UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

June 25, 2024

MEMORANDUM

SUBJECT: Environmental Risk Assessment for the Plant-Incorporated Protectants *Brevibacillus laterosporus* Mpp75Aa1.1 Protein and the Genetic Material Necessary for its Production (Vector PV-ZMIR525664); *Bacillus thuringiensis* Vpb4Da2 Protein and the Genetic Material Necessary for its Production (Vector PV-ZMIR525664); dsRNA Transcript Comprising a DvSnf7.1 Inverted Repeat Sequence Derived from *Diabrotica virgifera*, and the Genetic Material Necessary for its Production (Vector PV-ZMIR525664) in Corn Event MON 95275 (OECD Unique Identifier MON-95275-7). Data and Information Were Provided in Support of an Application for a FIFRA Section 5 Experimental Use Permit to Test Corn Event Mon 95275 with Short Stature Corn Event MON 94804 and Registered Insect-Protected Corn Traits and Controls.

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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 5 experimental use permit (EUP) for the plant-incorporated protectants (PIPs), Mpp75Aa1.1 and Vpb4Da2 proteins and DvSnf7.1 double-stranded RNA (dsRNA), as expressed in maize containing event MON-95275-7 (hereafter MON 95275 maize), which provides control of targeted coleopteran pests (corn rootworms; namely western corn rootworm [WCR] *Diabrotica virgifera virgifera* and northern corn rootworm *Diabrotica barberi*).

This ERA examines the potential for ecological risks associated with the use of Mpp75Aa1.1, Vpb4Da2, and DvSnf7.1 as expressed in MON 95275 maize on non-target organisms (NTOs). A study investigating the synergistic potential between Mpp75Aa1.1, Vpb4Da2, and DvSnf7.1 found no evidence for a greater than additive effect associated with the combined use of the three active ingredients (AIs) against the target pest. Thus, there is no expectation that the combined expression of the three AIs in MON 95275 maize would alter the risk characterization compared to the active ingredients in isolation and the EPA therefore provides separate risk characterizations for each active ingredient below.

Bridging risk characterization of DvSnf7 to DvSnf7.1

DvSnf7.1 was determined to be functionally equivalent to the previously registered DvSnf7 (MRID 52041504) and its concentration within MON 95275 maize tissue is similar to its concentration within previously registered products (MRID 52041403). As such, the primary conclusion reached in MON 87411 maize's ecological risk assessment regarding DvSnf7 has been bridged to support MON 95275 maize's Section 5 EUP:

> "Based on the data presented and anticipated minimal exposure in certain environments, adverse effects to nontarget organisms are not expected as a result of DvSnf7 as expressed in MON 89034 x TC1507 x MON 87411 x DAS-59122-7 corn" (U.S. EPA, 2016).

Due to the highly specific nature of DvSnf7 (i.e., DvSnf7.1), the EPA has determined that there is a reasonable expectation of no discernible effects to occur to any non-coleopteran NTO resulting from the use of DvSnf7.1 as expressed in MON 95275 maize. The endangered species assessment for this AI was therefore focused on coleopteran threatened and endangered species (TES). In total, two coleopteran TES are located within counties where experimental trials of MON 95275 maize may occur. The coleopteran TES have either dietary or habitat restrictions and do not utilize corn tissue as a food source nor corn fields as habitat, thus exposure to coleopteran TES is expected to be negligible to none (see **Section X** for a detailed analysis). Since the EPA has determined there is a reasonable expectation of no discernible effects to occur to any non-coleopteran NTO exposed to DvSnf7.1 and a reasonable expectation of negligible to no exposure for both coleopteran TES located within the proposed EUP locations, effects to TES and their designated critical habitats are not expected to result from the use of DvSnf7.1; therefore, the EPA is making a "No Effect" determination under the Endangered Species Act (ESA).

Risk characterization for new proteins Mpp75Aa1.1 and Vpb4Da2

For Mpp75Aa1.1 and Vpb4Da2, risk has been estimated in this assessment using comparisons of the no observed effect concentration (NOEC) in bioassays with representative NTOs as compared to relevant worst-case estimated environmental concentrations (EECs) of the two protein AIs. For MON 95275 maize, above-ground leaf tissues contained the highest concentrations of the novel proteins, and neither protein was detected in pollen. Twenty-one studies and data waiver rationales focus on the Mpp75Aa1.1 and Vpb4Da2 proteins (with the exception of a combined potency study that tested for synergism between the three AIs). These studies comprise toxicity assessments of non-target terrestrial invertebrates, including honey bees. Data waiver rationales, in lieu of studies, were provided for non-target plants, avians, wild mammals, freshwater fish, freshwater aquatic invertebrates, and estuarine/marine animals. Additionally, a spectrum of activity study was provided for both Mpp75Aa1.1 and Vpb4Da2.

Vpb4Da2

No adverse effects were observed for Vpb4Da2 in guideline Tier I studies of non-target insect species using honey bees (*Apis mellifera*), parasitoid wasps (*Pediobius foveolatus*), green lacewings (*Chrysoperla rufilabris*), and carabid beetles (*Poecilus cupreus*). The spectrum of activity study for Vpb4Da2 identified activity in two pest species – southern corn rootworm (*Diabrotica undecimpunctata*) and yellow fever mosquito (*Aedes aegypti*). However, activity was not seen in several lepidopteran and hemipteran species tested, thus the available data indicate that the toxicity of Vpb4Da2 is predominately specific to the coleopteran insect order. Finally, data waiver rationales for non-target plants, avians, wild mammals, freshwater fish, freshwater aquatic invertebrates, and estuarine/marine animals were sufficient to determine no adverse effects would be anticipated for these taxa based on a similar mode of action to previously commercialized Cry proteins, expected activity spectrum, and/or expected environmental exposures. Altogether, Vpb4Da2 is not anticipated to cause adverse effects to non-coleopteran NTOs in the proposed EUP locations, nor are indirect effects expected to any NTOs.

Based on the submitted scientific rationale and the lack of hazard observed in submitted studies for non-target species to Vpb4Da2, the EPA has determined that there is a reasonable expectation of no discernible effects to occur to any non-coleopteran NTO resulting from the use of Vpb4Da2 as expressed in MON 95275 maize. The endangered species assessment for this AI was therefore focused on coleopteran threatened and endangered species (TES). In total, two coleopteran TES are located within counties where experimental trials of MON 95275 maize may occur. The coleopteran TES have either dietary or habitat restrictions and do not utilize corn tissue as a food source nor corn fields as habitat, thus exposure to coleopteran TES is expected to be negligible to none (see **Section X** for a detailed analysis).

Since the EPA has determined there is a reasonable expectation of no discernible effects to occur to any non-coleopteran NTO exposed to Vpb4Da2 and a reasonable expectation of negligible to no exposure for both coleopteran TES located within the proposed EUP locations, effects to TES and their designated critical habitats are not expected to result from the use of Vpb4Da2; therefore, the EPA is making a “No Effect” determination under the ESA.

Mpp75Aa1.1

Effects were observed for Mpp75Aa1.1 in a guideline Tier I study of a pollinator species (*A. mellifera*); however, effects are not expected to occur in the field due to Mpp75Aa1.1’s lack of expression in MON 95275 maize pollen resulting in negligible exposure to pollinators. Effects were also observed for Mpp75Aa1.1 in guideline Tier 1 studies of two predatory species (*C. rufilabris* and *P. cupreus*); however, population-level effects to predatory species are not expected to occur in the field due to the limited acreage of the proposed EUP and the general expected lack of PIP bioaccumulation within prey species tissue (i.e., limited PIP exposure to predators; see **Section VI** for further detail). The spectrum of activity study for Mpp75Aa1.1 identified activity against four lepidopteran pest species – fall armyworm (*Spodoptera frugiperda*), corn earworm (*Helicoverpa zea*), soybean looper (*Chrysodeixis includens*), and European corn borer (*Ostrinia nubilalis*). However, activity was not seen against the valued species, the monarch butterfly (*Danaus plexippus*). Although Mpp75Aa1.1 is active against some insects in the lepidopteran order, given that Mpp75Aa1.1 has negligible expression in MON 95275 maize pollen, significant exposure to lepidopterans would require direct consumption of corn leaf or root tissue, with such a route of exposure being limited to corn pest species. Therefore, adverse effects are not anticipated for non-target lepidopterans. Finally, data waiver rationales for non-target plants, avians, wild mammals, freshwater fish, freshwater aquatic invertebrates, and estuarine/marine animals were sufficient to determine no adverse effects would be anticipated for these taxa based on a similar mode of action to previously commercialized Cry proteins, expected activity spectrum, and/or expected environmental exposures. Altogether, although hazard to some non-target insects was identified in Tier I studies, Mpp75Aa1.1 is not anticipated to cause direct population-level effects to non-pest non-target insects given its expected environmental exposures and the limited acreage of the EUP.

Although Mpp75Aa1.1 displayed a broader spectrum of activity relative to the two other AIs, given the submitted scientific rationale, the limited acreage of the EUP, and expected environmental exposures, the EPA has determined that there is a reasonable expectation of no discernible effects to any non-insect NTO and there is a reasonable expectation of no direct population-level effects to insect NTOs resulting from the use of Mpp75Aa1.1 as expressed in MON 95275 maize. Because Tier I studies featuring Mpp75Aa1.1 identified effects to several beneficial non-target insects (both coleopterans and non-coleopterans) at the lowest concentration of the protein tested, individual-level effects to non-target insects cannot be precluded and therefore the endangered species assessment evaluated the potential for effects associated with cultivation of MON 95275 maize that expresses Mpp75Aa1.1 to all insect TES in the proposed EUP locations. The assessment was limited to insects because no effects were observed against organisms outside of the class Insecta (*Folsomia candida* and *Mus musculus*), highlighting that the protein’s activity is likely limited to a subset of insect species/orders. In total, 30 insect TES are presently located within counties where experimental trials of MON 95275 maize may occur. Each of the 30 insect TES have either habitat and/or dietary restrictions and do not use corn fields as habitat nor corn tissue as a food source, thus exposure to insect TES is expected to be negligible to none and to not result in discernible effects (see **Section IX** for a detailed analysis).

Since the EPA has determined there is a reasonable expectation of no discernible effects to occur to any non-insect NTO exposed to Mpp75Aa1.1 and a reasonable expectation of negligible to no exposure for each insect TES located within the proposed EUP locations, effects to TES and their designated critical habitats are not expected to result from the use of Mpp75Aa1.1; therefore, the EPA is making a "No Effect" determination under the ESA.

II. Introduction

Bayer CropScience LP (hereafter "Bayer") submitted an application for a Section 5 EUP to test MON 95275 maize, and stack combinations with short stature corn MON 94804 and registered insect-protected corn traits and controls.

Insect-protected MON 95275 maize produces two insecticidal proteins, Mpp75Aa1.1 and Vpb4Da2, as well as the DvSnf7.1 dsRNA, which protect against feeding damage caused by corn rootworms (*Diabrotica* spp.). Mpp75Aa1.1 is a beta-pore forming protein derived from *Brevibacillus laterosporus* (Kouadio, Duff, et al., 2021), Vpb4Da2 is a Vegetative insecticidal protein (Vip) from the Vpb4 (formerly Vip4) protein family derived from *Bacillus thuringiensis* (Yin et al., 2020), and DvSnf7.1 is a dsRNA containing the same 240 base pair sequence responsible for silencing the Snf7 gene that is found in the previously registered DvSnf7 dsRNA (U.S. EPA, 2016).

Under the applied for EUP, MON 95275 maize is proposed to be tested with short stature corn MON 94804 and registered insect-protected corn traits and controls across 4,285 total acres in Alabama, Arkansas, California, Colorado, Florida, Georgia, Hawaii, Illinois, Indiana, Iowa, Kansas, Louisiana, Maryland, Michigan, Minnesota, Mississippi, Missouri, Nebraska, North Carolina, North Dakota, Ohio, Pennsylvania, South Carolina, South Dakota, Tennessee, Texas, and/or Wisconsin.

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

The 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. Given that the EPA has now successfully applied this approach to a more broad range of AIs, including insecticidal traits of non-*Bt* origin (e.g., proteins derived from non-*Bt* bacteria or ferns and dsRNA) and traits conferring disease resistance (e.g., R-proteins, viral coat protein genes), the current approach (see below) is sufficient for assessing the risks associated with Mpp75Aa1.1, Vpb4Da2, and DvSnf7.1 as expressed in MON 95275 maize as well.

This approach is described in several Biopesticide Registration Action Documents (BRADs) for Cry or Vip proteins (e.g., see U.S. 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, additional data are also considered regarding the environmental persistence of PIP pesticidal substances, as well as the potential for gene flow. The EPA requires 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, the 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

Vpb4Da2, which is derived from *Bacillus thuringiensis*, and Mpp75Aa1.1, which is derived from a spore-forming bacteria that also has entomopathogenic properties (*Brevibacillus laterosporus*) (Ruiu, 2013), are proteins with modes of action similar to that of several previously registered *Bt*-derived Cry and Vip proteins (e.g., U.S. EPA, 2008, 2010a, 2010b). In brief, their biological activity and specificity is the result of the combined effects of achieving solubilization after ingestion of the protein by the target insect(s) (i.e., corn rootworms), proteolytic activation, binding to specific receptors on the insect midgut cell membrane, oligomerization, and pore formation (Kouadio, Duff, et al., 2021; Kouadio, Zheng, et al., 2021). However, these novel proteins likely target a different binding site (*i.e.*, distinct receptors) within the insect midgut epithelium than previously registered Cry proteins given their effectiveness against strains of WCR resistant to Cry3Bb1 and Cry34Ab1/Cry35Ab1 (Bowen et al., 2021; Yin et al., 2020).

Given DvSnf7.1 contains the same 240 base pair sequence responsible for silencing the Snf7 gene that is found in the previously registered DvSnf7 dsRNA, the following description of DvSnf7 (largely taken from U.S. EPA (2016)) is applicable to DvSnf7.1. The DvSnf7 dsRNA expressed by MON 95275 maize results from expression of an inverted repeat sequence designed to match the sequence of WCR Snf7 gene. Expression of the sequence results in the formation of the dsRNA transcript containing a 240 bp fragment of the WCR Snf7 (DvSnf7). Snf7 is a vacuolar sorting protein belonging to the Endosomal Sorting Complex Required for Transport (ESCRT)-III complex, which is involved in sorting of transmembrane proteins *en route* to lysosomal degradation through the endosomal-autophagic pathway. Once consumed, the DvSnf7 dsRNA is recognized by the WCR's RNA interference (RNAi) machinery wherein it is cleaved into 21-24 mer small interfering RNAs (siRNAs). These siRNAs bind to an RNA induced silencing complex (RISC), which ultimately leads to down-regulation of the targeted DvSnf7 gene and mortality (MRID 48919004).

Mpp75Aa1.1, Vpb4Da2, and DvSnf7.1 have been genetically engineered to be expressed in MON 95275 maize and target corn rootworms.

III. Environmental Exposure Assessment

The environmental exposure assessment for the proposed EUP of MON 95275 maize is comprised of two major components: tissue specific expression of each AI 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 ecosystem.

A. Expression

Mpp75Aa1.1, Vpb4Da2, and DvSnf7.1 are expressed in MON 95275 maize tissues throughout multiple life stages of the plant and their tissue-specific expression levels largely drive the potential of exposure to NTOs (*e.g.*, pollen expression potentially exposes pollinators, grain/seed expression potentially exposes birds/wild mammals). The expression levels for Mpp75Aa1.1, Vpb4Da2, and DvSnf7.1 are described in Tables 1, 2, and 3, respectively. The highest expression levels for Mpp75Aa1.1, Vpb4Da2, and DvSnf7.1 in MON 95275 maize were found in leaf tissue, with early-stage leaf tissues having the highest concentrations of the AIs.

Table 1. Summary of Mpp75Aa1.1 protein levels in maize tissues collected from MON 95275 produced in five sites of United States field trials 2019. Data from MRID 51754506.

Tissue Type[1]	Development Stage[2]	Mean (SE) Range (µg/g fw)[3]	Mean (SE) Range (µg/g dw)[4]	LOQ/LOD (µg/g dw)[5]
OSL1	V2-V4	15 (1.1) 6.5 – 31	100 (7.0) 43 – 200	0.125/ 0.023
OSL4	VT-R1	8.6 (0.36) 6.1 – 12	37 (1.6) 27 – 50	0.125/0.023
OSR1	V2-V4	3.8 (0.48) 1.2 – 9.3	35 (4.3) 11 – 84	0.125/0.053
Forage	R5	4.9 (0.23) 3.5 – 7.5	16 (0.76) 12 – 25	0.125/0.039
Forage Root	R5	4.7 (0.44) 1.3 – 9.3	25 (2.3) 6.9 – 49	0.125/0.053

Table 1. Summary of Mpp75Aa1.1 protein levels in maize tissues collected from MON 95275 produced in five sites of United States field trials 2019. Data from MRID 51754506.

Grain	R6	1.1 (0.076) 0.59 – 1.7	1.3 (0.086) 0.67 – 1.9	0.125/0.065
Pollen	VT-R1	<LOQ (NA) NA – NA	<LOQ (NA) NA – NA	0.125/0.043
Silk	R1	12 (0.37) 8.9 – 14	120 (3.7) 89 – 140	0.125/0.053

[1] OSL = over season leaf, OSR = over season root

[2] The crop development stage at which each tissue was collected.

[3] Protein levels are expressed as the arithmetic mean and standard error (SE) as microgram (µg) of protein per gram (g) of tissue on a fresh weight basis (fw). The means, SE, and ranges (minimum and maximum values) were calculated for each tissue across all five sites (n=20).

[4] Protein levels are expressed as the arithmetic mean and standard error (SE) as microgram (µg) of protein per gram (g) of tissue on a dry weight basis (dw).

[5] LOQ=limit of quantitation defined as tissue LOD=limit of detection.

Table 2. Summary of Vpb4Da2 protein levels in maize tissues collected from MON 95275 produced in five sites of United States field trials 2019. Data from MRID 51754506.

Tissue Type	Development Stage	Mean (SE) Range (µg/g fw)	Mean (SE) Range (µg/g dw)	LOQ/LOD (µg/g dw)
OSL1	V2-V4	5.9 (0.27) 2.8 – 7.6	39 (1.8) 19 – 51	0.313/ 0.110
OSL4	VT-R1	1.3 (0.055) 0.99 – 1.9	5.6 (0.24) 4.3 – 8.2	0.313/ 0.110
OSR1	V2-V4	1.5 (0.15) 0.38 – 2.9	14 (1.3) 3.4 – 26	0.313/ 0.128
Forage	R5	1.0 (0.040) 0.75 – 1.4	3.3 (0.13) 2.5 – 4.8	0.313/ 0.124
Forage Root	R5	0.71 (0.062) 0.30 – 1.4	3.8 (0.33) 1.6 – 7.4	0.313/ 0.128
Grain	R6	1.0 (0.076) 0.37– 1.6	1.2 (0.086) 0.42 – 1.9	0.157/0.067
Pollen	VT-R1	<LOQ (NA) NA – NA	<LOQ (NA) NA – NA	0.157/0.082
Silk	R1	5.6 (0.19) 4.2 – 7.2	56 (1.9) 42 – 72	0.313/ 0.108

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 2019 United States field trials (Table 3) for Mpp75Aa1.1 and Vpb4Da2 will be used for risk characterization in this assessment. These field trials took place in Illinois (two different sites), Iowa, Indiana, and Missouri.

Table 3. 95th percentile values calculated for Mpp75Aa1.1 and Vpb4Da2 from MON 95275 maize produced in United States field trials across five sites in 2019. Data presented in MRID 52041502.

Tissue Type	μg/g fresh weight 95th Percentile	
	Mpp75Aa1.1	Vpb4Da2
OSL1	20.2	7.4
OSR1	7.8	2.7
OSL4	11.5	1.6
Silk	14.2	6.9
Pollen	NA[1]	NA[1]
Forage	6.3	1.3
Forage root	8.1	1.1
Grain	1.6	1.5

[1] 95th percentile values not calculated due to the protein concentration in the tissue being below the limit of quantitation.

The concentration of DvSnf7.1 within MON 95275 maize tissue (Table 4) is similar to the concentration of DvSnf7 within previously registered products (see Table 11 for updated margins of exposure for bridged studies).

Table 4. Summary of DvSnf7.1 RNA levels in maize tissues collected from MON 95275 in eight tissue types across five sites produced in the United States during 2019. Data from MRID 52041403.

