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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

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MEMORANDUM

SUBJECT: Revised Addendum to Environmental Risk Assessment for a FIFRA Section 3 Registration of MON 89034 x TC1507 x MON 87411 x DAS-59122-7 Combined Trait Maize Expressing Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34/35Ab1 *Bacillus thuringiensis* Derived Insecticidal Protein, and DvSnf7 Double Stranded RNA (dsRNA); Submitted by Monsanto Company; EPA File Symbols 524-AGE, 524-AGR; PC Codes 006514, 006515, 006481, 006490, 006580, 006566; Decision Nos. 514588, 514589; Submission Nos. 982159, 983961, 982149, 985448; DP Barcodes: 432075, 433101, 432074, 433105; MRIDs 49748501, 49781805, 49781806, 49886501, 49886502, 49886503

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EPA completed an ecological risk assessment for a FIFRA Section 3 registration of MON 89034 x TC1507 x MON 87411 x DAS-59122-7 combined trait corn (EPA File Symbols 524-AGE and 524-AGR), also known as SmartStax PRO (see USEPA 2016a). While MON 89034 x TC1507 x MON 87411 x DAS-59122-7 expresses several plant incorporated protectants (PIPs), the risk assessment focused on the DvSnf7 double stranded RNA (dsRNA) transcript expressed by event MON 87411. Because dsRNA is a new type of PIP and has some uncertainties that result from its unique mode of action, human health and ecological risk assessments for MON 87411 and MON 89034 x TC1507 x MON 87411 x DAS-59122-7 were reviewed by a FIFRA Science Advisory Panel (SAP) at a meeting held on September 27-28, 2016. For the ecological risk assessment, the SAP was asked to comment on several aspects of the risk assessment approach, including exposure assumptions, the completeness of the available environmental fate and nontarget effects data, and evaluation of nontarget toxicity and synergism. The minutes from the meeting provide details of the comments from the SAP (FIFRA SAP 2016), which include critique on the approach used in the ecological risk assessment and advice for improvement.

Additionally, EPA stated in the ecological risk assessment that an updated assessment for federally listed threatened and endangered ("listed") species would be forthcoming. This memorandum provides EPA's response to the SAP's comments and an updated assessment for listed species. This revised version corrects the previous version dated May 3, 2017, based on clarification provided by Monsanto Company regarding over season expression data. The revisions are reflected below in EPA's response to the SAP's comments for charge question 3a.

I. Response to SAP Comments

A. Charge Question 3

Charge Question 3 requested comment from the SAP regarding EPA's conclusions about environmental fate of DvSnf7 dsRNA. Generally, EPA had concluded that in terrestrial environments, exposure is primarily limited to organisms that directly consume corn plant material, and additional consideration was also given to the potential for secondary exposure through consumption of herbivorous arthropods. In aquatic environments, EPA concluded that exposure to DvSnf7 dsRNA in corn detritus is expected to be minimal, and while some exposure may occur in the water column, it will be minimal and also short lived. The SAP agreed with most of EPA's conclusions in general, but had specific recommendations, as described below.

Charge Question 3a

Charge Question 3a specifically requested the SAP to comment on the completeness of the data set considered for determining exposure and environmental fate of DvSnf7 dsRNA in both terrestrial and aquatic environments, taking into consideration the scope of EPA's needs for environmental risk assessment and the recommendations of the 2014 SAP (see FIFRA SAP 2014).

SAP Comment: The SAP referenced the 2014 SAP report, which specifically recommended a six step framework for performing risk assessment for dsRNA based PIPs. The second step of that framework involved first identifying species that are likely to be exposed, and then performing *in silico* evaluations to determine which species are likely to have some response to DvSnf7 dsRNA. The SAP noted that the organisms potentially at risk from exposure were not determined as required of the second step. The SAP stated that omission of that step diminished the utility of all data addressing recommendations in the remainder of the step and also subsequent steps.

