OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION
WASHINGTON, D.C. 20460

October 24, 2024

MEMORANDUM

SUBJECT: 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. Data and information were provided in support of an application for a FIFRA Section 3 seed increase registration.

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I. Executive Summary

The Environmental Protection Agency (EPA) has completed an environmental fate and ecological risk assessment (ERA) in support of a FIFRA Section 3 seed increase registration for the insecticidal plant-incorporated protectant (PIPs), Cry1Da2 protein, as expressed in maize containing event DAS-Ø1131-3 (hereafter DAS1131), which provides control of targeted lepidopteran pests.

This ERA examines the potential for ecological risks associated with the use of Cry1Da2 as expressed in DAS1131 maize on non-target organisms. Sixteen non-target organism toxicity studies or data waiver rationales were submitted to support the registration of Cry1Da2. Studies included toxicity assessments with avian species, non-target terrestrial invertebrates, including honeybees, and non-target freshwater invertebrates. Data waiver rationales, in lieu of studies, were provided for avian inhalation, non-target plants, wild mammals, freshwater fish, and estuarine/marine animals. Additionally, a spectrum of activity study was provided as well as a soil dissipation study.

No toxicity or sublethal effects were observed for Cry1Da2 in testing against avian species or non-target freshwater invertebrates. For non-target terrestrial invertebrate studies, toxicity was not observed for any of the species tested except for the monarch butterfly (*Danaus plexippus*) and the painted lady butterfly (*Vanessa cardui*), both of which had lethal and sublethal effects. Data waiver rationales for wild mammals, freshwater fish, avian inhalation, non-target plants, and estuarine/marine animals were sufficient to determine no adverse effect would be anticipated for these taxa. A submitted spectrum of activity study further confirmed that activity is limited to lepidopteran species with no effects seen in pest coleopteran species. Therefore, the available data indicate that the toxicity of Cry1Da2 protein exists only to a subset of the lepidopteran insect order.

Regarding non-target lepidopteran species, non-target lepidopteran organisms do not heavily utilize corn fields as habitat nor corn tissue as a food source. EPA therefore concludes that 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. Therefore, given these temporal and spatial limitations, population-level effects to non-target lepidoptera are not expected as a result of the Section 3 seed-increase for the Cry1Da2 protein as expressed in DAS1131.

Based on 1) the submitted scientific rationale, 2) data showing that effects are limited to lepidopteran species, 3) the expectation that population-level effects to lepidoptera will be limited to the pest species on field, 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.

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 in the states produced 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.

II. Introduction

Pioneer Hi-Bred International, Inc. (Pioneer), member of Corteva Agriscience group of companies, submitted an application to register the plant-incorporated protectant, Cry1Da2 protein, as expressed in maize containing event DAS1131 for a Section 3(c)(5) seed increase registration with a negotiated acreage cap.

Insect-protected maize DAS1131 produces the insecticidal protein Cry1Da2, which protects against feeding damage caused by targeted lepidopteran insect pests. The Cry1Da2 protein is encoded by a chimeric gene comprised of sequences from the *cry1Da2* gene encoding an insecticidal core toxin and a derivative of the *cry1Ab* gene, both of which are derived from *Bacillus thuringiensis*.

Under the application for seed increase registration, DAS1131 maize may be used for breeding purposes, agronomic testing, increasing inbred seed, and producing hybrid seed corn up to a total of 20,000 acres per county and up to a combined total of 250,000 acres per plant-incorporated protectant active ingredient per registrant per year in the United States.

The conclusions conveyed in this assessment 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/scientific-integrity/approaches-expressing-and-resolving-differing-scientific-opinions.

A. General Approach to Ecological Risk Assessment for PIPs

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. With nearly three decades of history indicating safe use, EPA considers the current approach sufficient for determining ecological risk of PIPs.

This approach is described in several Biopesticide Registration Action Documents (BRADs) for Cry or Vip proteins (e.g., see US EPA, 2010b). To summarize, the approach consists of a tiered testing scheme (Tiers I-IV) that is focused on hazard determination, and testing is based on the microbial pesticide data requirements published under 40 CFR 158.2150 and their associated 885 and 850 series OCSPP Harmonized Guidelines. At Tier I, studies are designed to be simplified and to estimate hazard to several non-target taxa under "worst-case" exposure conditions. A lack of adverse effects under these conditions would provide enough confidence that there is minimal risk, and no further data would be needed. Screening (Tier I) maximum hazard dose tests are conducted at exposure concentrations several times higher (e.g., 10X or greater when possible) than the highest concentrations expected to occur under realistic field exposure scenarios, with mortality as the toxicological endpoint. When screening tests indicate a need for additional data, the OCSPP Harmonized Guidelines call for testing at incrementally lower doses in order to establish a definitive LD_{50} or LC_{50} (defined as the dose or concentration required to kill 50 percent of the test organisms), and to quantify the hazard. Additional higher-tiered testing may be triggered when results with Tier I studies indicate potentially unacceptable risk, with Tiers II-IV designed to assess hazard under increasingly more realistic field exposure conditions. A risk determination is made by comparing the toxicological endpoint to the estimated environmental concentration (EEC).

In addition to the toxicity data, environmental persistence of PIP pesticidal substances, as well as the potential for gene flow are also considered. The EPA has historically received laboratory data demonstrating the degradation of the PIP pesticidal substance in soils typical of agronomic areas where the PIP crop is grown. To assess gene flow and potential for development of invasiveness, EPA considers several lines of evidence related to characteristics of the crop plant, including reproduction, presence of wild relatives, and containment or other mitigating measures.

B. Mode of Action

Cry proteins belong to a class of bacterial toxins known as pore-forming toxins (Bravo et al., 2007). They are produced during the sporulation phase by *Bacillus thuringiensis* (*Bt*), which are gram-positive spore-forming bacteria with entomopathogenic properties. *Bt* Cry proteins are known to have high specificity to a target insect order, with different proteins shown to be toxic to the insect orders Lepidoptera, Coleoptera, Hymenoptera and Diptera, and also to nematodes.

The mode of action of Cry toxins has been characterized principally in lepidopteran insects. It is widely accepted that the primary action of Cry toxins is to lyse midgut epithelial cells in the target insect via pore formation (Aronson and Shai, 2001; de Maagd et al., 2001, Bravo et al., 2005).

Cry1Da2 have been genetically engineered to be expressed in DAS1131 maize and target lepidopteran pests.

III. Environmental Exposure Assessment

The environmental exposure assessment of DAS1131 is comprised of three major components: tissue specific expression of the active ingredients, degradation profile of the active ingredients, and the general biology of corn. The information and data from each component are then combined in order to perform an exposure assessment for the representative taxa within their appropriate agroecosystem.

A. Expression

Cry1Da2 protein is expressed in DAS1131 maize tissues throughout multiple life stages of the plant and its tissue-specific expression levels largely drive the potential of exposure for non-target organisms (e.g., pollen expression potentially exposes pollinators, grain/seed expression potentially exposes birds/wild mammals). The protein expression levels for Cry1Da2, as determined by field conditions in the United States and Canada, are described in Table 1.

Table 1. Summary of Cry1Da2 protein level in maize tissues collected from DAS1131 maize produced in United States and Canadian field trials in 2020. Data from MRID 51887412.

Tissue (Growth Stage)	ng Cry1Da2/mg Tissue Dry Weight			Number of Samples <LLOQ / Number of Samples Reported
	Mean (S.D.)	Range	Sample LLOQ[1]	
DAS1131 Maize				
Leaf (V6)	44 (5.1)	35 – 52	0.27	0/23[2]
Leaf (V9)	32 (7.5)	21 – 49	0.27	0/24
Leaf (R1)	33(6.5)	20 – 48	0.27	0/24
Leaf (R4)	34 (5.6)	20 – 46	0.27	0/24
Root (V9)	29 (6.8)	19 – 42	0.14	0/24
Root (R1)	20 (5.2)	11 – 29	0.14	0/24
Root (R4)	19 (5.0)	9.6 – 27	0.14	0/24
Pollen (R1)	41 (4.1)	34 – 53	0.54	0/24
Stalk (R1)	21 (2.3)	17 – 24	0.090	0/24
Forage (R4)	24 (3.8)	11 – 30	0.090	0/24
Grain (R6)	8.1 (2.2)	4.2 – 12	0.14	0/24
Herbicide-Treated DAS1131 Maize				
Leaf (V6)	43 (5.4)	33 – 52	0.27	0/24
Leaf (V9)	31 (7.6)	19 – 52	0.27	0/24
Leaf (R1)	32 (4.4)	26 – 40	0.27	0/24
Leaf (R4)	37 (6.9)	26 – 60	0.27	0/24
Root (V9)	29 (7.8)	15 – 42	0.14	0/24
Root (R1)	19 (4.2)	13 – 30	0.14	0/24
Root (R4)	19 (5.6)	7.2 – 27	0.14	0/24
Pollen (R1)	46 (8.2)	36 – 62	0.54	0/24
Stalk (R1)	19 (2.3)	16 – 24	0.090	0/24
Forage (R4)	22 (3.3)	13 – 28	0.090	0/24
Grain (R6)	9.4 (2.4)	4.5 – 14	0.14	0/24

Note: Growth stages (Abendroth et al., 2011). Herbicide-treated refers to treatment with glufosinate.

[1] Lower limit of quantification (LLOQ) in ng/mg tissue dry weight.

[2] One sample was confirmed negative for the event of interest by polymerase chain reaction (PCR) analysis.

The level of expression of proteins, including expression of PIPs, normally varies in plants because of differences in environmental conditions. For example, variation is seen among plants in the same variety because of differences such as weather and soil condition (U.S. EPA, 2010a). Given the known impacts of the environment on expression levels, the 95th percentile values from the 2020 United States and Canada field trials (Table 2) will be used for risk characterization in this assessment. These field trials took place in Illinois, Iowa, Nebraska, Texas, and Ontario (Canada).

Table 2. 95th percentile values calculated for Cry1Da2 from DAS1131 maize produced in United States and Canada field trials in 2020. Calculations were based on raw data presented in MRID 51887412.

Tissue (Growth Stage)	95th percentile value (ng Cry1Da2/mg Tissue Dry

	Weight)
Leaf (V6)	51
Leaf (V9)	48
Leaf (R1)	42
Leaf (R4)	46
Root (V9)	42
Root (R1)	28
Root (R4)	27
Pollen (R1)	61
Stalk (R1)	24
Forage (R4)	28
Grain (R6)	13

Based on the 95th percentile values (Table 2), the highest expression levels for Cry1Da2 protein in event DAS1131 maize were found in the pollen tissue (61 ng Cry1Da2/mg dw).

B. Degradation Profile

The applicant submitted a terrestrial soil dissipation study for Cry1Da2. Table 3 summarizes the submitted study regarding the environmental fate of the AI, which is then followed by the Agency analysis of its fate profile.

Table 3. Summary of data submitted to support the registration of Cry1Da2 contained in DAS1131.

Study	OCSPP (OPPTS) Guideline No.	Results Summary and Classification	MRID No.
Environmental Fate: Dissipation Study	Non-guideline 885.ENV	A 28-day dissipation study was carried out with three different soils (loam, sandy clay loam, and silt loam). Each soil was spiked with Cry1Da2 protein. The western blot data demonstrated protein dissipation over 28 days, as well as a demonstrated loss of insecticidal activity to the sensitive insect bioassay. However, mean weight inhibition was still evident at the end of each study. Additionally, the western blot data demonstrated that the Cry1Da2 protein continued to dissipate with a faint band still evident at the end of the study. **Classification: Supplemental**	51887444

A soil dissipation study of Cry1Da2 protein from maize DAS1131 was conducted in three soil types (loam, sandy clay loam, and silt loam). The western blot data demonstrated that Cry1Da2 protein continued to dissipate over a 28-day period as progressively lighter intensity bands were observed over time in all three soil types. Faint bands were still visible at the conclusion of the of the 28-day period, although the western blot data did demonstrate progressive Cry1Da2 protein dissipation over the course of the study.

An insect bioassay was conducted with fall armyworm (*Spodoptera frugiperda*) due to its acute sensitivity to Cry1 class proteins. Similar to the western blot data, while insecticidal bioactivity declined over time, bioactivity was still present at the end of the observation period in all soils.

Despite the presence of faint band of Cry1Da2 protein in the western blots and bioactivity still evident in the bioassay in all three soil types at the conclusion of the test, the analyses did demonstrate progressive dissipation of the protein over time, and it is reasonable to believe microbial dissipation of the Cry1Da2 protein would continue after 28 days.

D. Environmental Exposure

1. Terrestrial Exposure

Because Cry1Da2 is contained within corn, movement of corn tissue was considered in the evaluation of NTO exposure. Corn is a wind-pollinated, monoecious, annual species with imperfect flowers (*i.e.*, spatially separate tassels [male flowers] and silks [female flowers] found on the same plant, a feature that limits inbreeding) (U.S. EPA, 2010b).

Movement of corn tissue influences the distribution and fate of Cry1Da2 in the environment since corn tissue will carry the AI to wherever it may move. How much Cry1Da2 moves within the environment depends on what organ or tissue is moved and when, since expression levels differ between corn tissues, and change within them over the course of the growing season.

Prior to harvest, most of the corn foliage expressing the AI will be contained within the planted field. Some breakage of foliage and other above ground plant parts may occur, which could result in their deposition outside field borders; however, movement of above-ground plant parts (excluding pollen) beyond the border is expected to be minimal prior to harvest. Within soil, exposure is expected to be primarily limited to the roots, although sloughing of roots cells into the surrounding soil will also occur. It is not known whether Cry1Da2 would be present in root exudates, though upon root cell lysis, small amounts could be released in the surrounding soil.

After harvest, corn tissue may be left on the field, where it may remain or be subject to movement by wind and water. The amount and distance moved is not known and is expected to vary, but ultimately corn tissue that remains in the terrestrial environment is expected to become a part of the plant detritus upon and within soil. Additionally, corn plant material left on the field may be tilled into the soil. Eventually, cells of corn tissue will lyse and release into the soil any Cry1Da2 that has not been broken down within the plant. Therefore, soil is expected to be the ultimate destination of Cry1Da2 in the terrestrial environment. As evidenced by the submitted soil dissipation study, rapid dissipation of the Cry1Da2 protein across multiple soil types is expected; thus, accumulation of the protein in soil is not anticipated.

Non-target invertebrates with the greatest exposure potential to protein in transgenic corn fields are beneficial insects, which feed on corn pollen or pest insects that have consumed corn tissue, and soil invertebrates, which feeds on detritus. Additionally, pollen from DAS1131 maize may land on non-target larval host plants found growing near corn fields and non-target insects may feed on leaves deposited with the pollen. During anthesis, corn pollen will shed and will be deposited on surfaces, including other plants, within the field and beyond the field borders. Corn pollen is of relatively large size among wind dispersed pollens (90 - 100 µg/m), which is thought to give it a greater tendency to settle (Pleasants et al., 2001; Raynor et al., 1972). While it is possible for corn pollen grains to travel significant distances (e.g., greater than 60 meters) away from a cornfield (Hofmann et al., 2014; Nielsen, 2020; Raynor et al., 1972), the overall amount of pollen traveling these distances is quite small. Indeed, evidence from several corn pollen dispersal studies has shown most pollen from a cornfield is deposited within a short distance (i.e., within 10 – 15 meters) of the corn plant (Aylor, 2003; Burris, 2001; Hofmann et al., 2014; Pleasants et al., 2001; Raynor et al., 1972; Wraight et al., 2000). More recent work has been done (e.g., see Gathman et al. 2006, Hoffman et al. 2014, Lang et al. 2015), and although sampling methods, sampling duration, and data analysis vary among all of these studies, they show similar deposition patterns, providing additional support for EPA's current understanding of this process as it affects PIP environmental fate.

For terrestrial vertebrate non-target organisms, birds and mammals are the non-target organisms most likely to consume corn grain, which would be the route of direct exposure to those taxa. Insectivorous birds and mammals could also be exposed via the consumption of insects that inhabit the corn agroecosystem; however, predation is not expected to be a meaningful route of exposure for vertebrates given the general lack of PIP concentrations within prey species (Li et al., 2017; Meissle & Romeis, 2018; Tian et al., 2014).

2. Aquatic Exposure

As with terrestrial environments, 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 Cry1Da2 into aquatic areas, though as described above, 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 AI. Therefore, pollen from DAS1131 is not a likely contributor to aquatic exposure.

Post-harvest corn plant residue can enter nearby waterways and may do so in large amounts in areas where corn is predominant within the landscape. Movement occurs by the action of wind and water (Griffiths et al. 2009, Tank et al. 2010) and inputs occur primarily in late fall and winter (Rosi-Marshall et al. 2007). All tissues and organs of corn plants that would be left over after harvest in field may be observed in nearby aquatic areas, including leaves, stems, and cobs (Tank et al. 2010).

Generally, insecticidal proteins from PIPs rapidly leach from corn tissue (Chambers et al. 2010, Bottger et al. 2015, Strain and Lydy 2015) and corn tissue is not suitable for consumption by invertebrates for one to two weeks while the tissue breaks down (Chambers et al. 2010, Jensen et al. 2010). After a two-week conditioning period into an aquatic system while the corn tissue is not suitable for consumption, the protein will likely be degraded beyond biologically relevant levels when the tissue is available as a food source to invertebrates.

Corn tissue (including plant foliage and pollen shed) movement into nearby aquatic habitats is possible but is expected to be minimal. It would then take approximately one to two weeks for leaching of the proteins to occur in the event pollen and other plant tissue does enter aquatic systems. Therefore, exposure to the active ingredient to aquatic non-target organisms is negligible.

IV. Non-Target Effects Data Submission Summary

EPA has historically used the microbial pesticide non-target data requirements under 40 CFR Part 158 as a guide for data needs for PIPs.

Scientific rationale to waive testing was submitted for wild mammal toxicity, fish acute toxicity, non-target plant, avian inhalation, and estuarine/marine animal toxicity. These scientific rationales were deemed to be acceptable, and the requests were therefore granted (Table 4). Toxicity studies with honeybee (*Apis mellifera*), collembola springtail (*Folsomia candida*), green lacewing (*Chrysoperla rufilabris*), pink spotted lady beetle (*Coleomegilla maculata*), parasitic wasp (*Pediobius foveolatus*), monarch butterfly (*Danaus plexippus*), fall armyworm (*Spodoptera frugiperda*), water flea (*Daphnia magna*), and northern bobwhite (*Colinus virginianus*) were conducted with the Cry1Da2 protein and were submitted to satisfy data requirements. A spectrum of analysis study was also submitted for Cry1Da2 as well as a catfish feeding study conducted with diet containing 30% DAS1131 maize grain. The studies submitted to support the registration of Cry1Da3 contained in DAS1131 are included in Table 5.

The information and data provided are sufficient to satisfy the Tier I non-target organism data needs for ecological risk assessment for the Section 3 seed increase registration. Further testing of non-target organisms at higher tiers is not required for the proposed Section 3 seed increase.

Table 4. Summary of waiver rationales submitted to support the registration Cry1Da2 contained in DAS1131.

Study	OCSPP (OPPTS) Guideline No.	Results Summary and Classification	MRID No.
Wild mammal toxicity/pathogenicity	885.4150	Tests required by 40 CFR § 158.2140 are adequate and appropriate for assessing hazards of Cry1Da2 to wild mammals. Acute oral toxicity/pathogenicity studies with laboratory rats indicates an oral LD_{50} 5000 mg/kg bw for Cry1Da2 (MRID 51887422). Additionally, the waiver request is based on the rationale that Cry1Da2 protein is derived from a	51887438

Study	OCSPP (OPPTS) Guideline No.	Results Summary and Classification	MRID No.
		bacterium that has a history of use in agriculture and has a narrow spectrum of activity. **Classification: Acceptable**	
Freshwater fish toxicity/pathogenicity	885.4200	The waiver request is based on the rationale that 1) Cry1Da2 is derived from a bacterium that has a history or safe use in agriculture, 2) Cry1Da2 protein has a narrow spectrum of activity, favorable degradation profile in soil, and expected exposure to freshwater fish is low, and 3) a study conducted with *Daphnia magna* shows low toxicity (NOEC was 4 mg/L)to freshwater invertebrates (MRID 52020602). Scientific rationale provides sufficient information to determine that adverse effects to freshwater fish are not expected as a result of the proposed uses Cry1Da2. **Classification: Acceptable**	52020603
Estuarine/marine animal testing	885.4280	The waiver request is based on the rationale that 1) Cry1Da2 is derived from a bacterium that has a history or safe use in agriculture, 2) Cry1Da2 protein has a narrow spectrum of activity, favorable degradation profile in soil, and expected exposure to estuarine/marine animals is low, and 3) a study conducted with *Daphnia magna* shows low toxicity (NOEC was 4 mg/L)to freshwater invertebrates (MRID 52020602). Scientific rationale provides sufficient information to determine that adverse effects to estuarine and marine animals are not expected as a result of the proposed uses Cry1Da2. **Classification: Acceptable**	52020604
Avian Inhalation	885.4100	The waiver request is based on the rationale that 1) Cry1Da2 is derived from a bacterium that has a history or safe use in agriculture and has a narrow spectrum of activity, 2) the lack of effects observed (LD_{50} >1250 mg/kg body weight) in the avian acute oral study conducted with the Cry1Da2 protein (MRID 51887436), and 3) limited exposure due to the intracellular nature of PIPs. Scientific rationale provides sufficient information to determine that adverse effects to non-target avian species expected as a result of the proposed uses Cry1Da2. **Classification: Acceptable**	51887439
Non-Target Plants	885.4300	The waiver request is based on the	51887440

Study	OCSPP (OPPTS) Guideline No.	Results Summary and Classification	MRID No.
		rationale that 1) Cry1Da2 is derived from a bacterium that has a history or safe use in agriculture and has a narrow spectrum of activity, 2) has a favorable degradation profile in soil. Scientific rationale provides sufficient information to determine that adverse effects to non-target plants expected as a result of the proposed uses Cry1Da2. **Classification: Acceptable**	

Table 5. Summary of data submitted to support the registration of Cry1Da2 contained in DAS1131.

Study	OCSPP (OPPTS) Guideline No.	Results Summary and Classification	MRID No.
Avian oral toxicity	885.4050	The LD_{50} for Cry1Da2 in Northern bobwhite > 1250 mg Cry1Da2 protein/kg bw. The NOEL and LOEL of Cry1Da2 protein to *Colinus virginianus*, based on mortality was 1250 mg/kg bw and >1250 mg/kg bw, respectively. **Classification: Acceptable**	51887436
Freshwater invertebrate toxicity/pathogenicity	885.4240	A 21-day toxicity and reproduction study with *Daphnia magna* determined the EC_{50} and NOEC values were >4 and 4 mg Cry1Da2 protein/L. **Classification: Supplemental** The study was classified as supplemental since only 20 organisms were tested per treatment group instead of 50 as stated in the 885.4240 guideline. Additionally, it is unclear if the nominal test concentration meets the minimal requirements specified in the guideline.	52020602
Non-target arthropod invertebrate testing Springtail (*Folsomia candida*)	885.4340	A 28-day oral toxicity laboratory study with springtails determined the EC_{50} and NOEC values were >1000 and 1000 ng/mg diet, respectively. **Classification: Acceptable**	52030305
Non-target insect testing Pink Spotted Lady Beetle (*Coleomegilla maculata*)	885.4340	A 21-day oral toxicity study with the ladybird beetle determined the LC_{50} to be >1000 ng a.i./mg. The NOEC and EC_{50} based on survival, development and adult weight was 1000 and >1000 ng/mg diet, respectively. **Classification: Acceptable**	52030303
Non-target insect testing Parasitic wasp (*Pediobius foveolatus*)	885.4340	A 7-day oral toxicity study with parasitic wasp adults determined the LC_{50} and NOEC values were >1000 and 1000 ng/mg diet, respectively. **Classification: Acceptable**	52020307
Non-target insect testing Green lacewing (*Chrysoperla*	885.4340	A 21-day oral toxicity laboratory study with green lacewings determined the	52030306

Study	OCSPP (OPPTS) Guideline No.	Results Summary and Classification	MRID No.
rufilabris)		LC_{50}, EC_{50}, and NOEC values were >1000, >1000 and 1000 ng/mg diet, respectively. **Classification: Acceptable**	
Evaluation of Biological Response Monarch (*Danaus plexippus*)	Non-Guideline	A 7-day oral toxicity laboratory study with monarch larvae determined the LC_{50} and NOEC (based on mortality) values were 0.00291 (95% confidence interval (CI) 0.00242 – 0.00339 ng Cry1Da2 protein/mg diet), and 0.002 ng/mg diet, respectively. The NOEC value for sublethal effects (weight) was <0.002 ng/mg diet. **Classification: Acceptable**	52408401
Evaluation of Biological Response Fall Armyworm (*Spodoptera frugiperda*)	Non-Guideline	A 7-day oral toxicity laboratory study with fall armyworm determined the mean LC_{50} value was 4.3 ng Cry1Da2 protein/mg diet (95% CI 3.1 and 5.6 ng Cry1Da2 protein/mg diet). **Classification: Acceptable**	51887429
Adult Honeybee testing	OECD 245	A 10-day oral toxicity laboratory study with honeybees determined the LOEC, LOEDD, NOEC and NOEDD values for body weight and mortality were >81 mg a.i./kg, >1700 ng a.i./bee/day, 81 mg a.i./kg and 1700 µg a.i./bee/day, respectively. **Classification: Acceptable**	51887433
Larval Honeybee testing	885.4380	A 22-day oral toxicity laboratory study with honeybee larvae determined the NOED and LOED values were 1250 and >1250 ng a.i./bee/day, respectively. **Classification: Acceptable**	51887432
Channel Catfish Feeding	Non-Guideline	A 8-week channel catfish dietary study (diets composed of 30% maize grain containing event DAS1131) determined there was no adverse effects observed in survival, diet consumption, weight gain, feed conversion, or behavior. **Classification: Supplemental** The study is classified as supplemental on the grounds that it isn't a required study.	51885338
Spectrum of Activity*	N/A	No adverse effects were observed for ECB or WCR at 3000 ng/mg diet. Cry1Da2 was highly active against CEW, CL, FAW, and SL, with EC_{50} values 5.1, 0.22, 2.8, and 3.4 ng/mg diet, respectively. Additional activity was detected in the non-target PLB, with a LC_{50} value of 2.0 ng/mg diet (95% CI 0.33 – 3.7 ng/mg diet), and a NOEC of 1.0 ng/mg diet.	51887428

Study	OCSPP (OPPTS) Guideline No.	Results Summary and Classification	MRID No.
		Weight reduction was observed for SB at all tested concentrations (0.3 to 3000 ng/mg diet) and for SCB and VBC at 3000 ng/mg. **Classification: Acceptable**	

*Fall Armyworm (FAW), *Spodoptera frugiperda*, Corn Earworm (CEW), *Helicoverpa zea*, European Corn Borer (ECB), *Ostrinia nubilalis*, Painted lady butterfly (PLB), *Vanessa cardui*, Western Corn Rootworm (WCR), *Diabrotica virgifera virgifera*, Cabbage looper (CL), *Trichoplusia ni*, Soybean looper (SL), *Chrysodeixis includens*, Sugarcane borer (SB), *Diatraea saccharalis*, Southwestern corn borer (SCB), *Diatraea grandiosella*, Velvetbean caterpillar (VBC), *Anticarsia gemmatalis*.

