades rapidly when exposed to simulated gastric fluid and completely digested within one minute when exposed to simulated intestinal fluid; (4) Cry1Da2 is inactivated when heated to high temperatures (≥ 75 °C) that are typical of food cooking; and (4) Cry1Da2 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 Cry1Da2 protein is not likely due to corn pollen being too large to be respirable. Inhalation from sources other than pollen is unlikely as Cry1Da2 proteins are contained within plant cells.

EPA has determined that there is a reasonable expectation of no discernible effects to occur to any non-lepidopteran non-target organisms exposed to Cry1Da2 as a result of the proposed Section 3 seed-increase application. This conclusion was reached after considering 1) submitted data and supporting

scientific rationale demonstrating that effects are limited to lepidopteran species, and 2) the expectation that any population-level effects will be limited to lepidopteran pest species in the field. Regarding listed lepidopteran species, EPA's analysis has determined that negligible to no exposure is expected for federally listed lepidopteran species from the cultivation of DAS1131 due to their life-cycle, habitat requirements, extremely limited temporal overlap with corn pollen shed, geographical isolation, host-plant specificity and distribution, feeding patterns, and flight dispersal characteristics, which would preclude the likelihood of the species to be found within or near maize fields. Based on this analysis, 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 the Cry1Da2 protein in event DAS1131 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.

After reviewing the submitted and publicly available data and information for Cry1Da2 protein, EPA concluded that there is a reasonable certainty of no harm from residues of Cry1Da2 protein, and its 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), DAS1131 Maize, containing the new active ingredient Cry1Da2 protein and the genetic materials necessary for its 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 under the Federal Food, Drug and Cosmetic Act (FFDCA) for residues of Cry1Da2 protein when used as a plant-incorporated protectant in or on corn.

2. Background

On April 29, 2022, EPA received an application from Pioneer Hi-Bred International, Inc. (Pioneer) that proposed to register the plant-incorporated protectant (PIP) DAS1131 Maize, containing the active ingredient Cry1Da2 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 Cry1Da2 protein in or on corn.

In the Federal Register of February 23, 2023 (88 FR 11433), EPA published a Notice of Receipt (NOR) that announced receipt of an application for registration of a product containing the new active ingredient Cry1Da2 protein. This Notice of Receipt can be found in the docket EPA-HQ-OPP-2023-0018. In the Federal Register of February 23, 2023 (88 FR 11401), EPA published a Notice of Filing (NOF) for the petition requesting the exemption from the requirement of a tolerance for residues of *Bacillus thuringiensis* Cry1Da2 protein in or on corn. This notice can be found in the docket EPA-HQ-OPP-2023-0022. No comments were received for either the Notice of Receipt or the Notice of Filing.

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

DAS1131 Maize is produced using *Agrobacterium*-mediated transformation on the public maize inbred line B104 with plasmid PHP88492. After herbicide and antibiotic selection of potential transformants, developing roots and shoots were analyzed via PCR for T-DNA insertion confirmation. Plants containing the T-DNA insert were selected for additional characterization.

Cry1Da2 is a chimeric protein consisting of sequences from *Bacillus thuringiensis* (Bt) crystal toxins, Cry1Ab and Cry1D. Cry1Da2 protein targets certain susceptible lepidopteran species. The mode of action is similar to other Bt PIPs: Cry1Da2 protein binds to receptors in the brush border membrane of susceptible insect pests (in this case certain insects of the order Lepidopteran) and results in cell and organismal death through the formation of ion conducting pores in the apical membrane of the midgut epithelial cells.

The data and information submitted to address the product analysis data requirements for the plant-incorporated protectant DAS1131 Maize containing Cry1Da2 protein have been classified as acceptable (U.S. EPA 2024a).