Tissue Type	Development Stage	Mean (SE) Range (μg/g fw)[1]	Mean (SE) Range (μg/g dw)[2]	LOQ/LOD (μg/g dw)[3]
OSL1	V2-V4	7.2×10^{-3} (0.51×10^{-3}) $4.3\times10^{-3} – 15\times10^{-3}$	48×10^{-3} (3.4×10^{-3}) $29\times10^{-3} – 100\times10^{-3}$	$3.7\times10^{-4}/0.85\times10^{-4}$
OSL4	VT-R1	11×10^{-3} (1.6×10^{-3}) $1.9\times10^{-3} – 27\times10^{-3}$	48×10^{-3} (6.8×10^{-3}) $8.3\times10^{-3} – 119\times10^{-3}$	$3.7\times10^{-4}/0.84\times10^{-4}$
OSR1	V2-V4	5.1×10^{-3} (1.0×10^{-3}) $0.41\times10^{-3} – 18\times10^{-3}$	46×10^{-3} (9.4×10^{-3}) $3.8\times10^{-3} – 166\times10^{-3}$	$1.3\times10^{-4}/0.30\times10^{-4}$
Forage root	R5	0.72×10^{-3} (0.076×10^{-3}) $0.15\times10^{-3} – 1.3\times10^{-3}$	3.8×10^{-3} (0.40×10^{-3}) $0.77\times10^{-3} – 6.7\times10^{-3}$	$0.76\times10^{-4}/0.17\times10^{-4}$
Forage	R5	6.0×10^{-3} (0.75×10^{-3}) $2.1\times10^{-3} – 13\times10^{-3}$	20×10^{-3} (2.5×10^{-3}) $7.1\times10^{-3} – 42\times10^{-3}$	$0.81\times10^{-4}/0.18\times10^{-4}$
Grain	R6	0.24×10^{-3} (0.019×10^{-3}) $0.099\times10^{-3} – 0.40\times10^{-3}$	0.28×10^{-3} (0.021×10^{-3}) $0.11\times10^{-3} – 0.46\times10^{-3}$	$0.21\times10^{-4}/0.047\times10^{-4}$
Pollen	VT-R1	0.27×10^{-3} (0.052×10^{-3}) $0.055\times10^{-3} – 0.85\times10^{-3}$	0.47×10-3 (0.091×10^{-3}) $0.097\times10^{-3} – 1.5\times10^{-3}$	$0.26\times10^{-4}/0.059\times10^{-4}$
Silk	R1	3.1×10^{-3} (0.25×10^{-3}) $1.8\times10^{-3} – 6.2\times10^{-3}$	31×10^{-3} (2.5×10^{-3}) $18\times10^{-3} – 62\times10^{-3}$	$0.17\times10^{-4}/0.040\times10^{-4}$

[1] The DvSnf7.1 RNA levels are calculated as microgram of DvSnf7.1 RNA per gram of tissue (μg/g) on a fresh weight (fw) basis. The sample means, SEs, and ranges (minimum and maximum values) were calculated for each tissue type across 5 sites (IAMP, ILHY, ILAG, MOFI, and INSH; n=20 except in pollen where n=17 due to three samples expressing <LOQ).
[2] The DvSnf7.1 RNA levels are calculated as microgram of DvSnf7.1 RNA per gram of tissue (μg/g) on a dry weight (dw) basis. The sample means, SEs, and ranges (minimum and maximum values) were calculated for each tissue type across 5 sites (IAMP, ILHY, ILAG, MOFI, and INSH; n=20 except in pollen where n=17 due to three samples expressing <LOQ).
[3] LOQ=limit of quantitation; LOD=limit of detection. The LOD and LOQ for each tissue type were calculated based on the QuantiGene® Plex 2.0 assay's LOD and LOQ in maize total RNA and the average total RNA/tissue ratio and dilution factor of each given tissue type.

B. Biology of Corn and Pollen Dispersal

Because Mpp75Aa1.1, Vpb4Da2, and DvSnf7.1 are 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). 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).

C. Environmental Exposure

1. Terrestrial Exposure

Movement of corn tissue influences the distribution and fate of Mpp75Aa1.1, Vpb4Da2, and DvSnf7.1 in the environment since corn tissue will carry the protein to wherever it may move. How much each AI 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 Mpp75Aa1.1, Vpb4Da2, and DvSnf7.1 will be contained within the planted field. Some breakage of foliage and other above ground plant parts may occur, which could result in its 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 Mpp75Aa1.1, Vpb4Da2, and/or DvSnf7.1 would be present in root exudates, though upon root cell lysis, small amounts of the protein 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 each of the AIs into the soil that have not been broken down within the plant. Therefore, soil is expected to be the ultimate destination of Mpp75Aa1.1, Vpb4Da2, and DvSnf7.1 in the terrestrial environment.

Non-target invertebrates with the greatest exposure potential to PIPs in transgenic corn fields are beneficial insects that feed on corn pollen and soil invertebrates that feed on detritus. Additionally, pollen from MON 95275 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. The EPA has previously evaluated the potential for distribution of PIPs due to corn pollen deposition (U.S. EPA, 2010). As discussed in **Section III.C**, most pollen from a cornfield is deposited within a short distance (i.e., within 10 – 15 meters) of the corn plant. More recent work has been done (*e.g.*, see Gathmann et al., 2006; Hofmann 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 the EPA's current understanding of this process as it affects PIP environmental fate. However, as the Mpp75Aa1.1 and Vpb4Da2 proteins exhibit negligible expression within MON 95275 maize pollen (see Tables 1 and 2 above), the DvSnf7.1 dsRNA is expected to be the sole AI expressed within MON 95275 maize that may present an exposure to non-target species via pollen.

For terrestrial vertebrate NTOs, birds and mammals are the NTOs most likely to consume corn grain, which would be the route of direct exposure to those taxa. However, insectivorous birds and mammals could also be exposed via the consumption of insects that inhabit the corn agroecosystem.

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 DvSnf7.1 into aquatic areas (expression of Mpp75Aa1.1 and Vpb4Da2 proteins in pollen is negligible) 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 the AIs. Therefore, pollen from MON 95275 maize is not a likely contributor of the protein 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 (Böttger et al., 2015; Chambers et al., 2010; Strain & 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. Therefore, exposure of Mpp75Aa1.1, Vpb4Da2, and DvSnf7.1 to aquatic NTOs is expected to be negligible.

IV. Non-Target Effects Data Submission Summary

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

Given DvSnf7.1 was shown to contain the same 240 base pair sequence responsible for silencing the Snf7 gene that is found in the previously registered DvSnf7 and to be functionally equivalent to the previously registered DvSnf7, four studies that supported the registration of DvSnf7 (U.S. EPA, 2016) have been bridged to MON 95275 maize's Section 5 EUP to fulfill the non-target data requirements for DvSnf7.1 (Table 5).

Table 5. Summary of DvSnf7 data submitted to comply with NTO data requirements published in 40 CFR § 158.2150 for support of MON 95275 maize's EUP.

Data Requirement	OCSPP (OPPTS) Guideline No.	Results Summary and Classification	MRID No.
Non-target insect testing Parasitic wasp (*Pediobius foveolatus*)	885.4340	No adverse effects were observed on adult survival in *P. foveolatus* fed a 30% honey/water solution containing 1000 ng DvSnf7 dsRNA/g diet in a 20-day study. **Classification: Acceptable**	49315115
Non-target insect testing Insidious flour bug (*Orius insidiosus*)	885.4340	No adverse effects were observed on survival or rate of adult emergence in *O. insidiosus* nymphs fed 1000 ng DvSnf7 dsRNA/g diet in a 10-day study. **Classification: Acceptable**	49315117
Non-target insect testing Carabid ground beetle (*Poecilus chalcites*)	885.4340	No adverse effects on survival, development to adult stage, time to emergence, or adult biomass were observed in carabid beetle larvae fed 1000 ng DvSnf7 dsRNA/g diet in a 35-day study. **Classification: Acceptable**	49315119
Larval honey bee testing	885.4380	No adverse effects were observed in survival or development of larvae provided a single dose equivalent to 11.3 ng DvSnf7 dsRNA per bee in a 14-day study. **Classification: Acceptable**	49315112

Scientific rationale to waive testing for Mpp75Aa1.1 and Vpb4Da2 was submitted for non-target plant toxicity, avian toxicity, wild mammal toxicity, freshwater fish toxicity, freshwater aquatic invertebrate toxicity, and estuarine/marine animal toxicity. Toxicity studies with honey bees (*Apis mellifera*), parasitic wasps (*Pediobius foveolatus*), ground-dwelling beetles (*Poecilus cupreus*), and green lacewings (*Chrysoperla rufilabris*) were submitted for both Mpp75Aa1.1 and Vpb4Da2 to satisfy data requirements. Toxicity studies with Collembola (*Folsomia candida*), big-eyed bugs (*Geocoris punctipes*), insidious flower bugs (*Orius insidiosus*), and ladybird beetles (*Coleomegilla maculata*) were also submitted solely for Mpp75Aa1.1. Additionally, spectrum of activity tests for both Mpp75Aa1.1 and Vpb4Da2 were conducted using three coleopterans (Colorado potato beetle [*Leptinotarsa decemlineata*], Mexican bean beetle [*Epilachna varivestis*], and southern corn rootworm [*Diabrotica undecimpunctata*]), five lepidopterans (corn earworm [*Helicoverpa zea*], European corn borer [*Ostrinia nubilalis*], fall armyworm [*Spodoptera frugiperda*], soybean looper [*Chrysodeixis includens*], and monarch [*Danaus plexippus*]), two hemipterans (western tarnished plant bug [*Lygus hesperus*] and neotropical brown stink bug [*Euschistus heros*]), a dipteran (yellow fever mosquito [*Aedes aegypti*]), and an acarid (red spider mite [*Tetranychus urticae*]). Finally, a synergy study was submitted to analyze the combined potency effect of Mpp75Aa1.1, Vpb4Da2, and DvSnf7.1. Information from the EPA's review of the submitted scientific rationale and studies is included in Tables 6, 7, and 8.

The information and data provided are sufficient to satisfy the Tier I NTO data requirements for ecological risk assessment for the Section 5 EUP. Further testing of NTOs at higher tiers is not required for the proposed Section 5 EUP.

Table 6. Summary of Mpp75Aa1.1's and Vpb4Da2's waiver rationales submitted to comply with NTO data requirements published in 40 CFR § 158.2150 for support of MON 95275 maize's EUP.

Data Requirement	OCSPP (OPPTS) Guideline No.	Results Summary and Classification	MRID No.
Non-target plant toxicity/pathogenicity	885.4300	The proposed use of Mpp75Aa1.1 and Vpb4Da2 in MON 95275 maize is not expected to result in significant exposure or adverse effects to non-target plant species based on the proteins' limited capacity to move off-field (i.e., limited pollen movement) and into new species (i.e., lack of sympatric sexually compatible species). **Classification: Acceptable**	52255311
Avian oral toxicity/pathogenicity	885.4050	The proposed use of Mpp75Aa1.1 and Vpb4Da2 in MON 95275 maize is not expected to result in significant exposure or adverse effects to avian species based on the proteins' insecticidal mode of action and low expression in tissues primarily consumed by vertebrates (i.e., birds). **Classification: Acceptable**	52255308
Wild mammal toxicity/pathogenicity	885.4150	The proposed use of Mpp75Aa1.1 and Vpb4Da2 in MON 95275 maize is not expected to result in significant exposure	52255310

Table 6. Summary of Mpp75Aa1.1's and Vpb4Da2's waiver rationales submitted to comply with NTO data requirements published in 40 CFR § 158.2150 for support of MON 95275 maize's EUP.

Data Requirement	OCSPP (OPPTS) Guideline No.	Results Summary and Classification	MRID No.
		or adverse effects to wild mammal species based on the proteins' insecticidal mode of action, low expression in tissues primarily consumed by vertebrates (i.e., mammals), and lack of effects observed in the acute oral toxicity testing in mice. **Classification: Acceptable**	
Freshwater fish toxicity/pathogenicity	885.4200	The proposed use of Mpp75Aa1.1 and Vpb4Da2 in MON 95275 maize is not expected to result in significant exposure or adverse effects to freshwater fish species based on the proteins' spatially and temporally limited exposure to aquatic environments, low expression in late season maize tissues, and lack of persistence in aquatic environments. **Classification: Acceptable**	52255306
Freshwater aquatic invertebrate toxicity/pathogenicity	885.4240	The proposed use of Mpp75Aa1.1 and Vpb4Da2 in MON 95275 maize is not expected to result in significant exposure or adverse effects to freshwater aquatic invertebrate species based on the proteins' spatially and temporally limited exposure to aquatic environments, low expression in late season maize tissues, and lack of persistence in aquatic environments. **Classification: Acceptable**	52255307
Estuarine/marine animal testing	885.4280	The proposed use of Mpp75Aa1.1 and Vpb4Da2 in MON 95275 maize is not expected to result in significant exposure or adverse effects to estuarine and marine animal species based on the proteins' spatially and temporally limited exposure to aquatic environments, low expression in late season maize tissues, and lack of persistence in aquatic environments. **Classification: Acceptable**	52255308

Table 7. Summary of Mpp75Aa1.1 data submitted to comply with NTO data requirements published in 40 CFR § 158.2150 for support of MON 95275 maize's EUP.

Data Requirement	OCSPP (OPPTS) Guideline No.	Results Summary and Classification	MRID No.
Non-target insect testing Collembola (*Folsomia candida*)	885.4340	A 28-day oral toxicity study with springtails determined the NOEC and LD_{50} values to be 500 and >500 µg Mpp75Aa1.1/g diet, respectively. **Classification: Acceptable**	52041542
Larval honey bee (*Apis*	885.4380	A 20-day oral toxicity study with larval	52041520

Table 7. Summary of Mpp75Aa1.1 data submitted to comply with NTO data requirements published in 40 CFR § 158.2150 for support of MON 95275 maize's EUP.

Data Requirement	OCSPP (OPPTS) Guideline No.	Results Summary and Classification	MRID No.
mellifera) testing		honey bees revealed >50% mortality at the lowest tested concentration of Mpp75Aa1.1 (250 mg/kg), thus neither an LD_{50} value nor a NOEC value could be determined. **Classification: Acceptable**	
Non-target insect testing parasitic wasp (*Pediobius foveolatus*)	885.4340	A 21-day oral toxicity study with *P. foveolatus* determined the NOEC and LD_{50} values to be 500 and >500 µg Mpp75Aa1.1/mL diet, respectively. **Classification: Acceptable**	52041529
Non-target insect testing green lacewing (*Chrysoperla rufilabris*)	885.4340	An oral toxicity study with green lacewings revealed >50% mortality at the lowest tested concentration of Mpp75Aa1.1 (250 mg/kg), thus neither an LD_{50} value nor a NOEC value could be determined. **Classification: Acceptable**	52041533
Non-target insect testing insidious flower bug (*Orius insidiosus*)	885.4340	An oral toxicity study with insidious flower bug determined the NOEC and LD_{50} values to be 500 and >500 µg Mpp75Aa1.1/g diet, respectively. **Classification: Acceptable**	52041527
Non-target insect testing big-eyed bug (*Geocoris punctipes*)	885.4340	A 21-day oral toxicity study with big-eyed bug determined the NOEC and LD_{50} values to be 500 and >500 µg Mpp75Aa1.1/g diet, respectively. **Classification: Acceptable**	52041525
Non-target insect testing ground-dwelling beetle (*Poecilus cupreus*)	885.4340	An oral toxicity study with ground-dwelling beetle revealed >50% mortality at the lowest tested concentration of Mpp75Aa1.1 (250 mg/kg), thus neither an LD_{50} value nor a NOEC value could be determined. **Classification: Acceptable**	52041538
Non-target insect testing spotted lady beetle (*Coleomegilla maculata*)	885.4340	An oral toxicity study with spotted lady beetle determined the NOEC and LD_{50} values to be 500 and >500 µg Mpp75Aa1.1/g diet, respectively. **Classification: Acceptable**	52041523
Spectrum of Activity*	N/A	No lethal or sub-lethal effects were observed for: SCR, CPB, MBB, MBF, WTP, NBS, YFM, or RSM. Mpp75Aa1.1 was active against FAW[1], SBL, ECB, and CEW, with both sub-lethal (in all four species) and lethal (in ECB and CEW) effects present. EC_{50} and LD_{50} values of SBL, ECB, and CEW were estimated to be 59, 130, and 2.7, and >500, 340, and 10 µg Mpp75Aa1.1/mL diet, respectively. **Classification: Acceptable**	52255303

* *Leptinotarsa decemlineata* – Colorado potato beetle (CPB), *Epilachna varivestis* – Mexican bean beetle (MBB),

Diabrotica undecimpunctata – southern corn rootworm (SCR), *Helicoverpa zea* – corn earworm (CEW), *Ostrinia nubilalis* – European corn borer (ECB), *Spodoptera frugiperda* – fall armyworm (FAW), *Chrysodeixis includens* – soybean looper (SBL), *Danaus plexippus* – monarch butterfly (MBF), *Lygus hesperus* – western tarnished plant bug (WTP), *Euschistus heros* – neotropical brown stink bug (NBS), *Aedes aegypti* – yellow fever mosquito (YFM), and *Tetranychus urticae* – red spider mite (RSM).

[1] An EC_{50} value could not be estimated for FAW due to limitations of adding protein to the artificial diet.

Table 8. Summary of Vpb4Da2 data submitted to comply with NTO data requirements published in 40 CFR § 158.2150 for support of MON 95275 maize's EUP.

Data Requirement	OCSPP (OPPTS) Guideline No.	Results Summary and Classification	MRID No.
Larval honey bee (*Apis mellifera*) testing	885.4380	A 20-day oral toxicity study with larval honey bees determined the NOEC and NOED values to be 124 µg Vpb4Da2/g diet and 19.8 µg Vpb4Da2/larva, respectively. **Classification: Acceptable**	52041521
Non-target insect testing parasitic wasp (*Pediobius foveolatus*)	885.4340	A 7-day oral toxicity study with *P. foveolatus* determined the NOEC and LD_{50} values to be 500 and >500 µg Vpb4Da2/mL diet, respectively. **Classification: Acceptable**	52041530
Non-target insect testing green lacewing (*Chrysoperla rufilabris*)	885.4340	A 26-day oral toxicity study with green lacewings determined the NOEC and LD_{50} values to be 80 and >80 mg Vpb4Da2/kg diet, respectively. **Classification: Acceptable**	52041534
Non-target insect testing ground-dwelling beetle (*Poecilus cupreus*)	885.4340	A 21-day oral toxicity study with ground-dwelling beetle determined the NOEC and LD_{50} values to be 500 and >500 µg Vpb4Da2/g diet, respectively. **Classification: Acceptable**	52041537
Spectrum of Activity*	N/A	No lethal or sub-lethal effects were observed for: CPB, MBB, FAW, CEW, SBL, ECB, WTP, NBS, or RSM. Vpb4Da2 was active against SCR[1] (sub-lethal effect) and YFM (lethal effect). The LC_{50} value of YFM was estimated to be 45.5 µg Vpb4Da2/mL diet. **Classification: Acceptable**	52255304

* The same species that were challenged with Mpp75Aa1.1, minus the monarch butterfly.

[1] An EC_{50} value could not be estimated for SCR due to limitations of adding protein to the artificial diet.

V. Non-Target Organism Hazard

The EPA risk assessment process is centered on determination of hazard at field exposure rates, which are typically based on expression levels within PIPs to calculate a safety factor (i.e., margin of exposure, MOE) to quantify risk. For the purposes of the NTO risk assessment of Mpp75Aa1.1 and Vpb4Da2 in MON 95275 maize, worst-case estimated environmental concentrations (EECs) are based on 95th percentile values of Mpp75Aa1.1 and Vpb4Da2 expressed in corn tissues(s) from protein expression studies in tissue types relevant to the taxa being considered (see Table 3). To calculate an MOE, the hazard level (i.e., the no observed effects concentration, NOEC) is divided by the EEC. Generally, an MOE ≥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. If, however, hazard testing indicates an MOE of <10x, comprehensive exposure characterization is further considered in the risk characterization section.

Below, hazard identification from Tier 1 bioassays with various taxa with Mpp75Aa1.1 and Vpb4Da2 (Tables 9 and 10) are presented alongside their respective EECs for worst-case expression in relevant tissues and MOEs.

Table 9. Worst case estimated EECs (fresh weight[1]) and MOEs for representative NTOs exposed to Mpp75Aa1.1.