V. Non-target Organism Hazard

The EPA risk assessment process is centered on a determination of hazard at field exposure rates, which are typically based on expression levels within PIPs to calculate a multiplicative safety factor (i.e., margin of exposure, MOE) to quantify risk. For the purposes of the non-target organism risk assessment of Cry1Da2 in DAS1131 maize, worst-case estimated environmental concentrations (EECs) are based on the 95th percentile values calculated from protein expression studies (see Table 2) in tissue types relevant to the taxa being considered. To calculate an MOE, the hazard level (i.e., the no observed effects concentration, NOEC) is divided by the EEC. Generally, an MOE of ≥10X is considered highly conservative to address a direct route of exposure (i.e., consumption of plant material) and to allow for extrapolation of an expectation of no discernible effects from the surrogate species tested to potentially more sensitive species within the same taxonomic and/or functional group. However, if hazard testing indicates an MOE of <10X, comprehensive exposure characterization is further considered in the risk characterization section.

Below, hazard identification from Tier I bioassays with various taxa with Cry1Da2 (Table 6) are presented alongside their respective EECs for worst-case expression in relevant tissues and MOEs.

Table 6. Worst case EECs (dry weight) and MOEs for representative non-target organisms exposed to Cry1Da2.

Species name	Tissue	Cry1Da2 worst-case EEC for relevant tissues	Cry1Da2 NOED/NOEC	Cry1Da2 MOE
Aquatic Invertebrates				
D. magna	95th percentile concentration in above ground tissue	0.57 mg/L[1]	4 mg/L	7.0X
Pollinators and Pollen Feeders				
A. mellifera – honey bee larvae	95th percentile concentration in pollen	122 ng/bee[2]	1250 ng/bee	10.2X
A. mellifera – honey bee adult	95th percentile concentration in pollen	262 ng/bee/day[2]	1700 ng/bee/day	6.4X

Species name	Tissue	Cry1Da2 worst-case EEC for relevant tissues	Cry1Da2 NOED/NOEC	Cry1Da2 MOE
V. cardui – painted lady butterfly	95th percentile concentration in pollen	0.0096 ng/mg[3]	1.0 ng/mg	208X
D. plexippus – monarch butterfly	95th percentile concentration in pollen	0.0096 ng/mg[3]	0.002 ng/mg	0.21X
Soil-Dwelling Non-Target Insects				
F. candida – Collembola	95th percentile concentration in forage	42 ng/mg	1000 ng/mg	24X
Non-Target Insects				
C. rufilabris – red-lipped green lacewing	95th percentile concentration in above ground tissue	51 ng/mg	1000 ng/mg	20X
P. foveolatus – parasitic wasp	95th percentile concentration in above ground tissue	51 ng/mg	1000 ng/mg	20X
Co. maculata – pink spotted lady beetle	95th percentile concentration in above ground tissue	51 ng/mg	1000 ng/mg	20X
Bird				
Coli. virginianus – northern bobwhite	95th percentile concentration in grain	13 ng/mg	1250 mg/kg bw	96X
	95th percentile concentration in above ground tissue	51 ng/mg[4]	1250 mg/kg bw	25X

[1] For *Daphnia*, the EEC considers the EPA standard agricultural field-farm pond model together with the maximum concentration in above ground tissue value listed in Table 2, although the EPA acknowledges that this EEC is highly conservative given the model's inflated exposure assumptions as they relate to PIPs. Based on the 95th percentile value of Cry1Da2 in DAS1131 maize leaf tissue (51 ng Cry1Da2/mg tissue dry weight; see Table 2) and the specifications outlined in EPA's pond model (20 million liter, 1-hectare pond receives 100% of protein from a 10-hectare field of DAS1131 maize; and 22,500 kg of dry maize tissue is present in a single hectare; with 100% of protein immediately bioavailable), the worst-case EEC for Cry1Da2 protein is 0.57 mg/L.

[2] For honeybees, the EEC is not calculated directly from the maximum pollen expression (Table 2) but also considers consumption. For example, based on the 95th percentile concentration of Cry1Da2 in DAS1131 maize pollen of 61 ng/mg (see Table 2), and the maximum amount of pollen consumed by honeybee larvae (2.0 mg) and adults (4.3 mg), the worst-case EEC for Cry1Da2 protein is 122 ng/larvae and 262 µg/adult bee/day (Babendreier et al. 2004; Crailsheim et al. 1992).

[3] This EEC represents the expected exposure from corn pollen deposition onto larval host plants one meter away from a corn field. To calculate the EEC, pollen deposition was first estimated using a recently updated model from EFSA (2016) (see **Section VI** for the formula and more information). This deposition value (12.6 grains/cm^2) was then incorporated into a larger formula that considers the number of pollen grains per gram of pollen (4,000,000; Miller, 1985), the 95th percentile value of

Cry1Da2 in DAS1131 maize pollen (61 ng/mg), and the surface density of corn (0.02 g/cm^2; Pleasants et al., 2001), producing a worst-case EEC of 0.0096 ng Cry1Da2/mg one meter away from a corn field.

[5] This EEC is based on highest levels found in above ground leaf tissue and represents worst case exposure for insectivorous birds consuming corn pest insects. It assumes 100% transfer of the protein to the predator through prey and assumes that predators are then exposed to the highest levels found in above ground leaf tissue.

A. Evaluation of the potential direct effects

No effects were seen from Cry1Da2 in guideline non-target organism studies using honeybee, springtail, pink spotted lady beetle, green lacewing, parasitoid wasp, daphnia, or quail. The LC_{50}/LD_{50} toxicity endpoints for all of the guideline non-target organism studies conducted for Cry1Da2 was greater than the highest concentration or level tested based on mortality.

A spectrum of activity study was performed utilizing Cry1Da2 protein to evaluate potential effects on survival or growth inhibition on 10 herbivorous insect species (Table 5). The species tested included; Lepidopteran pests (Fall Armyworm (FAW), *Spodoptera frugiperda*, Corn Earworm (CEW), *Helicoverpa zea*, European Corn Borer (ECB), *Ostrinia nubilalis*, Painted lady butterfly (PLB), *Vanessa cardui*, , Cabbage looper (CL), *Trichoplusia ni*, Soybean looper (SL), *Chrysodeixis includens*, Sugarcane borer (SB), *Diatraea saccharalis*, Southwestern corn borer (SCB), *Diatraea grandiosella*, Velvetbean caterpillar (VBC), *Anticarsia gemmatalis*) and the coleopteran pest species (Western Corn Rootworm (WCR), *Diabrotica virgifera virgifera*).

The results showed that Cry1Da2 was highly active only against the lepidopteran pest species CEW, CL, FAW and SL, with EC_{50} values 5.1, 0.22, 2.8, and 3.4 ng/mg diet, respectively. There was no activity detected with ECB or WCR. Weight reduction was observed for SB and SCB.

Biological activity was also detected in the non-target lepidopteran species, the painted lady butterfly (*V. cardui*), with LC_{50} and NOEC values of 2.0 and 1.0 of ng/mg diet, respectively. Additionally, a non-guideline bioassay was conducted, and biological activity was detected in the non-target lepidopteran species, the monarch butterfly (*D. plexippus*), with a LC_{50} and NOEC (based on mortality) values of 0.0029 (95% confidence interval of 0.00242 – 0.00339 ng/mg diet) and 0.002 ng/mg diet, respectively.

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. Evaluation of the potential indirect effects

EPA evaluated the potential for indirect effects, which generally include negative effects to non-target organisms from the reduction of a food source or habitat. Potential indirect effects from the proposed uses of Cry1Da2 include a reduction of a food source, specifically targeted lepidopteran pest species that are directly affected within the treatment area. Targeted lepidopteran pests are available in areas outside of the treated field and, subsequently, organisms that consume lepidoptera as a source of food will have opportunity off-field to encounter these organisms.

Indeed, lepidopteran species can be found in virtually all habitats that insects inhabit worldwide on every continent except for Antarctica (Culin, J., 2023).

Another consideration would be the potential of pollination impacts from the proposed uses of Cry1Da2. Because the Cry protein in DAS1131 maize has specific toxicity to lepidopteran species, they are expected to have no effect on non-lepidopteran species, including major pollinators (e.g., honeybees). Additionally, the targeted lepidopteran pests are not known pollinators themselves and its reduction is therefore not expected to alter pollination services. As discussed in more detail in **Section VI.A.2.d.**, population effects on non-target lepidopteran species are not expected, indicating that those non-target lepidopteran species that do provide pollination services would not show a measurable decrease in numbers and therefore pollination would not be reduced. Thus, the reduction of pollinators for plants that are food sources for non-target organisms are not expected, nor are effects expected for plants that rely on pollinators as part of their lifecycle.

Therefore, indirect effects to non-target organisms are not expected because any measurable 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-target organisms that consume lepidoptera, nor is the pest species a known pollinator for non-target plants.

VI. Ecological Risk Characterization

Above, hazard, soil degradation, and tissue expression data were presented for Cry1Da2. In this section, the likelihood of hazard will be placed in context of potential exposure as relevant to each taxon to assess potential risk from Cry1Da2 as expressed in DAS1131 corn. In cases where a scientific rationale was received in lieu of data, the information ruling out unreasonable hazard or exposure levels will be reviewed. Below, the taxa of interest will be presented as the header of each subsequent section then characterization will be provided individually for Cry1Da2.

A. Terrestrial Environments

1. Birds and Mammals

The EPA has determined that there is a reasonable expectation of no discernible effects to occur to avian and mammalian species as a result of the use of Cry1Da2 as expressed in DAS1131 maize.

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 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). Indeed, the non-target toxicity study described above demonstrate that no activity was seen against an avian species. The worst-case EECs for avian species in the relevant tissues likely to be consumed (i.e., 95th percentile of grain and above ground expression) is 13 ng/mg in grain and 51 ng/mg in above ground tissue for Cry1Da2. In the acute toxicity study conducted with a representative avian species, the LD_{50} for Cry1Da2 exceeded 1250 mg/kg bw, which exceeds the worst-case EEC by several orders of magnitude. No sublethal effects were observed at any dose level.

Additionally, Cry1Da2 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 avians 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.

2. Non-target Invertebrates and Honeybees

A series of representative non-target terrestrial invertebrates were assayed against Cry1Da2, and a spectrum of analysis test was initiated 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 (butterflies) (see Table 5 above).

Testing for the spectrum of analysis of the AI 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.

a. 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 (Table 5). The concentration of Cry1Da2 protein in maize pollen is 61 ng/mg dw. Considering the assumption that honeybee larvae consume 2.0 mg of pollen and 4.3 mg of pollen during each life stage (Babendreier et al. 2004; Crailsheim et al. 1992), the worst-case EEC is 122 ng/larvae and 262 ng/adult bee/day.

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 MOE for honeybee larvae is 10.2X and for adult bees 6.4X the worst-case EEC. 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.

b. Soil-Dwelling Organisms and Detritivores

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.

Soil-dwelling decomposers and detritivores are most likely to consume senescent maize tissues that are incorporated into the soil post-harvest. The worst-case EEC for soil-dwelling organisms that consume senescent plant material can be calculated based on the 95th percentile of expression concentration of Cry1Da2 protein in root-forage plant tissue. The representative non-target detritivore selected for hazard testing with Cry1Da2 was springtail (*F. candida*).

Folsomia candida was exposed to 1000 ng Cry1Da2 protein/mg diet. No effects were observed at this highest concentration this species (Table 5). The 95th percentile for Cry1Da2 protein measured in root-forage plant tissue was 42 mg Cry1Da2 protein/mg (Table 2). 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.

c. Predator and Parasitoids

A predator or parasitoid may be exposed to Cry1Da2 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 (Rose 2007), 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.

For worst-case assumptions, 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 (Table 2). Note, this is expected to exceed realistic exposure levels given that previous studies have shown that there is very low to non-detectable levels of PIPs in prey species fed this tissue (Meissle et al. 2017).

Hazard testing was conducted for three representative predators and parasitoids exposed to Cry1Da2 protein in isolation including green lacewing (*C. rufilabris*), pink spotted lady beetle (*C. maculata*), and a parasitic wasp (*P. foveolatus*).

For Cry1Da2, no adverse effects were seen at the highest test concentrations for any of the predator or parasitoid species tested. The MOE for all of the representative predator and parasitoid species was 20X the worst-case EEC.

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 predator and parasitoid species from Cry1Da2 as expressed in DAS1131.

d. Non-Target Lepidoptera

Possible routes of exposure for non-target lepidopteran species to transgenic proteins produced in plants (not maize specific) 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. Regarding exposure via the consumption of leaf tissue and/or nectar, it has been documented that lepidopteran species have a tight relationship with their host plant that has evolved for many thousands of year (Patiny 2012). Indeed, 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 (U.S. Forest Service 2024), 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 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.

For previously registered Cry proteins in corn, the EPA was able to make “reasonable expectation of no discernable effects” conclusions for non-target lepidopteran species due to the tight association of taxa with their host plants, the toxicity (i.e., LC_{50}) of previously registered events, the relatively low protein expression levels in pollen, and expected exposure from corn pollen (EPA, 2010a). Specifically, the 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 given the four 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 non-target lepidopterans more generally (given the monarch's heightened sensitivity).

Larvae of the monarch feed on milkweed plants (*Asclepias* spp.), which may be found within and/or nearby agriculture and agroecosystems. As with other non-target lepidopterans, exposure of non-target lepidopteran species to transgenic proteins would most likely occur via deposition of pollen on plants adjacent to the field, which may be frequented and utilized by lepidopteran species for foraging. As mentioned above in **Section III.C**, several studies have empirically shown that corn pollen has a limited ability to disperse (i.e., most corn pollen settles within 10 – 15 meters of the corn plant). Recent analysis of such corn pollen dispersal data (Hofmann et al., 2014, 2016), combined with expert scientific opinion (EFSA, 2015; EFSA, 2016), has facilitated the refinement of off-field exposure estimates for corn pollen, enabling more accurate and robust characterizations of risk. In short, the model identified by Hoffman et al. (2014) as the best fit for their corn pollen dispersal data was adjusted by EFSA (2015) to account for several abiotic factors, such as weather (e.g., wind, rain) and the three-dimensional shape of leaves, and several biotic factors, such as competition for pollen and changes in feeding behavior from pollen exposure. EFSA (2016) subsequently derived support for a model containing two of their adjustments (weather and the three-dimensional structure of leaves) to the Hoffman et al. (2014) model through validation with pollen density data generated by Lang et al. (2015). Based on its successful validation with empirical data, the EPA utilized EFSA's 2016 corn pollen dispersal model to help quantify the risk of Cry1Da2 to the monarch butterfly.

First, the amount of corn pollen movement off-field was estimated following EFSA (2016):

$$\mathrm{b} = (1.0 * 10^{1.1}) * (\mathrm{a}^{-0.585})$$

where:
a = distance from the field in meters
b = pollen density in grains/cm^2

Next, this deposition value was utilized to estimate the worst-case EEC of Cry1Da2 to *D. plexippus*:

$$\mathrm{EEC} = \{\frac{\left[\left(\frac{\mathrm{b}}{\mathrm{c}}\right) * \mathrm{d}\right]}{\mathrm{e}}\}$$

where:
b = pollen density in grains/cm^2
c = number of pollen grains per gram of fresh weight corn pollen (4,000,000; Miller 1985)
d = 95th percentile value of Cry1Da2 in DAS1131 maize pollen (61 ng Cry1Da2/mg)
e = surface density of corn pollen on milkweed (0.02 g/cm^2; Pleasants et al., 2001)

Based on this calculation, the EEC for monarch butterfly larvae is 0.0096 ng/mg^3 at one meter from the edge of a DAS1131 maize field. In the bioassay assessing biological activity with monarch butterfly larvae, the LC_{50} value for Cry1Da2 was determined to be 0.0029 ng/mg diet (CI 0.00242 – 0.00339 ng Cry1Da2 protein/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 and MOE 0.21X of for *D. plexippus*. At 15 meters off-field (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.

Therefore, 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. However, there is an important temporal aspect of pollen deposition to consider when placing the potential for risk into a realistic context. Although there is the potential for effects to non-target lepidopteran species from consumption of pollen deposition of DAS1131 corn onto larval host plants, this potential 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 to non-target lepidopteran species from Cry1Da2 as expressed in DAS1131 maize. In summary, although there is the potential for effects to non-target lepidopteran larvae from consumption of pollen containing Cry1Da2 deposited onto larval host plants surrounding DAS1131 maize fields, this potential for effects exists only 1-2 weeks of the year, and therefore is not expected to have population level impacts on non-target lepidopteran species.

B. Aquatic Environments

1. Freshwater Fish and Invertebrates

The EPA has determined that there is a reasonable expectation of no discernible effects to occur to freshwater aquatic invertebrate and fish species as a result of the use of Cry1Da2 as expressed in DAS1131 maize. This finding is based on bioassays and several lines of evidence that support both a lack of hazard and exposure to the protein for these two groups of freshwater species.

Exposure in aquatic environments is possible in bodies of freshwater near corn growing areas, but as discussed in **Section III.D.2.**, is expected to be negligible. Exposure 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 AI is anticipated to have largely leached out of the tissue (Carstens et al. 2012; US EPA 2010a). Finally, the rapid degradation that Cry1Da2 experienced in soil (see **Section III.B**) and the likely similar rapid degradation the protein would experience in aquatic environments (due to both abiotic [e.g., photodegradation, pH, temperature] and biotic [e.g., microbes] factors) 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 was conducted on the non-target freshwater invertebrate, *Daphnia magna*, to Cry1Da2 protein and no adverse effects were seen at the highest test concentration (4 mg/L diet). The MOE for *D. magna* was 7X the worst-case EEC. 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 (described in Table 6). 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 (Table 5).

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.

2. Marine/Estuarine Fish and Invertebrates

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.

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 (USEPA 2016a). At this time, there is no information to indicate that this assumption would not apply to Cry1Da2 as expressed in DAS1131 maize. Therefore, EPA accepted a waiver rationale from the applicant for this toxicity testing with these taxa.

The data waiver rationale for Tier 1 toxicity testing with marine/estuarine fish and invertebrates expounded on the specificity of the AI, soil degradation profile, likelihood of AI degradation in aquatic environments, and the lack of effects in the *Daphnia magna* toxicity test.

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 marine or estuarine fish and invertebrate species from Cry1Da2 as expressed in DAS1131.

C. Probability of Outcrossing and Weediness of DAS1131 Corn

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 2001). 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.

D. Environmental Risk Conclusions

EPA considered possible routes of exposure to Cry1Da2 as expressed in DAS1131, including the likelihood of a hazard from contact with or the consumption of all possible parts of the corn tissue, as well as the likelihood of a hazard from the possible reduction of on-field targeted lepidopteran pests leading to a possible reduction in a non-target organisms' food source or a reduction in pollination services. EPA then evaluated risk by examining the possible hazards and possible routes of exposure in conjunction. In events where exposure may be possible, but no hazard is identified, risk is concluded to be negligible.

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 non-target organisms based on toxicity studies indicating no biologically meaningful effects upon any taxa outside of the Lepidopteran order. Additionally, indirect effects to non-target organisms 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-target organisms that consume lepidoptera. Therefore, due to the lack of direct or indirect effects, there is a reasonable expectation of no discernible effect for non-lepidopteran non-target organisms as a result of the Section 3 seed-increase for the Cry1Da2 protein as expressed in DAS1131.

Regarding non-target lepidopteran species, given that standard management practices limit non-crop plant abundance (i.e., potential non-target lepidopteran host plants) within agricultural fields, EPA concludes that the potential for effects to any non-target lepidopteran organisms from Cry1Da2 in DAS1131 maize pollen (i.e., the main source of exposure for non-target lepidopterans as they do not consume maize leaf tissue) are limited to areas within 15 m of the edge during the 1-2 week timespan of the year in which pollen is shed (see **Section III.C., Section IV.D.1., and Section IV.A.2.d.**). In addition to being temporally limited, this potential effect is also geographically limited, as the proposed seed increase is limited to 250,000 acres across the United States, with 250,000 acres representing less than 0.1% of the total acreage of corn grown in the United States (2024 U.S. Census Bureau). In summary, although there is the potential for effects to non-target lepidopteran larvae from consumption of pollen containing Cry1Da2 deposited onto larval host plants surrounding DAS1131 maize fields, this potential for effects exists only 1-2 weeks of the year and is limited due to the limited dispersal of corn pollen off-field. Therefore, risk of population-level effects to non-target beneficial lepidopteran species are not expected as a result of the Section 3 seed-increase for the Cry1Da2 protein as expressed in DAS1131.

VII. Risk to Federally Listed Threatened and Endangered Species

"No Effect" finding for the 37 lepidopteran TES present in the continental United States, Hawaii, and Puerto Rico where production associated with the Section 3 seed increase for DAS1131 maize may occur.

A. Introduction

The combination of scientific rationale, bioassay results, and toxicity studies indicate the specificity of the Cry1Da2 protein in the DAS1131 maize event to lepidopteran species. Therefore, the purpose of the below evaluation is to assess the effects of the cultivation of DAS1131 maize that expresses Cry1Da2 on threatened and endangered species (TES), specifically in the order of Lepidoptera, and their designated critical habitat. As the applicant excluded California from the registration request, EPA only evaluated the potential impact(s) of DAS1131 maize to the 37 lepidopteran TES currently present in the continental United States, Hawaii, and Puerto Rico (USFWS, 2023a; USFWS, 2023b) (Table 7).

Table 7. Lepidopteran TES in the continental United States, Hawaii, and Puerto Rico

Common Name	Scientific Name	ESA Listing Status	ESA Listing Date
El Segundo blue butterfly	*Euphilotes battoides allyni*	Endangered	6/8/1976
Karner blue butterfly	*Lycaeides melissa samuelis*	Endangered	12/14/1992
Lange's metalmark butterfly	*Apodemia mormo langei*	Endangered	6/8/1976
Lotis blue butterfly	*Lycaeides argyrognomon lotis*	Endangered	6/8/1976
Mission blue butterfly	*Icaricia icarioides missionensis*	Endangered	6/8/1976
Mitchell's satyr butterfly	*Neonympha mitchellii mitchellii*	Endangered	6/25/1991
Myrtle's silverspot butterfly	*Speyeria zerene myrtleae*	Endangered	6/22/1992
Quino checkerspot butterfly	*Euphydryas editha quino* (=*E. e. wrighti*)	Endangered	1/16/1997
San Bruno elfin butterfly	*Callophrys mossii bayensis*	Endangered	6/8/1976
Smith's blue butterfly	*Euphilotes enoptes smithi*	Endangered	6/8/1976
Schaus swallowtail butterfly	*Heraclides aristodemus ponceanus*	Endangered	4/28/1976
Callippe silverspot butterfly	*Speyeria callippe callippe*	Endangered	12/5/1997
Oregon silverspot butterfly	*Speyeria zerene hippolyta*	Threatened	7/2/1980
Palos Verdes blue butterfly	*Glaucopsyche lygdamus palosverdesensis*	Endangered	7/2/1980
Kern primrose sphinx moth	*Euproserpinus euterpe*	Threatened	4/8/1980
Pawnee montane skipper	*Hesperia leonardus montana*	Threatened	9/25/1987

Common Name	Scientific Name	ESA Listing Status	ESA Listing Date
Uncompahgre fritillary butterfly	*Boloria acrocnema*	Endangered	6/24/1991
Bay checkerspot butterfly	*Euphydryas editha bayensis*	Threatened	9/18/1987
Behren's silverspot butterfly	*Speyeria zerene behrensii*	Endangered	12/5/1997
Blackburn's sphinx moth	*Manduca blackburni*	Endangered	2/1/2000
Fender's blue butterfly	*Icaricia icarioides fenderi*	Endangered	1/25/2000
Laguna Mountains skipper	*Pyrgus ruralis lagunae*	Endangered	1/16/1997
Saint Francis' satyr butterfly	*Neonympha mitchellii francisci*	Endangered	4/18/1994
Carson wandering skipper	*Pseudocopaeodes eunus obscurus*	Endangered	11/29/2001
Sacramento Mountains checkerspot butterfly	*Euphydryas anicia cloudcrofti*	Endangered	3/2/2023
Hermes copper butterfly	*Lycaena hermes*	Threatened	1/20/2022
Dakota skipper	*Hesperia dacotae*	Threatened	11/24/2014
Miami Blue butterfly	*Cyclargus* (=*Hemiargus*) *thomasi bethunebakeri*	Endangered	4/6/2012
Bartram's hairstreak butterfly	*Strymon acis bartrami*	Endangered	9/11/2014
Island marble butterfly	*Euchloe ausonides insulanus*	Endangered	6/4/2020
Bog buck moth	*Hemileuca maia menyanthevora*	Endangered	4/14/2023
Taylor's (=whulge) checkerspot	*Euphydryas editha taylori*	Endangered	11/4/2013
Florida leafwing butterfly	*Anaea troglodyta floridalis*	Endangered	9/11/2014
Mount Charleston blue butterfly	*Icaricia* (*Plebejus*) *shasta charlestonensis*	Endangered	10/21/2013
Puerto Rico harlequin butterfly	*Atlantea tulita*	Threatened	1/3/2023
Poweshiek skipperling	*Oarisma poweshiek*	Endangered	11/24/2014
Silverspot	*Speyeria nokomis nokomis*	Threatened	3/18/2024

Source: US FWS 2024(a) and (b); US EPA 2024 (a) and (b).

B. Exposure and Hazard Summary

1. Risk Characterization

Given its confinement within plant tissue, exposure of Cry1Da2 to lepidopteran TES is expected to be limited to direct consumption of DAS 1131 maize. Non-target lepidopteran species are generally not known to consume maize tissue, as their feeding and reproductive ecology is generally tightly associated with a preferred host plant (EPA 2008). Federally listed lepidopteran TES do not use maize for any of their life stages, nor do they inhabit corn fields. It is possible, however, that their host plants and/or habitat could be in proximity of corn fields, which may result in exposure to the event proposed for registration. Because host plants of listed lepidoptera are not located within cornfields (see Appendix A for details) exposure of lepidopteran TES to the Cry1Da2 protein would most likely occur via off-field movement and deposition of pollen on plants adjacent to the field, utilized by TES for foraging.

Although insect species susceptibility to an endotoxin protein can vary within a taxonomic order (Peterson et al. 2006), the monarch butterfly was used as a surrogate species since laboratory toxicity data were available for this species for use in the risk assessment, with the assumption that lepidopteran TES will have the same sensitivity.

In the bioassay assessing biological activity with monarch butterfly larvae, the LC_{50} value for Cry1Da2 was determined to be 0.0029 ng/mg diet (CI 0.00242 – 0.00339 ng Cry1Da2 protein/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 based on 15 m from the edge of the cornfield to produce the MOE of 1.02X the EEC for *D. plexippus*. Use of milkweed as a larval host plant 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.