EPA Response: EPA acknowledges that this approach may be useful in refining species that would be exposed in corn growing areas. The idea driving the suggestion of this step is that effects of dsRNA based pesticides were determined by the 2014 SAP to be potentially unpredictable, primarily due to unintended effects like off-target silencing, such that surrogate species may not reliably predict adverse effects. Therefore, to be truly functional as intended, the second step would have to include survey and subsequent *in silico* analyses of all nontarget species likely to be exposed in corn throughout all areas in which corn may be grown within the U.S. and its territories. This approach is problematic given the time scale of the pesticide registration process, since surveys of all areas where corn may be grown must be performed, all

nontarget species must be identified to the species level (including insects – an often difficult task), and tests with species not previously utilized in toxicity testing would need to be developed and validated. Such an approach would take many years to accomplish. Additionally, not all nontarget species can be reared in the laboratory. Therefore, to address risk concerns on a time scale that better meets the needs of EPA's process, subsets of the information required of this approach must be utilized (e.g., surveys available in the literature, currently available genetic databases), as well as tests utilizing proven test methods with reliable, but sometimes surrogate, test species.

Studies submitted in support of the MON 87411 and MON 89034 x TC1507 x MON 87411 x DAS-59122-7 registrations included tests with Northern bobwhite (*Colinus virginianus*), channel catfish (*Ictalurus punctatus*), lady beetle (*Coleomegilla maculata*) parasitic wasp (*Pediobus foveolatus*), insidious flower bug (*Orius insidiosus*), carabid beetle (*Poecilus chalcites*), green lacewing (*Chrysoperla carnea*), honey bee (*Apis mellifera*), earthworm (*Eisenia andrei*), and springtail (*Folsomia candida*). Several of these, including lady beetle, parasitic wasp, lacewing, and insidious flower bug, were found in surveys of trial corn plots planted with MON 87411 corn in the U.S. (MRID 44953304), and have been noted in other surveys of insects in corn (e.g., see Wold et al. 2001). Others are also known to be widespread in distribution such that there is reasonable likelihood that they would be found in corn growing areas. Therefore, species that have been tested largely are representatives of species found in corn growing areas.

The SAP's suggestion also makes two assumptions that may not be supported. First, it assumes that the presence of a nontarget species in the vicinity of corn fields is indicative of exposure, which is not necessarily true in all cases. For instance, EPA utilized available information on environmental fate and exposure levels known to be toxic to the target organism to conclude that exposure in aquatic environments is not likely to reach levels that would cause effects. Therefore, additional testing with aquatic organisms was not required. Second, it assumes that *in silico* searches are reliable indicators of susceptibility to all potential effects. As discussed in the 2016 ecological risk assessment, EPA recognizes that these analyses are not predictive of effects, and has not yet determined how such analyses would be used. Additionally, the unexpected effects that are largely driving the reasoning behind the second step of the SAP's six step process cannot be predicted from *in silico* searches. Therefore, EPA determined that a better approach was to test a wide range of nontarget species, with focus on nontarget arthropods, since they are more closely related to the target pest, and include an expanded examination of endpoints (e.g., survival, development, reproduction) to ensure that such effects are captured.

SAP Comment: The SAP commented that the tables showing expression levels of DvSnf7 dsRNA in MON 87411 and MON 89034 x TC1507 x MON 87411 x DAS-59122-7 corn indicate that the data may not be normally distributed and appear to be skewed toward higher concentrations (see Tables 1 and 2 on pages 6 and 7 of USEPA 2016a). Without seeing the underlying data, the SAP stated that it is difficult to assess the information and that the variance should be expressed as 90% or 95% confidence limits (CL). The SAP also recommended that for screening level assessments, an upper bound on the data, such as the upper 90% or 95% CL are more appropriate for determining exposure levels. The SAP also noted that the U.S. data shown in Table 1 did not include data showing the change in expression over the growing season, and that without these data, exposure estimates for nontarget organisms may be inaccurate. The panel

also noted that the concentration of DvSnf7 dsRNA was not measured for pollen in the MON 89034 x TC1507 x MON 87411 x DAS-59122-7 hybrid. The SAP stated that although concentrations are low, expression data for pollen are necessary to complete this data set.