Species name	Tissue	Mpp75Aa1.1 worst-case EEC for relevant tissues	Mpp75Aa1.1 NOED/NOEC or lowest concentration tested[2]	Mpp75Aa1.1 MOE
Pollinators and Pollen Feeders				
A. mellifera – honey bee larvae	Maximum concentration in pollen	0.00025 µg/larva[3]	<39.8 µg/larva	<159,200X
Soil-Dwelling Non-Target Insects				
F. candida – Collembola	Maximum concentration in forage	6.3 µg/g	500 µg/g	79X
Non-Target Insects				
C. rufilabris – green lacewing	Maximum concentration in above ground tissue	20.2 µg/g	<250 ng/mg	<12X
P. foveolatus – parasitic wasp	Maximum concentration in above ground tissue	20.2 µg/g	500 µg/mL	25X
O. insidiosus – insidious flower bug	Maximum concentration in above ground tissue	20.2 µg/g	500 µg/g	25X
G. punctipes – big-eyed bug	Maximum concentration in above ground tissue	20.2 µg/g	500 µg/g	25X
Po. cupreus – ground-dwelling beetle	Maximum concentration in above ground tissue	20.2 µg/g	<250 µg/g	<12X
Co. maculata – spotted lady beetle	Maximum concentration in above ground tissue	20.2 µg/g	500 µg/g	25X

[1] The honey bee larvae EEC is relative to the dry weight.

[2] Values including a less than (<) sign indicate effects were seen below the lowest tested concentration of the test substance.
[3] For honey bees, the EEC considers consumption in conjunction with the pollen expression value listed in Table 3. As the 95th percentile value of Mpp75Aa1.1 in MON 95275 maize pollen could not be determined due to the protein's concentration being below the limit of quantitation, the limit of quantitation was used as a conservative substitute value (0.125 µg Mpp75Aa1.1/g tissue dry weight). After then taking the maximum amount of pollen able to be consumed by honeybee larvae (0.002 g) into account, the worst-case EEC for Mpp75Aa1.1 protein was determined to be 0.00025 µg/larvae (Babendreier et al., 2004; Crailsheim et al., 1992).

Table 10. Worst case estimated EECs (fresh weight[1]) and MOEs for representative NTOs exposed to Vpb4Da2.

Species name	Tissue	Vpb4Da2 worst-case EEC for relevant tissues	Vpb4Da2 NOED/NOEC	Vpb4Da2 MOE
Pollinators and Pollen Feeders				
A. mellifera – honey bee larvae	Maximum concentration in pollen	0.000314 µg/larva[2]	19.8 µg/larva	63,057X
Non-Target Insects				
C. rufilabris – green lacewing	Maximum concentration in above ground tissue	7.4 µg/g	80 mg/kg	11X
P. foveolatus – parasitic wasp	Maximum concentration in above ground tissue	7.4 µg/g	500 µg/ml	68X
Po. cupreus – ground-dwelling beetle	Maximum concentration in above ground tissue	7.4 µg/g	500 µg/g	68X

[1] The honey bee larvae EEC is relative to the dry weight.
[2] For honey bees, the EEC considers consumption in conjunction with the pollen expression value listed in Table 3. As the 95th percentile value of Vpb4Da2 in MON 95275 maize pollen could not be determined due to the protein's concentration being below the limit of quantitation, the limit of quantitation was used as a conservative substitute value (0.157 µg Vpb4Da2/g tissue dry weight). After then taking the maximum amount of pollen able to be consumed by honeybee larvae (0.002 g) into account, the worst-case EEC for Vpb4Da2 protein was determined to be 0.000314 µg/larvae (Babendreier et al., 2004).

Updated MOEs have been calculated to account for the slightly different expressions of DvSnf7.1 in MON 95275 tissues relative to those of DvSnf7 in MON 87411 tissues (Table 11). As evidenced by the MOE values in Table 11, these slight differences in expression have not significantly altered any potential environmental exposure scenario to the extent that would require an updated risk characterization.

Table 11. Worst case estimated EECs (fresh weight) and MOEs for representative NTOs exposed to DvSnf7.1 (and, for comparison, to DvSnf7).

Species name	Tissue	DvSnf7.1 worst-case EEC for relevant tissues	DvSnf7 NOED/NOEC[1]	DvSnf7.1 MOE
Pollinators and Pollen Feeders				
A. mellifera – honey bee larvae	Maximum concentration in pollen	0.0017 ng/larva[2]	11.3 ng/larva	6,647X
Non-Target Insects				
O. insidiosus – insidious flower bug	Maximum concentration in above ground tissue	27 ng/g	1000 ng/g	37X
P. foveolatus – parasitic wasp	Maximum concentration in above ground tissue	27 ng/g	1000 ng/g	37X
Po. chalcites – woodland ground beetle	Maximum concentration in above ground tissue	27 ng/g	1000 ng/g	37X

[1] The NOED/NOEC values were pulled from the bridged studies (i.e., the test substance was DvSnf7).

[2] For honey bees, the EEC considers consumption in conjunction with the maximum pollen expression value listed in MRID 49315104 (0.224 ng DvSnf7/g tissue fresh weight) or Table 4 (0.85 ng DvSnf7.1/g tissue fresh weight). After then taking the maximum amount of pollen able to be consumed by honeybee larvae (0.002 g) into account, the worst-case EECs for DvSnf7 and DvSnf7.1 were determined to be 0.000448 and 0.0017 ng/larvae, respectively (Babendreier et al., 2004).

A. Evaluation of the potential direct effects

1. Mpp75Aa1.1

The LC_{50}/LD_{50} toxicity endpoints for all guideline NTO studies conducted for Mpp75Aa1.1 were greater than the highest concentration tested based on mortality, except for the honey bee (*A. mellifera*), green lacewing (*C. rufilabris*), and ground-dwelling beetle (*Po. cupreus*).

For *A. mellifera*, exposure to both treatment levels of Mpp75Aa1.1 (250 and 500 µg AI/g diet) triggered a significant increase in mortality (64 and 83% in the 250 and 500 µg/g treatments, respectively) relative to the control (8% mortality). For *C. rufilabris*, exposure to both treatment levels (250 and 500 µg/g diet) triggered significant increases in development time (23 days in both treatments) and mortality (84 and 85% in the 250 and 500 µg/g treatments, respectively) relative to the control (20 days and 23% mortality). Similarly, for *Po. cupreus*, both treatment levels (250 and 500 µg/g diet) triggered significant increases in development time (39 and 43 days in the 250 and 500 µg/g treatments, respectively) and mortality (97.5% in both treatments) and a significant decrease in adult weight (54.7 and 55.5 mg in the 250 and 500 µg/g treatments, respectively) relative to the control (37 days, 63.8 mg, and 10% mortality). As adverse effects and significant mortality (>50%) were observed at the lowest tested concentration of Mpp75Aa1.1 for all three species, NOEC and LC_{50} values could not be determined for *A. mellifera*, *C. rufilabris*, nor *Po. cupreus*.

A spectrum of activity study was also performed to evaluate potential effects of Mpp75Aa1.1 on survival or growth inhibition for 12 herbivorous arthropod species from five orders (Table 7). The species tested included three coleopterans (Colorado potato beetle [*Leptinotarsa decemlineata*], Mexican bean beetle [*Epilachna varivestis*], and southern corn rootworm [*Diabrotica undecimpunctata*]), five lepidopterans (corn earworm [*Helicoverpa zea*], European corn borer [*Ostrinia nubilalis*], fall armyworm [*Spodoptera frugiperda*], soybean looper [*Chrysodeixis includens*], and monarch butterfly [*Danaus plexippus*]), two hemipterans (western tarnished plant bug [*Lygus hesperus*] and neotropical brown stink bug [*Euschistus heros*]), a dipteran (yellow fever mosquito [*Aedes aegypti*]), and an acarid (red spider mite [*Tetranychus urticae*]). The results showed that Mpp75Aa1.1 was active only against insects within the order Lepidoptera. Specifically, Mpp75Aa1.1 was active against four pest lepidopterans, with *S. frugiperda* (EC_{50} could not be determined) and *Ch. includens* (EC_{50} = 59 µg/mL) exhibiting significant growth inhibition and *H. zea* and *O. nubilalis* exhibiting both significant growth inhibition and mortality (EC_{50}'s of 2.7 and 130 and LC_{50}'s of 10 and 340 µg/mL, respectively). However, Mpp75Aa.1 did not induce adverse effects in the monarch butterfly (*D. plexippus*).

Overall, analysis of the non-target effects and the spectrum of analysis information/data provided lead to the conclusion that Mpp75Aa1.1 has a broader spectrum of activity within insects relative to previously assessed *Bt*-derived Cry or Vip proteins. However, given that the mode of action and structure of Mpp75Aa1.1 is similar to those of several previously registered *Bt*-derived Cry and Vip proteins that have a history of safe use/consumption by non-insect species (Wang et al., 2022), there is no expectation that this activity extends beyond insects. Indeed, no effects were observed against the two organisms outside of the class Insecta (*Folsomia candida* and *Mus musculus*) that were challenged with the protein, highlighting that Mpp75Aa1.1's activity is expected to be limited to a subset of insect species/orders.

2. Vpb4Da2

The LC_{50}/LD_{50} toxicity endpoints for all guideline NTO studies conducted for Vpb4Da2 were greater than the highest concentration tested based on mortality. A spectrum of activity study was also performed to evaluate its potential effects on survival or growth inhibition for 11 herbivorous arthropod species from five insect orders (Table 8). The species tested included the same species that were assayed against Mpp75Aa1.1, minus the monarch butterfly *D. plexippus*. The results showed that Vpb4Da2 was active only against the coleopteran *D. undecimpunctata* (sub-lethal effect – growth inhibition) and the dipteran *A. aegypti* (lethal effect), with LD_{50} values of >500 and 45.5 µg/mL, respectively.

Overall, analysis of the non-target effects and the spectrum of analysis information/data provided lead to the conclusion that activity of Vpb4Da2 is largely limited to a subset of coleopteran species.

B. Evaluation of the potential indirect effects

The EPA evaluated the potential for indirect effects, which generally include negative effects to NTOs from the reduction of a food source or habitat.

1. Mpp75Aa1.1

Given the relatively broad activity spectrum of the protein (within insects), Mpp75Aa1.1 could potentially affect herbivorous insect pest species that consume maize tissue across multiple orders. Thus, predatory species that consume these pests may experience reductions in their source(s) of food; however, given the ubiquity and richness of insects in habitats worldwide (Samways et al., 2020), not only would these insect pests likely be present in areas outside of the treated field, but also many other types of non-susceptible insect species would likely be readily available as prey (both on- and off-field). As a result, predators utilizing these insect pest species as a source of food would still have ample opportunity to encounter potential prey. Furthermore, the limited acreage of the EUP minimizes the extent to which these food sources would potentially be impacted.

A reduction in susceptible predatory species (resulting from exposure to the protein via consuming prey species that have fed on MON 95275 maize tissue) could potentially alter functional services; however, this risk is expected to be negligible given the limited footprint of this EUP and that previous studies have shown PIP concentrations to become increasingly diluted as they move through the food chain (Li et al., 2017; Meissle & Romeis, 2018; Tian et al., 2014). Furthermore, agricultural fields are expected to contain several species belonging to the same functional group (e.g., Bellamy et al., 2018; Duelli et al., 1999), providing ecological redundancies.

Another consideration is the potential of pollination impacts from the proposed use of Mpp75Aa1.1. Because the concentration of Mpp75Aa1.1 in MON 95275 maize pollen is below its level of quantitation (i.e., the proteins are not detected in pollen), it is not expected to have an effect on pollinator species (e.g., honey bees). Additionally, the targeted coleopteran pest, WCR, is not a known pollinator itself and its reduction is therefore not expected to alter pollination services. Similarly, non-targeted corn pests are not known to be significant pollinators and thus their potential population decrease(s) would also not be expected to alter pollinator services.

Overall, indirect effects to NTOs are not anticipated because of the limited acreage of the proposed EUP, expected environmental exposures, and the lack of evidence for PIP bioaccumulation through the food chain.

2. Vpb4Da2

As the proposed use of Vpb4Da2 is intended to control coleopteran pests of corn, populations of these pests within MON 95275 maize fields are expected to be minimal. Thus, predatory species that consume these pests may experience reductions in their source(s) of food; however, given the ubiquity and richness of insects in habitats worldwide (Samways et al., 2020), especially the speciose Coleoptera (Stork et al., 2015), not only would these coleopteran pests likely be present in areas outside of the treated field, but also many other types of non-susceptible insect species would likely be readily available as prey (both on- and off-field). As a result, predators utilizing these coleopteran pest species as a source of food would still have ample opportunity to encounter potential prey. Furthermore, the limited acreage of the EUP minimizes the extent to which these food sources will be impacted and the protein's narrow activity spectrum limits the adverse effects to the targeted coleopteran pest species within the treatment area.

Another consideration is the potential of pollination impacts from the proposed use of Vpb4Da2. Because the concentration of Vpb4Da2 in MON 95275 maize pollen is below its level of quantitation (i.e., the proteins are not detected in pollen), it is not expected to have an effect on pollinator species (e.g., honey bees). Additionally, the targeted coleopteran pest, WCR, is not a known pollinator itself and its reduction is therefore not expected to alter pollination services. Thus, the reduction of pollinators for plants that are food sources for NTOs are not expected, nor are effects expected for plants that rely on pollinators as part of their lifecycle.

Therefore, indirect effects to NTOs are not expected because any measurable effects to Coleoptera are expected to be limited to the pest species in the treatment field, which is not a sole source of feeding for NTOs that consume Coleoptera, nor is the pest species a known pollinator for non-target plants.

C. Synergism

A synergism study was conducted to evaluate the combined potency of Mpp75Aa1.1, Vpb4Da2, and DvSnf7.1 in MON 95275 maize using WCR larvae (*Diabrotica virgifera virgifera*), an insect sensitive to the three AIs.

The observed EC_{50} value was estimated to be 1.32 μg/mL with a 95% confidence interval (CI) of 0.87 to 1.98 μg/mL for Mpp75Aa1.1, 2.69 μg/mL with a 95% CI of 2.15 to 3.38 μg/mL for Vpb4Da2, and 0.0066 μg/mL with a 95% CI of 0.0039 to 0.011 μg/mL for DvSnf7.1. The predicted EC_{50} value for the combined mixture of the three AIs was then estimated to be 2.00 μg/mL with a 95% CI of 1.44 to 2.78 μg/mL diet. The observed EC_{50} value for the combined mixture of the three AIs was estimated to be 2.24 μg/mL with a 95% CI of 1.80 to 2.79 μg/mL. Thus, the predicted EC_{50} value was captured by the 95% CI of the observed EC_{50} value for the combined mixture of the three AIs.

These results demonstrate that the combined activity of Mpp75Aa1.1, Vpb4Da2, and DvSnf7.1 is consistent with additivity in the combined trait product MON 95275 and support the conclusion that the AIs are not synergistic. Therefore, there is no expectation that the combined use of these AIs in MON 95275 maize would alter the hazard characterization compared to the AIs in isolation.

VI. Ecological Risk Characterization

Given DvSnf7.1 has been shown to be functionally equivalent to the previously registered DvSnf7 (MRID 52041504) and its concentration within MON 95275 maize tissue is similar to its concentration within previously registered products (MRID 52041403; and see Table 11 for the updated MOEs related to DvSnf7's bridged studies), the primary conclusion reached in MON 87411 maize's ecological risk assessment regarding DvSnf7 has been bridged to support MON 95275 maize's Section 5 EUP:

> "Based on the data presented and anticipated minimal exposure in certain environments, adverse effects to nontarget organisms are not expected as a result of DvSnf7 as expressed in MON 89034 x TC1507 x MON 87411 x DAS-59122-7 corn" (U.S. EPA, 2016).

Therefore, no new risk characterization has been performed for DvSnf7.1 and the risk characterizations below solely concern the novel Mpp75Aa1.1 and Vpb4Da2 proteins.

Above, hazard, soil degradation, and tissue expression data were presented for Mpp75Aa1.1 and Vpb4Da2. In this section, the likelihood of hazard will be placed in context of potential exposure as relevant to each taxon to assess potential risk of Mpp75Aa1.1 and Vpb4Da2 as expressed in MON 95275 maize. 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 risk characterization will be provided for Mpp75Aa1.1 and Vpb4Da2.

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 Mpp75Aa1.1 and Vpb4Da2 as expressed in MON 95275 maize. This finding is based on several lines of evidence that support both a lack of hazard and lack of significant exposure to the proteins for these two groups of vertebrate species. First, the NTO hazard studies described above demonstrate that the activity of both proteins is limited to insect species as effects were not induced by Mpp75Aa1.1 when assayed against a collembolan species (i.e., a non-insect species) and Vpb4Da2's activity was predominantly limited to subset of coleopteran species (i.e., activity specific to a small subset of insects). Hazardous effects would therefore not be expected in more distantly related species (i.e., avians and mammals). Indeed, both proteins were tested against mice for the associated human health risk assessment and no effects were observed. Additionally, the level of expected environmental exposure to the proteins is not considered to be hazardous as the expression of the proteins in tissues relevant to avians and mammals (e.g., grains, leaves) is much lower than protein concentrations used in toxicity studies which demonstrated no hazard to non-insect species. Therefore, due to a lack of both hazard and relevant environmental exposure, there is a reasonable expectation of no discernible effects to avian and mammalian species from the use of Mpp75Aa1.1 and Vpb4Da2.

2. Honey Bees and Non-Target Invertebrates

For Mpp75Aa1.1 and Vpb4Da2, a series of representative non-target invertebrates were assayed against the proteins to determine the activity spectrum of Mpp75Aa1.1 and Vpb4Da2.

a. Pollinators (honey bees)

The potential exposure of Mpp75Aa1.1 and Vpb4Da2 as expressed in MON 95275 maize to honey bees was assessed due to the species' importance as a beneficial organism in agriculture and agroecosystems.

i. Mpp75Aa1.1

The EPA has determined that there is a reasonable expectation of no discernible effects to occur to pollinator species as a result of the use of Mpp75Aa1.1 as expressed in MON 95275 maize. Although effects were observed when Mpp75Aa1.1 was assayed against larval honey bees (Table 7), the expression of the protein within MON 95275 maize pollen is considered to be negligible (Table 3), thus exposure to pollinator species is not expected. Therefore, due to a lack of environmental exposure, Mpp75Aa1.1 is anticipated to pose negligible risk to pollinator species.

ii. Vpb4Da2

The EPA has determined that there is a reasonable expectation of no discernible effects to occur to pollinator species as a result of the use of Vpb4Da2 as expressed in MON 95275 maize. No effects were observed when Vpb4Da2 was assayed against larval honey bees (Table 8). Additionally, the expression of the protein within MON 95275 maize pollen is considered to be negligible (Table 3), thus exposure to pollinator species is not expected. Therefore, due to a lack of both hazard and environmental exposure, Vpb4Da2 is anticipated to pose negligible risk to pollinator species.

b. Soil-Dwelling Organisms and Detritivores

Since soil is expected to be the ultimate destination in the terrestrial environment for PIPs, the potential for risk to soil-dwelling organisms and detritivores was evaluated. The potential exposure of Mpp75Aa1.1 and Vpb4Da2 as expressed in MON 95275 maize to *Folsomia candida* (Collembola) was assessed due to the group's ubiquitous presence in soils worldwide and close relatedness with insects (while not being insects themselves). Therefore, a lack of effects observed in collembolan studies can support the insect-specific activity of an AI.

i. Mpp75Aa1.1

The EPA has determined that there is a reasonable expectation of no discernible effects to occur to soil dwelling and detritivore species as a result of the use of

Mpp75Aa1.1 as expressed in MON 95275 maize. No effects were observed when Mpp75Aa1.1 was assayed against *Folsomia candida* (Table 7). Additionally, the level of expected environmental exposure to the protein is not considered to be hazardous as the expression of Mpp75Aa1.1 in tissues relevant to soil dwelling and detritivore species (e.g., forage tissues; Table 3) is much lower than protein concentrations used in the *F. candida* toxicity study which demonstrated no hazard to the non-insect species. Furthermore, proteins are expected to experience high rates of degradation in agricultural field conditions (e.g., soil pH's near or above neutrality [MRID 52255309]), further limiting exposure. Therefore, due to a lack of both hazard to Collembola and relevant environmental exposure, Mpp75Aa1.1 is anticipated to pose negligible risk to soil dwelling and detritivore species.

ii. Vpb4Da2

The EPA has determined that there is a reasonable expectation of no discernible effects to occur to soil dwelling and detritivore species as a result of the use of Vpb4Da2 as expressed in MON 95275 maize. While no collembolan study was submitted for Vpb4Da2, the protein's activity was predominantly limited to a subset of coleopteran species (i.e., activity specific to a subset of insects; Table 8), thus activity in non-insect species is not expected. Additionally, proteins are expected to experience high rates of degradation in agricultural field conditions (e.g., soil pH's near or above neutrality [MRID 52255309]), limiting any potential environmental exposure. Therefore, due to a lack of both hazard and relevant environmental exposure, Vpb4Da2 is anticipated to pose negligible risk to soil dwelling and detritivore species.

c. Predators and Parasitoids (lacewings, parasitic wasps, insidious flower bugs, big-eyed bugs, ground-dwelling beetles, and lady bird beetles)

The potential exposure of Mpp75Aa1.1 and Vpb4Da2 as expressed in MON 95275 maize to species belonging to the predator and parasitoid functional groups (lacewings, parasitic wasps, insidious flower bugs, big-eyed bugs, ground-dwelling beetles, and lady bird beetles) was assessed due to these species' importance as beneficial organisms in agriculture and agroecosystems. All six species mentioned above were assayed against Mpp75Aa1.1 (Table 7), while only three species (lacewings, parasitic wasps, and ground-dwelling beetles) were assayed against Vpb4Da2 (Table 8). As predators and parasitoids do not feed directly on maize tissue, an important factor to consider in the exposure and risk assessment for these taxa is the amount of the protein that can potentially transfer to and accumulate in prey.

i. Mpp75Aa1.1

The EPA has determined that there is a reasonable expectation of no discernible population-level effects to occur to predator and parasitoid species as a result of the use of Mpp75Aa1.1 as expressed in MON 95275 maize for the proposed EUP. No effects were observed when Mpp75Aa1.1 was assayed against *P. foveolatus*, *O.*

insidiosus, *G. punctipes*, or *Co. maculata* (Table 7). Although effects were observed when Mpp75Aa1.1 was assayed against two predatory species (*C. rufilabris* and *Po. cupreus*; Table 7), given the limited acreage of the EUP application, the predatory nature of these species (i.e., these species will not directly consume significant amounts of MON 95275 maize tissue), and the general lack of PIP concentrations within prey species (Li et al., 2017; Meissle & Romeis, 2018; Tian et al., 2014), hazardous levels of protein exposure to these species are not expected to occur in the field. However, given that NOEC and LC_{50} values were not determined for the two predatory species noted above, the EPA cannot rule out the possibility of effects in the field. That being said, due to limited environmental exposure both from the general lack of PIP concentrations within prey species and the limited acreage proposed for the EUP, Mpp75Aa1.1 is anticipated to pose negligible population-level risk to predator and parasitoid species. However, further characterization regarding the potential hazard that Mpp75Aa1.1 may pose to certain insect species (i.e., higher-tiered testing to determine NOEC or LC_{50}) would be necessary should the applicant seek a Section 3 Registration.

ii. Vpb4Da2

The EPA has determined that there is a reasonable expectation of no discernible effects to occur to predator and parasitoid species as a result of the use of Vpb4Da2 as expressed in MON 95275 maize. No effects were observed when Vpb4Da2 was assayed against *C. rufilabris*, *P. foveolatus*, or *Po. cupreus* (Table 8). Additionally, given the general lack of PIP concentrations within prey species (Li et al., 2017; Meissle & Romeis, 2018; Tian et al., 2014), hazardous levels of protein exposure to these species are not expected to occur in the field. Therefore, due to a lack of both hazard and relevant environmental exposure, Vpb4Da2 is anticipated to pose negligible risk to predator and parasitoid species.