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 toxicity for the Cry1Da2 protein concluded the following: (1) an acute oral toxicity study showed no toxicity to mice after exposure to a single dose of 5000 mg/kg bw; the oral LD_{50} was determined to be >5000 mg/kg (EPA Toxicity Category IV); (2) the mode of action has a narrow spectrum of activity which is specific to the target organisms in the order Lepidoptera, which lowers the likelihood that Cry1Da2 protein will pose a hazard to humans or livestock; and (3) a bioinformatic analysis revealed no sequence homology between Cry1Da2 protein and any known toxins that would raise a safety concern. Finally, pesticidal active ingredients derived from *Bacillus thuringiensis* have a long history of safe use in agriculture and have not been shown to exhibit toxicity to humans. Based on this analysis, the Cry1Da2 protein represents a negligible toxicity risk to humans or livestock who consume DAS1131 Maize.

EPA's assessment of allergenicity for the Cry1Da2 protein concluded the following: (1) *Bacillus thuringienisis* parasporal crystal (Cry) toxins have a long history of safe use in agriculture and are not known to be allergenic; (2) a bioinformatic analysis showed no sequence similarity with known allergens; (3) Cry1Da2 protein was completely degraded in a simulated gastric environment, indicating that the intact protein will not pass from the stomach into the intestinal tract; (4) heat treatment above 75 °C denatures the protein resulting in loss of activity; since corn products are typically cooked beyond 100 ℃ or higher, it is expected that the Cry1Da2 protein would become denatured during these processes; and (5) the Cry1Da2 protein is not glycosylated (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). Based on this analysis, the EPA concludes that Cry1Da2 protein presents a negligible allergenicity risk to humans or livestock who consume DAS1131 Maize.

3.1.3 Aggregate Exposure and Risk Characterization

No adverse effects of concern were observed in toxicological tests for Cry1Da2 protein 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 Cry1Da2 protein applies to all food and feed commodities when used as a PIP in corn: corn, field; corn, sweet; and corn, pop. Therefore, the EPA assessed Cry1Da2 protein dietary exposure for all corn products. Grain serves as a basis for many commodities consumed by humans, thus dietary exposure to Cry1Da2 protein in DAS1131 Maize is expected. However, this exposure is expected to be very low given the low levels of Cry1Da2 expression in grain. The greatest Cry1Da2 expression was determined to be in pollen (46 ng/mg tissue dry weight); comparatively, the acute oral toxicity study was conducted at 5000 mg/kg body weight. Importantly, even if exposure were to occur, due to the low toxicity of Cry1Da2 and the minimal potential for the protein to be allergenic, there is the reasonable expectation that Cry1Da2 poses no human health risk.

Drinking Water Exposure and Risk Characterization:

Oral exposure to the Cry1Da2 protein via drinking water is considered unlikely because the protein is 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 Cry1Da2 does enter drinking water, exposure to this protein would not be expected to result in a human health risk based on the low hazard profile of low toxicity and minimal potential for allergenicity.

Non-occupational, Residential Exposure and Risk Characterization:

The proposed registration is limited to breeding and seed increase activities, thus residential and non-occupational exposures are expected to be minimal given that the active ingredient is 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 respiratory route are expected to be minimal as the Cry1Da2 protein is embedded in the matrix of the plant, essentially eliminating the dermal and respiratory routes of exposure. Additionally, protein expression of Cry1Da2 in various corn tissues (leaf, forage, pollen) was determined to be low. Taken together, there are no risks associated with these exposure routes as the Cry1Da2 protein is present in the plant at low levels and was not found to be toxic to mammals and showed minimal potential for allergenicity.

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 Cry1Da2 protein have been identified as no toxicity has been shown in the submitted studies. Therefore, EPA has not assumed that the Cry1Da2 protein has a common mechanism of toxicity with other substances.

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

U.S. Population: Based upon the evaluation described above and in the human health risk assessment for the Cry1Da2 protein (U.S. EPA 2024a), 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 Cry1Da2 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 Cry1Da2 protein. As a result, the EPA concludes that no additional margin of safety is necessary to protect infants and children.

3.1.6 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 Cry1Da2 in the grain of transformed plants further supports the expectation that exposure via seed dust would be negligible. If exposure should occur, The EPA 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 validated enzyme linked immunosorbent assay (ELISA) to be used for the detection of Cry1Da2 protein in maize grain samples. This ELISA has been demonstrated to reliably detect the levels of proteins in the tissues of corn. However, this method is not currently commercially available. As a term of registration, Pioneer will be required to provide the test kit to the U.S. Food and Drug Administration (FDA) upon commercialization of the product and provide reference material, appropriate control substances, and technical support if requested by the FDA.