There is also an important temporal aspect of pollen deposition to consider when placing the potential for risk into a realistic context. Corn pollen shed is limited to 1-2 weeks of the year, so for the majority of the corn growing and harvest season, there is no expected exposure and therefore no reasonable expectation for discernible effects to lepidopteran TES species from Cry1Da2 as expressed in DAS1131 maize.

2. Overlap Analysis

The Agency has developed the Use Data Layer (UDL) Overlap Tool (US EPA 2024(a) and (b)) in order to support the assessment of TES by summarizing the co-occurrence between potential pesticide use sites and species location. This tool uses GIS based datasets when considering co-occurrence and results give an overlap percentage (*i.e.,* the area of potential use and off-site movement found within the species location divided by the total area for the species). Generated using a number of publicly available land cover datasets, the EPA UDL Overlap Tool delineates the potential pesticide use locations and areas where off-site movement could occur following application. The datasets delineating species range and designated critical habitat are provided by the U.S Fish and Wildlife Service (US FWS) and National Marine Fisheries Services (NMFS) of NOAA. This tool generates outputs for species ranges and designated critical habitats separately, with the resulting percent overlaps summarized by use and off-site distance.

When assessing impacts to TES, the outputs from this tool are utilized to set the overlap category, as low (<5%), medium (<10% but >5%) or high (>10%). Any overlap below 1% generally supports a “No Effect” determination, because 1% is within the error bounds of spatial datasets when considering accuracy and precision (US EPA 2024(a)). Per the guidance (US EPA 2024(a)), for the purposes of rounding, a percent overlap that is ≤0.44% is treated as 0%.

A dataset of known corn crop production within the continental United States (U.S. EPA(a) 2023) and a dataset of known agricultural production within Hawaii and Puerto Rico (U.S. EPA(b) 2023) was compared against the ranges of lepidopteran TES to identify potential range overlap (up to 30 meters away from corn/agricultural production sites to account for pollen dispersal as potential source of exposure) (Table 8). As the Hawaiian and Puerto Rican agricultural production dataset includes crops other than corn, this dataset represents an extremely conservative exposure scenario.

Table 8. Lepidopteran TES range overlap with corn crop production (within the continental United States) or agricultural production (within Hawaii and Puerto Rico).

Common Name	Scientific Name	30m Range Overlap (%)	30m Critical Habitat Overlap (%)
Lepidopterans with ≥1% (>0.44%) range or critical habitat overlap with areas of corn crop or agricultural production			
Karner blue butterfly	*Lycaeides melissa samuelis*	34.33	0.0000
Mitchell's satyr butterfly	*Neonympha mitchellii mitchellii*	31.46	NA
Dakota skipper	*Hesperia dacotae*	25.84	6.7287
Poweshiek skipperling	*Oarisma poweshiek*	21.22	8.3975
Bog buck moth	*Hemileuca maia menyanthevora*	8.34	NA
Blackburn's sphinx moth	*Manduca blackburni*	6.85[1]	0.0000
Lange's metalmark butterfly	*Apodemia mormo langei*	2.077	0.0000
Saint Francis' satyr butterfly	*Neonympha mitchellii francisci*	3.29	NA
Fender's blue butterfly	*Icaricia icarioides fenderi*	3.18	0.2364
Bay checkerspot butterfly	*Euphydryas editha bayensis*	1.06	0.136
Taylor's (=whulge) checkerspot	*Euphydryas editha taylori*	1.48	1.4808
Puerto Rican harlequin butterfly	*Atlantea tulita*	0.510[2]	0.4587
Island marble butterfly	*Euchloe ausonides insulanus*	0.196	0.8191
Silverspot butterfly	*Speyeria nokomis nokomis*	0.518	NA
Smith's blue butterfly	*Euphilotes enoptes smithi*	0.459	0.0000
Lepidopterans with <1% (≤0.44%) range and critical habitat overlap with areas of corn crop or agricultural production			
Kern primrose sphinx moth	*Euproserpinus euterpe*	0.347	0.0000
Callippe silverspot butterfly	*Speyeria callippe callippe*	0.254	0.0000
Oregon silverspot butterfly	*Speyeria zerene hippolyta*	0.145	0.0000
Florida leafwing butterfly	*Anaea troglodyta floridalis*	0.149	0.0000
Bartram's hairstreak butterfly	*Strymon acis bartrami*	0.140	0.0000
Mission blue butterfly	*Icaricia icarioides missionensis*	0.073	0.0000
Myrtle's silverspot butterfly	*Speyeria zerene myrtleae*	0.049	0.0000
Carson wandering skipper	*Pseudocopaeodes eunus obscurus*	0.049	NA
Sacramento Mountains checkerspot butterfly	*Euphydryas anicia cloudcrofti*	0.043	0.0245
Miami blue butterfly	*Cyclargus (=Hemiargus) thomasi bethunebakeri*	0.045	NA
San Bruno elfin butterfly	*Callophrys mossii bayensis*	0.019	0.0000
El Segundo blue butterfly	*Euphilotes battoides allyni*	0.012	0.0000
Pawnee montane skipper	*Hesperia leonardus montana*	0.012	0.0000
Behren's silverspot butterfly	*Speyeria zerene behrensii*	0.0097	0.0000
Uncompahgre fritillary butterfly	*Boloria acrocnema*	0.0082	NA

Common Name	Scientific Name	30m Range Overlap (%)	30m Critical Habitat Overlap (%)
Quino checkerspot butterfly	*Euphydryas editha quino (=E. e. wrighti)*	0.0071	0.0000
Lotis blue butterfly	*Lycaeides argyrognomon lotis*	0.0039	0.0000
Hermes copper butterfly	*Lycaena hermes*	0.0000	0.0000
Laguna Mountains skipper	*Pyrgus ruralis lagunae*	0.0000	0.0000
Palos Verdes blue butterfly	*Glaucopsyche lygdamus palosverdesensis*	0.0000	0.0000
Schaus swallowtail butterfly	*Heraclides aristodemus ponceanus*	0.0000	NA
Mount Charleston blue butterfly	*Icaricia (Plebejus) shasta charlestonensis*	0.0000	0.0000

NA = Critical habitat type data was either not available in the UDL or was listed as “not prudent.”
[1]0.17% overlap corn crop production (USDA(a) 2024).
[2]0.081% overlap corn crop production (USDA(b) 2024).

C. Individual TES Assessment

Based on the above analysis, 15 of the 37 lepidopteran TES ranges overlap more than 1% (>0.44% when accounting for rounding) with areas of known corn crop or agricultural production (within 30 meters of such production; Table 8). Conversely, no overlap was identified for five species (i.e., species with 0% overlap; Table 8). Additionally, negligible overlap was identified for 17 species (i.e., species with less than 1% (≤0.44%) overlap; Table 8), which, per the EPA’s guidance on interpretation of UDL overlaps, ≤0.44% is treated as 0% and supports a “No Effect” determination, because this is within the error bounds of spatial datasets when considering accuracy and precision (U.S. EPA, 2023c). Thus, the EPA determines that DAS1131 maize will have no effect on the 22 lepidopteran TES (designated in Table 8 as <1% overlap) with no or negligible overlap nor their critical habitats due to the expectation of no exposure.

The remaining 15 lepidopteran TES showed some degree of overlap and further in-depth biological evaluations were conducted on their life-cycle, habitat requirements, temporal overlap with corn pollen shed, geographical isolation, host-plant specificity and distribution, feeding patterns, and flight pattern dispersal. Summaries of the assessments of these lepidopteran TES are described in the sections below, and a detailed analysis of the 15 lepidopteran TES with overlap ≥1% that were evaluated can be found in Appendix A.

1. Karner blue butterfly (*Lycaeides melissa samuelis*) – 34.33% Range overlap

Karner blue butterflies once occurred in a nearly continuous narrow band across 12 states and the province of Ontario, Canada, but it has been eliminated from seven of those states and Ontario. Today it is found in portions of New Hampshire, New York, Michigan, Wisconsin, and Ohio. The spatial overlap analysis of the species’ range and maize production can be found in Appendix A, and the maize UDL overlap calculation is 34.33% (categorized as high overlap).

While the species is limited to small, fragmented ranges over New Hampshire, New York, Michigan, Wisconsin and Ohio, its habitat includes grasslands (USFWS(a) 2023) and its primary larval food source/host plants are typically found in dry sandy openings including woodland clearings or disturbed areas (Meyer, R. 2006). Previous EPA analyses determined that the potential exposure of Karner blue to PIP corn pollen is limited because corn and lupine do not generally overlap. EPA found that wild lupine does not occur at all in corn fields nor is wild lupine expected to grow adjacent to corn fields (EPA 2001, 2010). This determination was based on communications with state natural resources department and conservation groups, as well as a published survey which found that five Karner blue populations in Winona County, Minnesota were a mean distance of 660 meters from the nearest agricultural lands (Andow, et al. 1995)- a distance much further than the expected pollen deposition range. There is no new information to alter these conclusions.

Furthermore, temporal overlap analysis of Karner blue oviposition with corn pollen shed is minimal to nonexistent. Karner blue lifecycle includes two generations per year, with the first generation of Karner blue butterflies emerging in mid-April, prior to pollen anthesis. The second generation larvae emerge in June-July where there is some potential for pollen-shed overlap depending on geography/climate (EPA 2001). However, EPA's 2010 *Bt* corn reassessment summarized a data analysis of the comparison of overlap between pollen shed and larval stages in Wisconsin, Michigan, Minnesota, Indiana, and New York, with most locations having no overlap, and for other counties the potential for overlap being sporadic (i.e., "does not happen every year nor for more than a day or two in the life of the feeding larvae").

This extremely limited window of potential overlap between pollen shed and the larval lifestage, coupled with the expectation that the larval host plant is not found in or adjacent to corn fields, indicates exposure of Cry1Da2 pollen to one brood of the Karner blue butterfly's annual life-cycle is negligible.

Therefore, EPA's analysis has determined that negligible exposure is expected for the Karner blue butterfly from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the Karner blue butterfly and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

2. Mitchell's satyr butterfly (*Neonympha mitchellii mitchellii*) – 31.46% Range overlap

The Mitchell's Satyr Butterfly is one of the most geographically restricted butterflies in North America. (FR 1991). Today, the butterfly can be found in only nine locations in Michigan and one location in Indiana, along with a single county in Virginia and Ohio, and restricted areas within Mississippi and Alabama (USFWS 2021, USFWS(c) 2024; USFWS(d) 2024). The spatial overlap analysis of the species' range and maize production can be found in Appendix A, and the maize UDL overlap calculation is 31.46% (categorized as high overlap).

The Mitchell's Satyr Butterfly habitat is restricted to rare wetlands called *fens,* which are low nutrient wetlands that receive carbonate-rich ground water from seeps and springs. The southern populations are typically associated with beaver-influenced wetlands that are sedge dominated, and occasionally semi-open riparian or floodplain forest areas (USFWS 2020). In the most recent 5-year review of the species, Mitchell's satyr butterfly was found to only occupy areas with approximately 70% ground cover, 55% canopy cover, and 60% sedge cover in the rare wetland communities throughout its current species range (USFWS 2021). These types of habitat requirements are not conducive in or near corn cultivated areas. Mitchell's satyr butterfly is also very small in size (3.8 to 4.4 wingspan), limiting its ability to venture far from its required habitat. Furthermore, Mitchell's satyr caterpillars feed on one or more species of grass-like plants called sedges. Concern surrounding pollen deposition onto larval host plants is more relevant for lepidoptera whose host plants have wider, horizontal leaves. As the larval host plants of the Mitchell's satyr butterfly are grass-like, they are not a shape that is conducive to pollen accumulation.

Due to the severe geographical restriction, rare fen habitat with specialized requirements, limited ranges in small locations in only a few states, reduced flight range due to its relatively small size, and the shape of the larval host plants (grass-like sedges) not being conducive to pollen accumulation, the likelihood of larvae of the Mitchell's Satyr Butterfly coming into contact with pollen from a maize producing field is negligible

Therefore, EPA's analysis has determined that negligible exposure is expected for the Mitchell's satyr butterfly from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the Mitchell's satyr butterfly and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

3. Dakota skipper (*Hesperia dacotae*) – 25.84% Range overlap

The Dakota Skipper is located in Minnesota, North Dakota, and South Dakota (USFWS(a) 2023). The spatial overlap analysis of the species' range and maize production can be found Appendix A, and the maize UDL overlap calculation is 25.84% (categorized as high overlap).

The Dakota Skipper is not adapted to survive in corn fields or directly bordering corn fields, which are disturbed areas. Dakota skippers are obligate residents of undisturbed (remnant, untilled) high-quality prairie, ranging from wet-mesic tallgrass prairie to dry-mesic mixed-grass prairie (USFWS(e) 2024). Soils unsuitable for agriculture and steep topography have allowed remnant native prairie habitats inhabited by Dakota skippers to persist. Loss of native prairie and degradation of remaining habitat have led to the decline of the butterfly (USFWS 2018) Dakota Skipper caterpillars only on feed several native grass species; little bluestem (*Schizachyrium scoparium*) Panicum spp., Poa spp., and other native grasses (USFWS(e) 2024). Since Dakota skippers are obligate residents of undisturbed high-quality prarie, unable to survive in agricultural environments, and their larval food source is also found in the same type of habitat, compounded with the fact this species is not found in or adjacent to corn fields, the likelihood of this butterfly being exposed to pollen drift from a maize producing field is negligible (USFWS(e) 2024).

The Dakota skipper's life-cycle further limits any potential for temporal overlap of the timing of pollen shed. While the eggs are laid during June through July, once hatched, larvae build shelters near or underground and only emerge at night to feed, and as stated above, they only feed on the native grasses defined as their host plants (USFWS(e) 2024). Concern surrounding pollen deposition onto larval host plants is more relevant for lepidoptera whose host plants have wider, horizontal leaves. As the larval host plants of the Dakota skipper are grasses, they are not a shape that is conducive to pollen accumulation. Since peak corn pollen shed occurs in the morning and again in the late afternoon (Nielsen 2020), the larvae would not encounter active pollen shed during the evening when they are feeding.

Therefore, based on the Dakota skipper's larval host plants not being expected to be in or adjacent to corn fields coupled with the shape of the larval host plants not being conducive to pollen accumulation, EPA's analysis has determined that negligible exposure is expected for the Dakota skipper from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the Dakota skipper and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

4. Blackburn's sphinx (*Manduca blackburni*) – 6.85% Range overlap (all agriculture); 0.17% range overlap agricultural corn USDA(a) (2024)

The Blackburn's sphinx moth is likely more widespread than originally known at the time of listing. The species is found on three different islands in a variety of habitats using multiple larval host species, both native and non-native. Surveys on the island of Hawaii have found high densities of the species, which project to a large and robust population across the area of potential habitat. The spatial overlap analysis of the species' range and maize production can be found in Appendix A, and the UDL overlap calculation is 6.85% (categorized as medium overlap). Blackburn's sphinx moths diet consists of members of the nightshade family (Solanaceae), and to date have not been observed to feed on maize plants (USFWS(f) 2024, Federal Register(a) 2000).

While the UDL overlap calculation is 6.853% (categorized as medium overlap), this calculation encompasses a dataset that encompasses all agricultural production, not just maize production, and is therefore an extremely conservative exposure scenario. A dataset for all agricultural production was initially used because the corn crop data layer used in the UDL overlap percent calculations for the other TES assessments did not contain agricultural corn production for the state of Hawaii (or Puerto Rico). However, the most recent census of agriculture in Hawaii lists corn production (seed only) at 2176 acres (USDA(a) 2024). Using this value places the potential overlap well below the 1% threshold (0.17% overlap). Consequently, the likelihood of this species being exposed to pollen drift from event DAS1131 maize producing field is negligible.

Therefore, EPA's analysis has determined that negligible exposure is expected for Blackburn's sphinx moth from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the Blackburn's sphinx moth and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

5. Poweshiek skipperling (*Oarisma poweshiek*) – 21.22% Range overlap

Once found in in Iowa, Michigan, Minnesota, North Dakota, South Dakota, and Wisconsin, the Poweshiek Skipperling' range has now been reduced to just several small locations in Michigan and only one county in Wisconsin in the United States (USFWS(c) 2022). The spatial overlap analysis of the species' range and maize production can be found in Appendix A, and the maize UDL overlap calculation is 21.22% (categorized as high overlap).

The Poweshiek Skipperling's habitat includes prairie fens, grassy lake and stream margins, moist meadows, sedge meadow, and wet to dry prairie (FR 2014). Its primary larval food source/host plant is prairie dropseed (*Sporobolus heterolepis*), a prairie grass. However, observations have been made of ovipositing in Michigan on other prairie fen and grassland plants (*M. richardsonis, M. glomerata [marsh muhly], C. sterilis [dioecious sedge],* and *Dasiphora fruticosa [shrubby cinquefoil]*). (USFWS(h) 2024). Field observations and laboratory studies indicate that first instar larvae seem to require or prefer initial feeding on "very fine, threadlike blade tip[s of grasses]" (USFWS(h). 2024)

In addition to its limited geographical range, Poweshiek skipperling are also not known to disperse widely, with estimated maximum dispersal distance of approximately 0.6 miles between patches of prairie habitat. Additionally, considering the isolation of the species, these small populations need immigration corridors, and evidence shows that they will not travel across unsuitable habitat, which includes row crops such as corn (Federal Register(a) 2013). In fact, dispersal grassland habitat consists of "undeveloped open areas dominated by perennial grassland with limited or no barriers to dispersal including tree or shrub cover less than 25 percent of the area and no row crops such as corn, beans, potatoes, or sunflowers" (Federal Register(a) 2013).

Finally, the Poweshiek skipperling adults emerge mid-June to early-July and typically only have a flight period of about two to four weeks. This limited flight period combined with their low dispersal rate and avoidance of unsuitable habitat (such as row crops) indicates that the likelihood of adult Poweshiek skipperlings laying eggs on their host plant(s) in or adjacent to corn fields is negligible. Furthermore, concern surrounding pollen deposition onto larval host plants is more relevant for lepidoptera whose host plants have wider, horizontal leaves. As the larval host plants of the Poweshiek skipperling tend to be grasses, with early instars indicating a need or preference for threadlike thin blades of grass, these host plants are not a shape that is conducive to pollen accumulation.

Therefore, based on the Poweshiek skipperling's oviposition not being expected to be in or adjacent to corn fields coupled with the shape of the larval host plants not being conducive to pollen accumulation, EPA's analysis has determined that negligible exposure is expected Poweshiek skipperling from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the Poweshiek and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

6. Bog buck moth (*Hemileuca maia menyanthevora*) – 8.34% Range overlap

The bog buck moth's habitat consists of groundwater-fed wetlands in Oswego County, New York in the United states. Bog buck moth caterpillars' key food source is bog buckbean (*Menyanthes trifoliata*) (USFWS(i) 2024). The spatial overlap analysis of the species' range and maize production can be found in Appendix A, and the maize UDL overlap calculation is 8.34% (categorized as Medium overlap).

The bog buck moth is restricted to its specialized wetland habitat, located in only one county in New York, with its primary larval food source, bog buckbean, only located within this specialized wetland habitat. Given the limited location of this primary resource, coupled with the very small body size of the moth (wingspans of 5-7 cm), evidence suggests it rarely leaves its habitat. Given this habitat and larval food plant restriction, the likelihood of larvae of this moth coming into contact with pollen from a maize producing field is negligible.

Therefore, EPA's analysis has determined that negligible exposure is expected for bog buck moth from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the bog buck moth and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

7. Saint Francis' satyr butterfly (*Neonympha mitchellii francisci*) – 3.29% Range overlap

The Saint Francis' Satyr Butterfly is extremely restricted geographically. The habitat occupied by this satyr consists primarily of wide, wet meadows dominated by sedges and other wetland graminoids, maintained naturally by fire and beaver. The species is very dependent on disturbance and dynamic environments. Only a single metapopulation of Saint Francis' satyr is known to exist on training grounds of Ft. Bragg in the sandhills of North Carolina, in Cumberland and Hoke Counties (USFWS(c) 2021). The spatial overlap analysis of the species' range and maize production can be found in Appendix A, and the maize UDL overlap calculation is 3.29% (categorized as low overlap); however, the restriction of the species to its to a location on a military installation makes UDL overlap estimate unrealistic.

The extreme isolation and restriction solely to the training grounds of Ft. Bragg in the sandhills of North Carolina makes the likelihood of this butterfly being exposed to pollen drift from event DAS1131 maize practically nonexistent.

Therefore, EPA's analysis has determined that negligible exposure is expected for Saint Francis' satyr butterfly from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the Saint Francis' satyr butterfly and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

8. Fender's blue butterfly (*Icaricia icarioides fenderi*) – 3.18% Range overlap

The Fender's Blue Butterfly habitat is found only in the prairie and oak savannah habitats of the Willamette Valley of Oregon (USFWS(d) 2021). The caterpillar's primary food plant is the Kincaid's lupine (*Lupinus sulphureus ssp. kincaidii*) with only two other known host plants- *Lupinus arbustus* (longspur lupine) and *Lupinus albicaulis* (sickle-keeled lupine) (Federal Register(b) 2000). Kincaid's lupine is also endemic to the Willamette Valley and is relatively rare as it is listed as a threatened species under the Act (Federal Register (b) 2000). The spatial overlap analysis of the species' range and maize production can be found in Appendix A, and the maize UDL overlap calculation is 3.18% (categorized as low overlap).

The Fender’s Blue Butterfly has only one generation per year, with adult butterflies laying eggs during the month of May and larvae feeding briefly on host plants until the plants senesce in early July. After this time, the larvae drop to the base of the plants and enter diapause and do not feed on leaves until new plants emerge in February or March. Therefore, like the Karner blue butterfly which also feeds on a lupine species, the temporal overlap of Fender’s Blue Butterfly larval feeding and corn pollen shed is expected to be minimal to nonexistent.

Furthermore, the Fender’s Blue Butterfly range is highly restricted to specific locations within the Willamette Valley in Oregon. Additionally, as the butterfly is dependent on another endangered species, the Kincaid’s lupine, as its primary food source. Kincaid’s lupine is also restricted to its critical habitat, which has a maize UDL overlap of 0.0% and 0.057% at 0 and 30 m off-field, indicating that the host plant is not expected to be in or adjacent to corn fields. Finally, the potential flight range of this species is minimal, it’s expected range is limited to its critical habitat. This limited geographic range results in a negligible likelihood of this butterfly being exposed to pollen drift from event DAS1131 maize.

Therefore, the EPA’s analysis has determined that negligible exposure is expected for Fender’s blue butterfly from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a “No Effect” determination under the ESA for the Fender’s blue butterfly and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

9. Lange’s metalmark butterfly (*Apodemia mormo langei*) – 2.077% Range overlap

The Lange’s metalmark butterfly is extremely restricted geographically and can only be found in the Antioch Dunes National Wildlife Refuge (ADNWR). The habitat occupied by this butterfly consists of sand dunes, canyons, and valleys. The spatial overlap analysis of the species’ range and maize production can be found in Appendix A, and the maize UDL overlap calculation is 2.077% for the species (categorized as low overlap).

The species is entirely dependent on its host plant Antioch Dunes buckwheat for all of its life stages and is endemic to the Antioch Dunes. Antioch Dunes buckwheat is a perennial forb that requires sandy, well-drained soils and some form of disturbance, preferably by natural processes such as wind or erosion, to shift the sand for seedling establishment (USFWS 2020). Furthermore, adult butterflies typically emerge from early August to mid-or late September, during which time they lay eggs. The eggs remain dormant until the first part of the rainy season, which occurs in the fall after the corn pollen shed period. Therefore, there is no reasonable expectation of exposure to Lange’s metalmark butterfly larvae from pollen deposition.

The extreme isolation and restriction solely to the ADNWR, the specific habitat requirements of its host plant, and the lack of temporal overlap between larvae and corn pollen shed makes the likelihood of this butterfly being exposed to pollen drift from event DAS1131 maize negligible to nonexistent.

Therefore, EPA's analysis has determined that negligible exposure is expected for Lange's metalmark butterfly from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the Lange's metalmark butterfly and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

10. Taylor's (=whulge) checkerspot (*Euphydryas editha taylori*) – 1.48% Range overlap

The Taylor's (=whulge) Checkerspot butterfly was historically known to occur in Washington, and Oregon, and its current distribution represents a reduction from over 80 locations range-wide to 14. The spatial overlap analysis of the species' range and maize production can be found in Appendix A, and the maize UDL overlap calculation is 1.48% (categorized as low overlap).

Taylor's checkerspots produce one brood per year, with larvae entering diapause in mid-June to early July, indicating that the temporal overlap of larval feeding and corn pollen shed is expected to be minimal to nonexistent. Furthermore, Taylor's (=whulge) checkerspot butterfly is now only found in 14 isolated locations in Washington and Oregon, and their habitat is specific to open prairies and Garry oak meadows and balds, not agricultural fields. Given the habitat specialization and minimal to nonexistent temporal overlap between pollen shed and larval feeding, the likelihood of this butterfly being exposed to pollen drift from event DAS1131 maize is negligible.

Therefore, EPA's analysis has determined that negligible exposure is expected for Taylor's (=whulge) checkerspot from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the Taylor's (=whulge) checkerspot and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

11. Bay checkerspot butterfly (*Euphydryas editha bayensis*) – 1.061% Range overlap

The bay checkerspot butterfly was only found in Santa Clara County until recently when it was reintroduced to San Bruno Mountain and Edgewood County Park in San Mateo County, California. The Bay checkerspot butterfly requires areas with topographic diversity, which are defined as having warm south and west slopes, as well as cool north and east slopes, because some slopes become unfavorable depending on annual weather conditions and time of year. The spatial overlap analysis of the species' range and maize production can be found in Appendix A, and the maize UDL overlap calculation is 1.061% (categorized as low overlap).

Bay checkerspot caterpillars go through two different phases of feeding with a pause in between. The first is just after they hatch between March and May, where they will feed until they have molted three times. Larvae enter diapause and spend the summer in cracks and crevices or under rocks. Then after going dormant for the hot and dry months of summer and fall, they wake up sometime in November to February, and eat more until finally making their chrysalis in early spring.

Given the habitat specialization and nonexistent temporal overlap between pollen shed and larval feeding, the likelihood of this butterfly being exposed to pollen drift from event DAS1131 maize is negligible.

Therefore, EPA's analysis has determined that negligible exposure is expected for bay checkerspot from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the bay checkerspot and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

12. Puerto Rican harlequin butterfly (*Atlantea tulita*) – 0.510% Range overlap (all agriculture); 0.081% range overlap agricultural corn USDA(b) (2024)

The Puerto Rican harlequin butterfly has a specialized diet (prickly bush) that does not include the consumption of corn (Appendix A). While the UDL overlap calculation is 0.510% (categorized as low overlap), this calculation uses a dataset that encompasses all agricultural production, not just maize production, and is therefore an extremely conservative exposure scenario. A dataset for all agricultural production was initially used because the corn crop data layer used in the UDL overlap percent calculations for the other TES assessments did not contain agricultural corn production for Puerto Rico (or Hawaii). While there is no commercial corn data layer available for Puerto Rico, the most recent census of agriculture in Puerto Rico lists corn production (seed only) at 441 acres (USDA(b) 2024). Using this value places potential overlap well below the 1% threshold (0.081% overlap). Consequently, the likelihood of this butterfly being exposed to pollen drift from event DAS1131 maize producing field is negligible.