B. Aquatic Environments

1. Freshwater Invertebrates and Fish

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 Mpp75Aa1.1 and Vpb4Da2 as expressed in MON 95275 maize. This finding is based on several lines of evidence that support both a lack of hazard and exposure to the proteins for these two groups of freshwater species. First, the NTO hazard studies described above demonstrate that the activity of both proteins is limited to insect species as effects were not induced by Mpp75Aa1.1 when assayed against a collembolan species (i.e., a non-insect species) and Vpb4Da2's activity was predominantly limited to subset of coleopteran species (i.e., activity specific to a small subset of insects). Hazardous effects would therefore not be expected in more distantly related species (i.e., non-insect aquatic invertebrates and fish). Regarding exposure, while maize tissue inputs in aquatic environments are likely in bodies of freshwater near corn growing areas, these inputs are typically minimal prior to harvest (most inputs occur in late fall and winter [Rosi-Marshall et al., 2007]), thus exposure is transient rather than continuous across time. Additionally, given the proteins exhibit negligible expression within MON 95275 maize pollen (Table 3), post-harvest crop residues are the only viable source of exposure for these proteins to aquatic environments (e.g., forage tissues, which display reduced protein concentrations relative to other tissues; Table 3). As proteins, including insecticidal proteins from PIPs, rapidly leach from corn tissue (Böttger et al., 2015; Chambers et al., 2010; Strain & 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), PIPs are anticipated to have largely leached out of post-harvest crop residues by the time the tissue is suitable for consumption by aquatic organisms (Carstens et al., 2012; U.S. EPA, 2010b). Overall, the transient nature of maize tissue inputs, limited protein expression within post-harvest crop residues, and rapid aquatic protein degredation rate results in an expectation of negligible exposure for both Mpp75Aa1.1 and Vpb4Da2 to aquatic environments. Therefore, due to a lack of hazard and/or relevant environmental exposure, Mpp75Aa1.1 and Vpb4Da2 are anticipated to pose negligible risk to freshwater aquatic invertebrate and fish species.

A freshwater aquatic invertebrate toxicity study for Mpp75Aa1.1 was performed for another regulatory agency and subsequently submitted by the applicant pursuant to §6(a)(2) of FIFRA. While a statistically significant sub-lethal effect was observed in the study, the study's NOEC was approximately 3,000 times greater than the estimated aquatic exposure (based on the EPA standard pond model), highlighting the extreme unlikelihood that the protein will approach potentially toxic concentrations in aquatic settings. Furthermore, although the endpoint value was significantly different from the study's buffer control value, it was within the range of historic control values, indicating that the result may represent normal variation in the endpoint and therefore may not be biologically meaningful. Finally, the study's (MRID 52347501) failed positive control resulted in questions as to the validity of the study. This uncertainty surrounding whether the study's results are biologically meaningful, coupled with the expectation of negligible aquatic exposure to the protein in the environment (see above), resulted in this study not being considered useful for the risk assessment.

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 Mpp75Aa1.1 and Vpb4Da2 as expressed in MON 95275 maize. EPA has previously determined that exposure to PIPs in marine and estuarine environments is not significant and therefore adverse effects are not anticipated for fish or invertebrates from these environments (U.S. EPA, 2016). At this time, there is no information to indicate that this assumption would not apply to Mpp75Aa1.1 and Vpb4Da2. The same arguments that apply to the risk characterization of freshwater aquatic invertebrate and fish species (see previous section) hold true here. To summarize, hazardous effects are not expected for non-insect marine or estuarine fish and invertebrates, and the transient nature of maize tissue inputs, limited protein expression within post-harvest crop residues, and rapid aquatic protein degredation rate results in an expectation of negligible exposure for both Mpp75Aa1.1 and Vpb4Da2 to aquatic environments. Therefore, due to a lack of hazard and/or relevant environmental exposure, Mpp75Aa1.1 and Vpb4Da2 are anticipated to pose negligible risk to marine or estuarine fish and invertebrate species.

C. Probability of Outcrossing and Weediness of MON 95275 Maize

The EPA has previously determined that there is no significant risk of gene flow from corn PIPs to wild or weedy relatives in the U.S., its possessions, or territories, based on lack of sexually compatible relatives (U.S. EPA, 2001). As this determination is based on corn plant biology, and is not AI specific, there is no information to indicate that this assumption would not apply to Mpp75Aa1.1, Vpb4Da2, or DvSnf7.1. Thus, no risk of gene flow or weediness is anticipated for Mpp75Aa1.1, Vpb4Da2, or DvSnf7.1 as expressed in MON 95275 maize.

D. Environmental Risk Conclusions

The EPA considered possible routes of exposure to Mpp75Aa1.1 as expressed in MON 95275 maize, 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 non-target insects leading to a possible reduction in an NTO's food source or a reduction in pollination services. The 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 (or vice versa), risk is concluded to be negligible.

While the activity of Mpp75Aa1.1 appears limited to insects given that no effects were observed against organisms outside of the class Insecta (*Folsomia candida* and *Mus musculus*), some Tier I studies identified adverse effects to non-target insects at the lowest tested concentration of the protein. Therefore, the EPA cannot definitively state that Mpp75Aa1.1 does not pose a risk to non-target insects. However, given the limited acreage of the proposed EUP and the absent or limited route of exposure to beneficial non-target insects that exhibited hazard to the protein (i.e., negligible exposure for pollinators and limited exposure for predatory insects), the EPA concludes that discernable population-level effects are not expected for non-target insects from the use of Mpp75Aa1.1 as expressed in MON 95275 maize for the proposed EUP. Similarly, the EPA concludes that indirect effects to NTOs are not anticipated because the limited acreage of the EUP diminishes impacts to food sources (i.e., non-target insects) and the lack of previously elucidated PIP bioaccumulation through the food chain, as well as Mpp75Aa1.1's negligible expression in pollen, diminishes the impact to pollination networks (i.e., networks supported to some degree by predator pollinators). Therefore, due to the limited acreage of the proposed EUP, expected environmental exposures, and expected negligible indirect effects, there is a reasonable expectation of no discernible effects to any NTO outside the class Insecta and there is a reasonable expectation of no direct population-level effects for non-target insects as a result of the Section 5 EUP for Mpp75Aa1.1 as expressed in MON 95275 maize.

The EPA also considered possible routes of exposure to Vpb4Da2 as expressed in MON 95275 maize, 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 coleopteran pests leading to a possible reduction in an NTO's food source or a reduction in pollination services. The 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 (or vice versa), risk is concluded to be negligible. In addition, the primary risk conclusion reached for DvSnf7 as expressed in MON 87411 maize (U.S. EPA, 2016) was bridged to support DvSnf7.1 as expressed in MON 95275 maize.

The EPA concludes that the consumption of or contact with MON 95275 maize tissues containing Vpb4Da2 and DvSnf7.1 by NTOs is not expected to pose a hazard to any non-coleopteran NTO based on toxicity studies indicating adverse effects are largely limited to the coleopteran order. Additionally, indirect effects to NTOs are not expected because any measurable effects to Coleoptera are expected to be limited to the pest species in the treatment field, which is not a sole, or significant, source of feeding for NTOs that consume Coleoptera, nor a sole, or significant, source of pollination for non-target plants that are pollinated by Coleoptera. Therefore, due to the lack of direct and indirect effects, there is a reasonable expectation of no discernible effects for non-coleopteran NTOs as a result of the Section 5 EUP for Vpb4Da2 and DvSnf7.1 as expressed in MON 95275 maize.

Regarding non-target coleopteran species, the EPA concludes that the potential for adverse effects to coleopteran organisms from Vpb4Da2 and DvSnf7.1 is limited to a subset of herbivorous coleopterans (e.g., corn rootworms) and because Vpb4Da2 and DvSnf7.1 are contained within corn tissue, effects are expected to be limited to pest species. Thus, based on specificity to pest coleopteran species, risk to non-target beneficial coleopteran species is expected to be negligible as a result of the Section 5 EUP for Vpb4Da2 and DvSnf7.1 as expressed in MON 95275 maize.

VII. Risk to Threatened and Endangered Species

"No Effect" finding for the 30 TES in the proposed counties for the Section 5 EUP.

A. Introduction

The combination of scientific rationale, bioassay results, and guideline toxicity studies indicate the specificity of Mpp75Aa1.1 to insect species. Additionally, given the limited acreage of the proposed EUP, the expected environmental exposures of the protein, and the lack of evidence for PIP bioaccumulation through the food chain, indirect effects to any NTOs are not anticipated. However, since Tier I studies featuring Mpp75Aa1.1 found adverse effects to beneficial non-target insects (both coleopteran and non-coleopteran insects) at the lowest tested concentration of the protein, the EPA cannot definitively state that Mpp75Aa1.1 does not pose a direct hazard to any non-target insect. Thus, the purpose of Mpp75Aa1.1's endangered species assessment was to evaluate its potential to directly affect insect threatened and endangered species (TES). In total, 30 insect TES are presently located within counties where experimental trials of MON 95275 maize may occur (USFWS, 2023a; USFWS, 2023b) (Table 12).

The combination of scientific rationale, bioassay results, and guideline toxicity studies indicate the specificity of Vpb4Da2 and DvSnf7 (i.e., DvSnf7.1) to coleopteran species. Additionally, indirect effects to any NTOs are not expected because any measurable effects to Coleoptera are expected to be limited to the pest species in the treatment field, which is not a sole, or significant, source of feeding for NTOs that consume Coleoptera, nor a sole, or significant, source of pollination for non-target plants that are pollinated by Coleoptera. Thus, the purpose of Vpb4Da2's and DvSnf7.1's endangered species assessment was to evaluate their potential to directly affect coleopteran TES. In total, two coleopteran TES are located within counties where experimental trials of MON 95275 maize may occur (USFWS, 2023a; USFWS, 2023b) (Table 12). As the order Coleoptera falls under the class Insecta, the two coleopteran TES were the only species to be assessed for all three AIs.

Table 12. Insect TES present in counties where experimental trials of MON 95275 maize may occur.

Common Name	**Scientific Name**	**Order**	**ESA Listing Status**	**County of overlap**
American burying beetle	*Nicrophorus americanus*	Coleoptera	Threatened	Antelope NE; Dawson NE
Puritan tiger beetle	*Ellipsoptera puritana*	Coleoptera	Threatened	Kent MD
Blackburn's sphinx moth	*Manduca blackburni*	Lepidoptera	Endangered	Maui, HI
Dakota skipper	*Hesperia dacotae*	Lepidoptera	Threatened	Brookings SD; Lincoln MN; Moody SD; Richland ND; Swift MN
Karner blue butterfly	*Lycaeides melissa samuelis*	Lepidoptera	Endangered	Ionia MI; Kent MI; Columbia WI
Mitchell's satyr butterfly	*Neonympha mitchellii mitchellii*	Lepidoptera	Endangered	Van Buren MI
Rusty patched bumble bee	*Bombus affinis*	Hymenoptera	Endangered	See footnote[1]
Anthricinan yellow-faced bee	*Hylaeus anthracinus*	Hymenoptera	Endangered	Maui HI; Honolulu HI
Assimulans yellow-faced bee	*Hylaeus assimulans*	Hymenoptera	Endangered	Maui, HI
Easy yellow-faced bee	*Hylaeus facilis*	Hymenoptera	Endangered	Maui HI; Honolulu HI
Hawaiian yellow-faced bee	*Hylaeus longiceps*	Hymenoptera	Endangered	Maui HI; Honolulu HI
Hawaiian yellow-faced bee	*Hylaeus kuakea*	Hymenoptera	Endangered	Honolulu HI
Hawaiian yellow-faced bee	*Hylaeus mana*	Hymenoptera	Endangered	Honolulu HI
Hilaris yellow-faced bee	*Hylaeus hilaris*	Hymenoptera	Endangered	Maui, HI
Hawaiian picture-wing fly	*Drosophila aglaia*	Diptera	Endangered	Honolulu, HI
Hawaiian picture-wing fly	*Drosophila differens*	Diptera	Endangered	Maui, HI
Hawaiian picture-wing fly	*Drosophila hemipeza*	Diptera	Endangered	Honolulu, HI

Table 12. Insect TES present in counties where experimental trials of MON 95275 maize may occur.

Common Name	Scientific Name	Order	ESA Listing Status	County of overlap
Hawaiian picture-wing fly	*Drosophila montgomeryi*	Diptera	Endangered	Honolulu, HI
Hawaiian picture-wing fly	*Drosophila neoclavisetae*	Diptera	Endangered	Maui, HI
Hawaiian picture-wing fly	*Drosophila obatai*	Diptera	Endangered	Honolulu, HI
Hawaiian picture-wing fly	*Drosophila sharpi*	Diptera	Endangered	Honolulu, HI
Hawaiian picture-wing fly	*Drosophila substenoptera*	Diptera	Endangered	Honolulu, HI
Hawaiian picture-wing fly	*Drosophila tarphytrichia*	Diptera	Endangered	Honolulu, HI
Hine's emerald dragonfly	*Somatochlora hineana*	Odonata	Endangered	Kane IL; Rock WI
Flying earwig Hawaiian damselfly	*Megalagrion nesiotes*	Odonata	Endangered	Maui, HI
Pacific Hawaiian damselfly	*Megalagrion pacificum*	Odonata	Endangered	Maui, HI
Orangeblack Hawaiian damselfly	*Megalagrion xanthomelas*	Odonata	Endangered	Maui HI; Honolulu HI
Blackline Hawaiian damselfly	*Megalagrion nigrohamatum nigrolineatum*	Odonata	Endangered	Honolulu, HI
Crimson Hawaiian damselfly	*Megalagrion leptodemas*	Odonata	Endangered	Honolulu, HI
Oceanic Hawaiian damselfly	*Megalagrion oceanicum*	Odonata	Endangered	Honolulu, HI

[1] Benton IA; Black Hawk IA; Blue Earth MN; Boone IA; Buchanan IA; Bureau IL; Champaign IL; Columbia WI; Dakota MN; Dekalb IL; Delaware IA; Dodge MN; Fond du Lac WI; Freeborn MN; Goodhue MN; Hancock IA; Howard IA; Iowa IA; Jones IA; Kane IL; Lafayette WI; Linn IA; Macon IL; Mitchell IA; Ogle IL; Peoria IL; Rock WI; Story IA; Walworth WI; Waseca MN; Winneshiek IA; Woodford IL

B. Exposure and Hazard Summary

Given their confinement within plant tissue, exposure of Mpp75Aa1.1 to insect TES and exposure of Vpb4Da2 and DvSnf7.1 to coleopteran TES is expected to be limited to direct consumption of MON 95275 maize. Therefore, a dataset of known corn crop production within the continental United States (U.S. EPA, 2023a) and a dataset of known agricultural production within Hawaii (U.S. EPA, 2023b) was compared against the ranges of insect TES in proposed EUP locations to identify potential range overlap. As the Hawaiian agricultural production dataset includes crops other than corn, this dataset represents an extremely conservative exposure scenario.

Based on the above analysis, 13 of the 30 insect TES ranges overlap more than 1% (>0.44% when accounting for rounding) with areas of known corn crop production or agricultural production (*i.e.*, potential direct exposure; Table 13). No overlap was identified for 16 insect TES; therefore, the EPA determines that MON 95275 maize will have no effect on these 16 insect TES nor their critical habitats due to the expectation of no exposure (see Table 13). One additional species (*Megalagrion xanthomelas*) also had an overlap percentage of less than 1% (≤0.44%), which, per the EPA's guidance on interpretation of UDL overlaps, ≤0.44% is treated as 0% and generally 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 MON 95275 maize will have no effect on *M. xanthomelas* nor its critical habitat due to the expectation of no exposure.

The remaining 13 insect TES showed some degree of overlap (>0.44%), but further biological evaluation indicated that these species are habitat and/or dietary specialists and do not use corn fields as habitat nor corn tissue as a food source, therefore the EPA determines that MON 95275 maize will have no effect on these species nor their critical habitats due to the expectation of negligible to no exposure. A detailed analysis of all 30 insect TES evaluated can be found in Appendices A and B.

Table 13. Insect TES range overlap with corn crop production (within the continental United States) or agricultural production (within Hawaii) in counties where experimental trials of MON 95275 maize may occur.

Common Name	Scientific Name	Direct Range Overlap in Each Potential EUP County
Insect TES with <1% (≤0.44%) range overlap with areas of corn crop or agricultural production in all counties		
Anthricinan yellow-faced bee	*Hylaeus anthracinus*	Maui HI – 0% Honolulu HI – 0%
Assimulans yellow-faced bee	*Hylaeus assimulans*	Maui HI – 0%
Easy yellow-faced bee	*Hylaeus facilis*	Maui HI – 0% Honolulu HI – 0%
Hawaiian yellow-faced bee	*Hylaeus longiceps*	Maui HI – 0% Honolulu HI – 0%
Hawaiian yellow-faced bee	*Hylaeus kuakea*	Honolulu HI – 0%
Hawaiian yellow-faced bee	*Hylaeus mana*	Honolulu HI – 0%
Hilaris yellow-faced bee	*Hylaeus hilaris*	Maui HI – 0%
Hawaiian picture-wing fly	*Drosophila aglaia*	Honolulu HI – 0%
Hawaiian picture-wing fly	*Drosophila differens*	Maui HI – 0%
Hawaiian picture-wing fly	*Drosophila hemipeza*	Honolulu HI – 0%
Hawaiian picture-wing fly	*Drosophila montgomeryi*	Honolulu HI – 0%

Table 13. Insect TES range overlap with corn crop production (within the continental United States) or agricultural production (within Hawaii) in counties where experimental trials of MON 95275 maize may occur.