3.1.8 Human Health Conclusions

The Agency used a "weight-of-evidence" approach outlined in the Annex to the Codex Alimentarius "Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants" and considered evidence including the history of safe use of Bt pesticides, low protein expression in DAS1131 Maize, mode of action, acute oral toxicity, glycosylation potential, heat stability, gastric digestibility, and bioinformatics analyses to conclude that Cry1Da2 protein does not exhibit toxic or allergenic potential. Accordingly, EPA has determined that no unreasonable adverse effects to humans are expected from Cry1Da2 protein and the genetic material necessary for its production in DAS1131 Maize. Further, as outlined in the associated Cry1Da2 protein tolerance exemption, there is reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to the Cry1Da2 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 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 Cry1Da2 protein (U.S. EPA 2024a), please see the supporting documentation provided in the associated regulatory docket (search for "EPA-HQ-OPP-2023-0018" 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 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 DAS1131 Maize containing *Bacillus thuringiensis* Cry1Da2 protein. 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-lepidopteran, non-target species exposed to Cry1Da2 protein expressed within DAS1131 Maize as a result of the proposed Section 3 seed increase registration. Further, most listed lepidopteran species do not use corn fields as habitat nor corn tissue as a food source. Thus, the potential for effects to any non-target lepidopteran organisms from Cry1Da2 is limited to the potential for pollen deposition onto larval host plants in areas within or adjacent to a field planted with event DAS1131 maize. However, this potential effect is limited temporally, as corn pollen shed is limited to 1-2 weeks. As such, for the majority of the corn growing and harvest season, there is no expected exposure and therefore no expected risk to non-target lepidopteran species from Cry1Da2 as expressed in DAS1131 maize.

Regarding listed lepidopteran species, EPA's analysis has determined that negligible to no exposure is expected for federally listed lepidopteran species from the cultivation of DAS1131 Maize due to their life-cycle, habitat requirements, the extremely limited temporal overlap with corn pollen shed, geographical isolation, host-plant specificity and distribution, feeding patterns, and flight dispersal characteristics, which would preclude the likelihood of the species to be found within or near maize fields. Based on this analysis, 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 the Cry1Da2 protein in event DAS1131 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.

A summary of the data and information reviewed for Cry1Da2 protein 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-2023-0018" 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. It is possible that insectivorous birds and mammals that inhabit corn agroecosystems are exposed via other sources (e.g., insect prey), although predation is not expected to be a meaningful route of exposure for vertebrates given the general lack of PIP concentrations within prey species.

The Agency risk assessment for avian and mammalian species for Cry1Da2 as expressed in DAS1131 Maize is based on acute toxicity tests and several lines of evidence that support both a lack of hazard and lack of significant exposure to the protein for these two groups of vertebrate species. The activity of the Cry1Da2 protein is limited to lepidopteran species as effects were not induced by Cry1Da2 when assayed against non-lepidopteran insects or a collembolan species (i.e., a non-insect hexapod species). Hazardous effects would therefore not be expected in non-insect species groups (i.e., birds or

mammals). The worst-case estimated environmental concentrations (EEC) for avian species in the relevant tissues likely to be consumed (i.e., maximum-above ground expression) is 13 ng/mg in grain and 51 ng/mg in above ground tissue for Cry1Da2 protein. In an acute toxicity study conducted with a representative avian species, the LD_{50} for Cry1Da2 protein exceeded 1250 mg/kg body weight, which surpasses the worst-case EEC by several orders of magnitude. No sublethal effects were observed at any dose level.

Additionally, Cry1Da2 protein was tested against mice for the associated human health risk assessment and no effects were observed. Finally, the level of expected environmental exposure to the protein is not considered to be hazardous as the expression of the protein in tissues relevant to birds and mammals (e.g., grains, leaves) is lower than protein concentrations used in toxicity studies which demonstrated no hazard to non-insect species.