Therefore, EPA's analysis has determined that negligible exposure is expected for Puerto Rican harlequin butterfly from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the Puerto Rican harlequin butterfly and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

13. Smith's blue butterfly (*Eupholotes enoptes smithi*) – 0.459% Range overlap;

The Smith's blue butterfly is restricted to scattered colonies along approximately 93 mi of California's Central Coast from Monterey County to San Luis Obispo County. Its habitat consists of sand dunes and cliffside chaparral. The spatial overlap analysis of the species' range and maize production can be found in Appendix A, and the maize UDL overlap calculation is 0.459% (categorized as low overlap).

The Smith's blue butterfly adults are quite sedentary, traveling only on average 300 feet. Individual adult males and females live approximately one week, and both sexes spend the majority of their time on the buckwheat flowerheads. Adult Smith's blues use the flowerheads to perch, bask, forage for nectar, search for mates, and reproduce. Additionally, larvae are even more stationary and in most cases spend their entire development on a single host plant. Smith's blues rely primarily on coast and seacliff buckwheat as their host plants, with the butterflies' life-cycles synched up with the blooming of the buckwheat flowers. The buckwheat plants require appropriate disturbance regimes along coastal habitats to thrive. These types of habitat requirements are not conducive in or near corn cultivated areas.

Given the habitat specialization, geographical restriction, and host-plant habitat needs, the likelihood of this butterfly being exposed to pollen drift from event DAS1131 maize is negligible.

Therefore, EPA's analysis has determined that negligible exposure is expected for Smith's blue butterfly from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the Smith's blue butterfly and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

14. Island marble butterfly (*Euchloe ausonides insulanus*) – 0.196% Range overlap; 0.819% Critical habitat overlap

The island marble butterfly is extremely restricted geographically. The butterfly is only known to exist on the San Juan Islands in Washington State. (USFWS(b) 2021). The spatial overlap analysis of the species' range and maize production can be found in Appendix A, and the maize UDL overlap calculation is 0.196% for the species range and a critical habitat range of 0.819% (both categorized as low overlap).

The island marble butterfly lives its entire lifecycle in upland prairie-like habitat, sand dunes, or coastal lagoon habitat on the San Juan islands. The extreme isolation and restriction of the species range and critical habitat to solely these islands make the likelihood of this butterfly being exposed to pollen drift from event DAS1131 maize practically nonexistent. Furthermore, the life cycle of this butterfly is unique, in that it spends 10-12 months of the year in its chrysalis, bolstering the expectation that exposure to pollen drift from event DAS1131 maize is negligible to nonexistent.

Therefore, EPA's analysis has determined that negligible exposure is expected for island marble butterfly from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the island marble butterfly and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

15. Silverspot (*Speyeria nokomis nokomis*) – 0.518% Range overlap

The silverspot is limited to ten populations across southwestern Colorado, eastern Utah, and northern New Mexico. The spatial overlap analysis of the species' range and maize production can be found in Appendix A, and the maize UDL overlap calculation is 0.518% for the species (categorized as low overlap).

The restriction of this species is to its wet meadow habitat, which is not typically conducive to agricultural production, and larvae feed exclusively on the bog violet located in these wet meadow habitats. Microhabitat conditions for the bog violet include soggy soil and shade, often under shrubs such as willows (Selby, G. 2007). This restricted habitat and conditions of the larval food plant result in a reasonable expectation of negligible exposure of larvae to corn pollen.

Therefore, EPA's analysis has determined that negligible exposure is expected for silverspot from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the silverspot and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

D. Endangered Species Conclusions

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 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 (Appendix A) 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.

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.

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USFWS(c). 2021. Saint Francis' satyr butterfly (*Neonympha mitchellii francisci*) Species Range Shapefile. Available through: https://ecos.fws.gov/docs/species/shapefiles/usfws_I0MZ_I01_Neonympha_mitchellii_francisci_current_range.zip

USFWS(d). 2021. Fender's blue butterfly (*Icaricia icarioides fenderi*) Species Range Shapefile. Available through: https://ecos.fws.gov/docs/species/shapefiles/usfws_I0IS_I01_Icaricia_icarioides_fenderi_current_range.zip

USFWS(a). 2022. Karner blue butterfly (*Lycaeides melissa samuelis*) Species Range Shapefile. Available through: https://ecos.fws.gov/docs/species/shapefiles/usfws_I00F_I01_Lycaeides_melissa_samuelis_current_range.zip

USFWS(b). 2022. Blackburn's sphinx moth (*Manduca blackburni*) Species Range Shapefile. Available through: https://ecos.fws.gov/docs/species/shapefiles/usfws_I0AL_I01_Manduca_blackburni_current_range.zip

USFWS(c). 2022. Poweshiek skipperling (*Oarisma poweshiek*) Species Range Shapefile. Available through: https://ecos.fws.gov/docs/species/shapefiles/usfws_I0W1_I01_Oarisma_poweshiek_current_range.zip

USFWS(d). 2022. Island marble butterfly (*Euchloe ausonides insulanus*) Species Range. Available through: https://ecos.fws.gov/docs/species/shapefiles/usfws_I0T7_I01_Euchloe_ausonides_insulanus_current_range.zip

USFWS(a). 2023. Dakota skipper (*Hesperia dacotae*) Species Range Shapefile. Available through https://ecos.fws.gov/docs/species/shapefiles/usfws_I011_I01_Hesperia_dacotae_current_range.zip

USFWS(b). 2023. Bog buck moth (*Hemileuca maia menyanthevora*) Species Range Shapefile. Available through: https://ecos.fws.gov/docs/species/shapefiles/usfws_I0NM_I01_Hemileuca_maia_menyanthevora_(=H._iroquois)_current_range.zip

USFWS(a). 2024. Species Profile: Karner blue butterfly (*Lycaeides melissa samuelis*). Available through: https://ecos.fws.gov/ecp/species/6656

USFWS(b). 2024. Karner blue butterfly (*Lycaeides melissa samuelis*). Available through: https://www.fws.gov/species/karner-blue-butterfly-lycaeides-melissa-samuelis

USFWS(c). 2024. Species Profile: Mitchell's satyr butterfly (*Neonympha mitchellii mitchellii*). Available through: https://ecos.fws.gov/ecp/species/8062

USFWS(d). 2024. Mitchell's satyr butterfly (*Neonympha mitchellii mitchellii*). Available through: https://ecos.fws.gov/ecp/species/8062

USFWS(e). 2024. Species Profile: Dakota skipper (*Hesperia dacotae*). Available through: https://ecos.fws.gov/ecp/species/1028

USFWS(f). 2024. Species Profile: Blackburn's sphinx moth (*Manduca blackburni*). Available through: https://ecos.fws.gov/ecp/species/4528

USFWS(g). 2024. Species Profile: Poweshiek skipperling (*Oarisma poweshiek*). Available through: https://ecos.fws.gov/ecp/species/9161

USFWS(h). 2024. Poweshiek skipperling (*Oarisma poweshiek*). Available through: https://www.fws.gov/species/poweshiek-skipperling-oarisma-poweshiek

USFWS(i). 2024. Species Profile: Bog buck moth (*Hemileuca maia menyanthevora*). Available through: https://ecos.fws.gov/ecp/species/8023

USFWS(j). 2024. Species Profile: Saint Francis' satyr butterfly (*Neonympha mitchellii francisci*). Available through: https://ecos.fws.gov/ecp/species/5419

USFWS(k). 2024. Saint Francis' satyr butterfly (*Neonympha mitchellii francisci*). Available through: https://www.fws.gov/species/saint-francis-satyr-butterfly-neonympha-mitchellii-francisci

USFWS(l). 2024. Species Profile: Fender's blue butterfly (*Icaricia icarioides fenderi*). Available through: https://ecos.fws.gov/ecp/species/6659

USFWS(m). 2024. Species Profile: Taylor's (=whulge) checkerspot (*Euphydryas editha taylori*). Available through: https://ecos.fws.gov/ecp/species/5907

USFWS(n). 2024. Taylor's (=whulge) checkerspot (*Euphydryas editha taylori*). Available through: https://www.fws.gov/species/taylors-checkerspot-euphydryas-editha-taylori

USFWS(o). 2024. Taylor's (=whulge) checkerspot (*Euphydryas editha taylori*) Species Range Shapefile. Available through: https://ecos.fws.gov/docs/species/shapefiles/usfws_I0T6_I01_Euphydryas_editha_taylori_current_range.zip

USFWS(p). 2024. Species Profile: Puerto Rican harlequin butterfly (*Atlantea tulita*). Available through: https://ecos.fws.gov/ecp/species/9005

USFWS(q). 2024. Puerto Rican harlequin butterfly (*Atlantea tulita*) Species Range Shapefile. Available through https://ecos.fws.gov/docs/species/shapefiles/usfws_I0VK_I01_Atlantea_tulita_current_range.zip

USFWS(r). 2023. Species Profile: Island marble butterfly (*Euchloe ausonides insulanus*). Available through: https://ecos.fws.gov/ecp/species/3285

USFWS(s). 2023. Island marble butterfly (*Euchloe ausonides insulanus*). Available through: https://www.fws.gov/species/island-marble-butterfly-euchloe-ausonides-insulanus

USFWS(s). 2023. Island marble butterfly (*Euchloe ausonides insulanus*). Available through: https://www.fws.gov/species/island-marble-butterfly-euchloe-ausonides-insulanus

Wraight CL, Zangerl AR, Carroll MJ, and Berenbaum MR. 2000. Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions. Proceedings of the National Academy of Sciences (PNAS) 97, pp. 7700 – 7703.

Wolt, J .D. and Peterson, R.K.D. 2010. Prospective formulation of environmental risk assessments: Probabilistic screening for CrylA(b) maize risk to aquatic insects. Ecotoxicology and Environmental Safety 73: 1182-1188.

Zangerl, A.R., McKenna, D., and Wraight, C.L. 2001. Effects of exposure to event 176 *Bacillus thuringiensis* corn pollen on monarch and black swallowtail caterpillars under field conditions. Proceedings of the National Academy of Sciences (PNAS) 98, pp. 11908 – 11912.

IX. Appendix A: Evaluation of DAS1131 Maize's Potential to Affect Lepidopteran Threatened and Endangered Species (TES)

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Karner Blue Butterfly (*Lycaeides melissa samuelis*)
Status: Endangered (USFWS (a) 2024)
Listed: 12/14/1992
UDL Spatial Analysis Overlap: 34.33%
Overlap Category: High

Critical habitat designated:

The following excerpts are from the Federal Register (1992); consult original reference for citations:

The Karner Blue Butterfly is a small butterfly with a wingspan of about one inch (Figure 1). The habitat of the Karner blue butterfly is characterized by the presence of wild blue lupine (*Lupinus perennis*), a member of the pea family. Wild lupine is the only known larval food plant for the Karner blue butterfly and is, therefore, closely tied to the butterfly's ecology and distribution. In eastern New York and New Hampshire, the habitat typically includes sandplain communities, and grassy openings within very dry, sandy pitch pine/ scrab oak barrens. In the Midwest, the habitat is also dry and sandy, including oak savanna and jack pine areas, and dune/ sandplain communities.

Figure 1. Female Karner Blue Butterfly (*Lycaeides melissa samuelis*).

Photo Credit. Female Karner Blue Butterfly, Jill Utrup/USFWS, Public Domain, https://www.fws.gov/banner/female-karner-blue-butterfly.

The Karner blue butterfly usually has two broods each year. Eggs that have overwintered from the previous year hatch in April. The larvae feed on wild lupine leaves and mature rapidly. Near the end of May, they pupate, and adult butterflies emerge very late in May in most years. The adults are typically in flight for the first 10 to 15 days of June, when the wild lupine is in bloom. Females lay eggs on or near the wild lupine plants. The eggs hatch in about one week and the larvae feed for about three weeks. They then pupate and the second brood adults appear in the second or third week of July. This time, the eggs are laid among plant litter or on grass blades at the base of the lupines, or on lupine pods or stems. By early August, no adults remain, and these eggs do not hatch until the following spring (Schweitzer 1989, Ding 1979).

The distribution of the Karner blue butterfly is very discontinuous and generally follows the northern limits of wild lupine. Eight major population clusters of the Karner blue butterfly were known historically from portions of Wisconsin, Michigan, Minnesota, Indiana, Illinois, Ohio, Massachusetts, New Hampshire, Pennsylvania, New York, and Ontario. Over the past 100 years, Karner blue butterfly numbers ave apparently declined range-wide by 99 percent or more. Over 90 percent of the decline occurred in the last 10 to 15 years. It is now extirpated from Massachusetts, Pennsylvania, and Ohio (Schweitzer 1989; in litt., 1990). Unconfirmed reports indicate that one or two Karner blues may have been sighted at an historic Ontario site in 1990 or 1991.

The following excerpt is from (USFWS(b) 2024); consult the original reference for citations:

Wild blue lupine (*Lupinus perennis*) is the only plant Karner blue butterflies lay eggs on, the only plant larvae, or caterpillars, can eat. Even so, the range of these butterflies and that of their host plant do not completely overlap. Instead, Karner blue butterfly are found predominantly along the northern band of wild lupine's range.

At the time of listing under the Endangered Species Act, the most important factor causing the decline of the Karner blue butterfly across its range was the loss of habitat due to suppression of wild fires, clearing land for farming and developing land for commercial and residential purposes. Without disturbance activities like fire and grazing, shrubs and trees invade the open savanna and barrens, shading out grass and herbaceous plants like wild lupine. When this happens, only pockets of suitable space remain, which make it hard for butterflies to find more areas with wild lupine. It also limits the amount of suitable habitat that's available. This results in small, isolated populations of Karner blue butterflies. Because the Karner blue butterfly's habitat is very specific, the butterfly is unable to adapt to these changes in its environment. Habitat loss and isolation of these populations, combined with the extremely small size of many of the remaining population, puts many populations at high risk of winking out of existence.

Assessment:

Karner blue butterflies once occurred in a nearly continuous narrow band across 12 states and the province of Ontario, Canada, but it has been eliminated from seven of those states and Ontario. Today it is found in portions of New Hampshire, New York, Michigan, Wisconsin, and Ohio. The spatial overlap analysis of the species’ range and maize production can be found in Figure 2, and the maize UDL overlap calculation is 34.33% (categorized as high overlap).

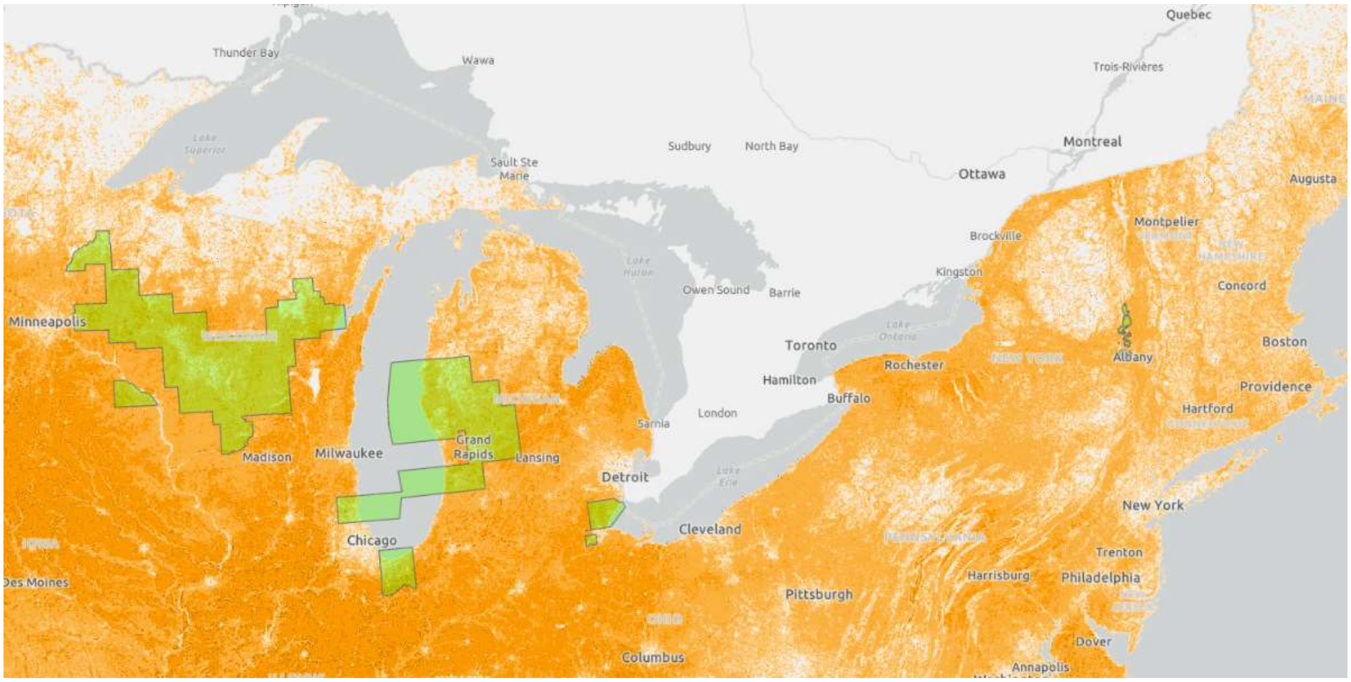
While the species is limited to small, fragmented ranges over New Hampshire, New York, Michigan, Wisconsin and Ohio, its habitat includes grasslands (USFWS(a) 2023) and its primary larval food source/host plants are typically found in dry sandy openings including woodland clearings or disturbed areas (Meyer, R. 2006). Previous EPA analyses determined that the potential exposure of Karner blue to PIP corn pollen is limited because corn and lupine do not generally overlap. EPA found that wild lupine does not occur at all in corn fields nor is wild lupine expected to grow adjacent to corn fields (EPA 2001, 2010). This determination was based on communications with state natural resources department and conservation groups, as well as a published survey which found that five Karner blue populations in Winona County, Minnesota were a mean distance of 660 meters from the nearest agricultural lands (Andow, et al. 1995)- a distance much further than the expected pollen deposition range. There is no new information to alter these conclusions.

Furthermore, temporal overlap analysis of Karner blue oviposition with corn pollen shed is minimal to nonexistent. Karner blue lifecycle includes two generations per year, with the first generation of Karner blue butterflies emerging in mid-April, prior to pollen anthesis. The second generation larvae emerge in June-July where there is some potential for pollen-shed overlap depending on geography/climate (EPA 2001). However, EPA's 2010 *Bt* corn reassessment summarized a data analysis of the comparison of overlap between pollen shed and larval stages in Wisconsin, Michigan, Minnesota, Indiana, and New York, with most locations having no overlap, and for other counties the potential for overlap being sporadic (i.e., "does not happen every year nor for more than a day or two in the life of the feeding larvae").

This extremely limited window of potential overlap between pollen shed and the larval lifestage, coupled with the expectation that the larval host plant is not found in or adjacent to corn fields, indicates exposure of Cry1Da2 pollen to one brood of the Karner blue butterfly's annual life-cycle is negligible.

Therefore, EPA's analysis has determined that negligible exposure is expected for the Karner blue butterfly from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the Karner blue butterfly and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

Figure 2. Overlap of the species range of the Karner Blue Butterfly (*Lycaeides melissa samuelis)* and agriculturally cultivated corn.



Current species range is indicated by the green polygons (USFWS(a) 2022) and corn crop is indicated by orange polygons

(U.S. EPA (a) 2023).

Mitchell's satyr Butterfly (*Neonympha mitchellii mitchellii*)
Status: Endangered (USFWS(c) 2024)
Listed: 6/25/1991
UDL Spatial Analysis Overlap: 31.46%
Overlap Category: High

Biology and Habitat Requirements:
The following excerpts are from the Federal Register (1991); consult original reference for citations:

Mitchell's satyr is a medium sized (38- 44 millimeter wingspan) butterfly with an overall rich brown coloration. A distinctive series of submarginal yellow- ringed black circular eyespots (ocelli) with silvery centers are found on the lower surfaces of both pairs of wings (Figure 3). The number of ocelli on the forewing varies between the sexes, with males generally having 4 (range 2-4) and females having 6 (range 5-6). The eyespots are accented by two orange bands along the posterior wing edges, as well as two fainter orange bands across the central portion of each wing. It is distinguishable from its North American congener N. areolata by the latter's well-marked ocelli on the upper wing surfaces, as well as the fighter coloration and stronger flight of N. areolata (French 1889: McAIpfne et al I960; Wilsmann and1 Schweitzer 1991).

Figure 3. Mitchell’s Satyr Butterfly (*Neonympha mitchellii mitchellii*).

Photo Credit. Mitchell’s Satyr Butterfly, USFWS Midwest Region, Public Domain, https://ecosphere-documents-production-public.s3.amazonaws.com/sams/public_docs/species_nonpublish/2218.pdf.

N. m. mitchellii is one of the most geographically restricted butterflies in North America. Historical records exist for approximately 30 locations in four States, ranging from southern Michigan, adjacent counties of northern Indiana, and a single Ohio county, with several disjunct populations in New Jersey. Hie species has been documented from a total of 18 counties (Badger 1958: Martin 1987: PaBister 1927; Rutkowskf 1988; Shuey et al 1987b; Wilsmann and Schweitzer 1991).

Although *N. m. mitchellii* has been reported from Maryland, the lack of suitable habitat makes it more likely that those 1940's specimens were misidentified members of a *Neonympha areolatus* subspecies. Suitable habitat may exist in New York, Connecticut Massachusetts, and Pennsylvania. However, searches in these States have failed to locate any *N. m. mitchellii* populations (Schweitzer 1989; Wilsmann and Schweitzer 1991).

The habitat occupied by *N. m. mitchellii* consists solely of wetlands known as fens. This is an uncommon wetland habitat type characterized by calcareous soils and fed by carbonate- rich water from seeps and springs. Fens are most frequently components of larger wetland complexes. Due to the superficial resemblance of fens to bogs, the habitat of Mitchell's satyr has sometimes been erroneously described in earlier literature as acid bogs (MeAlpme et al I960: Shuey 1985; Shuey et al 1987a; Wilsmann and Schweitzer 1991).

Assessment:

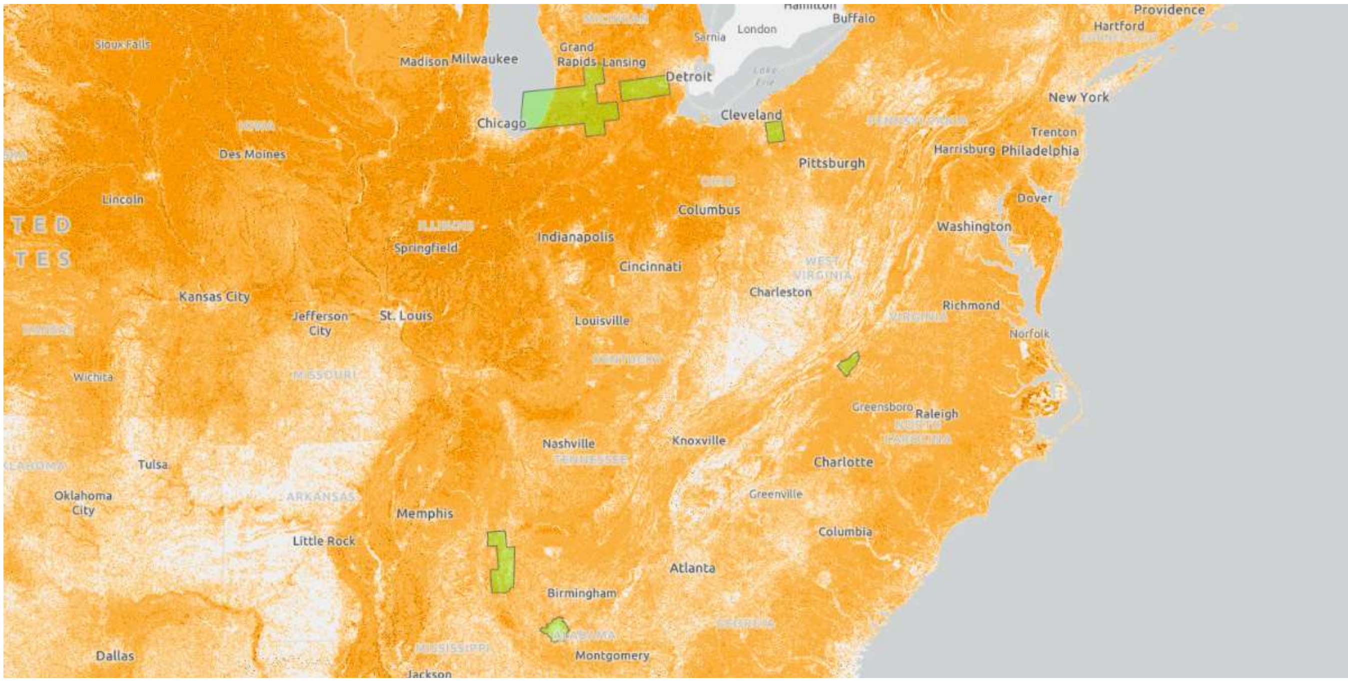
The Mitchell's Satyr Butterfly is one of the most geographically restricted butterflies in North America. (FR 1991). Today, the butterfly can be found in only nine locations in Michigan and one location in Indiana, along with a single county in Virginia and Ohio, and restricted areas within Mississippi and Alabama (USFWS 2021, USFWS(c) 2024; USFWS(d) 2024). The spatial overlap analysis of the species' range and maize production can be found in Appendix A, and the maize UDL overlap calculation is 31.46% (categorized as high overlap).

The Mitchell's Satyr Butterfly habitat is restricted to rare wetlands called *fens,* which are low nutrient wetlands that receive carbonate-rich ground water from seeps and springs. The southern populations are typically associated with beaver-influenced wetlands that are sedge dominated, and occasionally semi-open riparian or floodplain forest areas (USFWS 2020). In the most recent 5-year review of the species, Mitchell's satyr butterfly was found to only occupy areas with approximately 70% ground cover, 55% canopy cover, and 60% sedge cover in the rare wetland communities throughout its current species range (USFWS 2021). These types of habitat requirements are not conducive in or near corn cultivated areas. Mitchell's satyr butterfly is also very small in size (3.8 to 4.4 wingspan), limiting its ability to venture far from its required habitat. Furthermore, Mitchell's satyr caterpillars feed on one or more species of grass-like plants called sedges. Concern surrounding pollen deposition onto larval host plants is more relevant for lepidoptera whose host plants have wider, horizontal leaves. As the larval host plants of the Mitchell's satyr butterfly are grass-like, they are not a shape that is conducive to pollen accumulation.