Common Name	Scientific Name	Direct Range Overlap in Each Potential EUP County
Hawaiian picture-wing fly	*Drosophila neoclavisetae*	Maui HI – 0%
Hawaiian picture-wing fly	*Drosophila obatai*	Honolulu HI – 0%
Hawaiian picture-wing fly	*Drosophila sharpi*	Honolulu HI – 0%
Hawaiian picture-wing fly	*Drosophila substenoptera*	Honolulu HI – 0%
Hawaiian picture-wing fly	*Drosophila tarphytrichia*	Honolulu HI – 0%
Orangeblack Hawaiian damselfly	*Megalagrion xanthomelas*	Maui HI – 0.128% Honolulu HI – 0%
Insect TES with ≥1% (>0.44%) range overlap with areas of corn crop or agricultural production in at least one county		
American burying beetle	*Nicrophorus americanus*	Dawson NE – 0.516% Antelope NE – 0.407%
Dakota skipper	*Hesperia dacotae*	Richland ND – 0.865% Brookings SD – 0.279% Moody SD – 0.093% Swift MN – 0.044% Lincoln MN – 0.043%
Hine's emerald dragonfly	*Somatochlora hineana*	Kane IL – 0.803% Rock WI – 0.290%
Karner blue butterfly	*Lycaeides melissa samuelis*	Ionia MI – 1.126% Kent MI – 0.594% Columbia WI – 0.001%
Pacific Hawaiian damselfly	*Megalagrion pacificum*	Maui HI – 1.391%
Mitchell's satyr butterfly	*Neonympha mitchellii mitchellii*	Van Buren MI – 1.863%
Puritan tiger beetle	*Ellipsoptera puritana*	Kent MD – 2.139%
Blackburn's sphinx moth	*Manduca blackburni*	Maui HI – 4.215%
Oceanic Hawaiian damselfly	*Megalagrion oceanicum*	Honolulu, HI – 6.516%
Blackline Hawaiian damselfly	*Megalagrion nigrohamatum nigrolineatum*	Honolulu HI – 6.599%
Crimson Hawaiian damselfly	*Megalagrion leptodemas*	Honolulu HI – 6.599%
Flying earwig Hawaiian damselfly	*Megalagrion nesiotes*	Maui HI – 7.603%
Rusty patched bumble bee	*Bombus affinis*	See footnote[1]

[1] Benton IA – 0%; Black Hawk IA – 0.068%; Blue Earth MN – 0.050%; Boone IA – 0.077%; Buchanan IA – 0.040%; Bureau IL – 0.033%; Champaign IL – 0.001%; Columbia WI – 0.321%; Dakota MN – 0.368%; Dekalb IL – 0.076%; Delaware IA – 0.049%; Dodge MN – 0%; Fond du Lac WI – 0.160%; Freeborn MN – 0.080%; Goodhue MN – 0.175%; Hancock IA – 0.033%; Howard IA – 0.099%; Iowa IA – 0.002%; Jones IA – 0.037%; Kane IL – 0.250%; Lafayette WI – 0.191%; Linn IA – 0.065%; Macon IL – 0.007%; Mitchell IA – 0.047%; Ogle IL – 0.141%; Peoria IL – 0.097%; Rock WI – 0.278%; Story IA – 0.066%; Walworth WI – 0.191%; Waseca MN – 0.001%; **Winneshiek IA – 0.530%**; Woodford IL – 0.015%

C. Endangered Species Conclusions

For Mpp75Aa1.1, the EPA concludes that the consumption of or contact with MON 95275 maize tissues by NTOs, including TES, is not expected to pose a hazard to any non-insect TES based on toxicity studies indicating an activity spectrum limited to species within the class Insecta. Additionally, indirect effects to TES are not expected given the limited acreage of the proposed EUP, the expected environmental exposures of the protein, and the lack of evidence for PIP bioaccumulation through the food chain. Therefore, due to the lack of direct effects to any non-insect TES or indirect effects to any TES, there is a reasonable expectation of no discernible effect for non-insect TES as a result of the Section 5 EUP for Mpp75Aa1.1 as expressed in MON 95275 maize.

Regarding the possibility of direct effects to insect TES, the EPA's analysis has determined that negligible to no exposure to MPP75Aa1.1 is expected for the 30 insect TES present in counties where experimental trials of MON 95275 maize may occur (Appendix A). Most of the insect TES are habitat specialists and do not use corn or corn fields as habitat; thus, exposure to such species is not anticipated to occur. Additionally, even where exposure to the insect TES may occur at the county-level, the EPA does not reasonably expect such exposure to result in discernible effects to the insect TES because these species do not consume corn or corn tissue, nor are they expected to consume corn pests as prey in significant quantities. Therefore, the EPA has determined that negligible to no exposure is expected for the 30 insect TES from the EUP of MON 95275 maize due to the habitat and/or dietary requirements of each species.

For Vpb4Da2 and DvSnf7.1, the EPA concludes that the consumption of or contact with MON 95275 maize tissues by NTOs, including TES, is not expected to pose a hazard to any non-coleopteran TES based on toxicity studies indicating an activity spectrum predominantly limited to a subset of coleopteran species. Additionally, indirect effects to TES are not expected because any measurable effects to non-TES are expected to be limited to the pest species in the treatment field, which is not a sole, or significant, source of feeding for TES that consume Coleoptera, nor are pollination impacts expected as the targeted coleopteran pest species is not a known pollinator. Therefore, due to the lack of direct effects to any non-coleopteran TES or indirect effects to any TES, there is a reasonable expectation of no discernible effect for non-coleopteran TES as a result of the Section 5 EUP for Vpb4Da2 and DvSnf7.1 as expressed in MON 95275 maize.

Regarding the possibility of direct effects to coleopteran TES, the EPA's analysis has determined that negligible to no exposure to Vpb4Da2 and DvSnf7.1 is expected for the two coleopteran TES present in counties where experimental trials of MON 95275 maize may occur (Appendix B). One coleopteran TES is a dietary specialist and does not use corn as a food source and the other coleopteran TES is a beach dwelling habitat specialist and therefore is not expected to be found in corn fields; thus, even where county-level exposure to the coleopteran TES may occur, the EPA does not reasonably expect such exposure to result in discernible effects. Therefore, the EPA has determined that negligible to no exposure is expected for the two coleopteran TES from the EUP of MON 95275 maize due to the dietary requirements of each species.

Based on this analysis, the EPA is making a "No Effect" determination under the Endangered Species Act (ESA) for all TES and their designated critical habitats resulting from the proposed uses of Mpp75Aa1.1, Vpb4Da2, and DvSnf7.1 in MON 95275 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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IX. Appendix A: Evaluation of Mpp75Aa1.1's Potential to Affect Insect Threatened and Endangered Species (TES)

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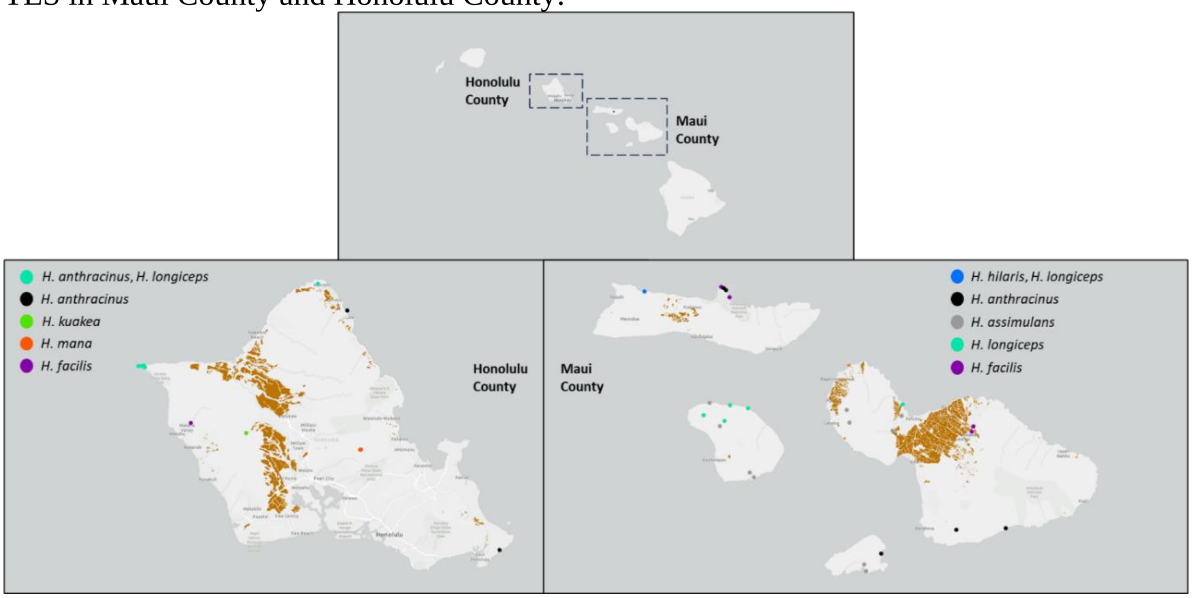
Insect TES with <1% (≤0.44%) range overlap with areas of agricultural production

1 – 7) Anthricinan yellow-faced bee (*Hylaeus anthracinus*), Assimulans yellow-faced bee (*H. assimulans*), Easy yellow-faced bee (*H. facilis*), Hawaiian yellow-faced bees (*H. kuakea*, *H. longiceps*, *H. mana*), Hilaris yellow-faced bee (*H. hilaris*) – 0% (Maui County, HI and Honolulu County, HI)

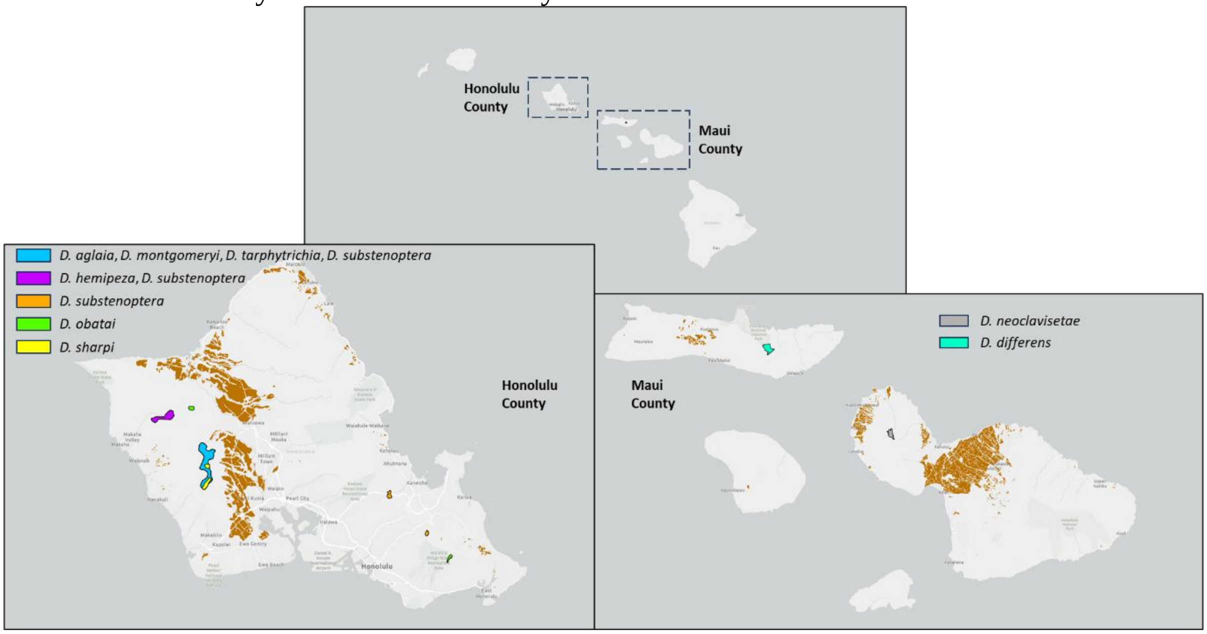
Figure 1. Agricultural production as it relates to the species ranges of the seven *Hylaeus* TES in Maui County and Honolulu County.



Current species ranges (indicated by the colored dots (USFWS, 2023a, b, c, d, e, f, g)) are overlayed with agricultural production (indicated by the burnt orange shaded areas (U.S. EPA, 2023a)), and there is no overlap of agricultural production and the ranges of the seven species.

8 – 16) Hawaiian picture-wing flies (*Drosophila aglaia*, *D. differens*, *D. hemipeza*, *D. montgomeryi*, *D. neoclavisetae*, *D. obatai*, *D. sharpi*, *D. substenoptera*, *D. tarphytrichia*) – 0% (Maui County, HI and Honolulu County, HI)

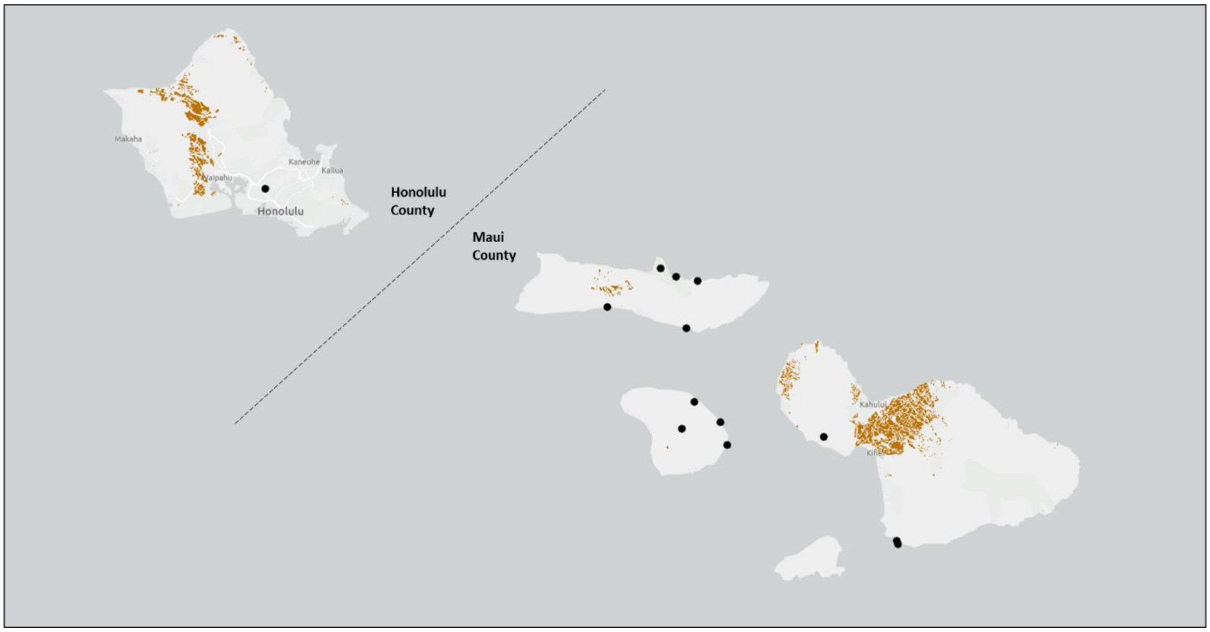
Figure 2. Agricultural production as it relates to the species ranges of the nine *Drosophila* TES in Maui County and Honolulu County.



Current species ranges (indicated by the colored polygons (USFWS, 2023h, i, j, k, l, m, n, o, p)) are overlayed with agricultural production (indicated by the burnt orange shaded areas (U.S. EPA, 2023a)), and there is no overlap of agricultural production and the ranges of the nine species.

17) Orangeblack Hawaiian damselfly (*Megalagrion xanthomelas*) – 0.128% (Maui County, HI)

Figure 3. Agricultural production as it relates to the species range of *Megalagrion xanthomelas* in Maui County and Honolulu County.



Current species range (indicated by the black dots (USFWS, 2023q)) is overlayed with agricultural production (indicated by the burnt orange shaded areas (U.S. EPA, 2023a)), and there is negligible overlap (0.128% in Maui County) of agricultural production and the range of the species.

Assessment

Less than 1% (≤0.44%) range overlap was identified for seven *Hylaeus* TES (Figure 1), nine *Drosophila* TES (Figure 2), *Megalagrion nesiotes* (Figure 3) and *M. xanthomelas* (Figure 4). Per the EPA's guidance on interpretation of UDL overlaps, ≤0.44% is treated as 0% and generally supports a No Effect determination, because this is within the error bounds of spatial datasets when considering accuracy and precision (U.S. EPA, 2023c). Therefore, EPA is making a "No Effect" determination under the ESA for the above 17 species and their designated critical habitats resulting from the proposed use of Mpp75Aa1.1 in MON 95275 maize.

Insect TES with ≥1% (>0.44%) overlap with areas of corn crop production or agricultural production

In order to characterize the potential exposure of these species to MON 95275 maize, critical habitat and updated species range information were evaluated based on information taken directly from or slightly modified from the USFWS ECOS and Federal Register Notices, unless otherwise cited.

18) American burying beetle (*Nicrophorus americanus*) – 0.516% (Dawson County, NE); 0.407% (Antelope County, NE)

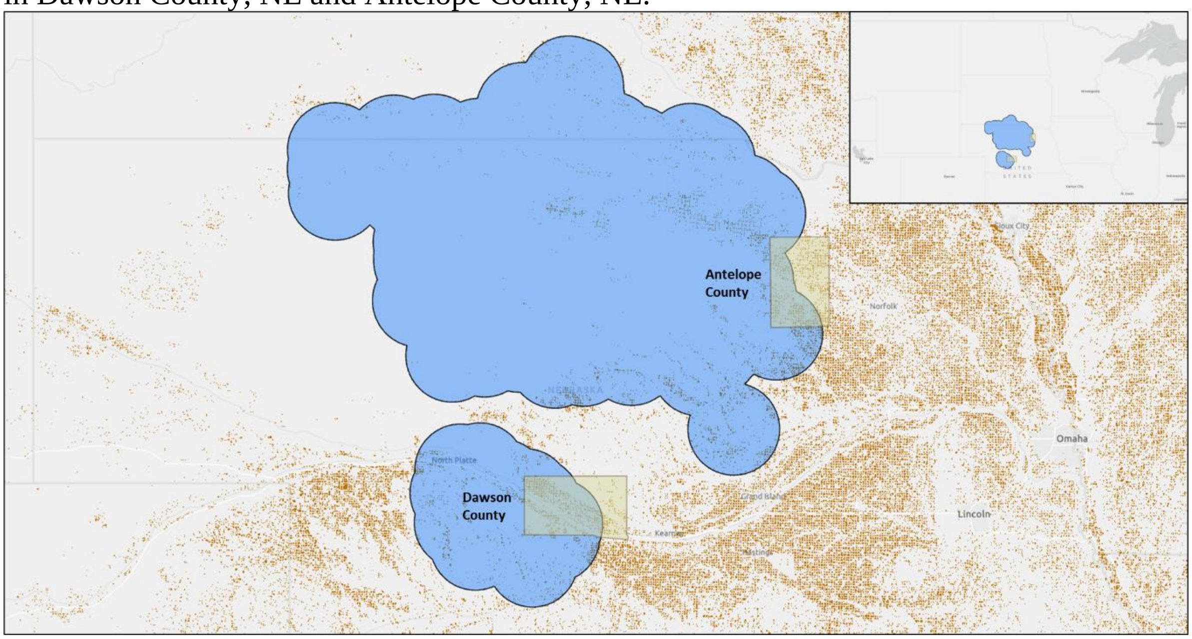
Biology and Habitat Requirements

The following excerpts are from the Federal Register (2020); consult original reference for citations:

The American burying beetle is native to at least 35 States in the United States, covering most of temperate eastern North America, and the southern borders of three eastern Canadian provinces. The species is believed to be extirpated from all but nine States in the United States and is likely extirpated from Canada. However, the current range is much larger than originally thought when the species was listed in 1989.

Adults and larvae depend on dead animals (carrion), e.g., cotton rats, pheasants, prairie dogs, ground squirrels, etc., for food and moisture. Adults also require adequate soil moisture, appropriate soil temperatures, and appropriate soil particle size to allow them to bury themselves and/or a carcass (see chapter 2 of the SSA Report; Service 2019). Adequate soil moisture levels appear to be critical for American burying beetles, and they show a strong preference for moist, sandy loam soil with organic matter (Hoback 2008, unpublished), but a specific threshold for soil moisture is unknown.

Figure 4. Corn crop production as it relates to the species range of *Nicrophorus americanus* in Dawson County, NE and Antelope County, NE.



Current species range (indicated by the blue polygons (USFWS, 2023r)) is overlayed with corn crop planting (indicated by the burnt orange areas (U.S. EPA, 2023b)) and the county boundaries (indicated by the gold polygons), and there is overlap (0.516% and 0.407% in Dawson County, NE and Antelope County, NE, respectively) of corn crop planting and the range of the species.