In conclusion, due to a lack of both hazard and relevant environmental exposure, the EPA has determined there is a reasonable expectation of no discernable effects to avian or mammalian species from the use of Cry1Da2 as expressed in DAS1131 Maize.

B. Honeybees and Nontarget Insects

The highest expression levels for Cry1Da2 protein in event DAS1131 maize were found in the pollen tissue (36 – 62 ng Cry1Da2/mg dry weight). The 95th percentile values from the protein expression trials were used for risk characterization in the assessment, including the following plant tissues: V6 leaf (51 ng Cry1Da2/mg dry weight), forage (28), grain (13), and pollen (61).

A series of representative non-target terrestrial invertebrates were assayed against Cry1Da2, and a spectrum of analysis test was conducted to determine the host range of the protein. The representative non-target invertebrates tested included pollinators (honeybees), predators and parasitoids (lacewings, ladybird beetles, and wasps), detritivores (springtails), and non-target lepidopteran (painted lady and monarch butterflies). Testing for the spectrum of analysis for Cry1Da2 was conducted with 10 species across the coleopteran and lepidopteran orders. The nontarget insect testing is summarized below and fully described in the ecological effects risk assessment for Cry1Da2 (U.S. EPA 2024b).

i. Honeybees

The potential exposure of honeybees to the Cry1Da2 protein in DAS1131 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 DAS1131 Maize.

No adverse effects on survival, behavior, or appearance were observed when Cry1Da2 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; concentration of Cry1Da2 protein in maize pollen is 61 ng/mg dw. Based on these assumptions the worst-case EECs for Cry1Da2 protein are 122 ng/larvae and 262 ng/adult bee. The maximum concentration larvae were exposed to was 1250 ng Cry1Da2 protein/larva and a mean daily dose of 1700 ng Cry1Da2 protein per adult bee per day. As there were no observed effects at any dose tested, the margin of exposure

(MOE) for honeybee larvae is 10.2X and for adult bees 6.4X the worst-case EECs. While the MOE for adult bees is <10X the EEC, it is important to note that the MOE was calculated based on the highest concentration tested, which did not cause any lethal or sublethal effects to adult honeybees. Additionally, the juvenile (i.e., larval) life stage of organisms tends to be the life stage most sensitive to potential toxins, and as such the 10.2X MOE from the honeybee larval study is considered sufficiently conservative.

Therefore, due to a lack of both hazard and relevant environmental exposure, there is a reasonable expectation of no discernable effects to honeybees from Cry1Da2 as expressed in DAS1131.

ii. Non-target Insects

a. Spectrum of Activity

A submitted spectrum of activity study further confirmed that activity is limited to lepidopteran species with no effects seen in pest coleopteran species. Testing for the spectrum of analysis for Cry1Da2 was conducted with 10 species across the coleopteran and lepidopteran orders. 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. The species tested included nine Lepidopteran species (fall armyworm, corn earworm, European corn borer, painted lady butterfly, cabbage looper, soybean looper, sugarcane borer, southwestern corn borer, and velvetbean caterpillar) and one coleopteran pest species (western corn rootworm).

The results showed that Cry1Da2 was highly active only against the lepidopteran pest species corn earworm, cabbage looper, fall armyworm, and soybean looper, with EC_{50} values 5.1, 0.2, 2.8, and 3.4 ng/mg diet, respectively. Weight reduction was observed for sugarcane borer and southwestern corn borer. Biological activity was also detected in the non-target lepidopteran species, the painted lady butterfly, with LC_{50} and NOEC values of 2.0 and 1.0 of ng/mg diet, respectively. There was no activity detected against European corn borer or western corn rootworm.