Due to the severe geographical restriction, rare fen habitat with specialized requirements, limited ranges in small locations in only a few states, reduced flight range due to its relatively small size, and the shape of the larval host plants (grass-like sedges) not being conducive to pollen accumulation, the likelihood of larvae of the Mitchell's Satyr Butterfly coming into contact with pollen from a maize producing field is negligible

Therefore, EPA's analysis has determined that negligible exposure is expected for the Mitchell's satyr butterfly from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the Mitchell’s satyr butterfly and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

Figure 4. Overlap of the species range of the Mitchell's satyr Butterfly (*Neonympha mitchellii mitchellii)* and agriculturally cultivated corn.



Current species range is indicated by the blue (USFWS(a) 2021) and corn crop is indicated by orange polygons (U.S. EPA (a) 2023).

Dakota Skipper (*Hesperia dacotae*)
Status: Threatened (USFWS(e) 2024)
Listed: 11/24/2014
UDL Spatial Analysis Overlap: 25.84%
Overlap Category: High

Biology and Habitat Requirements:
The following information is directly or slightly modified from USFWS (2018).

The adult Dakota skipper is a small to medium-sized butterfly with a wingspan of about an inch (2–3 cm) with hooked antennae (Figure 5). Dakota skipper larvae feed only on several native grass species; little bluestem (*Schizachyrium scoparium*) Panicum spp., Poa spp., and other native grasses. There are areas where the Dakota skipper species’ range overlaps with agricultural production and corn is a primary crop in the areas the skipper could potentially be found. However, the habitat for the skipper is not in corn fields or directly bordering corn fields, which are disturbed areas. Dakota skippers are obligate residents of undisturbed (remnant, untilled) high-quality prairie, ranging from wet-mesic tallgrass prairie to dry-mesic mixed-grass prairie. Soils unsuitable for agriculture and steep topography have allowed remnant native prairie habitats inhabited by Dakota skippers to persist.

Figure 5. Dakota Skipper (*Hesperia dacotae*).

Photo Credit. Dakota skipper, aecole2010, Creative Commons Attribution 2.0, https://www.fws.gov/banner/dakota-skipper https://ecosphere-documents-production-public.s3.amazonaws.com/sams/public_docs/species_nonpublish/2218.pdf

The following excerpt is from (USFWS(e) 2024); consult the original reference for citations:

Dakota skippers live in two types of prairies. One type is moist bluestem prairie in which three wildflower species are usually blooming when Dakota skippers are adults: wood lily (*Lilium philadelphicum*), harebell (*Campanula rotundifolia*) and smooth camas (*Zygadenus elegans*). The second type is upland prairie that is relatively dry and often found on ridges and hillsides. Bluestem grasses and needlegrasses dominate these prairies; purple coneflower (*Echinacea angustifolia*) is typical of high quality sites that support this skipper, although it also uses other flowers for nectar. Both of these habitat types are unlikely to be re-established on a site that has been plowed. Therefore, activities that maintain the original native grass habitat are fundamental to the species' conservation according to the U.S. Fish and Wildlife Service.

Although some native prairie plants and animals have adaptations that allow them to survive in modern agricultural landscapes, the Dakota skipper does not. Dakota skippers need high-quality prairie that has retained a large part of its original plant diversity. The future of many prairies where this butterfly persists is not secure because of threats from conversion to row crops, herbicide use, woody and non-native plant invasion, road construction, over-grazing, and gravel mining.

Dakota skippers have four basic life stages: egg, larva, pupa and adult. During the brief adult period in June and July, females lay eggs on the underside of leaves. Eggs take about 10 days to hatch into larvae, or caterpillars. After hatching, larvae build shelters at or below the ground surface and emerge at night to feed on their native grass leaves. This continues until fall when larvae become dormant. They overwinter in shelters at or just below ground level, usually in the base of native bunchgrasses. The following spring, larvae emerge to continue developing. Pupation takes about 10 days and usually happens in June, according to the U.S. Fish and Wildlife Service.

Adult males emerge from pupae about five days before females, and the adults live for three weeks, at most. This brief period is the only time that Dakota skippers can reproduce. If a female Dakota skipper lives for the full three weeks and adequate flowers for nectar are available, she may lay up to 250 eggs. Nectar, providing both water and food, is crucial for survival of both sexes during the adult flight period, which often occurs during the hottest part of summer. according to the U.S. Fish and Wildlife Service.

The following excerpts are from the Federal Register(a) (2013); consult original reference for citations:

Dakota skippers are not known to disperse widely and have low mobility; experts estimate Dakota skipper has a mean mobility of 3.5 (standard deviation = 0.71) on a scale of 0 (sedentary) to 10 (highly mobile) (Burke et al. 2011, Fitzsimmons 2012, pers. comm.). Five Dakota skipper experts interviewed in 2001 indicated that it was unlikely that Dakota skippers were capable of moving greater than 1 kilometer (km) (0.6 miles (mi)) between patches of prairie habitat separated by structurally similar habitats (e.g., perennial grassland, but not necessarily native prairie) (Cochrane and Delphey 2002, p. 6). The species will not likely disperse across unsuitable habitat, such as certain types of row crops (e.g., corn, beets), or anywhere not dominated by grasses.

Assessment:

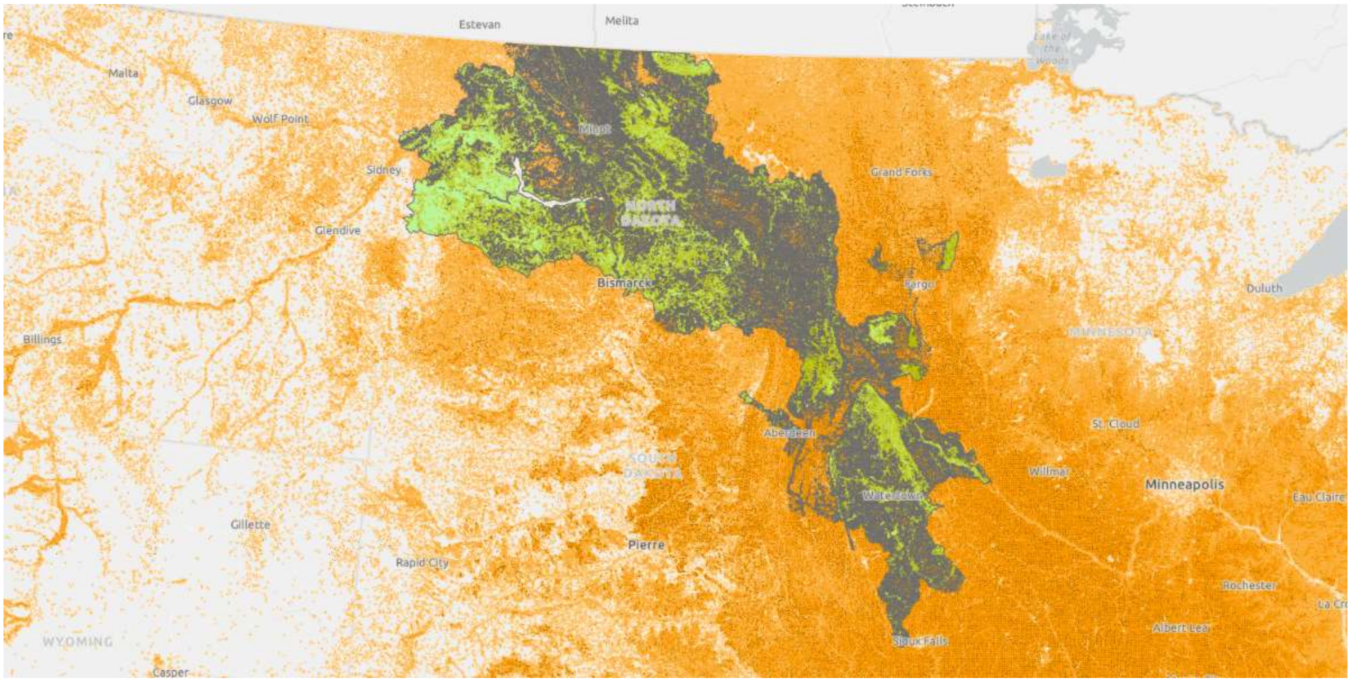
The Dakota Skipper is located in Minnesota, North Dakota, and South Dakota (USFWS(a) 2023). The spatial overlap analysis of the species' range and maize production can be found Figure 6, and the maize UDL overlap calculation is 25.84% (categorized as high overlap).

The Dakota Skipper is not adapted to survive in corn fields or directly bordering corn fields, which are disturbed areas. Dakota skippers are obligate residents of undisturbed (remnant, untilled) high-quality prairie, ranging from wet-mesic tallgrass prairie to dry-mesic mixed-grass prairie (USFWS(e) 2024). Soils unsuitable for agriculture and steep topography have allowed remnant native prairie habitats inhabited by Dakota skippers to persist. Loss of native prairie and degradation of remaining habitat have led to the decline of the butterfly (USFWS 2018) Dakota Skipper caterpillars only on feed several native grass species; little bluestem (*Schizachyrium scoparium*) Panicum spp., Poa spp., and other native grasses (USFWS(e) 2024). Since Dakota skippers are obligate residents of undisturbed high-quality prarie, unable to survive in agricultural environments, and their larval food source is also found in the same type of habitat, compounded with the fact this species is not found in or adjacent to corn fields, the likelihood of this butterfly being exposed to pollen drift from a maize producing field is negligible (USFWS(e) 2024).

The Dakota skipper's life-cycle further limits any potential for temporal overlap of the timing of pollen shed. While the eggs are laid during June through July, once hatched, larvae build shelters near or underground and only emerge at night to feed, and as stated above, they only feed on the native grasses defined as their host plants (USFWS(e) 2024). Concern surrounding pollen deposition onto larval host plants is more relevant for lepidoptera whose host plants have wider, horizontal leaves. As the larval host plants of the Dakota skipper are grasses, they are not a shape that is conducive to pollen accumulation. Since peak corn pollen shed occurs in the morning and again in the late afternoon (Nielsen 2020), the larvae would not encounter active pollen shed during the evening when they are feeding.

Therefore, based on the Dakota skipper's larval host plants not being expected to be in or adjacent to corn fields coupled with the shape of the larval host plants not being conducive to pollen accumulation, EPA's analysis has determined that negligible exposure is expected for the Dakota skipper from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the Dakota skipper and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

Figure 6. Overlap of the species range of the Dakota skipper (*Hesperia dacotae*) and agriculturally cultivated corn.



Current species range is indicated by the light green polygons (USFWS(a) 2023) and corn crop is indicated by (U.S. EPA (b) 2023) orange polygons.

Blackburn's sphinx moth (*Manduca blackburni*)
Status: Endangered (USFWS(f) 2024)
Listed: 2/1/2000
UDL Spatial Analysis Overlap: 6.853%
Overlap Category: Medium

Biology and Habitat Requirements:
The following excerpts are from the Federal Register (2000(a)); consult original reference for citations:

Blackburn's sphinx moth (*Manduca blackburni*) is Hawaii's largest native insect, with a wingspan of up to 12 cm (5 in) (Figure 7). Like other sphinx moths (family Sphingidae), it has long, narrow forewings and a thick, spindle-shaped body tapered at both ends. It is grayish brown in color, with black bands across the apical (top) margins of the hind wings and five orange spots along each side of the abdomen. The larva is a typical, large "hornworm" caterpillar, with a spinelike process on the dorsal (upper) surface of the eighth abdominal segment. Caterpillars occur in two color forms, a bright green or a grayish phase. Both color forms have scattered white speckles throughout the dorsum (back), with the lateral (side) margin of each segment bearing a horizontal white stripe, and segments four to seven bearing diagonal stripes on the lateral margins (Zimmerman 1958; Betsy Gagne , Hawaii Department of Land and Natural Resources, pers. comm. 1998).

Figure 7. Blackburn's sphinx moth (*Manduca blackburni*).

Photo Credit. Blackburn's sphinx moth, Department of Land and Natural Resources
https://dlnr.hawaii.gov/ecosystems/files/2013/07/Blackburns-Sphinx-moth-adult-from-Makawao-Maui.jpg .

Larvae of Blackburn's sphinx moth feed on plants in the nightshade family (Solanaceae). The natural host plants are native shrubs in the genus *Solanum* (popolo), and the native tree, *Nothocestrum latifolium* (‘aiea) (Riotte 1986), on which the larvae consume leaves, stems, flowers, and buds (B. Gagne , pers. comm. 1994). However, many of the host plants recorded for this species are not native to the Hawaiian Islands, and include *Nicotiana tabacum* (commercial tobacco), *Nicotiana glauca* (tree tobacco), *Solanum melongena* (eggplant), *Lycopersicon esculentum* (tomato), and possibly *Datura stramonium* (Jimson weed) (Riotte 1986). Development from egg to adult can take as little as 56 days (Williams 1947), but pupae may remain in a state of torpor (inactivity) in the soil up to a year (Williams 1931; B. Gagne , pers. comm. 1994). Adult moths can be found throughout the year (Riotte 1986). Historically, Blackburn's sphinx moth has been recorded from the islands of Kauai, Oahu, Molokai, Maui, and Hawaii, and collected from sea level to 760 meters (m) (2,500 feet (ft)) (Riotte 1986). Most historical records were from coastal, lowland, and dryland forest habitats in areas receiving less than 120 cm (50 in) annual rainfall. It appears that this moth was historically most common on Maui (Riotte 1986).

The following excerpt is from (USFWS(f) 2024); consult the original reference for citations:

Sphinx moths usually mate quickly after reaching adulthood. The female sphinx moth may lay as many as 1,000 eggs, usually on the surface of an aiea, a native Hawaiian plant that is its preferred host plant. A few days later, the eggs hatch. The male and female die after reproducing. In its larval (caterpillar) stage, the Blackburn's sphinx moth is a 3.5- to 4-inch (9- to 10-centimeter) caterpillar. The caterpillars can be either bright green or gray with white spots or lines. They are usually called "hornworms" because they have a red or black horn on their abdomen. The caterpillars feed on plants from the nightshade family, from which they eat the leaves, stems, and flowers. Blackburn's sphinx moths also eat plants that have been introduced to Hawaii, such as a variety of tobacco plants, as well as eggplant and tomato. After the larval stage, the insect goes through the pupal (cocoon) stage, for which it goes underground. In this stage, it transforms, to eventually rise up to the surface as an adult moth. Nothocestrum latifolium (aiea) and N. breviflorum (aiea) are both known larval host plants for the species (Riotte 1986). Other host plants include non-native Nicotiana tabacum (commercial tobacco), Nicotiana glauca (tree tobacco), Solanum melongena (eggplant), Lycopersicon esculentum (tomato), and possibly Datura stramonium (Jimson weed)(Riotte 1986). Recent work on moth captive propagation indicates that tomato may be a suboptimal larval host plant (Rubinoff 2007).

Blackburn's sphinx moths are found in dry to mesic forest habitats. When listed in 2000, BSM was known from 2 sites on Maui, 2 sites on the island of Hawaii, and on Kahoolawe (USFWS 2000). We now know that it is more widespread on at least Maui and Hawaii. While key sites on Maui and Hawaii were associated with the largest concentrations of aiea on the respective islands, at least one of the original Maui sites and the population on Kahoolawe had no aiea present. At these sites, the species appeared entirely dependent on tree tobacco; or on Maui, possibly on naturalized commercial tobacco (Nicotiana tabacum) (USFWS 2000). Our current knowledge of the overall distribution of BSM is based largely on incidental sightings. On Maui, observations of BSM have been made from the Kanaio area on leeward Haleakala, Ulupalakua, Wailea/Makena, Makawao, Launiupoko on west Maui, along Kuihelani Highway in the central valley, and along the north coast from Waihee to Kanaha (USFWS 2005, USFWS unpubl. data). BSM has been recently documented from surveys on Kahoolawe, which were conducted in 2018 and 2019. Similar to previous documentation, BSM was found to be not uncommon on tree tobacco (C. King pers. comm. 2018); however data from this survey are not yet available. On the island of Hawaii, BSM are known from the Puu Anahulu & Puu Waawaa areas, as well as along Saddle Road. BSM presence is not currently known from Lanai, though there were reports of moths present in 2011 (USFWS unpubl. data). None have been reported since that time, though no comprehensive surveys have been conducted. No recent sightings have been made on Molokai (last observed in 1940s), Oahu (1931), or Kauai (1940).

Assessment:

The Blackburn's sphinx moth is likely more widespread than originally known at the time of listing. The species is found on three different islands in a variety of habitats using multiple larval host species, both native and non-native. Surveys on the island of Hawaii have found high densities of the species, which project to a large and robust population across the area of potential habitat. The spatial overlap analysis of the species' range and maize production can be found in Figure 8, and the UDL overlap calculation is 6.85% (categorized as medium overlap). Blackburn's sphinx moths diet consists of members of the nightshade family (Solanaceae), and to date have not been observed to feed on maize plants (USFWS(f) 2024, Federal Register(a) 2000).

While the UDL overlap calculation is 6.853% (categorized as medium overlap), this calculation encompasses a dataset that encompasses all agricultural production, not just maize production, and is therefore an extremely conservative exposure scenario. A dataset for all agricultural production was initially used because the corn crop data layer used in the UDL overlap percent calculations for the other TES assessments did not contain agricultural corn production for the state of Hawaii (or Puerto Rico). However, the most recent census of agriculture in Hawaii lists corn production (seed only) at 2176 acres (USDA(a) 2024). Using this value places the potential overlap well below the 1% threshold (0.17% overlap). Consequently, the likelihood of this species being exposed to pollen drift from event DAS1131 maize producing field is negligible.

Therefore, EPA's analysis has determined that negligible exposure is expected for Blackburn's sphinx moth from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the Blackburn's sphinx moth and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

Figure 8. Agricultural production as it relates to the species range of the Blackburn's sphinx moth (*Manduca blackburni*) in Hawaii.

Current species range is indicated by the purple polygons (USFWS(b) 2022) and agricultural production is indicated by (U.S. EPA (b) 2023) orange polygons.

Poweshiek Skipperling (*Oarisma poweshiek*)
Status: Endangered (USFWS (g) 2024)
Listed: 11/24/2014
UDL Spatial Analysis Overlap: 21.22%
Overlap Category: High

Biology and Habitat Requirements:
The following excerpts are from the Federal Register (2014); consult original reference for citations:

The Poweshiek skipperling is a small and slender-bodied butterfly (Figure 9) and its habitats include prairie fens, grassy lake and stream margins, moist meadows, sedge meadow, and wet-to-dry prairie. McCabe and Post (McCabe and Post 1977) describe the species' habitat in North Dakota as ''. . . high dry prairie and low, moist prairie stretches as well as old fields and meadows.'' Royer and Marrone (1992b) describe Poweshiek skipperling habitat in North Dakota and South Dakota as moist ground in undisturbed native tallgrass prairies. Poweshiek skipperling habitat throughout Iowa and Minnesota is described as both ''high dry'' and ''low wet'' prairie (McCabe and Post 1977). Southern dry prairies in Minnesota are described as having sparse shrub cover (less than 5%) composed primarily of leadplant, with prairie rose, wormwood sage, or smooth sumac present and few, if any, trees (Minnesota DNR, 2012). Southern mesic prairies also have sparse shrubs (5–25% cover) consisting of leadplant and prairie rose with occasional wolfberry (*Symphoricarpos occidentalis*) and few, if any, trees (Minnesota DNR, 2012b), [Federal Register, 2014].

Figure 9. Poweshiek Skipperling (*Oarisma poweshiek*).

Photo Credit. Poweshiek skipperling, Vince Cavalieri/USFWS, Public Domain, https://www.fws.gov/banner/poweshiek-skipperling.

Until recently, the larval food plant was presumed to be elliptic spikerush (*Eleocharis elliptica*) or sedges, but this was based on limited observations, primarily from the Michigan populations (e.g., Holzman, 1972). More recent observations show that the preferred larval food plant for some populations of Poweshiek skipperling is prairie dropseed (*Sporobolus heterolepis*) (Borkin, 1995); larvae have also been observed feeding on little bluestem (*Schizachyrium scoparium*) (Borkin, 1995) and sideoats grama (*Bouteloua curtipendula*) (Dana 2005a, pers. comm.). Poweshiek skipperling larvae have been observed feeding on Carex sp. (Borkin 1994, Borkin 1996), although not through the entire larval development (Borkin 2014, pers. comm.). Poweshiek skipperling have been observed laying eggs (ovipositing) on mat muhly (*Muhlenbergia richardsonis*) (Cuthrell 2012a, pers. comm.), a grass in Michigan's prairie fens (Penskar and Higman 1999). Poweshiek skipperlings were observed to oviposit on big bluestem in Wisconsin (Borkin 2012a, pers. comm.), although indiscriminate oviposition on unsuitable larval plants has been observed during high summer temperatures (Borkin 1995). Borkin (1995) also observed oviposition on an unidentified sedge (Eleocharis sp.), but only 2 eggs were found on the sedge in comparison to more than 100 eggs found on prairie dropseed. [Federal Register, 2014]

The following excerpts are from the Federal Register(a) (2013); consult original reference for citations:

Poweshiek skipperling are not known to disperse widely. The maximum dispersal distance for male Poweshiek skipperling travelling across contiguous suitable habitat is estimated to be approximately 1.6 km (1.0 mi) (Dana 2012a, pers. comm.). The species was evaluated among 291 butterfly species in Canada and is thought to have relatively low mobility, lower mobility than that of the Dakota skipper (Burke et al. 2011; Fitzsimmons 2012, pers. comm.). Therefore, a more conservative estimated dispersal distance would be that of the Dakota skipper, approximately 1 km (0.6 mi) (Cochrane and Delphey 2002, p. 6). Poweshiek skipperling frequently perch on vegetation, but males will occasionally patrol in search of mating opportunities (Royer and Marrone 1992b, p. 15). Poweshiek skipperling may move between patches of prairie habitat separated by structurally similar habitats (e.g., perennial grasslands but not necessarily native prairie); small populations need immigration corridors for dispersal from nearby populations to prevent genetic drift and to reestablish a population after local extirpation. The species will not likely disperse across unsuitable habitat, such as certain types of row crops, or anywhere not dominated by grasses (Westwood 2012, pers. comm.; Dana 2012a, pers. comm.). Dispersal grassland habitat consists of the following physical characteristics appropriate for supporting Poweshiek skipperling dispersal: undeveloped open areas dominated by perennial grassland with limited or no barriers to dispersal including tree or shrub cover less than 25 percent of the area and no row crops such as corn, beans, potatoes, or sunflowers.

The following excerpt is from (USFWS(b) 2021); consult the original reference for citations:

The Poweshiek skipperling occurs or once occurred in Iowa, Michigan, Minnesota, North Dakota, South Dakota, and Wisconsin. Its range is now substantially reduced such that the Poweshiek skipperling is restricted to small patches of fragmented native- prairie remnants in portions of two States (Wisconsin and Michigan) and one Canadian province. The species is presumed extirpated from Illinois and Indiana, and the status of the species is unknown in four of the six States with relatively recent records (within the last 20 years) (Federal Register, 2014). In Michigan, Poweshiek skipperling habitat suitability generally increases along with increasing prairie fen area and increasing surrounding natural land cover and decreases with increasing surrounding road density and surrounding developed land cover.

The disjunct populations of Poweshiek skipperlings in Michigan have more narrowly defined habitat preferences than most of its historical range, which are variously described as prairie fens in the Michigan Natural Features Inventory of 2011 and 2012. Poweshiek skipperling have been described as occupying peat domes within larger prairie fen complexes in areas that are either dominated by mat muhly or prairie dropseed, as documented by Cuthrell and others in 2013. Historical Poweshiek skipperling populations in Wisconsin were also disjunct from the population to the west and are associated with areas that contain intermixed wet prairie, wet-mesic and dry-mesic prairie habitats, as documented by S.S. Borkin in 1995 and later confirmed by Swengel in 2013.

Poweshiek skipperlings are *univoltine*, meaning that they have one brood or single flight period a year, with adults emerging from mid-June to early July. The actual flight period varies somewhat across the species' range and can also vary significantly from year to year, depending on weather patterns, as documented by R.A. Royer and G.M. Marrone in 1992 and later confirmed by A.B. Swengel and S.R. Swengel in 1999. The flight period in a given locality lasts two to four weeks, and mating occurs throughout this period, as documented by T.L. McCabe and R.L. Post in 1977 and later confirmed by A.B. Swengel and S.R. Swengel in 1999.

Poweshiek skipperlings lay their eggs near the tips of leaf blades and overwinter as larvae on the host plants, as documented by the Bureau of Endangered Resources in A.B. Swengel and S.R. Swengel in 1999. In 1972, W.S. McAlpine observed hatching of larval Poweshiek skipperling after about 9 days. The number of instars can be influenced by many factors, for example larvae reared at the Minnesota Zoo, typically have six instars, as documented by C. Nordmeyer in 2021. Captive Poweshiek skipperling eggs hatched eight to nine days after oviposition, as documented by Runquist in 2013. After hatching, Poweshiek skipperling larvae crawl out near the tip of grasses and may remain stationary, with their head usually pointing downward, as documented by W.S. McAlpine in 1972. W.S. McAlpine also observed that Poweshiek skipperling do not form shelters underground and S. Borkin later confirmed in 1995. Instead, the larvae overwinter up on the blades of grasses and on the stem near the base of the plant, as documented by S. Borkin in 2008 and later confirmed by Dana in 2008.

Assessment:

Once found in in Iowa, Michigan, Minnesota, North Dakota, South Dakota, and Wisconsin, the Poweshiek Skipperling' range has now been reduced to just several small locations in Michigan and only one county in Wisconsin in the United States (USFWS(c) 2022). The spatial overlap analysis of the species' range and maize production can be found in Figure 10, and the maize UDL overlap calculation is 21.22% (categorized as high overlap).