Assessment

While there is overlap of the range of *N. americanus* and corn crop planting, the beetle is unlikely to be found in agricultural settings given its soil preferences. Additionally, as a carrion specialist, any exposure to Mpp75Aa1.1 would be limited to exposure via dead vertebrate species that consumed MON 95275 maize tissue while still alive and previous studies have shown PIP concentrations to become increasingly diluted as they move through the food chain (Li et al., 2017; Meissle & Romeis, 2018; Tian et al., 2014). Furthermore, expression of Mpp75Aa1.1 in MON 95275 maize in parts of the plant most likely to be consumed by vertebrate species is low (e.g., grain, leaf; see Table 1) and the nature of carrion (i.e., decaying/rotten flesh) means that Mpp75Aa1.1 would likely be degraded beyond biological relevancy. Given these habitat and dietary needs, exposure of Mpp75Aa1.1 to this beetle is expected to be negligible. Therefore, the EPA is making a "No Effect" determination under the ESA for *N. americanus* and its designated critical habitat resulting from the proposed use of Mpp75Aa1.1 in MON 95275 maize.

19) Dakota skipper (*Hesperia dacotae*) – 0.865% (Richland County, ND); 0.279% (Brookings County, SD); 0.093% (Moody County, SD); 0.044% (Swift County, MN); 0.043% (Lincoln County, MN)

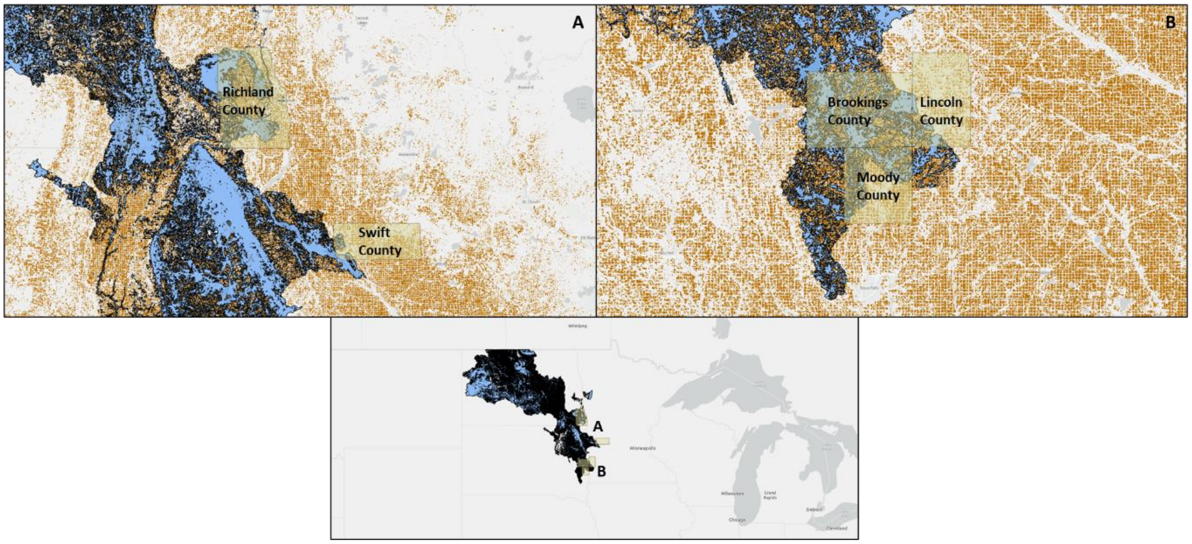
Biology and Habitat Requirements

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

Dakota skippers are obligate residents of undisturbed (remnant, untilled) high-quality prairie, ranging from wet-mesic tallgrass prairie to dry-mesic mixed-grass prairie (Royer and Marrone 1992a, pp. 8, 21). High-quality prairie contains a high diversity of native plant species, including flowering herbaceous plants (forbs).

Nectar and water sources for adult Dakota skippers vary regionally and include purple coneflower (*Echinacea angustifolia*), bluebell bellflower (*Campanula rotundifolia*), white prairie clover (*Dalea candida*), upright prairie coneflower (*Ratibida columnifera*), fleabanes (*Erigeron* spp.), blanketflowers (*Gaillardia* spp.), black-eyed Susan (*Rudbeckia hirta*), groundplum milkvetch (*Astragalus crassicarpus*), and yellow sundrops (*Calylophus serrulatus*) (McCabe and Post 1977b, p. 36; Royer and Marrone 1992a, p. 21). Dakota skipper larvae feed only on several native grass species; little bluestem (*Schizachyrium scoparium*) is a frequent food source of the larvae (Dana 1991, p. 17; Royer and Marrone 1992a, p. 25), although they have been found on *Panicum* spp., *Poa* spp., and other native grasses (Royer and Marrone 1992a, p. 25)

Figure 5. Corn crop production as it relates to the species range of *Hesperia dacotae* in Richland County, ND, Brookings County, SD, Moody County, SD, Swift County, MN, and Lincoln County, MN.



Current species range (indicated by the blue polygons (USFWS, 2023s)) is overlayed with corn crop planting (indicated by the burnt orange shaded areas (U.S. EPA, 2023b)) and the county boundaries (indicated by the gold polygons), and there is overlap (0.865%, 0.279%, 0.093%, 0.044%, and 0.043% in Richland County, ND, Brookings County, SD, Moody County, SD, Swift County, MN, and Lincoln County, MN, respectively) of corn crop planting and the range of the species.

Assessment

While there is overlap of the range of *H. dacotae* and corn crop planting, this butterfly is restricted to undisturbed high-quality prairie habitat and not known to utilize agricultural lands as habitat. Thus, any exposure to Mpp75Aa1.1 would be limited to exposure via pollen drift from MON 95275 maize. Given that the expression level of Mpp75Aa1.1 in MON 95275 maize pollen was undetectable, exposure of Mpp75Aa1.1 to this butterfly is expected to be negligible. Therefore, the EPA is making a “No Effect” determination under the ESA for *H. dacotae* and its designated critical habitat resulting from the proposed use of Mpp75Aa1.1 in MON 95275 maize.

20) Hine’s emerald dragonfly (*Somatochlora hineana*) – 0.803% (Kane County, IL); 0.290% (Rock County, WI)

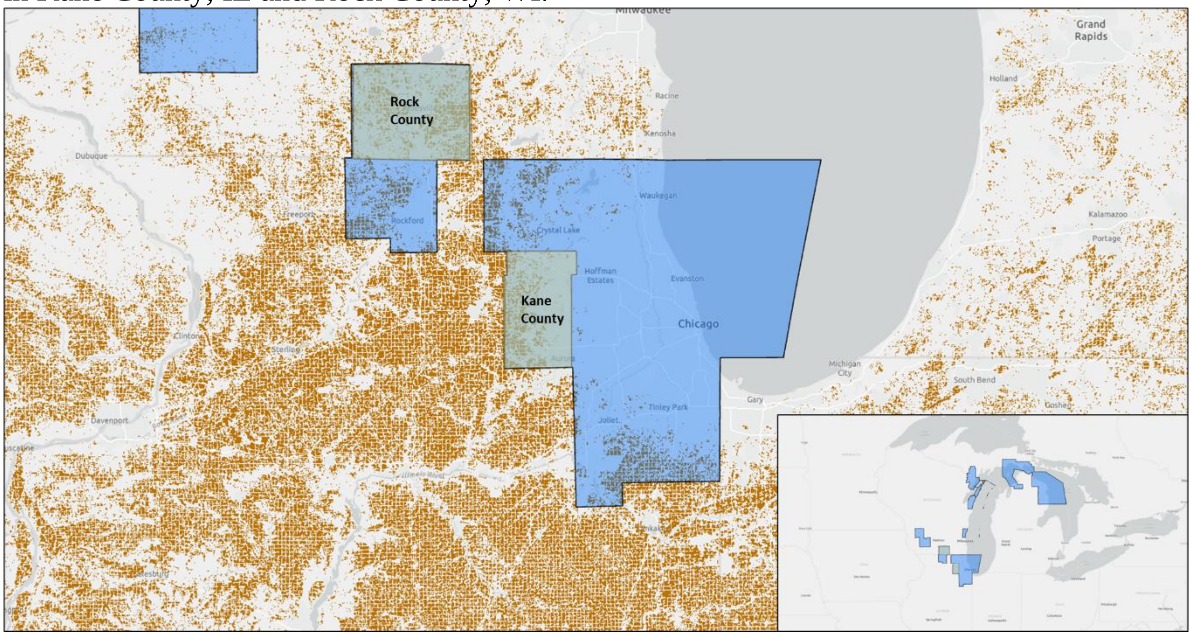
Biology and Habitat Requirements

Somatochlora hineana is a member of Odonata (i.e., dragonflies and damselflies) and is thus an obligate predator (predominantly within/near aquatic environments) in both its larval and adult forms (May, 2019).

The following excerpts regarding the habitat requirements of *S. hineana* are from the Federal Register (1995); consult original reference for citations:

Cashatt and Vogt (1990) indicated that the Illinois habitat of the Hine’s emerald dragonfly consists of complex wetlands with small, calcareous or underlying limestone bedrock, and shallow, spring-fed streams that drain into wet meadows and cattail marshes. These marshes are found primarily along the Des Plaines River drainage in Illinois. Wisconsin habitat consists of small, calcareous, marshy streams and associated cattail marshes on dolomite bedrock.

Figure 6. Corn crop production as it relates to the species range of *Somatochlora hineana* in Kane County, IL and Rock County, WI.



Current species range (indicated by the blue polygons (USFWS, 2023t)) is overlayed with corn crop planting (indicated by the burnt orange shaded areas (U.S. EPA, 2023b)) and the county boundaries (indicated by the gold polygons), and there is overlap (0.803% and 0.290% in Kane County, IL and Rock County, WI, respectively) of corn crop planting and the range of the species.

Assessment

While there is overlap of the range of *S. hineana* and corn crop planting, this dragonfly has specific habitat requirements and dietary habitats that render exposure to Mpp75Aa1.1 negligible. First, like most odonates, larvae of this species are strictly aquatic, therefore exposure to its larval stage is expected to be negligible (see **Section VI** above). Additionally, larvae require specific habitat conditions (calcareous fens containing crayfish burrows) to complete their maturation (Cashatt & Vogt, 2001; Pintor & Soluk, 2006). Adults therefore maintain a close relationship with their larval habitat and forage nearby (Foster & Soluk, 2006), thus their exposure to agriculture is expected to be limited. Also, as an obligate predator, any exposure to Mpp75Aa1.1 would be limited to exposure via prey species that have consumed MON 95275 maize tissue. Given the expectation that their exposure in agriculture is limited and that dragonflies are opportunistic feeders (i.e., generalists that may consume a variety of prey species) (Corbet, 1999), there is no reasonable expectation of the dragonflies consuming corn pest prey species in significant quantities. Furthermore, previous studies have shown PIP concentrations to become increasingly diluted as they move through the food chain (Li et al., 2017; Meissle & Romeis, 2018; Tian et al., 2014), resulting in the reasonable expectation that potential incidental exposure to Mpp75Aa1.1 via the consumption of corn pest prey species would not result in discernible effects. Therefore, the EPA is making a "No Effect" determination under the ESA for *S. hineana* and its designated critical habitat resulting from the proposed use of Mpp75Aa1.1 in MON 95275 maize.

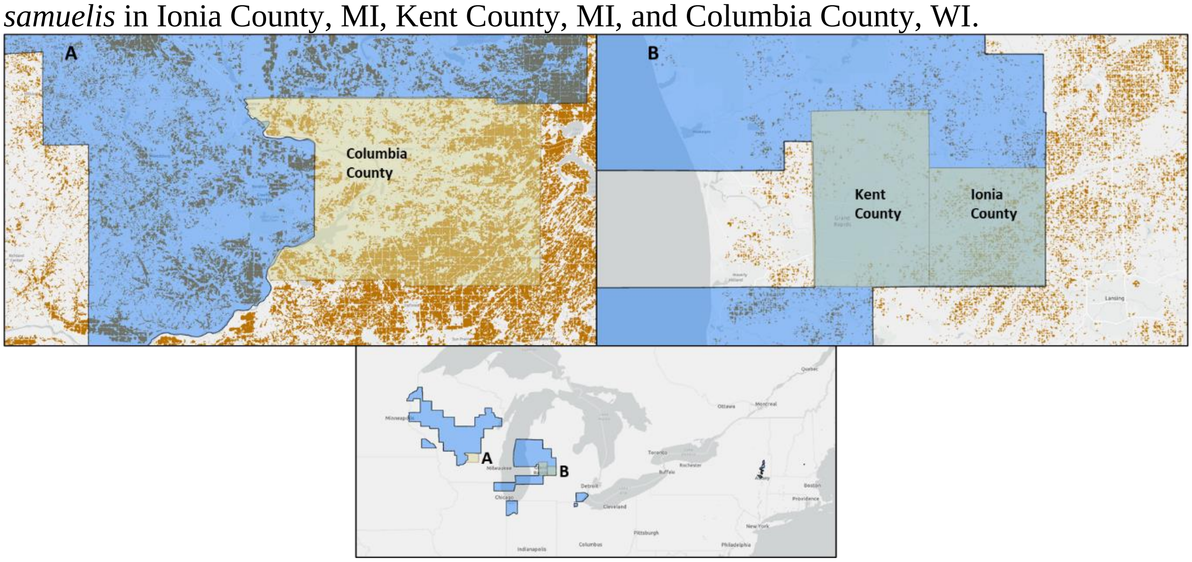
21) Karner blue butterfly (*Lycaeides melissa samuelis*) – 1.126% (Ionia County, MI); 0.594% (Kent County, MI); 0.001% (Columbia County, WI)

Biology and Habitat Requirements

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

The habitat of the Karner blue butterfly is characterized by the presence of wild 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 sand plain unities, 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/sand plain communities.

Figure 7. Corn crop production as it relates to the species range of *Lycaeides melissa samuelis* in Ionia County, MI, Kent County, MI, and Columbia County, WI.



Current species range (indicated by the blue polygons (USFWS, 2023u)) is overlayed with corn crop planting (indicated by the burnt orange shaded areas (U.S. EPA, 2023b)) and the county boundaries (indicated by the gold polygons), and there is overlap (1.126%, 0.594%, and 0.001% in Ionia County, MI, Kent County, MI, and Columbia County, WI, respectively) of corn crop planting and the range of the species.

Assessment

While there is overlap of the range of *L. melissa samuelis* and corn crop planting, larvae of this butterfly are specialists of wild lupine and not known to utilize maize as a food source. Additionally, like most other adult Lepidoptera, adult Karner blue butterflies solely consume nectar (which, being wind-pollinated, corn does not produce). Thus, any exposure to Mpp75Aa1.1 would be limited to exposure via pollen drift from MON 95275 maize.

Given that the expression level of Mpp75Aa1.1 in MON 95275 maize pollen was undetectable, exposure of Mpp75Aa1.1 to this butterfly is expected to be negligible. Therefore, the EPA is making a "No Effect" determination under the ESA for *L. melissa samuelis* and its designated critical habitat resulting from the proposed use of Mpp75Aa1.1 in MON 95275 maize.

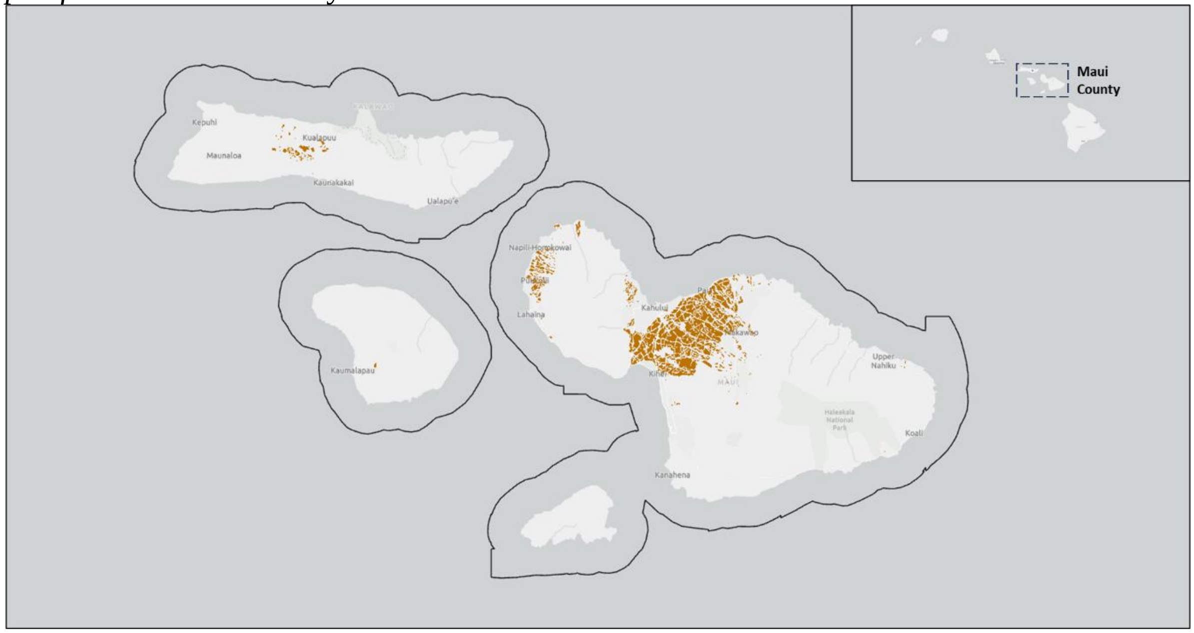
22) Pacific Hawaiian damselfly (*Megalagrion pacificum*) – 1.391% (Maui County, HI)

Biology and Habitat Requirements
Megalagrion pacificum is a member of Odonata (i.e., dragonflies and damselflies) and is thus an obligate predator (predominantly within/near aquatic environments) in both its larval and adult forms (May, 2019).

The following excerpts regarding the habitat requirements of *M. pacificum* are from the Federal Register (2010); consult original reference for citations:

The Pacific Hawaiian damselfly was historically found on all of the main Hawaiian Islands except Kahoolawe and Niihau. Currently, the Pacific Hawaiian damselfly is known only from the islands of Hawaii, Maui and Molokai. This species was known to breed primarily in lentic (standing water) systems such as marshes, seepage-fed pools, large ponds at higher elevations, and small, quiet pools in gulches that have been cut off from the main stream channel (Moore and Gagne 1982, p. 4; Polhemus and Asquith 1996, p. 83). The Pacific Hawaiian damselfly is currently found in at least seven streams on Molokai and may possibly be extant in other unsurveyed streams on Molokai's northern coast that have not been invaded by nonnative fish (Englund 2008). On the island of Maui, the species is currently known from 14 streams.

Figure 8. Agricultural production as it relates to the species range of *Megalagrion pacificum* in Maui County.



Current species range (area within the black outlines (USFWS, 2023v)) is overlayed with agricultural production (indicated by the burnt orange shaded areas (U.S. EPA, 2023a)), and there is overlap (1.391%) of agricultural production and the range of the species.

Assessment

While there is overlap of the range of *M. pacificum* and agricultural production, this damselfly has specific habitat requirements and dietary habitats that render exposure to Mpp75Aa1.1 negligible. First, like most odonates, larvae of this species are strictly aquatic, therefore exposure to its larval stage is expected to be negligible (see **Section VI** above). Also, the species is now restricted to a fraction of its historical habitat, with both larvae and adults only found in and near seepage-fed pools surrounded by thick vegetation (Federal Register, 2010), thus their exposure to agriculture is expected to be limited. Additionally, as an obligate predator, any exposure to Mpp75Aa1.1 would be limited to exposure via prey species that have consumed MON 95275 maize tissue. Given the expectation that their exposure in agriculture is limited and that damselflies are opportunistic feeders (i.e., generalists that may consume a variety of prey species) (e.g., Kaunisto et al., 2020), there is no reasonable expectation of the damselflies consuming corn pest prey species in significant quantities. Furthermore, previous studies have shown PIP concentrations to become increasingly diluted as they move through the food chain (Li et al., 2017; Meissle & Romeis, 2018; Tian et al., 2014), resulting in the reasonable expectation that potential incidental exposure to Mpp75Aa1.1 via the consumption of corn pest prey species would not result in discernible effects. Therefore, the EPA is making a "No Effect" determination under the ESA for *M. pacificum* and its designated critical habitat resulting from the proposed use of Mpp75Aa1.1 in MON 95275 maize.