In summary, analysis of the non-target effects and the spectrum of analysis information/data provided lead to the conclusion that the activity spectrum of Cry1Da2 is specific to lepidopteran species.

b. Predators and Parasitoids

Although predators and parasitoids do not feed directly on maize tissue, they may be exposed to Cry1Da2 protein via the consumption of prey that has previously consumed tissue from DAS1131 Maize. Predators and parasitoids do not feed directly on maize leaf tissue, thus, one factor to consider in the exposure assessment for these taxa is the amount of AI that transfers and accumulates in prey. Secondary exposures via prey are influenced not only by the rates of ingestion, digestion, and excretion of plant material by the prey, but also the stability of the AI within the prey. The worst-case EEC assumes that: 1) 100% of the AI from the PIP transfers to the predator/parasitoid through the prey, and that, 2) predators/parasitoids are exposed to the maximum AI concentration expressed in the PIP. Accordingly, predators and parasitoids were assumed to be exposed to the 95th percentile expression levels in above ground tissues in DAS1131, 51 ng Cry1Da2/mg dw.

Hazard testing was conducted for three representative predators and parasitoids exposed to Cry1Da2 protein: green lacewing, pink spotted lady beetle, and a parasitic wasp. These assays showed no adverse effects at the highest test concentrations for green lacewing (1000 ng/mg), the parasitic wasp (1000 ng/mg), nor the pink spotted lady beetle (1000 ng/mg). The MOE for all of the representative predator and parasitoid species was 20x the worst-case EEC.

Due to a lack of both hazard and relevant environmental exposure, EPA has determined there is a reasonable expectation of no discernable effects to representative predator and parasitoid species.

c. Soil-dwelling Organisms and Detritivores

Soil dissipation and soil bioactivity studies were conducted for Cry1Da2 protein. These studies demonstrated progressive Cry1Da2 protein dissipation and reduced bioactivity over the study timeframe (28 days). However, the Cry1Da2 protein in DAS1131 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 Cry1Da2 protein via ingestion of DAS1131 senescent maize tissues. Decomposers and detritivores are most likely to consume senescent maize tissues that are incorporated into the soil post-harvest.

A springtail species (*Folsomia candida*) was selected as a representative non-target detritivore for hazard testing with Cry1Da2. The species was exposed to 1000 ng Cry1Da2 protein/mg diet. No effects were observed at this highest concentration this species. The 95th percentile for Cry1Da2 protein measured in root-forage plant tissue was 42 mg Cry1Da2 protein/mg. Thus, the MOE was 24X the worst-case EEC for the representative detritivores for Cry1Da2.

Therefore, due to a lack of both hazard and relevant environmental exposure, EPA has determined there is a reasonable expectation of no discernable effects to representative detritivore species from Cry1Da2 as expressed in DAS1131 Maize.

d. Non-Target Lepidoptera

Possible routes of exposure for non-target lepidopteran species to transgenic proteins produced in plants include consuming leaf tissue, nectar, and pollen that has deposited on plants adjacent to the field utilized by the non-target species for foraging during the larval stage. Lepidopterans known to consume corn leaf tissue are pest species, such as the European corn borer, corn earworm, southwestern corn borer, and fall armyworm, not non-target lepidopteran species. Therefore, the likelihood of non-target lepidopteran species consuming corn leaf tissue is considered to be negligible to none. Additionally, wind pollinated plants, such as maize, do not produce nectar, thus no exposure is possible to non-target lepidopteran species through nectar in the case of maize. Therefore, the only remaining route of exposure of transgenic proteins produced in maize to non-target lepidopteran species is the deposition of pollen on larval host-plants adjacent to the field.

The potential exposure of monarch butterflies (*Danaus plexippus*) to the Cry1Da2 protein in DAS1131 Maize pollen was assessed as a representative sensitive lepidopteran species whose larvae feed on milkweed plants (*Asclepias* spp.), which may be found within and/or adjacent to agriculture and

agroecosystems. In previous assessments (EPA, 2010), the Agency concluded that a combination of several factors indicated a low probability of exposure and therefore demonstrable adverse effects of Bt corn pollen on monarch larvae: 1) the distribution of corn pollen within and around corn fields, 2) the distribution of milkweeds within and around corn fields, 3) monarch oviposition and feeding behavior, and 4) limited temporal overlap between monarch larvae and corn pollen shed. However, in contrast with most previously registered Cry proteins in corn, Cry1Da2 appears to be highly toxic to representative non-target lepidopterans, and Cry1Da2 has relatively high protein expression levels in the DAS 1131 Maize pollen. Therefore, despite the expectation of minimal exposure of Cry1Da2 within DAS1131 Maize's pollen to monarchs due to the factors listed above, exposure of the protein to the monarch was newly assessed to infer the potential risk of Cry1Da2 to the monarch butterfly, as well as to nontarget lepidopterans more generally (given the monarch's heightened sensitivity).