EPA Response: EPA utilized the expression data to confirm that exposure levels used in nontarget organism hazard testing were high enough to account for any exposures in the environment. Of the expression data presented, EPA selected the highest mean value for dry weight expression (0.097 µg DvSnf7 dsRNA/g dry weight leaf tissue, see Table 1, page 6 of USEPA 2016a) for comparison. Regarding expression data collected over the season, Monsanto confirmed in correspondence dated May 8, 2017 that all expression data have been submitted for the U.S. trials as shown in Table 1 of the risk assessment, and that additional over season data had not been collected. EPA notes that the data collected in the U.S. and Argentina trials are highly comparable. Additionally, among the available data, EPA can utilize the highest individual data point measured as a "worst case" estimate for potentially higher expression levels that may have been shown at other time points, which would be 0.213 µg DvSnf7 dsRNA/g dry weight for whole plant, as indicated in Table 2 on page 7 of the ecological risk assessment. Assuming this is the worst case exposure, the levels tested for most nontarget organisms would still be 4.7 times this maximum level. As explained in the ecological risk assessment, dry weight levels for most plant tissues and organs during the growing season are already considered high estimates, since in reality the nontarget organisms would be exposed to levels comparable to fresh weight expression levels. According to MRID 49315104 mean fresh weight values range from 12% – 25% percent of the mean dry weight values, with most below 20% of the dry weight value. Therefore, while EPA utilized the mean of the dry weight values, these values still confirm that the exposure levels in hazard testing are much higher than those likely to be consumed in the field. Collection of additional over season data, as suggested by the SAP, is unlikely to change EPA's analysis and are therefore not necessary.

EPA indicated in its ecological risk assessment that all pollen samples from MON 89034 x TC1507 x MON 87411 x DAS-59122-7 in U.S. trials were below the limit of detection (0.065×10^{-4} µg DvSnf7 dsRNA/g) and the level of quantitation (0.29×10^{-4} µg DvSnf7 dsRNA/g). These levels are extremely low, and the 1000 ng DvSnf7 dsRNA/g diet test level used in most nontarget organism tests is approximately 34,000 – 154,000 times higher than either of these levels. Based on these calculations, EPA is confident that the levels tested adequately cover exposures to DvSnf7 dsRNA from consumption of pollen, and that exposure levels from only pollen are extremely low. EPA does not see a need to ask for additional analyses for pollen expression.

SAP Comment: The SAP stated that the soil degradation data suggested residual insecticidal activity after appreciable degradation. Based on these data and data presented in what was stated to be Fisher et al. (2016) in Chemosphere, the SAP concluded that 1) there may be residual although diminished activity of degraded dsRNA, 2) the nature and extent of microbial degradation of dsRNA is likely to be variable, and 3) without *in situ* measurements of DvSnf7 dsRNA in soils, there remains an unanswered question of how much DvSnf7 dsRNA is present in root zone soils.

EPA Response: EPA notes that on page 28 of the SAP report, the SAP noted that excellent information was provided concerning in-field and off-site movement of parts of the corn plant during the growing season, and that post-harvest information was also useful. EPA had assumed that most plant material would remain on the planted field, and that little plant material would enter the soil until after harvest, which would occur after senescence of the corn plants. Based on data in Tables 1 and 2 of the ecological risk assessment, residues of DvSnf7 dsRNA in senescent plant material are several orders of magnitude lower compared to expression during the growing season. EPA concluded that the concentration would be very low, based on calculations using expression levels during the growing season and afterward. Unlike the issue described above about use of means versus upper bounds for estimating exposure, the differences in mean and maximum expression levels for stover and forage are very small, so use of an upper bound estimate is still expected to return a low expected concentration. Additionally, the soil degradation study (MRID 49315122) required not only an amount of plant material equivalent to 3 times the expected maximum to be incorporated into the soil, it also required spiking the soil samples with additional naked DvSnf7 dsRNA so that DvSnf7 dsRNA could be detected and quantified sufficiently to determine the degradation kinetics.

The SAP mentions the upper limit of DvSnf7 dsRNA persistence in what is believed to be Fischer et al. (2016) in Chemosphere (the reference is not provided in the SAP report), but the upper limit does not appear to be specifically described in this paper. It is noted that Fischer et al. (2016) describes development of a method for measuring degradation rates of dsRNA using molecular analysis, part of which involved determining the influence of extraction chemicals on background mortality in bioassays (see section 3 of the paper, first paragraph). EPA initially noted what appeared to be insecticidal persistence of DvSnf7 dsRNA in MRID 49315122, but stated in the updated data evaluation record (DER) that additional information from Monsanto Company provided better description of this background mortality. The mortality that appeared to persist was within the bounds of control mortality.