23) Mitchell's satyr butterfly (*Neonympha mitchellii mitchellii*) – 1.863% (Van Buren County, MI)

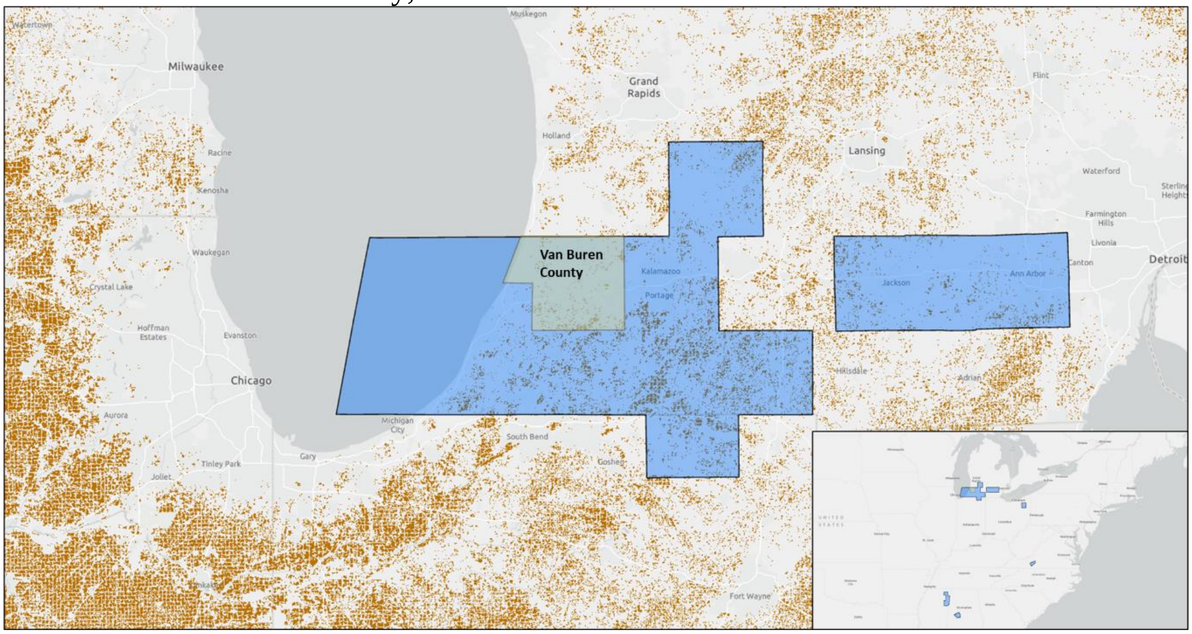
Biology and Habitat Requirements

The following excerpts are from the USFWS (1998); consult original reference for citations:

Although isolated populations of this species are known from northern New Jersey, northeastern Ohio, and perhaps Maryland, the majority of population sites are clustered in southern Michigan and adjacent northern Indiana. Known habitats are all peatlands but range along a continuum from prairie/bog fen to sedge meadow/swamp. All historical and active habitats have an herbaceous community which is dominated by sedges, usually *Carex stricta*, with scattered deciduous and/or coniferous trees, most often *L. laricina* or *Juniperus virginiana* (red cedar).

Despite a few historical studies, the biology of Mitchell's satyr is poorly documented. Although Mitchell's satyr has not been observed ovipositing in nature, its hostplants are almost certainly sedges, and *C. stricta* is probably the primary hostplant… in the field, adult Mitchell's satyr are almost always found in close association with dense stands of *C. stnicta*.

Figure 9. Corn crop production as it relates to the species range of *Neonympha mitchellii mitchellii* in Van Buren County, MI.



Current species range (indicated by the blue polygons (USFWS, 2023w)) is overlayed with corn crop planting (indicated by the burnt orange shaded areas (U.S. EPA, 2023b)) and the county boundary (indicated by the gold polygon), and there is overlap (1.863%) of corn crop planting and the range of the species.

Assessment

While there is overlap of the range of *N. mitchellii mitchellii* and corn crop planting, this butterfly is restricted to sedge-dominated fen habitats (where its primary host plant is abundant) and not known to utilize agricultural lands as habitat. Thus, any exposure to Mpp75Aa1.1 would be limited to exposure via pollen drift from MON 95275 maize. Given that the expression level of Mpp75Aa1.1 in MON 95275 maize pollen was undetectable, exposure of Mpp75Aa1.1 to this butterfly is expected to be negligible. Therefore, the EPA is making a "No Effect" determination under the ESA for *N. mitchellii mitchellii* and its designated critical habitat resulting from the proposed use of Mpp75Aa1.1 in MON 95275 maize.

24) Puritan tiger beetle (*Ellipsoptera puritana*) – 2.139% (Kent County, MD)

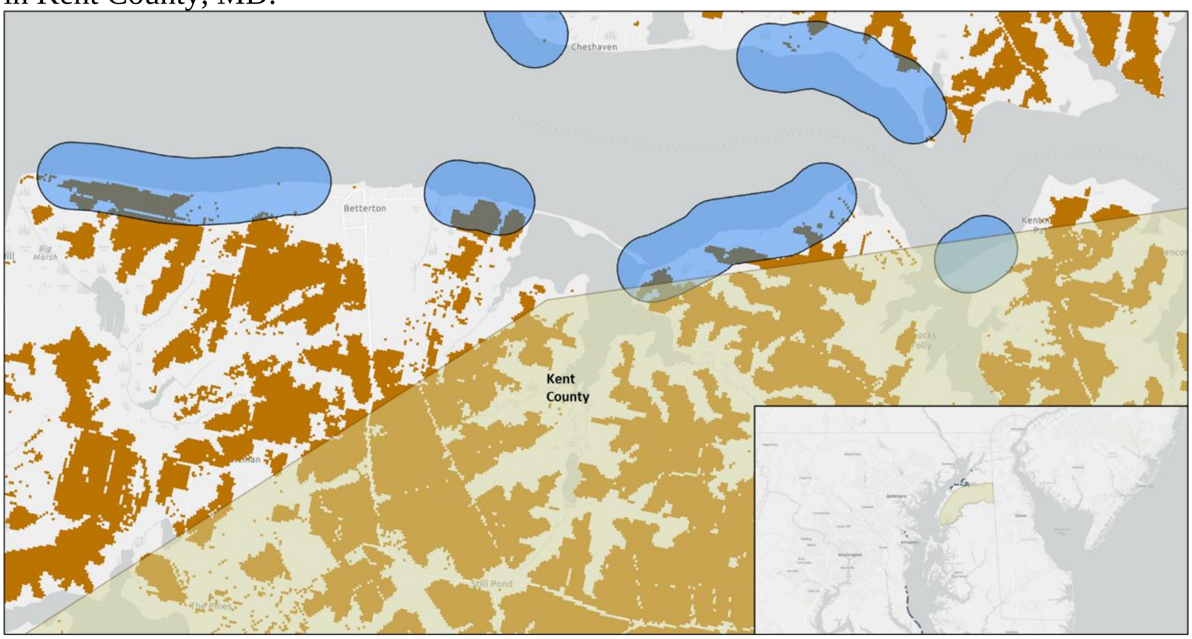
Biology and Habitat Requirements

The following excerpt is from the Federal Register (1990); consult original reference for citations:

Tiger beetles (genus; *Cicindela* [this species was previously known as *Cicindela puritana*]) are day-active, predatory insects that capture small arthropods in a "tigerlike" manner, grasping prey with their mandibles (mouthparts). Tiger beetle larvae, which live in burrows in the ground, are also voracious predators, fastening themselves near the tops of the burrows by means of abdominal hooks and rapidly extending from their burrows to seize passing invertebrate prey.

The Puritan tiger beetle was known historically from numerous sites along the Connecticut River in Vermont, New Hampshire, Massachusetts and Connecticut, and from along the Chesapeake Bay in Maryland; it is now restricted to Maryland and two Connecticut River sites, one in Massachusetts and one in Connecticut. Within the Chesapeake Bay, its habitat is characterized by the presence of narrow sandy beaches with adjacent, well-developed bluffs of sand and clay (Glaser 1984, Knisley 1987, Knisley and Hill, 1990). Habitat of the Connecticut River population in Massachusetts is similar, with steep, clay banks adjacent to a wider (10 meters or greater) sandy beach (Nothnagel 1987).

Figure 10. Corn crop production as it relates to the species range of *Ellipsoptera puritana* in Kent County, MD.



Current species range (indicated by the blue polygons (USFWS, 2023x)) is overlayed with corn crop planting (indicated by the burnt orange shaded areas (U.S. EPA, 2023b)) and the county boundary (indicated by the gold shaded area), and there is overlap (2.139%) of corn crop planting and the range of the species.

Assessment

While there is overlap of the range of *E. puritana* and corn crop planting, the beetle's sole habitat consists of sandy beaches and bluffs, which are not conducive to corn crop production. Furthermore, given that the Puritan tiger beetle is predatory, any possibility of exposure to Mpp75Aa1.1 would be limited to exposure via prey species that have consumed MON 95275 maize tissue. Given the lack of presence of the Puritan tiger beetle within agricultural environments and that previous studies have shown PIP concentrations to become increasingly diluted as they move through the food chain (Li et al., 2017; Meissle & Romeis, 2018; Tian et al., 2014), there is a reasonable expectation of no exposure to Mpp75Aa1.1. Therefore, the EPA is making a "No Effect" determination under the ESA for *E. puritana* and its designated critical habitat resulting from the proposed use of Mpp75Aa1.1 in MON 95275 maize.

25) Blackburn's sphinx moth (*Manduca blackburni*) – 4.215% (Maui County, HI)

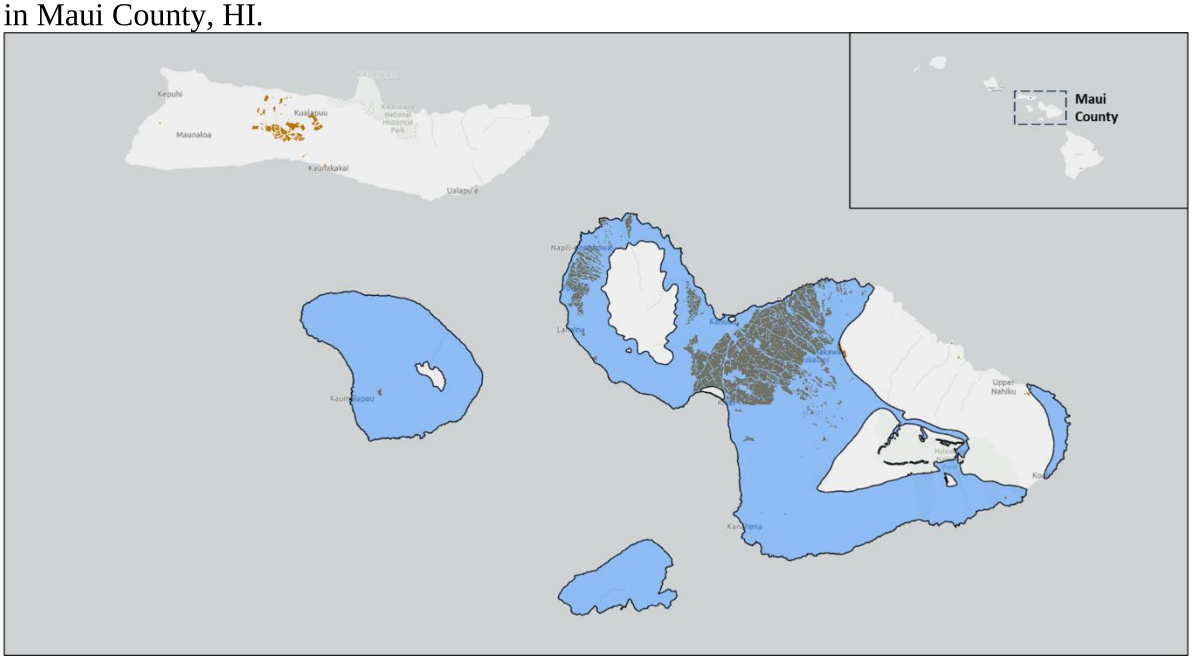
Biology and Habitat Requirements

The following excerpt is from the Federal Register (2000); consult original reference for citations:

Historically, this species occurred on the Hawaiian islands of Kauai, Oahu, Molokai, Maui, and Hawaii, but until recently, was known only from one population on Maui. Researchers observed a second population on Maui in 1992, and populations are now known to also occur on the islands of Kahoolawe and Hawaii.

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).

Figure 11. Agricultural production as it relates to the species range of *Manduca blackburni* in Maui County, HI.



Current species range (indicated by the blue polygons (USFWS, 2023y)) is overlayed with corn crop planting (indicated by the burnt orange shaded areas (U.S. EPA, 2023a)), and there is overlap (4.215%) of agriculture production and the range of the species.

Assessment

While there is overlap of the range of *M. blackburni* and agricultural production, larvae of this moth are specialists of Solanaceae (which does not include maize) and not known to utilize maize as a food source. Additionally, like most other adult Lepidoptera, adult Blackburn's sphinx moths solely consume nectar (which, being wind-pollinated, corn does not produce). Thus, any exposure to Mpp75Aa1.1 would be limited to exposure via pollen drift from MON 95275 maize. Given that the expression level of Mpp75Aa1.1 in MON 95275 maize pollen was undetectable, exposure of Mpp75Aa1.1 to this moth is expected to be negligible. Therefore, the EPA is making a "No Effect" determination under the ESA for *M. blackburni* and its designated critical habitat resulting from the proposed use of Mpp75Aa1.1 in MON 95275 maize.

26) Oceanic Hawaiian damselfly (*Megalagrion oceanicum*) – 6.516% (Honolulu County, HI)

27) Blackline Hawaiian damselfly (*Megalagrion nigrohamatum nigrolineatum*) – 6.599% (Honolulu County, HI)

28) Crimson Hawaiian damselfly (*Megalagrion leptodemas*) – 6.599% (Honolulu County, HI)

Biology and Habitat Requirements

Megalagrion oceanicum, *M. nigrohamatum nigrolineatum*, and *M. leptodemas* are members of Odonata (i.e., dragonflies and damselflies) and are thus obligate predators (predominantly within/near aquatic environments) in both their larval and adult forms (May, 2019).

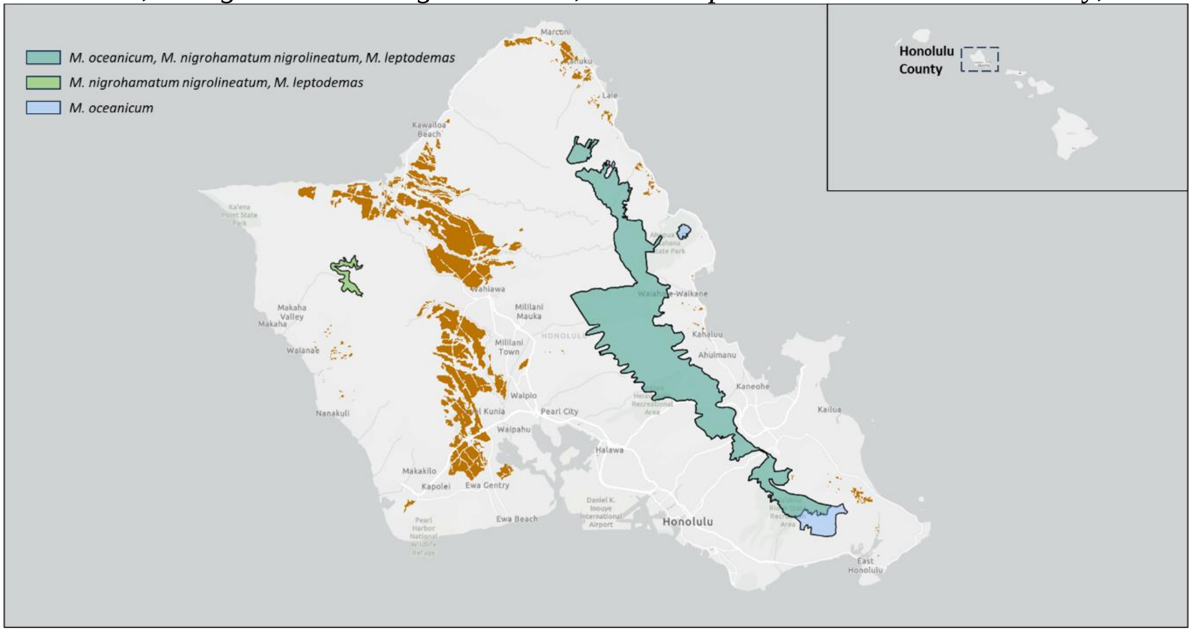
The following excerpts regarding the habitat requirements of *M. oceanicum*, *M. nigrohamatum nigrolineatum*, and *M. leptodem* are from the Federal Register (2012); consult original reference for citations:

The oceanic Hawaiian damselfly is found in swiftly flowing sections of streams, usually amid rocks and gravel in stream riffles (stream sections with sufficient gradient to create small standing waves) and small cascades on waterfalls (Williams 1936, pp. 321–322; Polhemus and Asquith 1996, p. 106). The species now currently occupies 12 sites above 300 ft (100 m) in elevation on the windward side of the Koolau Mountains at Kahawainui, Wailele, Koloa, Kaipapau, Maakua, upper Kaluanui, Kawaiiki, Opaeula, upper Helemano, Makaua, Waihee, and Kahaluu, in the lowland mesic, lowland wet, and wet cliff ecosystems (TNC 2007; Polhemus 2007, pp. 237–239; HBMP 2008; Preston 2011, in litt.).

The blackline Hawaiian damselfly…occurs in the slow sections or pools along mid-reach and headwater sections of perennial upland streams and in seep-fed pools along overflow channels bordering such streams. Currently, this species is found in the lowland wet ecosystem on the windward and leeward sides of the Koolau Mountains, in the headwaters and upper reaches of 17 streams: Koloa, Kaipapau, Maakua, upper Kaluanui, Palaa, Helemano headwaters, Poamoho, Kahana, Waiahole, Waiawa, Kaalaea, Waihee, Kahaluu, north Halawa, Heeia, Kalihi, and Maunawili (TNC 2007; Polhemus 2008a, in litt.; Wolff 2008, in litt.; HBMP 2008; Preston 2011, in litt.)

The crimson Hawaiian damselfly breeds in the slow reaches of streams and seep-fed pools (Williams 1936, p. 306; Zimmerman 1948a, p. 369; Polhemus 1994a, p. 7; Polhemus 1994b, p. 37). Currently, only three occurrences of the crimson Hawaiian damselfly are known, all from the Koolau Mountains in the lowland wet and wet cliff ecosystems at Moanalua, north Halawa, and Maakua (TNC 2007; Polhemus 2008a, in litt.; HBMP 2008; Preston 2011, in litt.).

Figure 12. Agricultural production as it relates to the species range of *Megalagrion oceanicum*, *M. nigrohamatum nigrolineatum*, and *M. leptodemas* in Honolulu County, HI.



Current species ranges (indicated by the blue and green polygons (USFWS, 2023z, aa, ab)) are overlayed with agricultural production (indicated by the burnt orange shaded areas (U.S. EPA, 2023a)), and there is overlap (6.516% for *M. oceanicum* and 6.599% for *M. nigrohamatum nigrolineatum* and *M. leptodemas*) of agricultural production and the range of the three species.

Assessment

While there is overlap of the range of the three damselflies and agricultural production, these damselflies have specific habitat requirements and dietary habitats that render exposure to Mpp75Aa1.1 negligible. First, like most odonates, larvae of these three species are strictly aquatic, therefore exposure to their larval stages is expected to be negligible (see **Section VI** above). Also, all three species are now restricted to portions of mountainous streams above geologic or manmade barriers (Federal Register, 2012), thus their exposure to agriculture is expected to be limited. Additionally, as obligate predators, any exposure to Mpp75Aa1.1 would be limited to exposure via prey species that have consumed MON 95275 maize tissue. Given the expectation that their exposure in agriculture is limited and that damselflies are opportunistic feeders (i.e., generalists that may consume a variety of prey species) (e.g., Kaunisto et al., 2020), there is no reasonable expectation of the damselflies consuming corn pest prey species in significant quantities.

Furthermore, previous studies have shown PIP concentrations to become increasingly diluted as they move through the food chain (Li et al., 2017; Meissle & Romeis, 2018; Tian et al., 2014), resulting in the reasonable expectation that potential incidental exposure to Mpp75Aa1.1 via the consumption of corn pest prey species would not result in discernible effects. Therefore, the EPA is making a "No Effect" determination under the ESA for *M. oceanicum*, *M. nigrohamatum nigrolineatum*, and *M. leptodemas* and their designated critical habitats resulting from the proposed use of Mpp75Aa1.1 in MON 95275 maize.