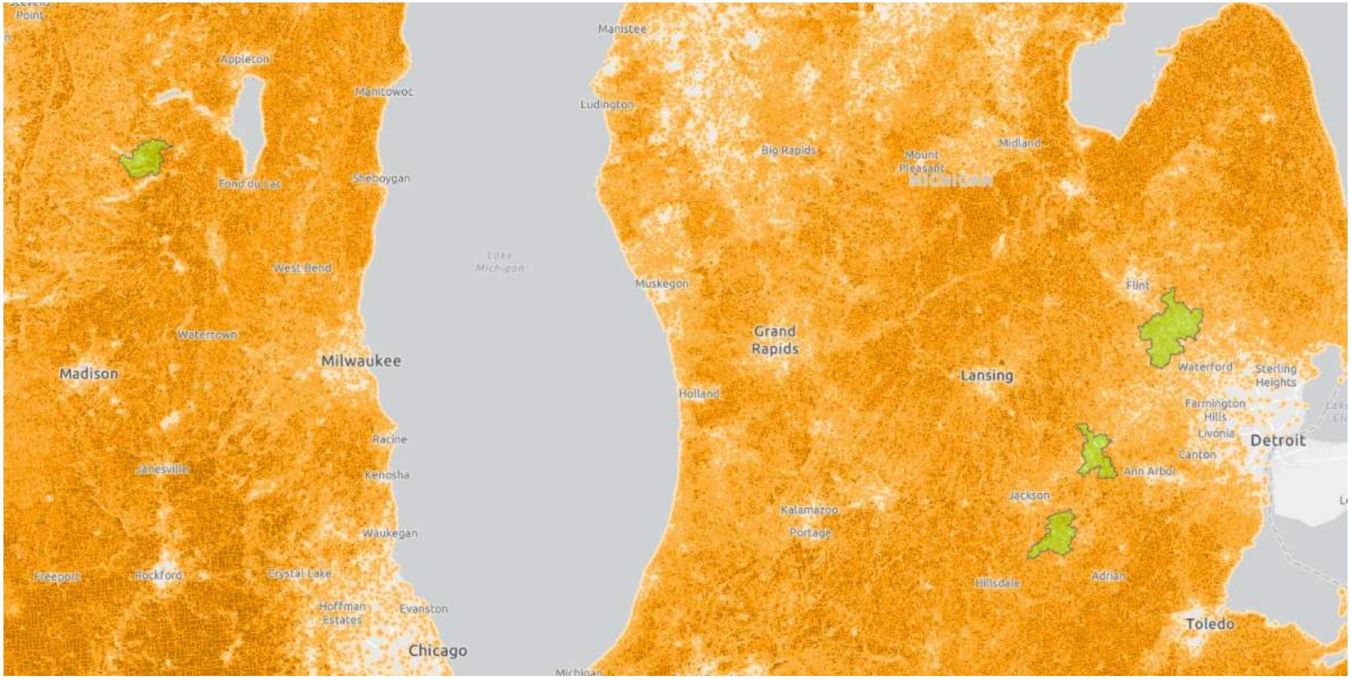
The Poweshiek Skipperling's habitat includes prairie fens, grassy lake and stream margins, moist meadows, sedge meadow, and wet to dry prairie (FR 2014). Its primary larval food source/host plant is prairie dropseed (*Sporobolus heterolepis*), a prairie grass. However, observations have been made of ovipositing in Michigan on other prairie fen and grassland plants (*M. richardsonis, M. glomerata [marsh muhly], C. sterilis [dioecious sedge],* and *Dasiphora fruticosa [shrubby cinquefoil]*). (USFWS(h) 2024). Field observations and laboratory studies indicate that first instar larvae seem to require or prefer initial feeding on "very fine, threadlike blade tip[s of grasses]" (USFWS(h). 2024)

In addition to its limited geographical range, Poweshiek skipperling are also not known to disperse widely, with estimated maximum dispersal distance of approximately 0.6 miles between patches of prairie habitat. Additionally, considering the isolation of the species, these small populations need immigration corridors, and evidence shows that they will not travel across unsuitable habitat, which includes row crops such as corn (Federal Register(a) 2013). In fact, dispersal grassland habitat consists of "undeveloped open areas dominated by perennial grassland with limited or no barriers to dispersal including tree or shrub cover less than 25 percent of the area and no row crops such as corn, beans, potatoes, or sunflowers" (Federal Register(a) 2013).

Finally, the Poweshiek skipperling adults emerge mid-June to early-July and typically only have a flight period of about two to four weeks. This limited flight period combined with their low dispersal rate and avoidance of unsuitable habitat (such as row crops) indicates that the likelihood of adult Poweshiek skipperlings laying eggs on their host plant(s) in or adjacent to corn fields is negligible. Furthermore, concern surrounding pollen deposition onto larval host plants is more relevant for lepidoptera whose host plants have wider, horizontal leaves. As the larval host plants of the Poweshiek skipperling tend to be grasses, with early instars indicating a need or preference for threadlike thin blades of grass, these host plants are not a shape that is conducive to pollen accumulation.

Therefore, based on the Poweshiek skipperling's oviposition not being expected to be in or adjacent to corn fields coupled with the shape of the larval host plants not being conducive to pollen accumulation, EPA's analysis has determined that negligible exposure is expected Poweshiek skipperling from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the Poweshiek and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

Figure 10. Overlap of the species range of the Poweshiek skipperling (*Oarisma poweshiek)* and agriculturally cultivated corn.



Current species range is indicated by the green polygons (USFWS(c) 2022) and corn crop is indicated by orange polygons (U.S. EPA (a) 2023).

Bog Buck Moth (Hemileuca maia menyanthevora (=H. Iroquois))
Status: Endangered (
Listed: 4/14/2023
UDL Spatial Analysis Overlap: 8.34%
Overlap Category: Medium

Biology and Habitat Requirements:
The following excerpts are from the Federal Register(a) (2022); consult original reference for citations:

Bog buck moth adults have black bodies and black/gray translucent wings with wide, white wing bands and an eyespot (COSEWIC 2009, p. 5; NatureServe 2015, p. 4) (Figure 11). Bog buck moths have forewing lengths of 22 to 36 millimeters (mm) (0.9 to 1.4 inches (in)) (Tuskes et al. 1996, p. 121; Pavulaan 2020, p. 9). Males and females are generally similar in appearance with a few morphological differences. Similar to all saturniids, males have highly branched, feather-like antennae with receptors that respond to female pheromones (Tuskes et al. 1996, p. 14), and females have simple antennae. Males also have a red-tipped abdomen while females do not; males are also slightly smaller than females (COSEWIC 2009, p. 5). In addition, both male and female adults are larger than other Hemileuca maia and have similar highly translucent wings as H. lucina. White wing bands are much larger than other H. maia (Cryan and Dirig 2020, p. 26; Pavulaan 2020, p. 9).

Figure 11. Male Bog Buck Moth (*Hemileuca maia menyanthevora*).

Photo Credit. Male bog buck moth (center) by J. Jaycox, NYNHP and instar larvae (top right) by K. Sime, USFWS New York Office, Public Domain, https://ecos.fws.gov/docs/recovery_plan/BogBuckMoth%20Recovery%20Outline_final_27%20April%202023%20signed.pdf

The bog buck moth is sedentary (nonmigratory) and therefore present within suitable habitat year-round with small movements of 0.5 kilometers (km) (0.3 miles (mi)) within suitable habitat described as ‘‘common’’ (NatureServe 2015, p. 5). While bog buck moth populations were previously described as individuals separated by areas of unsuitable habitat greater than 2 km (1.24 mi) or areas of suitable habitat greater than 10 km (6.2 mi) with some infrequent dispersal events at slightly longer distances between unsuitable patches (NatureServe 2015, p. 5), movements are now described as ‘‘should be capable of flying several to many kilometers, but seldom leaves habitat’’ NatureServe (2020, p. 5). In New York, some movement likely occurs between sites that are close together. Isolation of populations is likely increased by the short-lived adult stage (not much time for adults to fly far) (COSEWIC 2009, p. 15). Adult females that do make short flights are laden with hundreds of eggs.

Late instar larvae are dark with reddish orange branched urticating (stinging) spines dorsally, and a reddish-brown head capsule and prolegs (COSEWIC 2009, p. 6). Initially egg rings are light green (Cryan and Dirig 2020, p. 26) and fade to light brown or tan (Sime 2020, pers. comm.). Mature larvae are usually predominantly black with small white dots and lack yellow markings compared to other Hemileuca maia (COSEWIC 2009, p. 6; NatureServe 2015, p. 4; Cryan and Dirig 2020, p. 26). The bog buck moth is restricted to open, calcareous, low shrub fens containing large amounts of *Menyanthes trifoliata* (COSEWIC 2009, p. 10) (referred to herein as bog buckbean, but also known as bogbean or buckbean). Fens are classified along a gradient that ranges from rich fens to poor fens based on their water chemistry and plant community structure. Rich fens receive more mineral-rich groundwater than poor fens, which results in higher conductivity, pH, and calcium and magnesium ion concentrations (Vitt and Chee 1990, p. 97). The sites in New York are considered medium fens (New York Natural Heritage Program [NYNHP] 2020a, p. 3). Medium fens are fed by waters that are moderately mineralized, with pH values generally ranging from 4.5 to 6.5 (Olivero 2001, p. 15). Medium fens often occur as a narrow transition zone between a stream or lake and either a swamp or an upland community (Olivero 2001, p. 15).

In the United States, the Lakeside population occurs along the eastern shore of Lake Ontario in Oswego County, NY, and comprises five sites or subpopulations (referred to as Lakeside 1 to Lakeside 5). To the southwest, the Oswego Inland Site population occurs in Oswego County, NY, and is a single site with two fen openings with metapopulation dynamics operating at a smaller scale. It is unlikely that other bog buck moth populations exist besides the ones mentioned above. In New York State, researchers sought out additional populations during years of exploring the bed of former glacial Lake Iroquois and its tributaries and outlets, and while they found some fens with bog buckbean, they found no additional sites with bog buck moths (Cryan and Dirig 2020, pp. 4–5). In addition, researchers have visited fens in New York for many years and likely would have observed the highly conspicuous larvae on bog buckbean or adult male moths, which are readily visible due to their lengthy, localized flight pattern, had they been present.

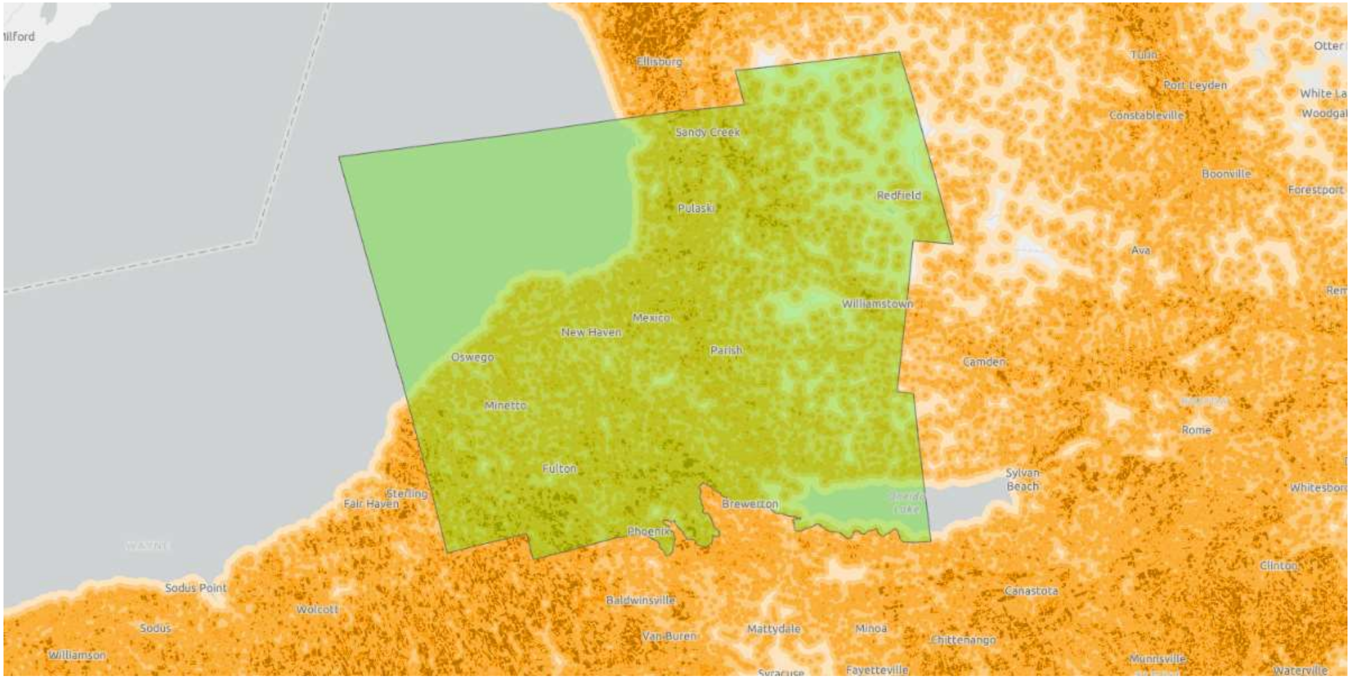
Assessment:

The bog buck moth's habitat consists of groundwater-fed wetlands in Oswego County, New York in the United states. Bog buck moth caterpillars' key food source is bog buckbean (*Menyanthes trifoliata*) (USFWS(i) 2024). The spatial overlap analysis of the species' range and maize production can be found in Figure 12, and the maize UDL overlap calculation is 8.34% (categorized as Medium overlap).

The bog buck moth is restricted to its specialized wetland habitat, located in only one county in New York, with its primary larval food source, bog buckbean, only located within this specialized wetland habitat. Given the limited location of this primary resource, coupled with the very small body size of the moth (wingspans of 5-7 cm), evidence suggests it rarely leaves its habitat. Given this habitat and larval food plant restriction, the likelihood of larvae of this moth coming into contact with pollen from a maize producing field is negligible.

Therefore, EPA's analysis has determined that negligible exposure is expected for bog buck moth from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the bog buck moth and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

Figure 12. Overlap of the species range of the Bog Buck Moth (*Hemileuca maia menyanthevora (=H. Iroquois)* and agriculturally cultivated corn.



Current species range is indicated by the green polygons (USFWS(b) 2023) and corn crop is indicated by orange polygons (U.S. EPA (a) 2023).

Saint Francis' Satyr Butterfly (*Neonympha mitchellii francisci*)
Status: Endangered (USFWS(j) 2024)
Listed: 4/18/1994
UDL Spatial Analysis Overlap: 3.29%
Overlap Category: Low

Biology and Habitat Requirements:
The following excerpts are from the Federal Register (1995); consult original reference for citations:

Saint Francis' satyr is a fairly small, dark brown butterfly and is a typical member of the Satyrinae, a subfamily of the Nymphalidae family, which includes many species commonly called satyrs and wood nymphs (Figure 13). The wingspan for the species ranges from 34 to 44 mm (Opler and Malikul 1992). Saint Francis' satyr and Mitchell's satyr (N. m. mitchellii), the northern subspecies, which was listed as endangered on May 20, 1992 (57 FR 21569), are nearly identical in size and show only a slight degree of sexual size dimorphism (Hall 1993, Parshall and Kral 1989). Like most species in the wood nymph group, Saint Francis' satyr has conspicuous ``eyespots'' on the lower surfaces of the wings.

Figure 13. Saint Francis’ Satyr Butterfly (*Neonympha mitchellii francisci*).

Photo Credit. Saint Francis’ satyr butterfly, Becky Harrison, USFWS, https://ecosphere-documents-production-public.s3.amazonaws.com/sams/public_docs/species_nonpublish/2972.pdf

Saint Francis' satyr is extremely restricted geographically. The northern subspecies has been eliminated from approximately half its known range, primarily due to collecting (Refsnider 1991). Saint Francis' satyr is now known to exist as a single population in North Carolina. The habitat occupied by this satyr consists primarily of wide, wet meadows dominated by sedges and other wetland graminoids. In the North Carolina sandhills, such meadows are often relicts of beaver activity. Unlike the habitat of Mitchell's satyr, the North Carolina species' habitat cannot properly be called a fen because the waters of this sandhills region are extremely poor in inorganic nutrients.

Larval host plants are believed to be graminoids such as grasses, sedges, and rushes. Little else is known about the life history of this butterfly.

Hall (1993) states: Under the natural regime of frequent fires ignited by summer thunderstorms, the sandhills were once covered with a much more open type of woodland, dominated by longleaf pine, wiregrass, and other fire-tolerant species. The type of forest that currently exists along [the creek inhabited by Saint Francis' satyr] can only grow up under a long period of fire suppression. The dominance on this site of loblolly pine, moreover, is due primarily to past forestry management practices, not any form of natural succession. Extensive searches have been made of suitable habitat in North Carolina and South Carolina, but no other populations of this butterfly have been found (Hall 1993, Schweitzer 1989).

The following excerpt is from (USFWS(k) 2024); consult the original reference for citations:

Soon after its discovery in the 1980s, scientists believed that this small, dark brown butterfly had been collected to extinction, but it was rediscovered in 1992. Its habitat is open grassy wetlands maintained naturally by fire and beaver. The species is very dependent on disturbance and dynamic environments. Military activities on the artillery ranges also maintain the open wetland habitats in the absence of beaver. Disturbance can both create new habitats but also destroy existing habitats, so finding the ideal levels is necessary to help butterfly populations. Currently all known subpopulations are found on Ft. Bragg, an active military installation in central North Carolina.

The historic range for the butterfly consists solely of the area currently known to be occupied by the species within Ft. Bragg. Only a single metapopulation of Saint Francis’ satyr is known to exist on training grounds of Ft. Bragg in the sandhills of North Carolina, in Cumberland and Hoke Counties (USFWS(c) 2021). Efforts are currently underway to augment existing populations at Ft. Bragg with releases of captive-reared adults.

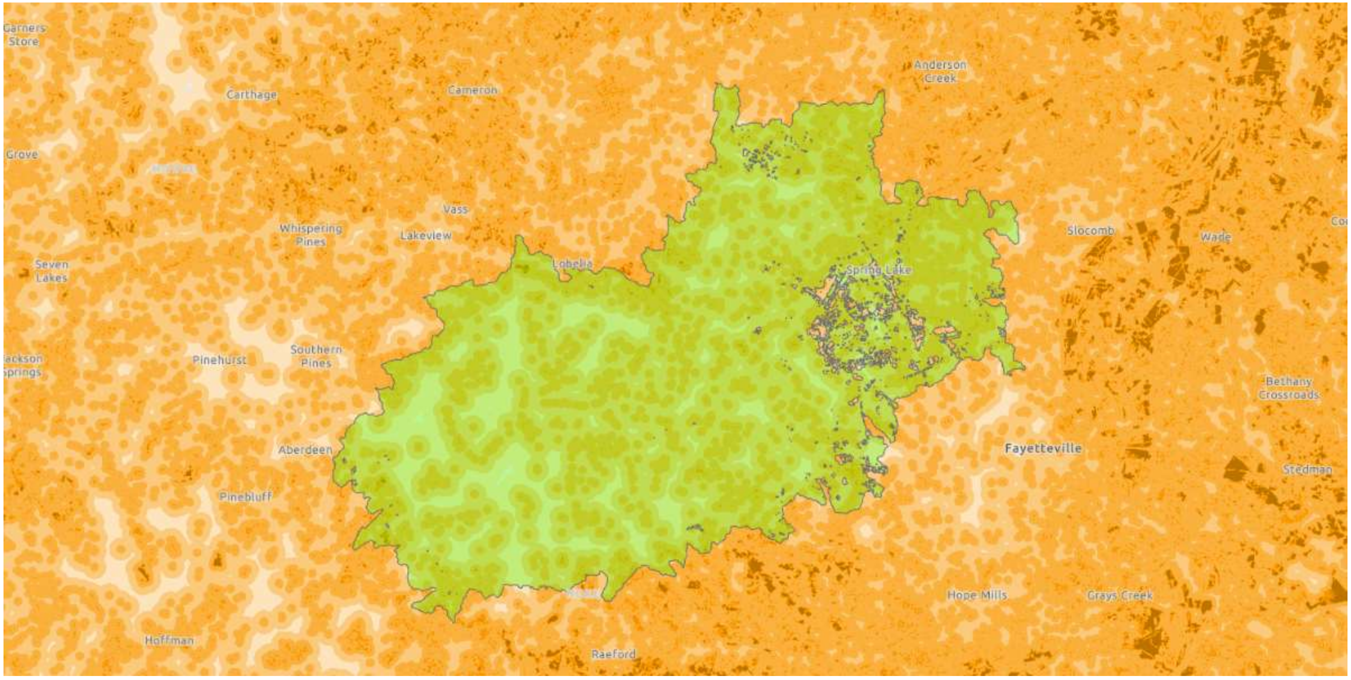
Assessment:

The Saint Francis' Satyr Butterfly is extremely restricted geographically. The habitat occupied by this satyr consists primarily of wide, wet meadows dominated by sedges and other wetland graminoids, maintained naturally by fire and beaver. The species is very dependent on disturbance and dynamic environments. Only a single metapopulation of Saint Francis' satyr is known to exist on training grounds of Ft. Bragg in the sandhills of North Carolina, in Cumberland and Hoke Counties (USFWS(c) 2021). The spatial overlap analysis of the species' range and maize production can be found in Figure 14, and the maize UDL overlap calculation is 3.29% (categorized as low overlap); however, the restriction of the species to its to a location on a military installation makes UDL overlap estimate unrealistic.

The extreme isolation and restriction solely to the training grounds of Ft. Bragg in the sandhills of North Carolina makes the likelihood of this butterfly being exposed to pollen drift from event DAS1131 maize practically nonexistent.

Therefore, EPA's analysis has determined that negligible exposure is expected for Saint Francis' satyr butterfly from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the Saint Francis' satyr butterfly and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

Figure 14. Overlap of the species range of the Saint Francis' Satyr Butterfly (*Neonympha mitchellii francisci)* and agriculturally cultivated corn.



Current species range is indicated by the blue (USFWS(c) 2021) and corn crop is indicated by orange polygons (U.S. EPA (a) 2023).

Fender's Blue Butterfly (*Icaricia icarioides fenderi*)
Status: Endangered (USFWS
Listed: 1/25/2000
UDL Spatial Analysis Overlap: 3.18%
Overlap Category: Low

Biology and Habitat Requirements:
The following excerpts are from the Federal Register(b) (2000); consult original reference for citations:

The upper wings of males of the Fender's blue butterfly are brilliant blue in color with black borders and basal areas, whereas the upper wings of females are brown (Figure 15). Fender's blue butterfly is found only in the prairie and oak savannah habitats of the Willamette Valley of Oregon. Fender's blue butterfly relies primarily upon a relatively uncommon lupine plant, the Kincaid's lupine (*Lupinus sulphureus ssp. kincaidii*), also endemic to the Willamette Valley and listed as a threatened species under the Act (65 FR 3875; January 25, 2000), as the host plant for the larval (caterpillar) life stage (Hammond and Wilson 1993, p. 2). The only other host plants known for Fender's blue butterflies are *Lupinus arbustus* (longspur lupine) and Lupinus albicaulis (sickle-keeled lupine) (Schultz et al. 2003, pp. 64–67). Females lay single eggs, up to approximately 350 eggs in total, on the underside of the leaves of one of these three lupine species. Eggs hatch from mid-May to mid-July, and the larvae feed on the lupine until the plants senesce and the larvae go into diapause for the fall and winter. The larvae break diapause in early spring, feed exclusively on the host lupine, and metamorphose into adults, emerging as butterflies between mid-April and the end of June. Adult Fender's blue butterflies only live 7 to 14 days, and feed exclusively on nectar from flowering plants (Schultz 1995, p. 36; Schultz et al. 2003, pp. 64–2012;65).

Given its short adult lifespan, Fender's blue butterfly has limited dispersal ability. Butterflies are estimated to disperse approximately 0.75 kilometers (km) (0.5 miles (mi)) if they remain in their natal lupine patch, and approximately 2 km (1.2 mi) if they disperse between lupine patches (Schultz 1998, p. 290).

Figure 15. Fender's Blue Butterfly (*Icaricia icarioides fenderi*).

Photo Credit. Fender's Blue Butterfly, ECOS USFWS, https://ecos.fws.gov/ecp/species/6659 https://ecosphere-documents-production-public.s3.amazonaws.com/sams/public_docs/species_nonpublish/2218.pdf

Assessment:

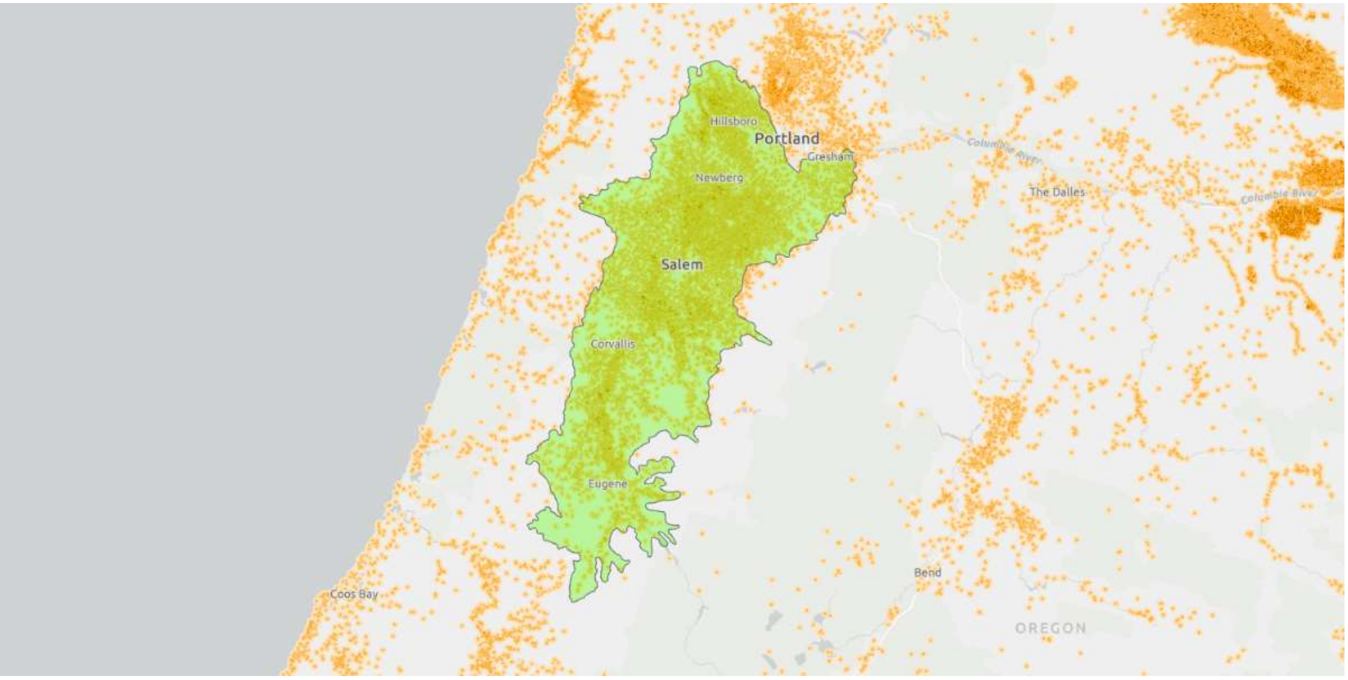
The Fender's Blue Butterfly habitat is found only in the prairie and oak savannah habitats of the Willamette Valley of Oregon (USFWS(d) 2021). The caterpillar's primary food plant is the Kincaid's lupine (*Lupinus sulphureus ssp. kincaidii*) with only two other known host plants- *Lupinus arbustus* (longspur lupine) and *Lupinus albicaulis* (sickle-keeled lupine) (Federal Register(b) 2000). Kincaid's lupine is also endemic to the Willamette Valley and is relatively rare as it is listed as a threatened species under the Act (Federal Register (b) 2000). The spatial overlap analysis of the species' range and maize production can be found in Figure 16, and the maize UDL overlap calculation is 3.18% (categorized as low overlap).

The Fender's Blue Butterfly has only one generation per year, with adult butterflies laying eggs during the month of May and larvae feeding briefly on host plants until the plants senesce in early July. After this time, the larvae drop to the base of the plants and enter diapause and do not feed on leaves until new plants emerge in February or March. Therefore, like the Karner blue butterfly which also feeds on a lupine species, the temporal overlap of Fender's Blue Butterfly larval feeding and corn pollen shed is expected to be minimal to nonexistent.