Based on the above information, EPA concludes that *in situ* soil measurements of DvSnf7 dsRNA are likely to result in extremely low concentrations below the limit of detection by molecular analysis and bioassay. Data confirming these assumptions would resolve any uncertainties; however, these data are not needed to make a risk determination for nontarget organisms.

SAP Comment: The SAP agreed with EPA that concentrations in aquatic environments would largely reflect movement of plant debris post-harvest, though the panel stated that it was unclear whether exposure estimates reflected movement of plant debris and run-off at multiple time points. The SAP disputed EPA's conclusions regarding persistence of DvSnf7 dsRNA in sediment, stating that data from Fischer et al. (2017) indicated potential extended persistence. The SAP also disagreed that toxicity data were not needed for aquatic organisms, particularly those dwelling in sediment in estuarine environments. The SAP stated that EPA had discounted estuarine organisms from further analysis because these areas were assumed not to have opportunity to receive runoff or plant debris from corn fields.

EPA Response: Regarding exposure estimates and the potential for multiple "pulses" of debris entering waterways, it is important to point out that the model used is a worst case screening

model that assumes an amount of corn equivalent to that growing on 10 hectares enters a 1 ha pond that is 2 m deep. While corn debris may be realistically deposited in nearby aquatic areas at multiple time points, it is expected that the sum of debris deposited by such events will not exceed the number of plants reasonably expected to be within the area drained by the aquatic habitat. More refined exposure estimates are generally not needed unless this screening level calculation indicates exposure levels above a level of concern, since models providing more refined estimates are expected to return lower concentrations. Such models also are developed to determine exposure resulting from residues of chemicals moving around in the environment, not plant material, so current models utilized by EPA for other types of pesticides are not appropriate for DvSnf7 dsRNA expressed in plants without modification of the models. It is unclear that data on corn plant debris movement into aquatic habitats exists such that a reliable model specific to this scenario can be developed without further research. Given that the calculation used is expected to provide the worst case assumption for exposure, EPA concludes that use of more refined models is not necessary in this case.

Regarding persistence, the SAP noted that based on the data in Fisher et al. (2016b), DvSnf7 dsRNA was detectable for a period between 14 and >28 days in sediment samples treated with no overlaying water. It should be made clear that while it was detectable by molecular analysis for >28 days, it was not detected by bioassay, an indication of insecticidal activity, beyond 14 days. Molecular analyses tend to be more sensitive because they can detect molecular fragments that do not necessarily have insecticidal activity and their presence is not as relevant to the risk assessment. Additional information has become available since the SAP meeting on the dissipation of dsRNA in aquatic environments with publication of Albright III et al. (2016). This paper examined dissipation of a 100 bp non-insecticidal dsRNA surrogate, which was also used in Fischer et al. (2016), in three different microcosms (laboratory water over sterilized sediment, sterilized pond water over sterilized sediment, and active pond water over active sediment). The study concluded that the dsRNA degraded rapidly within all three microcosms, and was undetectable by 96 h. Additionally, they concluded that the dsRNA did not partition to sediment in this cases, though the sediment used had a high sand content, so partitioning to sediment was not a major factor in rapid dissipation from water. Based on these results, the authors concluded that dsRNA is not expected to persist in aquatic environments or have long-term environmental impact.

Using the screening level calculation, the estimate for how much DvSnf7 dsRNA could enter water was determined to be very low (up to 0.0087 ng DvSnf7 dsRNA/mL), which is well below the level at which DvSnf7 dsRNA is expected to cause adverse effects in the sensitive target organisms (LC_{50} as low as 1.2 ng/g diet, Bachman et al. 2013). As noted on page 30 of EPA's ecological risk assessment, the target organisms are expected to have the greatest sensitivity, since they have the gene sequence homology targeted by the DvSnf7 dsRNA. Adverse effects did not occur in even closely related insects at exposure levels of 500 ng/g diet to 5000 ng/g diet. EPA concluded that concentrations of DvSnf7 dsRNA are not expected to be deposited into aquatic habitats at levels known to cause adverse effects in the target organisms, and given the rapid dissipation of DvSnf7 dsRNA in aquatic environments, it is very unlikely that DvSnf7 dsRNA will accumulate to such levels.