EPA utilized a corn pollen dispersal model (EFSA 2016) to help quantify the risk of Cry1Da2 to the monarch butterfly. An estimate of the amount of corn pollen movement off-field was dervied from the model and used to calculate the worst-case EEC of Cry1Da2 to the monarch butterfly. Assuming the 95th percentile value of Cry1Da2 in DAS1131 Maize pollen (61 ng Cry1Da2/mg), the EEC for monarch butterfly larvae is 0.0096 ng/mg^3 at one meter from the edge of a DAS1131 Maize field. In a bioassay assessing biological activity with monarch butterfly larvae, the LC_{50} value for Cry1Da2 was determined to be 0.0029 ng/mg diet, and a NOEC of 0.002 ng/mg diet based on mortality. The mortality NOEC of 0.002 ng Cry1Da2/mg diet was divided by the above EEC estimate to produce an MOE of 0.21X for *D. plexippus*. At 15 meters offield (i.e., the approximate outer limit of most corn pollen deposition), the MOE becomes 1.02X the worst-case EEC. Use of milkweed in the model provides a conservative estimate for larval host plant deposition, as milkweed leaves tend to be large, oval shaped, and horizontally positioned - all attributes increasing the likelihood of pollen deposition.

The low MOE of 1.02X for the monarch butterfly at 15 meters off-field indicates that it is possible for Cry1Da2 to adversely affect non-target lepidopteran species from consumption of DAS1131 Maize pollen deposited corn onto larval host plants. However, this potential for adverse effects is highly limited temporally, as corn pollen shed is limited to 1-2 weeks of the year. Therefore, for the majority of the corn growing and harvest season, there is no expected exposure and therefore no expected risk or population impacts to non-target lepidopteran species from Cry1Da2 as expressed in DAS1131 Maize.

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 (U.S. 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 Cry1Da2. Thus, no risk of gene flow or weediness is anticipated for Cry1Da2 as expressed in DAS1131 Maize.

3.2.2 Aquatic Animals and Plants

A. Freshwater Fish and Invertebrates

Aquatic exposure to the Cry1Da2 protein may result from pollen drift and movement of leaf or other

post-harvest crop residue off cultivated fields with the most likely source being post-harvest crop residues that enter water. Like previously evaluated Cry proteins, aquatic exposure from the Cry1Da2 is predicted to be lower than levels that would elicit adverse effects due to the approximately two-week timeframe for corn to degrade sufficiently for consumption by aquatic taxa during which time the protein is anticipated to have largely leached out of the tissue (U.S. EPA 2010). Further, Cry1Da2 was shown to rapidly degrade in soil and is likely to have similar rapid degradation in aquatic environments (due to both abiotic [e.g., photodegradation, pH, temperature] and biotic [e.g., microbes] factors), which further minimizes any potential aquatic exposure to the protein. Overall, the transient nature of maize tissue inputs, reduced protein expression within post-harvest crop residues, and rapid protein degredation rate results in an expectation of negligible exposure for Cry1B.34 to aquatic environments.

Hazard testing to the Cry1Da2 protein was conducted on the non-target freshwater invertebrate, *Daphnia magna*, and channel catfish. For *D. magna*, no adverse effects were seen at the highest test concentration (4 mg/L diet) and the MOE was 7X the worst-case EEC (0.57 mg/L). While the MOE for is <10X the EEC, it is important to note that the MOE was calculated based on the highest concentration tested, which did not cause any lethal or sublethal effects to daphnids. Additionally, the EEC is estimated based on the EPA standard agricultural field-farm pond model. This is a highly conservative, worst-case calculation that assumes all of the corn tissue from the field enter the water and that the PIP will be immediately bioavailable, which is highly unrealistic. Additionally, while the EPA recognizes the limitations of a nutritional equivalence study for assessing toxicity of a protein, no adverse effects were observed in a dietary study conducted with channel catfish fed an experimental diet containing 30% DAS1131 Maize grain.