Furthermore, the Fender's Blue Butterfly range is highly restricted to specific locations within the Willamette Valley in Oregon. Additionally, as the butterfly is dependent on another endangered species, the Kincaid's lupine, as its primary food source. Kincaid's lupine is also restricted to its critical habitat, which has a maize UDL overlap of 0.0% and 0.057% at 0 and 30 m off-field, indicating that the host plant is not expected to be in or adjacent to corn fields. Finally, the potential flight range of this species is minimal, it's expected range is limited to its critical habitat. This limited geographic range results in a negligible likelihood of this butterfly being exposed to pollen drift from event DAS1131 maize.

Therefore, the EPA's analysis has determined that negligible exposure is expected for Fender's blue butterfly from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the Fender’s blue butterfly and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

Figure 16. Overlap of the species range of the Fender's blue butterfly (*Icaricia icarioides fenderi)* and agriculturally cultivated corn.



Current species range is indicated by the green polygons (USFWS(d) 2021) and corn crop is indicated by orange polygons (U.S. EPA (a) 2023).

Lange’s Metalmark Butterfly (*Apodemia mormo langei*)
Status: Threatened (USFWS(v) 2024)
Listed: 6/7/1976
UDL Spatial Analysis Overlap: Species range 2.077%
Overlap Category: Low

Biology and Habitat Requirements:
The following excerpt is from (USFWS(b) 2020); consult the original reference for citations:

The Lange’s metalmark butterfly (Figure 17) is a relatively small butterfly, with a wing expanse from 18-27 millimeters [mm.] (0.06 -0.71-inches [in.]), mostly black and orange colored with white spotting, and primarily identified by orange scaling of the discal spot (Comstock, 1939). It is a univoltine subspecies (producing one brood per year) that lays eggs in small clusters on the larval host plant in late summer. The first larval instar emerges from these eggs following the first rain events of the fall, and the leaves of the larval host plant provide both food and shelter throughout the larval instar phases (Arnold, 1978; Arnold, 1980). Adult butterflies begin emerging from pupation in early August, and may be observed until mid- or late-September, with females laying eggs throughout this adult flight period (Service, 1984). Adult butterflies may live on average a week, and though little is known about the duration of the larval stages in the wild, pupation is thought to last 6-18 days (Arnold, 1980).

All life stages of Lange's metalmark are closely tied to *Eriogonum nudum* var. *psychicola* (formerly var. *auriculatum*), hereafter referred to as Antioch Dunes buckwheat. This host plant, also endemic to the Antioch Dunes, serves as the primary nectar source for adult butterflies, as sites for oviposition, and as the larval foodplant (Arnold, 1978). However, Antioch Dunes buckwheat may not be utilized by the Lange's metalmark until plants are about three years old, when it is able to produce robust flowers (Arnold, 1983). Antioch Dunes buckwheat is a perennial forb that requires sandy, well-drained soils and some form of disturbance, preferably by natural processes such as wind or erosion, to shift the sand for seedling establishment (Arnold, 1978). Flowering of Antioch Dunes buckwheat begins in July or August, depending on the climate, and just prior to the emergence of Lange's metalmarks. The species is found in several habitat types, from canyons and valleys to sandy dunes.

Currently, Lange's metalmark can only be found within the Antioch Dunes National Wildlife Refuge (ADNWR), the last remnants of the Antioch Dunes following commercial mining and land development. The ADNWR boundary encompasses 67 acres; the 41-acre Stamm Unit, owned by the Service, and the 26-acre Sardis Unit, of which 14 acres are owned by the Service and 12 acres are owned by Pacific Gas and Electric (Service, 2002). Lange's metalmark is associated with Antioch Dunes, a riverbank dune system that historically reached heights of over 100 feet, but specimens collected from Oakley suggest the subspecies may not have been confined to the Antioch Dunes (Howard and Arnold, 1980; Stanford et al., 2011).

Figure 17. Lange's Metalmark Butterfly (*Apodemia mormo langei*)

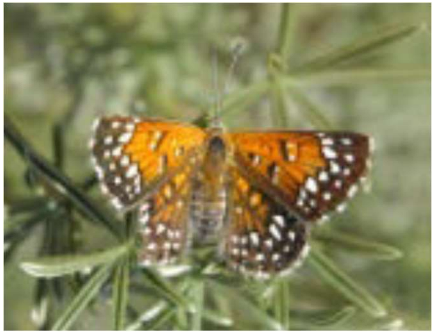


Photo credit https://ecosphere-documents-production-public.s3.amazonaws.com/sams/public_docs/species_nonpublish/3078.pdf

The only extant population of Lange's metalmark is located at ADNWR, a very limited geographic area representing the entirety of the known range-wide native population. The refuge itself is bifurcated by the Georgia Pacific Gypsum Plant, separating the Sardis and Stamm Units by greater than 600 meters. Annual surveys have been conducted in at least some portion of the Stamm and Sardis Units since about 1986. Prior to this, Arnold and Powell conducted ecological studies of the Lange's metalmark and its habitat from 1977 to 1985 (Arnold, 1978; Arnold, 1980; Arnold, 1983; Arnold and Powell, 1983; Arnold 1986; Powell, 1983). During this time, Arnold used capture-mark-recapture methods to estimate the population of Lange's metalmark, and studied the flight patterns of individuals (Arnold, 1978; Arnold, 1986). These studies estimated that the population size was approximately 2,100 in 1976, but declined rapidly to an estimate of 560 in 1982, before partially rebounding to an estimate of 1,286 in 1985 (Arnold, 1983; Arnold, 1986). These studies were discontinued due to a determination by the Service that capture-mark-recapture methods adversely affected the species (Service, 1986).

Assessment:

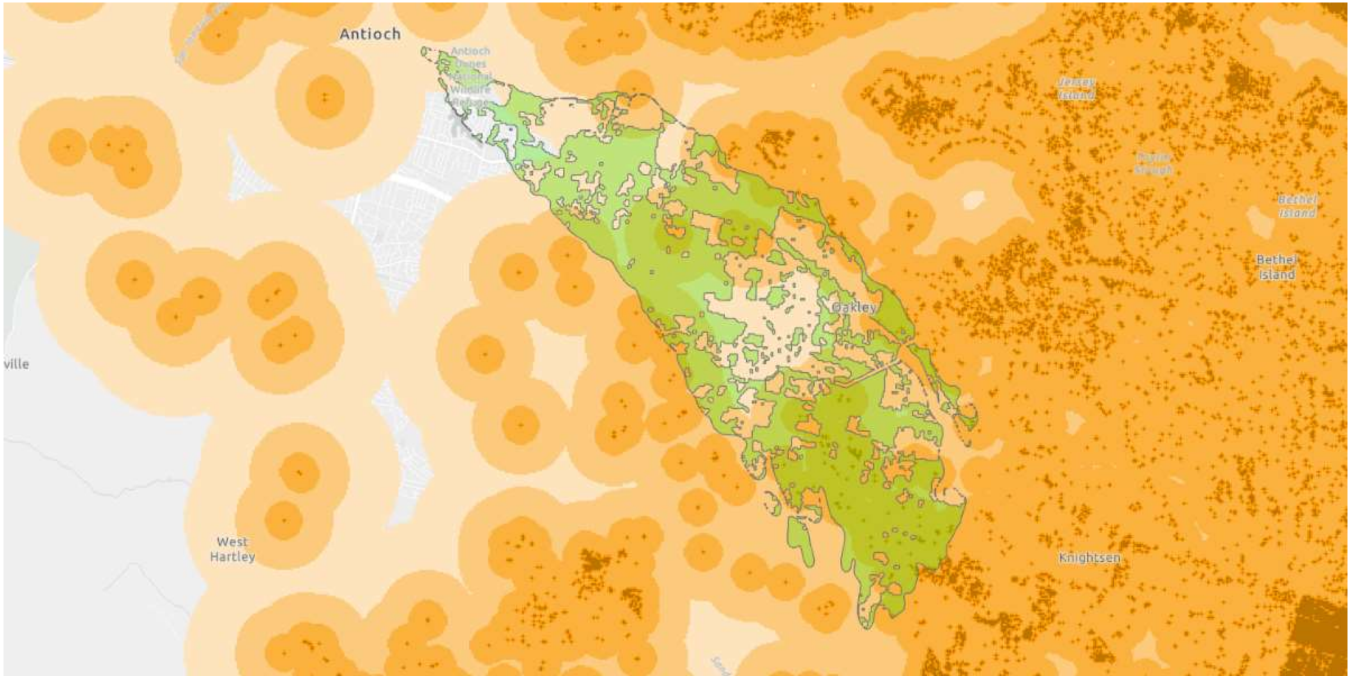
The Lange's metalmark butterfly is extremely restricted geographically and can only be found in the Antioch Dunes National Wildlife Refuge (ADNWR). The habitat occupied by this butterfly consists of sand dunes, canyons, and valleys. The spatial overlap analysis of the species' range and maize production can be found in Appendix A, and the maize UDL overlap calculation is 2.077% for the species (categorized as low overlap).

The species is entirely dependent on its host plant Antioch Dunes buckwheat for all of its life stages and is endemic to the Antioch Dunes. Antioch Dunes buckwheat is a perennial forb that requires sandy, well-drained soils and some form of disturbance, preferably by natural processes such as wind or erosion, to shift the sand for seedling establishment (USFWS 2020). Furthermore, adult butterflies typically emerge from early August to mid-or late September, during which time they lay eggs. The eggs remain dormant until the first part of the rainy season, which occurs in the fall after the corn pollen shed period. Therefore, there is no reasonable expectation of exposure to Lange's metalmark butterfly larvae from pollen deposition.

The extreme isolation and restriction solely to the ADNWR, the specific habitat requirements of its host plant, and the lack of temporal overlap between larvae and corn pollen shed makes the likelihood of this butterfly being exposed to pollen drift from event DAS1131 maize negligible to nonexistent.

Therefore, EPA's analysis has determined that negligible exposure is expected for Lange's metalmark butterfly from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the Lange's metalmark butterfly and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

Figure 18. Overlap of the species range of the Lange's metalmark butterfly (*Apodemia mormo langei*) and agriculturally cultivated corn.



Current species range is indicated by the green polygons (USFWS(d) 2021), and corn crop is indicated by orange polygons

(U.S. EPA (a) 2023).

Taylor's (=whulge) Checkerspot (*Euphydryas editha taylori*)
Status: Endangered (USFWS(m) 2024)
Listed: 11/4/2013
UDL Spatial Analysis Overlap: 1.48%
Overlap Category: Low

Biology and Habitat Requirements:
The following excerpts are from the Federal Register(b) (2013); consult original reference for citations:

Taylor's checkerspot butterfly is a medium-sized, colorfully marked butterfly with a checkerboard pattern on the upper (dorsal) side of the wings (Pyle 2002, p. 310) (Figure 19). Their wings are orange with black and yellowish (or white) spot bands, giving them a checkered appearance (Pyle 1981, p. 607; Pyle 2002, p. 310).

Figure 19. Taylor's Checkerspot (*Euphydryas editha taylori*).

Photo Credit. Taylor's checkerspot, USFWS - Endangered Species/USFWS, https://www.fws.gov/banner/taylors-checkerspot

Historically, the Taylor's checkerspot butterfly was likely distributed throughout grassland habitat found on prairies, shallow-soil balds (a bald is a small opening on slopes in a treeless area, dominated by herbaceous vegetation), grassland bluffs, and grassland openings within a forested matrix in south Vancouver Island, northern Olympic Peninsula, the south Puget Sound, and the Willamette Valley. The historical range and abundance of the subspecies are not precisely known because extensive searches for the Taylor's checkerspot butterfly did not occur until recently. Northwest prairies were formerly more common, larger, and interconnected, and would likely have supported a greater distribution and abundance of the Taylor's checkerspot butterflies than prairie habitat does today.

In Washington and Oregon, the adults lay eggs and larvae feed on native plants from the broomrape (*Orobanchaceae*) family (*Castilleja hispida, C. levisecta*) in addition to the non-native ribwort plantain. Several annual species are documented as larval host foods, including several species of speedwell (Veronica spp), blue-eyed Mary (*Collinsia grandiflora* and *C. parviflora*) and sea blush (*Plectritus congesta*). The two remaining populations in Oregon also depend upon *P. lanceolata*, however, golden paintbrush (*C. levisecta*) has been planted where Taylor's checkerspots are found and they have been documented using the species for egg laying and as a larval food resource. Adults will feed on numerous species that bloom during their flight period, but *Balsamorhiza deltoidea*, *Lomatium triternatum*, *Micranthes integrifolia* and *Camassia quamash* are particularly important (USFWS 2019)

The Taylor's checkerspot butterfly was historically known to occur in British Columbia, Washington, and Oregon, and its current distribution represents a reduction from over 80 locations range-wide to 14. Nearly all localities for the Taylor's checkerspot butterflies in British Columbia have been lost; the only location currently known from British Columbia was discovered in 2005 (COSEWIC 2011, p. iv). In Oregon, although many surveys have been conducted at a variety of historical and potential locations within the Willamette Valley, many of those have failed to detect the species; the number of locations occupied by Taylor's checkerspot butterflies in Oregon has declined from 13 to 2 (Ross 2011, in litt., p. 1). In Washington State, more than 43 historical locales were documented for the Taylor's checkerspot butterfly. In 2012, there were 11 documented locations for the Taylor's checkerspot butterflies with only 1 of the localities harboring more than 1,000 individuals, and the majority of known sites have daily counts of fewer than 100 individual butterflies.

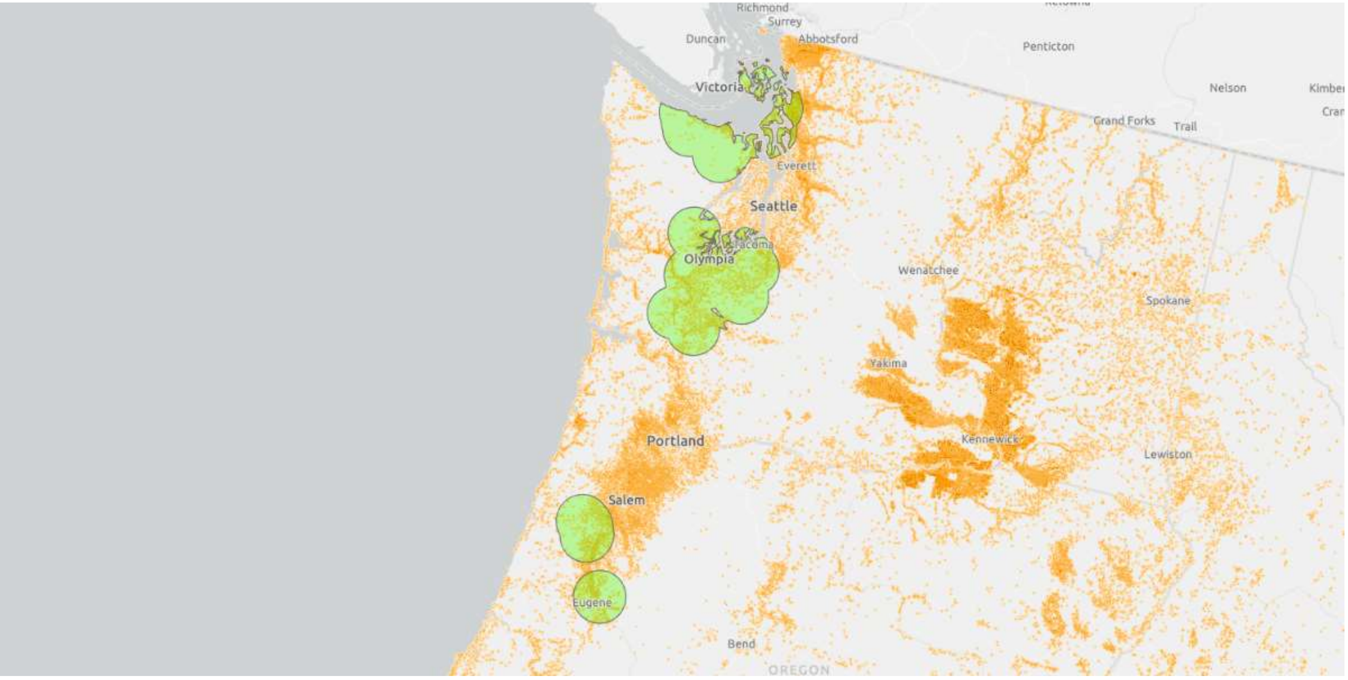
Assessment:

The Taylor's (=whulge) Checkerspot butterfly was historically known to occur in Washington, and Oregon, and its current distribution represents a reduction from over 80 locations range-wide to 14. The spatial overlap analysis of the species' range and maize production can be found in Figure 20, and the maize UDL overlap calculation is 1.48% (categorized as low overlap).

Taylor's checkerspots produce one brood per year, with larvae entering diapause in mid-June to early July, indicating that the temporal overlap of larval feeding and corn pollen shed is expected to be minimal to nonexistent. Furthermore, Taylor's (=whulge) checkerspot butterfly is now only found in 14 isolated locations in Washington and Oregon, and their habitat is specific to open prairies and Garry oak meadows and balds, not agricultural fields. Given the habitat specialization and minimal to nonexistent temporal overlap between pollen shed and larval feeding, the likelihood of this butterfly being exposed to pollen drift from event DAS1131 maize is negligible.

Therefore, EPA's analysis has determined that negligible exposure is expected for Taylor's (=whulge) checkerspot from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the Taylor's (=whulge) checkerspot and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

Figure 20. Overlap of the species range of the Taylor's (=whulge) Checkerspot (*Euphydryas editha taylori)* and agriculturally cultivated corn.



Current species range is indicated by the green polygons (USFWS(o) 2024) and corn crop is indicated by orange polygons (U.S. EPA (a) 2023).

Bay checkerspot (*Euphydryas editha bayensis*)
Status: Endangered (USFWS(e) 2021)
Listed: 6/9/2021
UDL Spatial Analysis Overlap: 1.061%
Overlap Category: Low

Biology and Habitat Requirements:
The following excerpts are from the (USFWS(v) 2024); consult original reference for citations:

Bay checkerspot butterflies are medium-sized insects and have brilliant markings in a mosaic of white, black and reddish-orange (Figure 21). This butterfly was only found in Santa Clara County until recently when it was reintroduced to San Bruno Mountain and Edgewood County Park in San Mateo County, California.

Figure 21. Bay checkerspot (*Euphydryas editha bayensis*).

Photo Credit. https://www.fws.gov/species/bay-checkerspot-butterfly-euphydryas-editha-bayensis

Historically, the subspecies occurred in the vicinity of the San Francisco Bay area from San Bruno Mountain, west of the bay, Mount Diablo, east of the bay, to Coyote Reservoir, south of the bay. The current range of the subspecies is limited to Santa Clara and San Mateo counties, California and all occurrences are on serpentine or serpentine-like grasslands except for San Bruno Mountain where it occurs on non-serpentine nonnative annual grasslands. Since 2009, Bay checkerspot butterfly reintroductions or translocations continued in Santa Clara County at Tulare Hill and in San Mateo County at Edgewood Natural Preserve and San Bruno Mountain. The Edgewood and Tulare Hill reintroductions have had limited success, while the San Bruno Mountain reintroduction has the potential for success.

The adult butterfly's average life span is about 10 days with some individuals living over three weeks. The Bay checkerspot butterfly reaches sexual maturity each year and generally reproduces and dies within a single year. Adults emerge from pupae in early spring from late February to early May and have an average life span of about 10 days with some individuals living over three weeks. Eggs are laid during the 4 to 6-week flight season near the base of the larval host plant and hatch within 10 days. Bay checkerspot caterpillars go through two different phases of feeding with a pause in between. The first is just after they hatch between March and May, where they will feed until they have molted three times. Larvae enter diapause and spend the summer in cracks and crevices or under rocks. Then after going dormant for the hot and dry months of summer and fall, they wake up sometime in November to February, and eat more until finally making their chrysalis in early spring. After mating, females lay 1 to 5 egg masses on the larval host plant containing anywhere from 5 to 250 eggs each. Eggs hatch in 13 to 15 days.

The primary larval host plant for the butterfly is a small, annual, native dwarf plantain (*Plantago erecta*). The butterfly also frequently requires the presence of a secondary host plant, either purple owl's-clover (*Castilleja densiflora*) or exserted paintbrush (*Castilleja exserta*), since owl's clover and the paintbrush remain edible longer than the plantain. At San Bruno Mountain, the butterfly also utilizes the non-native English plantain (*Plantago lanceolata*) as a larval host plant, which is more abundant and remains edible longer than the dwarf plantain. The Bay checkerspot butterfly requires areas with topographic diversity, which are defined as having warm south and west slopes, as well as cool north and east slopes, because some slopes become unfavorable depending on annual weather conditions and time of year. The delayed senescence of host plants on cool, moist slopes allows larvae to reach their fourth instar, which is the larval development stage or molt, and enter diapause, a stage of dormancy, before host plants become inedible. Larvae that are not able to enter diapause prior to host plant senescence starve and die. Warm temperatures in the spring accelerate the senescence of the host plants resulting in fewer larvae surviving to the adult phase.

Assessment:

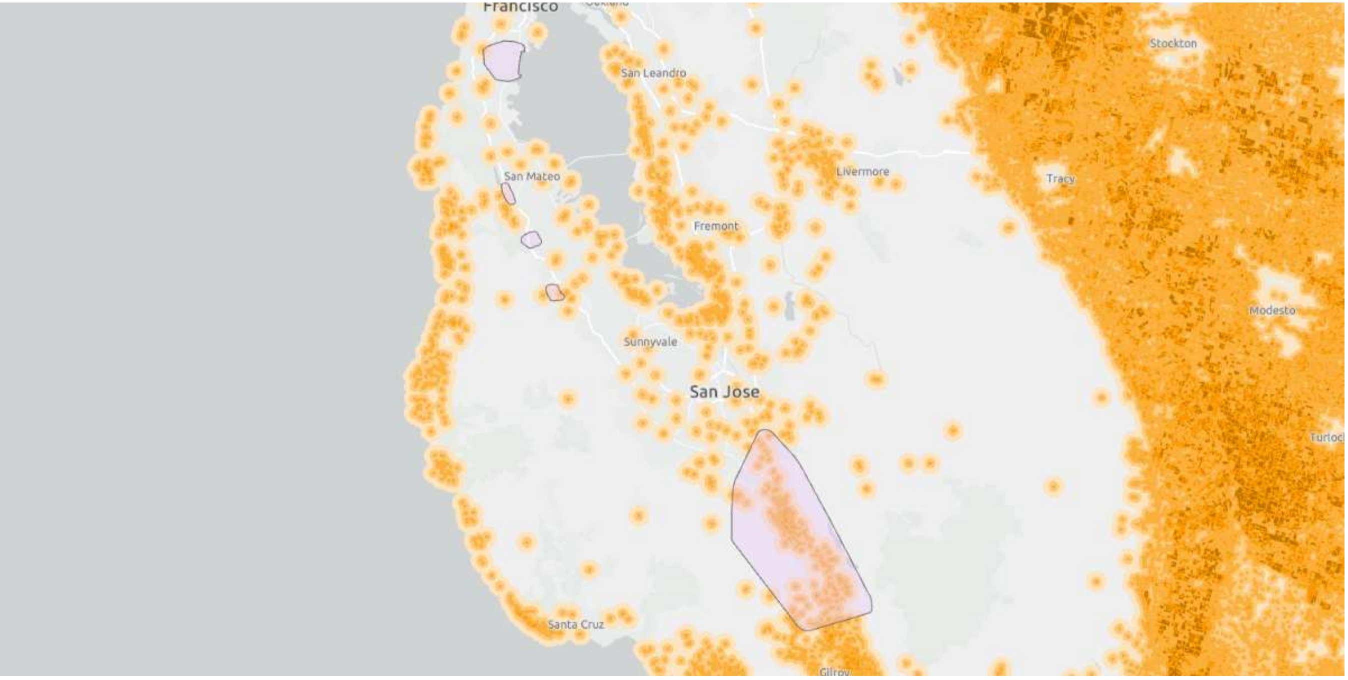
The bay checkerspot butterfly was only found in Santa Clara County until recently when it was reintroduced to San Bruno Mountain and Edgewood County Park in San Mateo County, California. The Bay checkerspot butterfly requires areas with topographic diversity, which are defined as having warm south and west slopes, as well as cool north and east slopes, because some slopes become unfavorable depending on annual weather conditions and time of year. The spatial overlap analysis of the species' range and maize production can be found in Appendix A, and the maize UDL overlap calculation is 1.061% (categorized as low overlap).

Bay checkerspot caterpillars go through two different phases of feeding with a pause in between. The first is just after they hatch between March and May, where they will feed until they have molted three times. Larvae enter diapause and spend the summer in cracks and crevices or under rocks. Then after going dormant for the hot and dry months of summer and fall, they wake up sometime in November to February, and eat more until finally making their chrysalis in early spring.

Given the habitat specialization and nonexistent temporal overlap between pollen shed and larval feeding, the likelihood of this butterfly being exposed to pollen drift from event DAS1131 maize is negligible.

Therefore, EPA's analysis has determined that negligible exposure is expected for bay checkerspot from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the bay checkerspot and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

Figure 22. Overlap of the species range of the Bay checkerspot (*Euphydryas editha bayensis)* and agriculturally cultivated corn.



Current species range is indicated by the pink polygons (USFWS(e) 2021) and corn crop is indicated by orange polygons (U.S. EPA (a) 2023).

Puerto Rican harlequin butterfly (*Atlantea tulita*)
Status: Threatened (USFWS(p) 2024)
Listed: 11/4/2013
UDL Spatial Analysis Overlap: 0.510%
Overlap Category: Low

Biology and Habitat Requirements:

The following excerpts are from the Federal Register(b) (2022); consult original reference for citations:

The Puerto Rican harlequin butterfly (Figure 23) is endemic to Puerto Rico, occurring in the western portion of the island, in the Northern Karst region and in the West-central Volcanic-serpentine region. Eggs and larvae are found only on *Oplonia spinosa* (prickly bush). First instars feed only on this plant. While prickly bush is essential to Puerto Rican harlequin butterfly viability, the plant occurs throughout the species' range and, unless removed for land clearing, is not a limited resource. Active during the daytime, the butterflies feed on the nectar of several tree species and also drink water. Puerto Rican harlequin butterflies have been found only within 1 km (0.6 mi) of a water source (e.g., creek, river, pond, puddle).

Figure 23. Puerto Rican harlequin butterfly (*Atlantea tulita*).