29) Flying earwig Hawaiian damselfly (*Megalagrion nesiotes*) – 7.603% (Maui County, HI)

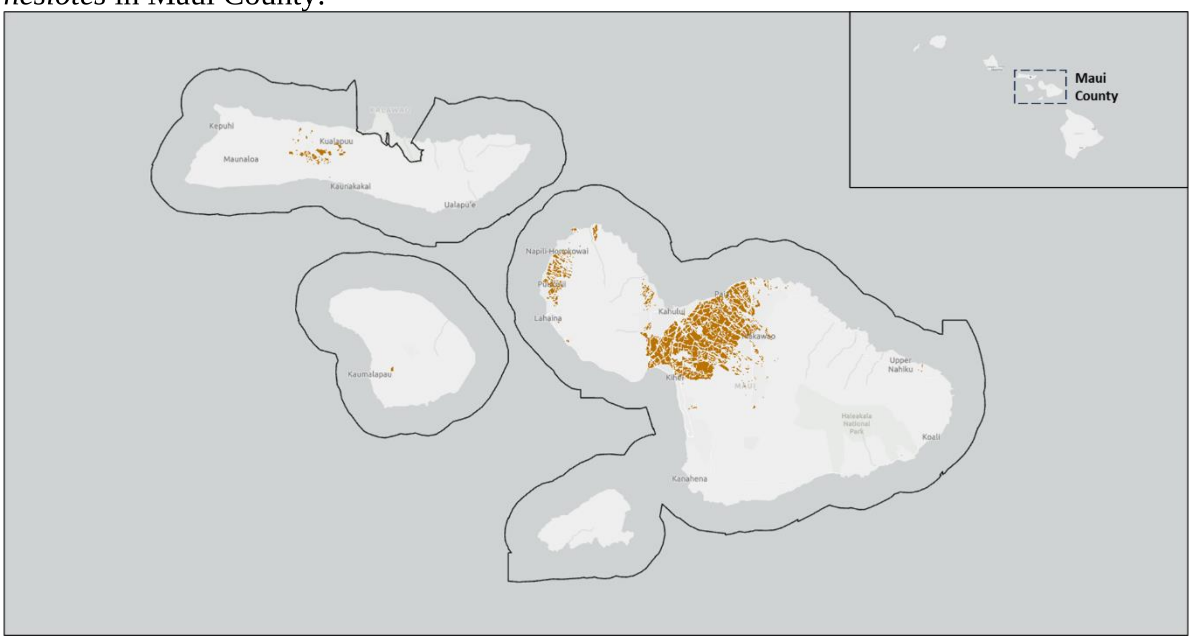
Biology and Habitat Requirements

Megalagrion nesiotes is a member of Odonata (i.e., dragonflies and damselflies) and is thus an obligate predator (predominantly within/near aquatic environments) in both its larval and adult forms (May, 2019).

The following excerpts regarding the habitat requirements of *M. nesiotes* are from the Federal Register (2010); consult original reference for citations:

Historically, the flying earwig Hawaiian damselfly was known from the islands of Hawaii and Maui. Since the 1930s, however, the flying earwig Hawaiian damselfly has only been observed in a single area along a particular stream on the windward side of east Maui, despite surveys from 1993 through 2008 at several of its historically occupied sites. The only confirmed population found in the last 6 years occurs along a single East Maui stream and the adjacent steep, moist, riparian talus slope (a slope formed by an accumulation of rock debris), which is densely covered with *Dicranopteris linearis* (uluhe), a native fern.

Figure 13. Agricultural production as it relates to the species range of *Megalagrion nesiotes* in Maui County.



Current species ranges (indicated by the black outlines (USFWS, 2023ac)) are overlayed with agricultural production (indicated by the burnt orange shaded areas (U.S. EPA, 2023a)), and there is overlap (7.603%) of agricultural production and the range of the three species.

Assessment

While there is overlap of the range of *M. nesiotes* and agricultural production, this damselfly has specific habitat requirements and dietary habitats that render exposure to Mpp75Aa1.1 negligible. Adults and larvae of the species are now only found alongside a single East Maui stream within a rocky riparian habitat (Federal Register, 2010), thus their exposure to agriculture is expected to be limited. Additionally, as an obligate predator, any exposure to Mpp75Aa1.1 would be limited to exposure via prey species that have consumed MON 95275 maize tissue. Given the expectation that their exposure in agriculture is limited and that damselflies are opportunistic feeders (i.e., generalists that may consume a variety of prey species) (e.g., Kaunisto et al., 2020), there is no reasonable expectation of the damselflies consuming corn pest prey species in significant quantities. Furthermore, previous studies have shown PIP concentrations to become increasingly diluted as they move through the food chain (Li et al., 2017; Meissle & Romeis, 2018; Tian et al., 2014), resulting in the reasonable expectation that potential incidental exposure to Mpp75Aa1.1 via the consumption of corn pest prey species would not result in discernible effects. Therefore, the EPA is making a "No Effect" determination under the ESA for *M. nesiotes* and its designated critical habitat resulting from the proposed use of Mpp75Aa1.1 in MON 95275 maize.

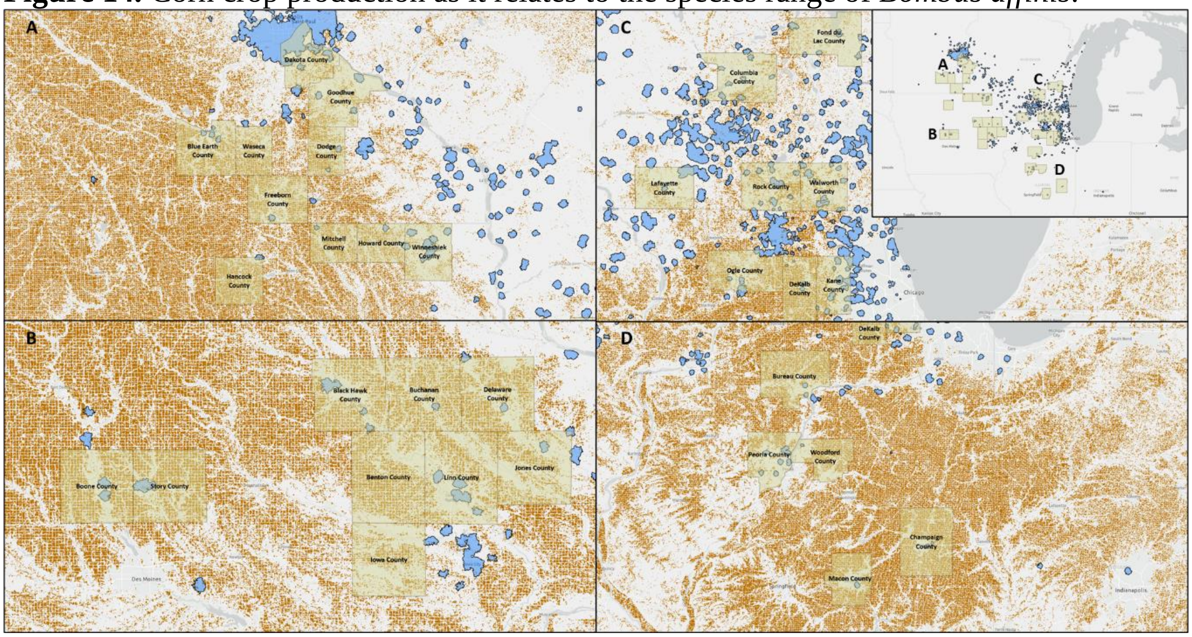
30) **Rusty patched bumble bee (*Bombus affinis*) – 0% (Benton County, IA); 0.068% (Black Hawk County, IA); 0.050% (Blue Earth County, MN); 0.077% (Boone County, IA); 0.040% (Buchanan County, IA); 0.033% (Bureau County, IL); 0.001% (Champaign County, IL); 0.321% (Columbia County, WI); 0.368% (Dakota County, MN); 0.076% (Dekalb County, IL); 0.049% (Delaware County, IA); 0% (Dodge County, MN); 0.160% (Fond du Lac County, WI); 0.080% (Freeborn County, MN); 0.175% (Goodhue County, MN); 0.033% (Hancock County, IA); 0.099% (Howard County, IA); 0.002% (Iowa County, IA); 0.037% (Jones County, IA); 0.250% (Kane County, IL); 0.191% (Lafayette County, WI); 0.065% (Linn County, IA); 0.007% (Macon County, IL); 0.047% (Mitchell County, IA); 0.141% (Ogle County, IL); 0.097% (Peoria County, IL); 0.278% (Rock County, WI); 0.066% (Story County, IA); 0.191% (Walworth County, WI); 0.001% (Waseca County, MN); 0.530% (Winneshiek County, IA); 0.015% (Woodford County, IL)**

Biology and Habitat Requirements

The following excerpt is from the Federal Register (2017); consult original reference for citations:

Since 2000, the rusty patched bumble bee has been reported from 14 States/Provinces: Illinois, Indiana, Iowa, Maine, Maryland, Massachusetts, Minnesota, North Carolina, Ontario, Ohio, Pennsylvania, Tennessee, Virginia, and Wisconsin. The rusty patched bumble bee has been observed and collected in a variety of habitats, including prairies, woodlands, marshes, agricultural landscapes, and residential parks and gardens (Colla and Packer 2008, p. 1381; Colla and Dumesh 2010, p. 46; USFWS rusty patched bumble bee unpublished geodatabase 2016). The species requires areas that support sufficient food (nectar and pollen from diverse and abundant flowers), undisturbed nesting sites in proximity to floral resources, and overwintering sites for hibernating queens (Goulson et al. 2015, p. 2; Potts et al. 2010, p. 349). Bumble bees are generalist foragers, meaning they gather pollen and nectar from a wide variety of flowering plants (Xerces 2013, pp. 27–28)

Figure 14. Corn crop production as it relates to the species range of *Bombus affinis*.



Current species range (indicated by the blue polygons (USFWS, 2023ad)) is overlayed with corn crop planting (indicated by the burnt orange shaded areas (U.S. EPA, 2023b)) and the county boundaries (indicated by the gold polygons), and there is overlap (see above for the overlap percentage in each county) of corn crop planting and the range of the species.

Assessment

While there is overlap of the range of *B. affinis* and corn crop planting and *B. affinis* is known to utilize agricultural landscapes, this bee is a pollinator species thus any exposure to Mpp75Aa1.1 would be limited to pollen from MON 95275 maize. Adverse effects were found at the lowest concentration of Mpp75Aa1.1 tested against the honey bee (250 mg Mpp75Aa1.1/kg); however, this concentration is 2,000X greater than the maximum concentration that *B. affinis* could realistically encounter in the environment given that the expression level of Mpp75Aa1.1 in MON 95275 maize pollen was not detectable (<0.125 mg/kg). Additionally, the most susceptible pest species to Mpp75Aa1.1 has an EC_{50} of 2.7 mg/L (approximately 22X greater than the maximum concentration in MON 95275 maize pollen). Given the extreme unlikelihood that *B. affinis* is 22X more susceptible to Mpp75Aa1.1 than the most sensitive pest species, exposure of Mpp75Aa1.1 to this bee is expected to be negligible. Therefore, the EPA is making a “No Effect” determination under the ESA for *B. affinis* and its designated critical habitat resulting from the proposed use of Mpp75Aa1.1 in MON 95275 maize.

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X. Appendix B: Evaluation of Vpb4Da2's and DvSnf7.1's Potential to Affect Coleopteran Threatened and Endangered Species (TES)

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Coleopteran TES with >1% (≥0.44%) overlap with areas of corn crop production or agricultural production

In order to characterize the potential exposure of these species to MON 95275 maize, critical habitat and updated species range information were evaluated based on information taken directly from or slightly modified from the USFWS ECOS and Federal Register Notices, unless otherwise cited.

1) American burying beetle (*Nicrophorus americanus*) – 0.516% (Dawson County, NE); 0.407% (Antelope County, NE)

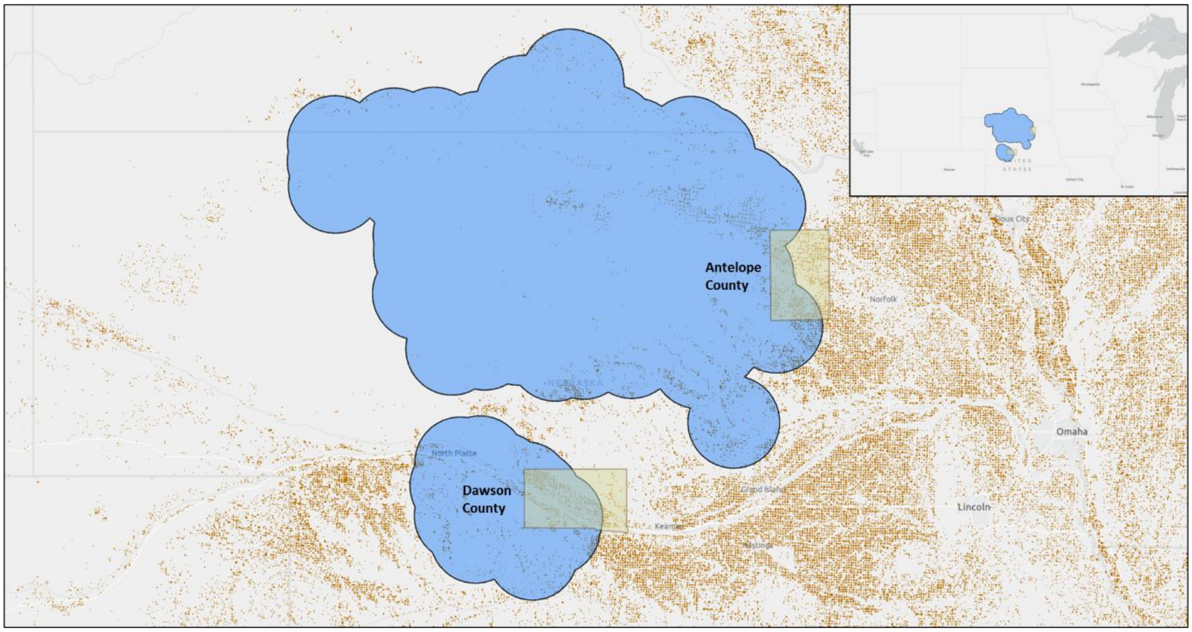
Biology and Habitat Requirements

The following excerpts are from the Federal Register (2020); consult original reference for citations:

The American burying beetle is native to at least 35 States in the United States, covering most of temperate eastern North America, and the southern borders of three eastern Canadian provinces. The species is believed to be extirpated from all but nine States in the United States and is likely extirpated from Canada. However, the current range is much larger than originally thought when the species was listed in 1989.

Adults and larvae depend on dead animals (carrion), e.g., cotton rats, pheasants, prairie dogs, ground squirrels, etc., for food and moisture. Adults also require adequate soil moisture, appropriate soil temperatures, and appropriate soil particle size to allow them to bury themselves and/or a carcass (see chapter 2 of the SSA Report; Service 2019). Adequate soil moisture levels appear to be critical for American burying beetles, and they show a strong preference for moist, sandy loam soil with organic matter (Hoback 2008, unpublished), but a specific threshold for soil moisture is unknown.

Figure 1. Corn crop production as it relates to the species range of *Nicrophorus americanus* in Dawson County, NE and Antelope County, NE.



Current species range (indicated by the blue polygons (USFWS, 2023a)) is overlayed with corn crop planting (indicated by the burnt orange areas (U.S. EPA, 2023)) and the county boundaries (indicated by the gold polygons), and there is overlap (0.516% and 0.407% in Dawson County, NE and Antelope County, NE, respectively) of corn crop planting and the range of the species.

Assessment
While there is overlap of the range of *N. americanus* and corn crop planting, the beetle is unlikely to be found in agricultural settings given its soil preferences. Additionally, as a carrion specialist, any exposure to Vpb4Da2 or DvSnf7.1 would be limited to exposure via dead vertebrate species that consumed MON 95275 maize tissue while still alive and previous studies have shown PIP concentrations to become increasingly diluted as they move through the food chain (Li et al., 2017; Meissle & Romeis, 2018; Tian et al., 2014). Furthermore, expression of Vpb4Da2 and DvSnf7.1 in MON 95275 maize in parts of the plant most likely to be consumed by vertebrate species is low (e.g., grain, leaf; see Table 1) and the nature of carrion (i.e., decaying/rotten flesh) means that Vpb4Da2 and DvSnf7.1 would likely be degraded beyond biological relevancy. Given these habitat and dietary needs, exposure of Vpb4Da2 and DvSnf7.1 to this beetle is expected to be negligible. Therefore, the EPA is making a "No Effect" determination under the ESA for *N. americanus* and its designated critical habitat resulting from the proposed use of Vpb4Da2 and DvSnf7.1 in MON 95275 maize.

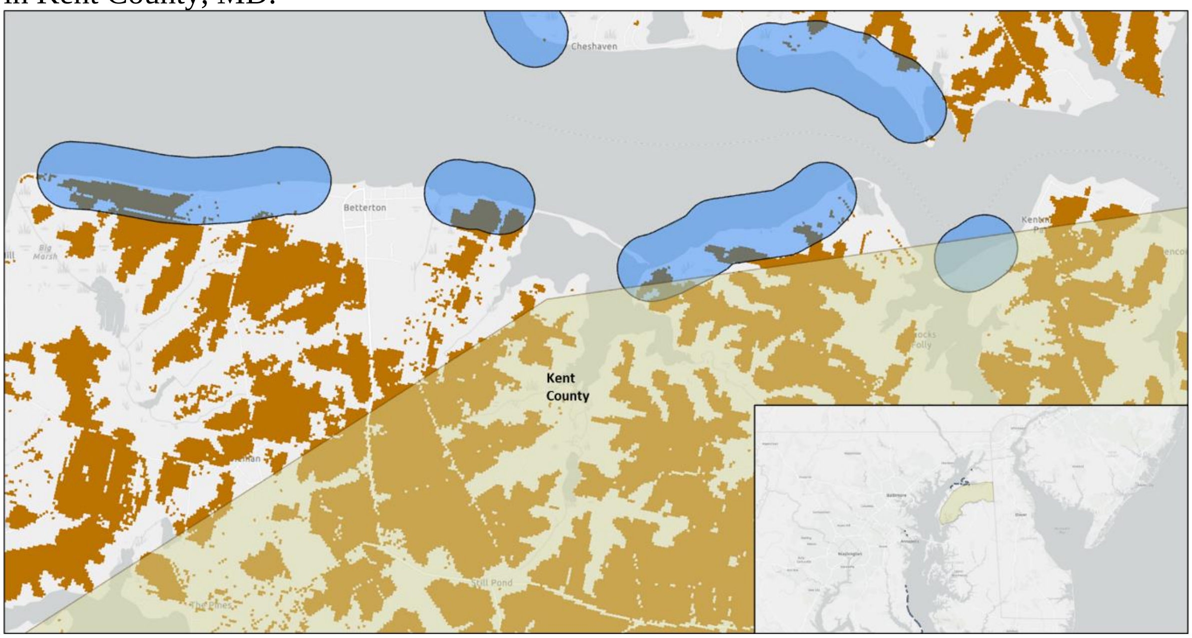
2) **Puritan tiger beetle (*Ellipsoptera puritana*) – 2.139% (Kent County, MD)**

Biology and Habitat Requirements
The following excerpt is from the Federal Register (1990); consult original reference for citations:

Tiger beetles (genus; *Cicindela* [this species was previously known as *Cicindela puritana*]) are day-active, predatory insects that capture small arthropods in a "tigerlike" manner, grasping prey with their mandibles (mouthparts). Tiger beetle larvae, which live in burrows in the ground, are also voracious predators, fastening themselves near the tops of the burrows by means of abdominal hooks and rapidly extending from their burrows to seize passing invertebrate prey.

The Puritan tiger beetle was known historically from numerous sites along the Connecticut River in Vermont, New Hampshire, Massachusetts and Connecticut, and from along the Chesapeake Bay in Maryland; it is now restricted to Maryland and two Connecticut River sites, one in Massachusetts and one in Connecticut. Within the Chesapeake Bay, its habitat is characterized by the presence of narrow sandy beaches with adjacent, well-developed bluffs of sand and clay (Glaser 1984, Knisley 1987, Knisley and Hill, 1990). Habitat of the Connecticut River population in Massachusetts is similar, with steep, clay banks adjacent to a wider (10 meters or greater) sandy beach (Nothnagel 1987).

Figure 2. Corn crop production as it relates to the species range of *Ellipsoptera puritana* in Kent County, MD.



Current species range (indicated by the blue polygons (USFWS, 2023b)) is overlayed with corn crop planting (indicated by the burnt orange shaded areas (U.S. EPA, 2023)) and the county boundary (indicated by the gold shaded area), and there is overlap (2.139%) of corn crop planting and the range of the species.

Assessment

While there is overlap of the range of *E. puritana* and corn crop planting, the beetle's sole habitat consists of sandy beaches and bluffs, which are not conducive to corn crop production. Furthermore, given that the Puritan tiger beetle is predatory, any possibility of exposure to Vpb4Da2 or DvSnf7.1 would be limited to exposure via prey species that have consumed MON 95275 maize tissue. Given the lack of presence of the Puritan tiger beetle within agricultural environments and that previous studies have shown PIP concentrations to become increasingly diluted as they move through the food chain (Li et al., 2017;

Meissle & Romeis, 2018; Tian et al., 2014), there is a reasonable expectation of no exposure to Vpb4Da2 and DvSnf7.1. Therefore, the EPA is making a "No Effect" determination under the ESA for *E. puritana* and its designated critical habitat resulting from the proposed use of Vpb4Da2 and DvSnf7.1 in MON 95275 maize.

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