Therefore, due to a lack of both hazard and relevant environmental exposure, EPA has determined there is a reasonable expectation of no discernable effects to freshwater invertebrate and fish species from Cry1Da2 as expressed in DAS1131 Maize.

B. Marine/Estuarine Fish and Invertebrates

EPA has previously determined that exposure to maize contained PIPs in marine and estuarine environments is not significant and therefore adverse effects are not anticipated for fish or invertebrates inhabiting these environments (U.S. EPA 2016). At this time, there is no information to indicate that this assumption would not apply to Cry1Da2 as expressed in DAS1131 Maize. Therefore, the EPA has determined that there is a reasonable expectation of no discernible effects to occur to marine or estuarine fish and invertebrate species as a result of the use of Cry1Da2 as expressed in DAS1131 Maize.

3.2.3 Endangered Species Conclusion

The EPA concludes that the consumption of or contact with DAS1131 corn tissues containing Cry1Da2 by non-target organisms is not expected to pose a hazard to any non-lepidopteran listed species based on toxicity studies indicating no biologically meaningful effects upon any taxa outside of the Lepidopteran order. Additionally, indirect effects to non-lepidopteran listed species are not expected because any measurable population-level effects to lepidoptera are expected to be limited to the pest species in the treatment field, which is not a sole, or significant, source of feeding for non-lepidopteran listed species that consume lepidoptera, nor are pollination impacts expected as the target

lepidopteran pest species is not a known pollinator. Therefore, due to the lack of direct effects for listed non-lepidopteran threatened or endangered species (TES) or indirect effects to any TES, there is a reasonable expectation of no discernible effects to listed non-lepidopteran species as a result of the Section 3 seed-increase for the Cry1Da2 protein as expressed in DAS1131 maize.

Regarding the possibility of direct effects to lepidopteran TES, the EPA's analysis has determined that negligible to no exposure to Cry1Da2 is expected for each lepidopteran TES (Table 1) within the area where Cry1Da2 in DAS1131 maize is proposed to be used. The evaluations of these lepidopteran TES demonstrate that their life-cycle, habitat requirements, extremely limited temporal overlap with corn pollen shed, geographical isolation, host-plant specificity and distribution, feeding patterns, and flight dispersal result in the reasonable expectation that such negligible exposure would not result in discernible effects. Therefore, the EPA has determined that negligible to no exposure is expected for these 22 lepidopteran TES from the cultivation of DAS1131 maize due to the habitat and/or dietary requirements of the lepidopteran TES. For the full endangered species analysis for each threatened or endangered lepidoptera, please refer to the environmental risk assessment (U.S. EPA, 2024b) in the docket EPA-HQ-OPP-2023-0018 on Regulations.gov.

Based on this analysis, 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 the Cry1Da2 protein in event DAS1131 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.

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

Common Name	**Scientific Name**	**ESA Listing Status**
Karner blue butterfly	*Lycaeides melissa samuelis*	Endangered
Mitchell's satyr butterfly	*Neonympha mitchellii mitchellii*	Endangered
Dakota skipper	*Hesperia dacotae*	Threatened
Poweshiek skipperling	*Oarisma poweshiek*	Endangered
Bog buck moth	*Hemileuca maia menyanthevora*	Endangered
Blackburn's sphinx moth	*Manduca blackburni*	Endangered
Saint Francis' satyr butterfly	*Neonympha mitchellii francisci*	Endangered
Fender's blue butterfly	*Icaricia icarioides fenderi*	Endangered
Taylor's (=whulge) checkerspot	*Euphydryas editha taylori*	Endangered
Puerto Rican harlequin butterfly	*Atlantea tulita*	Threatened
Island marble butterfly	*Euchloe ausonides insulanus*	Endangered
Silverspot butterfly	*Speyeria nokomis nokomis*	Threatened
Oregon silverspot butterfly	*Speyeria zerene hippolyta*	Threatened