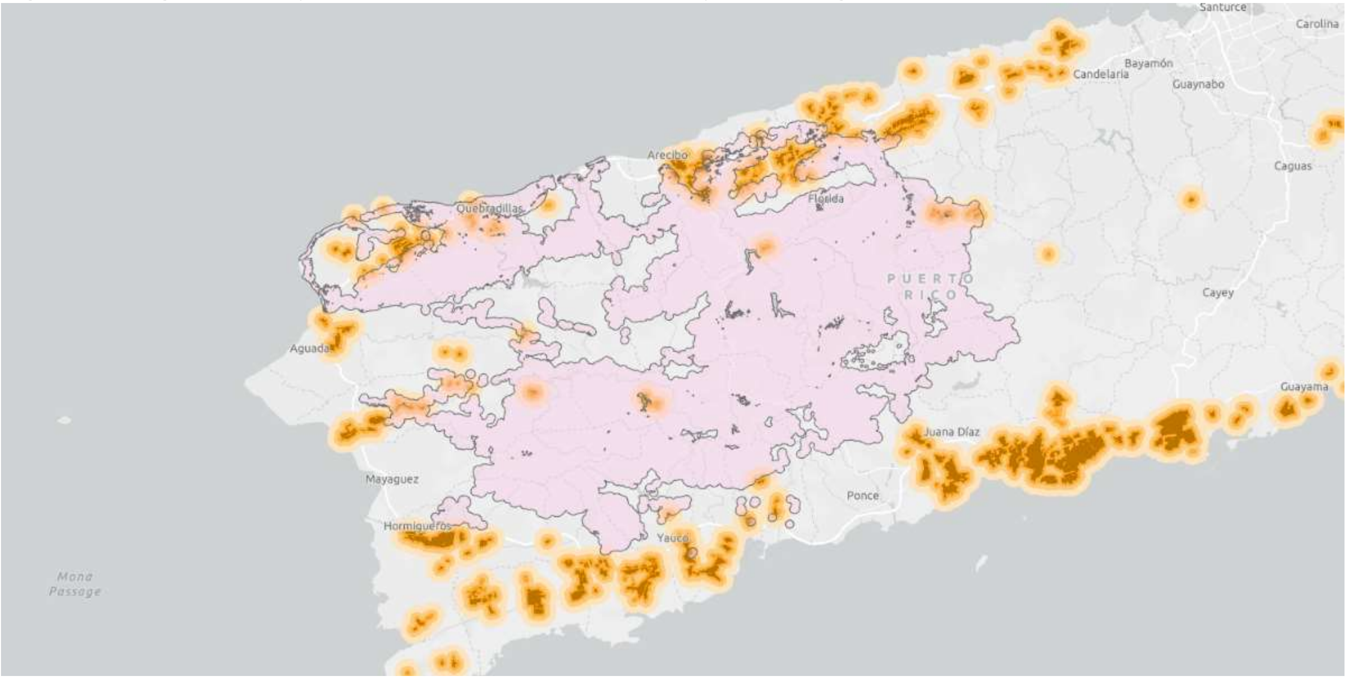
Photo credit: https://www.fws.gov/sites/default/files/2021-07/%283%29%20Credit_USFWS_Jan%20Zegarra_USFWS.JPG

Assessment:

The Puerto Rican harlequin butterfly has a specialized diet (prickly bush) that does not include the consumption of corn. While the UDL overlap calculation is 0.510% (categorized as low overlap), this calculation uses a dataset that encompasses all agricultural production (Figure 24), not just maize production, and is therefore an extremely conservative exposure scenario. A dataset for all agricultural production was initially used because the corn crop data layer used in the UDL overlap percent calculations for the other TES assessments did not contain agricultural corn production for Puerto Rico (or Hawaii). While there is no commercial corn data layer available for Puerto Rico, the most recent census of agriculture in Puerto Rico lists corn production (seed only) at 441 acres (USDA(b) 2024). Using this value places potential overlap well below the 1% threshold (0.081% overlap). Consequently, the likelihood of this butterfly being exposed to pollen drift from event DAS1131 maize producing field is negligible.

Therefore, EPA's analysis has determined that negligible exposure is expected for Puerto Rican harlequin butterfly from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the Puerto Rican harlequin butterfly and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

Figure 24. Agricultural production as it relates to the species range of *Atlantea tulita* in Puerto Rico.



Current species range is indicated by the pink polygons (USFWS(q) 2024) and agricultural crop is indicated by orange polygons (U.S. EPA (b) 2023).

Smith's blue butterfly (*Euphilotes enoptes smithi*)
Status: Endangered (USFWS(c) 2023)
Listed: 12/28/2023
UDL Spatial Analysis Overlap: 0.459%
Overlap Category: Low

Biology and Habitat Requirements:
The following excerpts are from the (USFWS(c) 2020); consult original reference for citations:

Smith's blues have a wingspan of less than one inch (0.80.9679 [in]; 2.02–2.42 centimeters [cm]) (Mattoni 1954, pp. 160–161). Like other members of this tribe, Smith's blues have blue on the dorsal (upper) sides of their wings. Males are bright blue, whereas females are more brown-colored with a band of orange marks across the hind wings. The wings of both species have a checkered fringe along the outer edge, which is more pronounced on the forewings (front wings closer to the head) than the hindwings (rear wings). The ventral (under) sides of both sexes are whitish-gray, speckled with black dots, and have a narrow and wavy orange hindwing band (Figure 25).

Figure 25. Smith's blue butterfly (*Euphilotes enoptes smithi*).

Photo Credit Lara Drizd/U.S. Fish and Wildlife Service

The Smith's blue is univoltine. The life cycle of the butterfly and seasonal activity is synchronized with the blooming period of two buckwheat host species, coast buckwheat (*E. latifolium*) and seacliff buckwheat (E. parvifolium). A third species known as naked buckwheat (*E. nudum*) is used to a lesser extent by adults only for nectaring (Arnold 1991, p. 4). Each summer, adult Smith's blues are active for about four to ten weeks. The flight season extends from mid-June to early September, but the activity period and duration can vary dramatically from year to year and from one location to another (Figure 3; Arnold 2002, p. 15). Smith's blues in the northern metapopulation tend to be active from mid-June to early August, while those in the southern metapopulation are active later, between mid-July and early September (Arnold 1983b, p. 51). Females lay single eggs on the buckwheat flowerheads, typically on sepals of the newly opened flowers or on the late bud stage (Arnold 1978, p. 52). Oviposition occurs after females probe flowers with their abdomens, repeatedly walking over the flowers and "dancing" on the flowerheads. Larvae hatch four to eight days after oviposition and begin feeding. Young larvae feed on the pollen and developing flower parts, while older larvae feed on the seeds. Larvae grow and molt through five instars, maturing after approximately one month. They pupate between mid-August and September, and drop into the leaf litter and sand at the base of the buckwheat where they overwinter as pupae and emerge as adults the following summer.

Movement data from capture-recapture studies indicate that most adults are quite sedentary, with most movements averaging 300 feet (ft; 90 meters [m]) (Arnold 1983b, p. 69; Arnold 1986, p. 10). However, a small percentage of adults move farther and exhibited movements greater than 400 ft (120 m), and one was observed flying 3770 ft (1150 m) from where it was originally captured (Arnold 1986, p. 10). In contrast to the adult butterflies, larvae are more stationary and will complete their development on a single plant if there is adequate food.

Currently, the Smith's blue occurs in scattered colonies along approximately 93 mi (150 km) of California's Central Coast from Monterey County to San Luis Obispo County. The Smith's blue utilizes inland and coastal sand dunes and cliffside chaparral communities along central California; however, the habitats of the northern and southern metapopulations of Smith's blue have significant differences. The northern metapopulation, which covers the coastal strip next to Monterey Bay, is distinguished by dune habitats occupied by both the coast and seacliff buckwheats. The portion of this metapopulation close to the Salinas River is dominated by the coast buckwheat, while the portion near

Sand City is more of a mix of the two species. The southern metapopulation, which is defined as the Carmel Valley south into Big Sur, is distinguished by scrub, chaparral, and grassland plant communities. This area is rockier, with cliffs and more dramatic changes in elevation. The Smith's blues in the southern metapopulation utilize seacliff buckwheat, but adults have also been observed nectaring on the naked buckwheat, which grows in the more inland areas of the Carmel Valley (Arnold 1991, p. 9).

Assessment:

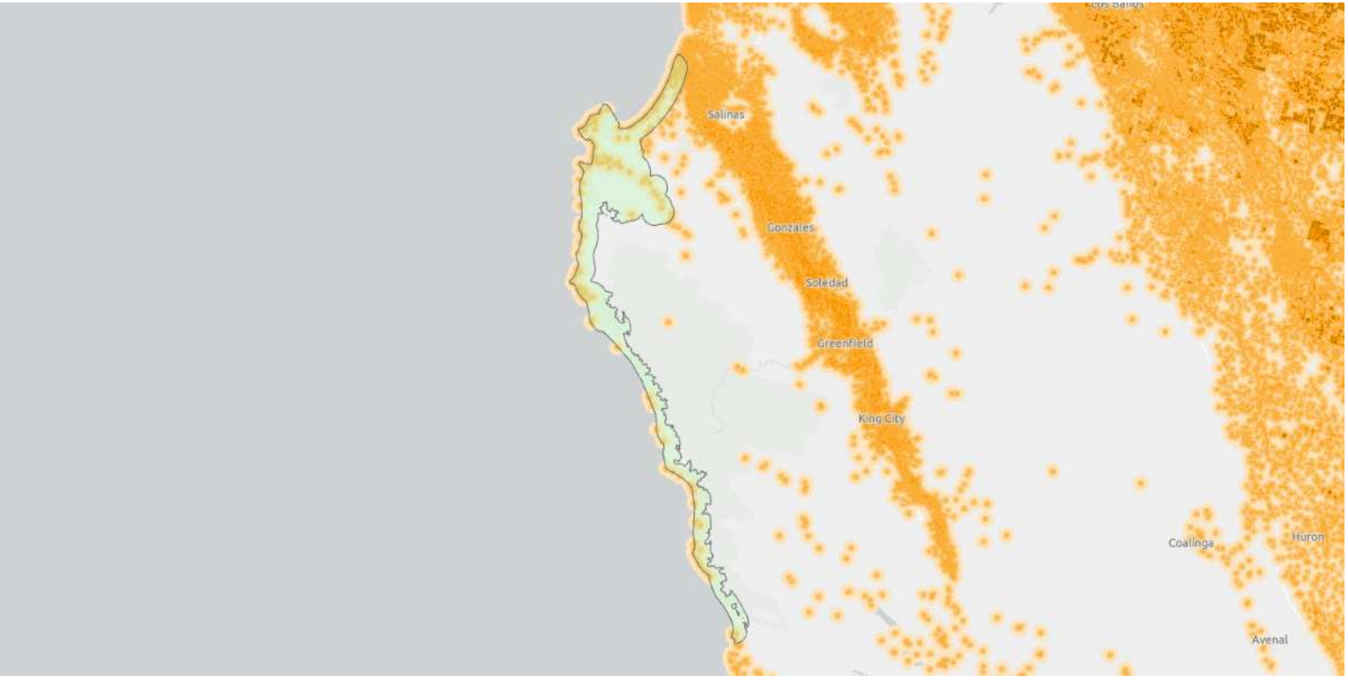
The Smith's blue butterfly is restricted to scattered colonies along approximately 93 mi of California's Central Coast from Monterey County to San Luis Obispo County. Its habitat consists of sand dunes and cliffside chaparral. The spatial overlap analysis of the species' range and maize production can be found in Appendix A, and the maize UDL overlap calculation is 0.459% (categorized as low overlap).

The Smith's blue butterfly adults are quite sedentary, traveling only on average 300 feet. Individual adult males and females live approximately one week, and both sexes spend the majority of their time on the buckwheat flowerheads. Adult Smith's blues use the flowerheads to perch, bask, forage for nectar, search for mates, and reproduce. Additionally, larvae are even more stationary and in most cases spend their entire development on a single host plant. Smith's blues rely primarily on coast and seacliff buckwheat as their host plants, with the butterflies' life-cycles synched up with the blooming of the buckwheat flowers. The buckwheat plants require appropriate disturbance regimes along coastal habitats to thrive. These types of habitat requirements are not conducive in or near corn cultivated areas.

Given the habitat specialization, geographical restriction, and host-plant habitat needs, the likelihood of this butterfly being exposed to pollen drift from event DAS1131 maize is negligible.

Therefore, EPA's analysis has determined that negligible exposure is expected for Smith's blue butterfly from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the Smith's blue butterfly and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

Figure 26. Overlap of the species range of the Smith's blue butterfly (*Euphydryas editha taylori)* and agriculturally cultivated corn.



Current species range is indicated by the green polygons (USFWS(c) 2023) and corn crop is indicated by orange polygons (U.S. EPA (a) 2023).

Island marble butterfly (*Euchloe ausonides insulanus*)
Status: Endangered (USFWS(r) 2024)
Listed: 3/1/2022
UDL Spatial Analysis Overlap: Species range 0.196%; Critical Habitat range 0.819%
Overlap Category: Low

Biology and Habitat Requirements:
The following excerpt is from (USFWS(s) 2024); consult the original reference for citations:

The island marble butterfly (Figure 27) does not migrate and is only known from the San Juan Islands in Washington State. It lives its entire lifecycle in upland prairie-like habitat, sand dunes or coastal lagoon habitat. The butterfly primarily uses two non-native plants and one native plant of the mustard family as their larval host plants. The species appears to have developed the capacity to use non-native mustard plants that readily colonize the open prairie-like habitat and sand dunes in addition to the native mustard plant. Regardless of how this development may have occurred, the use of non-native plants like field mustard and tumble mustard has contributed to the survival of the island marble in upland habitat and is expected to continue to play a significant role in the species continued existence.

Island marble butterfly larvae feed on their three known larval host plants. While adult island marble primarily feed on those plants, they also forage on a variety of nectar plants such as yarrow, small-flowered fiddleneck, field chickweed, common lomatium, California buttercup and native prairie plants.

Until 1998, the island marble butterfly was only known from historical collections made on Vancouver Island and the Canadian Gulf Islands in British Columbia, with 14 specimens collected from 1859 to 1908. These islands are part of the same geologic formation as the San Juan Archipelago, which is located immediately to the south of the Canadian Gulf Islands. The island marble butterfly had been last collected on Gabriola Island, Canada, in 1908 and was considered extirpated throughout its known range before it was rediscovered in 1998 in San Juan Island National Historical Park. Although the island marble was not previously documented on the San Juan Islands, it has likely been present in isolated locations in the islands throughout the last century. Extensive surveys were conducted from 2005 to 2010 that included six northern counties and 16 islands in Washington State. During this time period, 26 locations were determined to be occupied, representing five core populations, all on San Juan and Lopez Islands.

Figure 27. Island Marble Butterfly (*Euchloe ausonides insulanus*).

Photo credit: https://ecos.fws.gov/docs/species_images/doc5766-250px-thumbnail.jpg

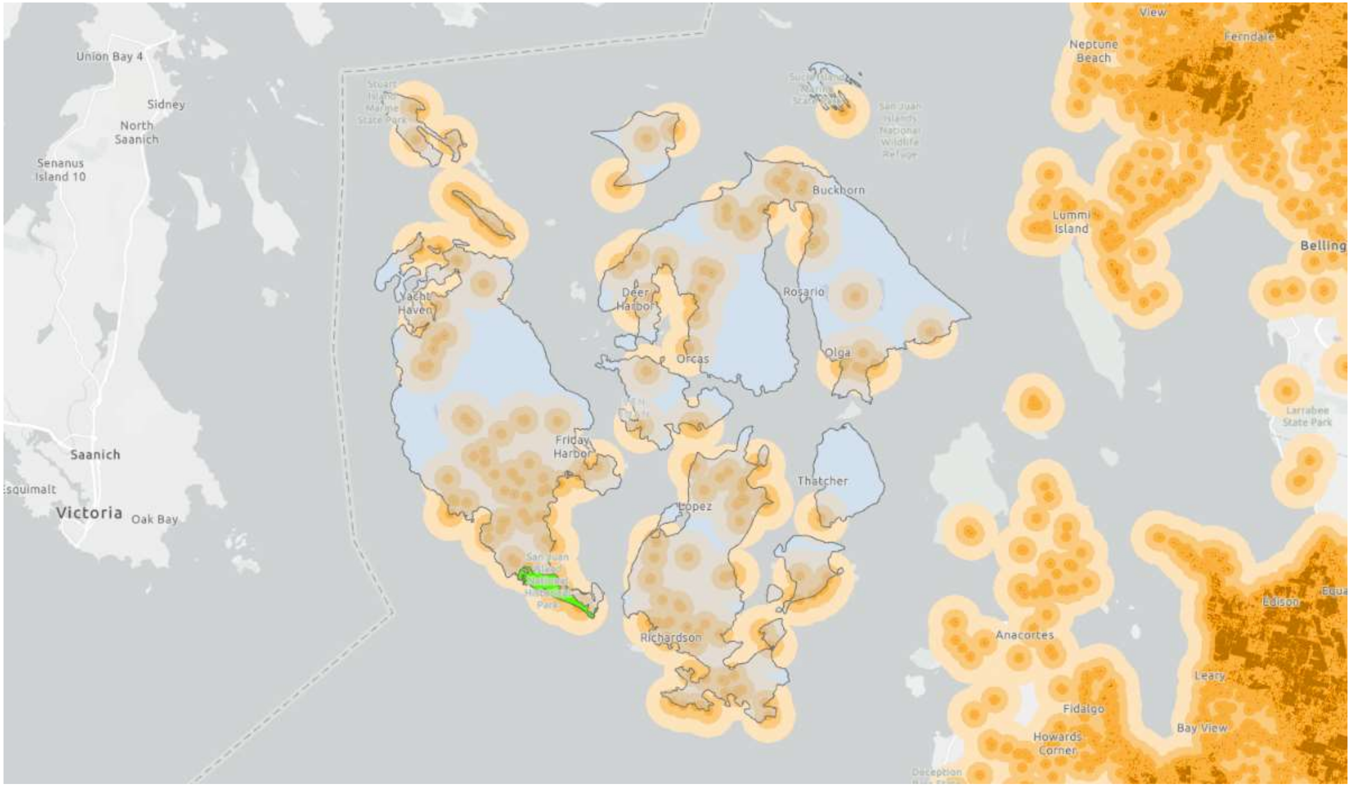
Assessment:

The island marble butterfly is extremely restricted geographically. The butterfly is only known to exist on the San Juan Islands in Washington State. (USFWS(b) 2021). The spatial overlap analysis of the species' range and maize production can be found in Figure 28, and the maize UDL overlap calculation is 0.196% for the species range and a critical habitat range of 0.819% (both categorized as low overlap).

The island marble butterfly lives its entire lifecycle in upland prairie-like habitat, sand dunes, or coastal lagoon habitat on the San Juan islands. The extreme isolation and restriction of the species range and critical habitat to solely these islands make the likelihood of this butterfly being exposed to pollen drift from event DAS1131 maize practically nonexistent. Furthermore, the life cycle of this butterfly is unique, in that it spends 10-12 months of the year in its chrysalis, bolstering the expectation that exposure to pollen drift from event DAS1131 maize is negligible to nonexistent.

Therefore, EPA's analysis has determined that negligible exposure is expected for island marble butterfly from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the island marble butterfly and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

Figure 28. Overlap of the species range of the Island marble butterfly (*Euchloe ausonides insulanus)* and agriculturally cultivated corn.



Current species range is indicated by the blue polygons (USFWS(d) 2022), critical habitat is indicated by the green polygons (USFWS 2020), and corn crop is indicated by orange polygons (U.S. EPA (a) 2023).

Silverspot (*Speyeria nokomis nokomis*)
Status: Threatened (USFWS(t) 2024)
Listed: 3/18/2024
UDL Spatial Analysis Overlap: Species range 0.518%
Overlap Category: Low

Biology and Habitat Requirements:
The following excerpt is from (Federal Register 2024); consult the original reference for citations:

Individual silverspot (Figure 29) needs include wet meadows supported by springs, seeps, streams, or irrigated areas that contain the bog violet host plant for eggs and larvae, and other herbaceous vegetation for cover and food resources. The butterflies may benefit from a light interspersion of willow or other shrubs for shade and for larval shelter. More dense willow and shrubs often surround open meadows where the silverspot occurs and, if the woody vegetation does not take over the meadows, the margins of denser stands can be beneficial for shade and shelter as well.

Although there are various types of "silverspot" butterflies, this particular subspecies is documented in ten populations across southwestern Colorado, eastern Utah, and northern New Mexico, at elevations ranging from 5,200 to 8,300 feet. A relatively large butterfly with up to a 3-inch wingspan, silverspot butterflies are known for distinctive silvery-white spots on the underside of their wings. On their upper side, females have a cream or light-yellow coloring with brown or black, and males have a bright orange upper side.

Figure 29. Silverspot (*Speyeria nokomis nokomis*).

Photo credit
https://www.fws.gov/sites/default/files/documents/20210211%20Final%20S.%20n.%20nokomis%20SSA%20Version%201_508.pdf

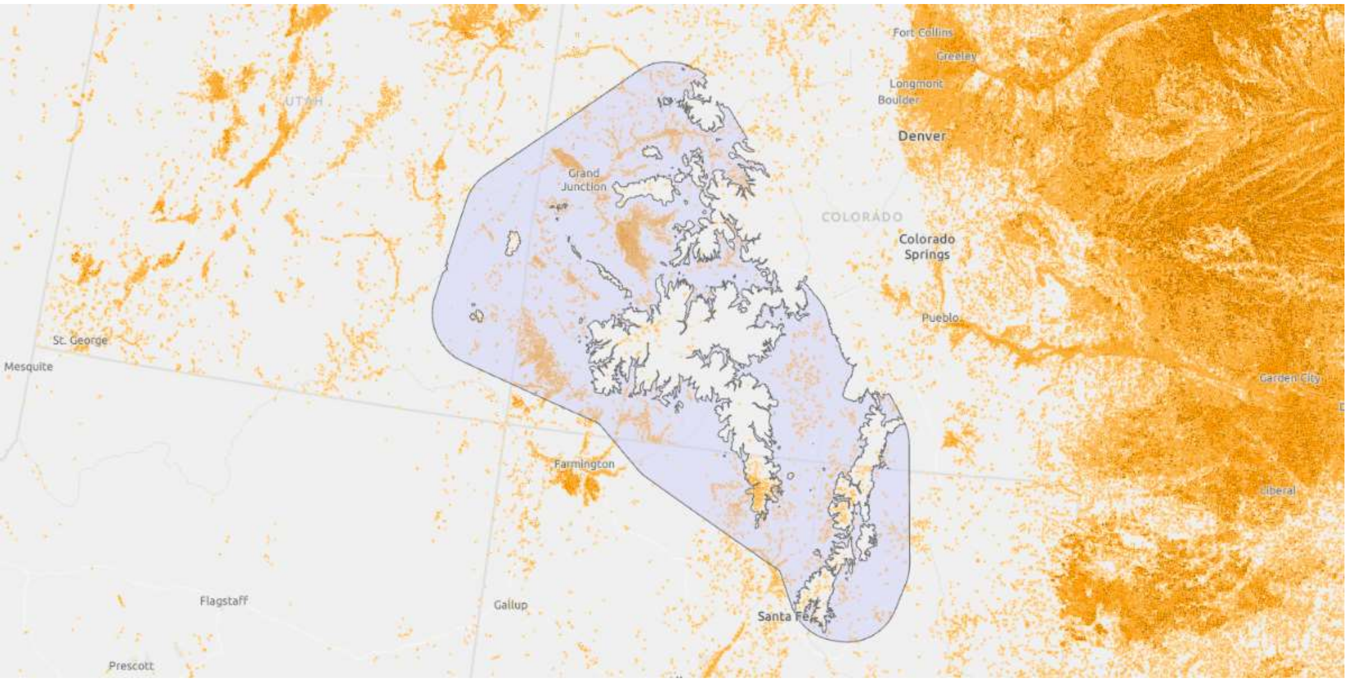
Assessment:

The silverspot is limited to ten populations across southwestern Colorado, eastern Utah, and northern New Mexico. The spatial overlap analysis of the species' range and maize production can be found in Figure 30, and the maize UDL overlap calculation is 0.518% for the species (categorized as low overlap).

The restriction of this species is to its wet meadow habitat, which is not typically conducive to agricultural production, and larvae feed exclusively on the bog violet located in these wet meadow habitats. Microhabitat conditions for the bog violet include soggy soil and shade, often under shrubs such as willows (Selby, G. 2007). This restricted habitat and conditions of the larval food plant result in a reasonable expectation of negligible exposure of larvae to corn pollen.

Therefore, EPA's analysis has determined that negligible exposure is expected for silverspot from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the silverspot and its designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

Figure 30. Overlap of the species range of the silverspot (*Speyeria nokomis nokomis)* and agriculturally cultivated corn.



Current species range is indicated by the purple polygons (USFWS(u) 2023), and corn crop is indicated by orange polygons (U.S. EPA (a) 2023).

TES Lepidopterans with <1% UDL Overlap

The lepidopteran TES in Table 1 all had calculated maize UDL overlap of <1% (defined as <0.445%). As stated previously, any overlap below 1% generally supports a "No Effect" determination, and therefore in-depth biological evaluations are not necessary for these species.

Table 1. Lepidopteran species with <1% UDL overlap.

Common Name	Scientific Name
Kern primrose sphinx moth	*Euproserpinus euterpe*
Callippe silverspot butterfly	*Speyeria callippe callippe*
Oregon silverspot butterfly	*Speyeria zerene hippolyta*
Florida leafwing butterfly	*Anaea troglodyta floridalis*
Bartram's hairstreak butterfly	*Strymon acis bartrami*
Mission blue butterfly	*Icaricia icarioides missionensis*
Myrtle's silverspot butterfly	*Speyeria zerene myrtleae*
Carson wandering skipper	*Pseudocopaeodes eunus obscurus*
Sacramento Mountains checkerspot butterfly	*Euphydryas anicia cloudcrofti*
Miami blue butterfly	*Cyclargus (=Hemiargus) thomasi bethunebakeri*
San Bruno elfin butterfly	*Callophrys mossii bayensis*
El Segundo blue butterfly	*Euphilotes battoides allyni*
Pawnee montane skipper	*Hesperia leonardus montana*
Behren's silverspot butterfly	*Speyeria zerene behrensii*
Uncompahgre fritillary butterfly	*Boloria acrocnema*

Common Name	**Scientific Name**
Quino checkerspot butterfly	*Euphydryas editha quino (=E. e. wrighti)*
Lotis blue butterfly	*Lycaeides argyrognomon lotis*
Hermes copper butterfly	*Lycaena hermes*
Laguna Mountains skipper	*Pyrgus ruralis lagunae*
Palos Verdes blue butterfly	*Glaucopsyche lygdamus palosverdesensis*
Schaus swallowtail butterfly	*Heraclides aristodemus ponceanus*
Mount Charleston blue butterfly	*Icaricia (Plebejus) shasta charlestonensis*

Therefore, EPA's analysis has determined that negligible exposure is expected for those species listed in Table 1 from the cultivation of DAS1131 maize. Based on this analysis, EPA is making a "No Effect" determination under the ESA for the species listed in Table 1 and their designated critical habitat resulting from the proposed uses of Cry1Da2 protein in event DAS1131 maize.

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