The SAP is incorrect in stating that EPA specifically discounted marine/estuarine areas because they were assumed not to have opportunity to receive runoff or plant debris from corn fields. EPA's analysis of DvSnf7 dsRNA in aquatic habitats was focused on freshwater areas, since most understanding about corn debris in aquatic systems comes from studies in freshwater. However, the calculations were general in nature and did not require consideration of physical or chemical qualities of freshwater versus brackish or salt water. EPA concluded that significant exposure to DvSnf7 dsRNA is not expected in aquatic environments, which applied to freshwater, marine, and estuarine environments.

SAP Comment: The SAP commented on pages 30-33 of the report that corn grown in coastal areas of the U.S. do not support EPA's conclusion that estuarine organisms would not be exposed to DvSnf7 dsRNA expressed in MON 89034 x TC1507 x MON 87411 x DAS-59122-7 corn. Given the SAP's concerns over potential persistence in sediment, the report suggests that sediment dwelling organisms could be tested. Since EPA does not have these data and has not utilized the six step process suggested by the 2014 SAP, specifically to determine the intersection of estuarine species with corn growing areas, conclusions of the assessment, including those for endangered species, are incomplete.

This section also included comments about deficiencies in the 28-day rodent study. Since this study is relevant to the human health risk assessment and is discussed in detail there, no further comment about it will be presented below. Additionally, the SAP commented on the *in silico* evaluation provided by Monsanto Company, and stated that an evaluation included fewer than 25 species and did not necessarily target potentially sensitive species occupying corn growing regions. The SAP stated that the target species should have been assessed according to the 2014 SAP recommendations.

EPA Response: EPA believes that the SAP's comments regarding exclusion of nontarget estuarine organisms in coastal areas result from a misread of EPA's ecological risk assessment. As discussed above, EPA did not discount these areas because it was assumed they would not receive plant debris. EPA's analysis was for all aquatic habitats, and determined that exposure in aquatic areas would be low even with deposits of large amounts of plant debris. These conclusions apply to freshwater, as well as brackish and salt water. As noted above, EPA does not assume that proximity of nontarget organisms to corn growing areas necessarily indicates their exposure. Therefore, since DvSnf7 dsRNA is not expected to be present in aquatic environments at levels that would cause adverse effects to nontarget organisms and is not expected to persist, the suggested toxicity testing is not necessary. EPA concluded that because exposure is expected to be very low, adverse effects to aquatic nontarget organisms are not expected, and the data continue to support this conclusion, as well as EPA's "no effect" determination for federally listed threatened and endangered ("listed") species.

Regarding the *in silico* evaluation, the significance of 25 species is unclear from the SAP reports of both 2014 and 2016. The ecological risk assessment explained that *in silico* evaluations are not considered to be predictive of adverse effects, and that EPA is still evaluating their application to risk assessment for dsRNA based pesticides. Currently, *in silico* evaluations are used as supplemental information providing an additional line of evidence for risk determination.

Charge Question 3b

Charge Question 3b described assumptions used in determining the environment fate of DvSnf7 dsRNA in aquatic environments, which were based on those developed for *Bacillus thuringiensis* derived Cry proteins. The question requested the SAP to comment on the applicability of the assumptions and describe any additional or alternative information and/or analyses that EPA should consider.

SAP Comment: The SAP was uncertain whether the assumptions for Cry proteins would apply to DvSnf7 dsRNA, and suggested that this uncertainty could be addressed by measuring dissipation of DvSnf7 dsRNA in plant material in aquatic systems in controlled laboratory or field studies. The SAP commented that these could be required post registration if EPA decides that this uncertainty is of minimal concern. The SAP reiterated its comments about exclusion of nontarget organisms in estuarine areas.