Florida leafwing butterfly	*Anaea troglodyta floridalis*	Endangered
Bartram's hairstreak butterfly	*Strymon acis bartrami*	Endangered
Carson wandering skipper	*Pseudocopaeodes eunus obscurus*	Endangered
Sacramento Mountains checkerspot butterfly	*Euphydryas anicia cloudcrofti*	Endangered
Miami blue butterfly	*Cyclargus* (=*Hemiargus*) *thomasi bethunebakeri*	Endangered
Pawnee montane skipper	*Hesperia leonardus montana*	Threatened
Uncompahgre fritillary butterfly	*Boloria acrocnema*	Endangered
Schaus swallowtail butterfly	*Heraclides aristodemus ponceanus*	Endangered
Mount Charleston blue butterfly	*Icaricia* (*Plebejus*) *shasta charlestonensis*	Endangered

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, DAS1131 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, the Cry1Da2 protein is not toxic or allergenic to humans and does not pose unreasonable risks to nontarget species.

EPA has provided the public two opportunities to comment on the Cry1Da2 protein pesticide product

and its associated Cry1Da2 protein tolerance exemption petition through information presented in the Federal Register and/or on www.regulations.gov. No comments were received either for the Notice of Filing or Notice of Receipt.

Because Pioneer's pesticide product contains Cry1Da2 protein, a new active ingredient, 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 Cry1Da2 protein database is comprised of studies and information that meet the data requirements and support the labeled use. In considering the assessed risk to human health and the environment, EPA concludes that *Bacillus thuringiensis* Cry1Da2 protein and the genetic material (PHP88492) necessary for its production in DAS1131 Maize (OECD Unique Identifier: DAS-Ø1131-3) meets the regulatory standard under FIFRA. Therefore, EPA is proposing to grant the seed increase registration of the Cry1Da2 protein active ingredient in pesticide product DAS1131 Maize under FIFRA section 3(c)(5).

EPA is proposing to register one end use product, DAS1131 Maize, for use in corn against targeted lepidopteran 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 provide reference material, appropriate control substances and technical support if requested by the Food and Drug Administration to support the analytical method for the Cry1Da2 protein.

The risk assessments and label supporting this proposed decision can be found in the associated regulatory docket (search for "EPA-HQ-OPP-2023-0018" 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-2023-0018" at www.regulations.gov).

U.S, EPA, 2010. Cry1Ab and Cry1F *Bacillus thuringiensis* (Bt) Corn Plant-Incorporated Protectants. Biopesticides Registration Action Document. Available at: https://www3.epa.gov/pesticides/chem_search/reg_actions/pip/cry1f-cry1ab-brad.pdf

U.S. EPA, 2016. Environmental Risk Assessment for a FIFRA Section 3 Registration of MON 89034 x TC1507 x MON 87411 x DAS-59122-7, combined trait maize expressing Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34/35Ab1 Bacillus thuringiensis derived insecticidal protein, and DvSnf7 double stranded RNA (dsRNA); submitted by Monsanto Company. Memorandum from S. Borges through C. Wozniak to

J. Kausch, dated August 16, 2016.

U.S. EPA, 2024a. Product Characterization Review and Human Health Risk Assessment of the Insecticidal Plant-Incorporated Protectant Active Ingredient, *Bacillus thuringiensis* Cry1Da2 and Plant-Incorporated Inert Ingredient DGT-28 EPSPS, and the Genetic Material Necessary (PHP88492) for their Production in Event DAS-1131-3 Maize, and Establishment of a Permanent Tolerance Exemption for Residues of these Proteins when used as a Plant-incorporated Protectant in Corn. Memorandum from N. Ortiz through W. Striegel and M. Mendelsohn to M. Weiner, dated October 28, 2024.

U.S. EPA, 2024b. Environmental risk assessment for the plant-incorporated protectant *Bacillus thuringiensis* Cry1Da2 protein and the genetic material necessary for their production in Event DAS-Ø1131-3. Memorandum from M.B. Claude through A. Pierce and M. Mendelsohn to M. Weiner, dated October 24, 2024.