EPA Response: EPA addressed concerns for nontarget organisms in estuarine areas in responses above. EPA based its decision to use assumptions on aquatic environmental fate for Cry proteins for those used for DvSnf7 dsRNA on the high degree of polarity for dsRNA molecules. Given this quality, it was assumed that as plant material broke down in aquatic environments, DvSnf7 dsRNA would leach into water, but that concentrations in aquatic environments will be extremely low and will not cause adverse effects in nontarget organisms. The SAP cited lack of empirical data, but did not comment on EPA's reasoning behind application of the assumption. EPA believes that this assumption is reasonable. Even if all DvSnf7 dsRNA does not leach out of the plant material, by the time the plant material can be consumed by aquatic detritivores (approximately two weeks, as discussed in the ecological risk assessment), much of the DvSnf7 dsRNA in plant material will be degraded by RNases, physical forces, and microorganisms present in the environment. Additionally, the potential for effects to aquatic organisms was discussed extensively in EPA's ecological risk assessment, and EPA determined that adverse effects are not expected to occur in aquatic environments (USEPA 2016a). The SAP's comments do not change these conclusions. . As suggested by the SAP, data showing degradation of DvSnf7 dsRNA within plant material in aquatic environments would be useful to confirm assumptions used in the exposure analysis.

Charge Question 3c

Charge Question 3c inquired about alternative analyses that may be used to estimate exposure to nontarget organisms, such as consideration of exposure above a certain threshold of dsRNA molecules required to induce RNAi and gene silencing.

SAP Comment: The SAP commented that this question was mostly addressed in response to Charge Question 1. Uptake of plant miRNA is limited to < 1 copy per cell and is considered insufficient for mediating RNAi (it was unclear whether this comment was in reference to humans, specifically, and if by "miRNA" the SAP was referring to siRNA from DvSnf7). Additionally, barriers that exist in terrestrial vertebrates provide significant protection and RNAs are rapidly degraded. The SAP also identified no evidence of bioaccumulation of dsRNA, so this aspect of risk assessment did not need consideration. The SAP also concluded that current data

suggests a low probability that exposures would exceed any toxic threshold for terrestrial organisms and for aquatic vertebrates.

The SAP also commented that EPA has taken the position that "cessation of exposure is expected to result in reduction and eventual cessation of effects." The SAP pointed out that one-time exposure can have durable effects, so additional testing in nontarget organisms representing aquatic biota and experiments designed to address off-target and other unintended effects related to dsRNA exposure are warranted to conclude this question.

EPA Response: EPA assumes, based on the SAP's comments, that the approach to estimating exposure to dsRNAs based on environmental concentrations is sufficient for ecological risk assessment. In stating that "cessation of exposure is expected to result in reduction and eventual cessation of effects," EPA was not discounting potential effects that may become apparent at a later time. The point of this statement was that gene silencing was expected to be reduced and eventually cease after cessation of exposure. This conclusion is reasonable, given what is understood about breakdown of dsRNA and siRNA *in vivo*, and is confirmed by information pointed out by the SAP that indicate that dsRNA does not bioaccumulate. It is clear in the ecological risk assessment that EPA recognizes the potential for latent effects, and required additional nontarget testing to include additional toxicity endpoints thought to capture potential latent effects most relevant to the risk assessment. EPA has required testing to address off-target and other unintended effects, none of which indicated any effects in the organisms tested.

B. Charge Question 4

Charge Question 4 requested comment from the SAP regarding EPA's conclusions about the completeness of nontarget organism hazard data, which were addressed with data typically submitted for Cry protein based PIPs, with additional data on nontarget insect reproduction. Part of the risk analysis for vertebrates also included assumptions about barriers to dsRNA uptake and bioinformatic analysis as additional lines of evidence supporting a conclusion of no expected adverse effects to nontarget organisms. The SAP generally agreed that the hazard data are adequate, with some concerns as described below.

Charge Question 4a

Charge Question 4a requested comment on the completeness of the non-target organism hazard data reviewed for DvSnf7 dsRNA as it pertains to the needs of the ecological risk assessment and the recommendations for testing made by the 2014 SAP.

SAP Comment: The SAP concluded that nontarget hazard data were largely adequate, but noted some concerns. The SAP agreed with EPA's conclusions regarding the supplemental status of the broiler chicken and channel catfish nutritional equivalence studies, as well as the acceptable status of the study with Northern bobwhite. The SAP determined that additional surrogate species testing representing the soil biota should be included in the nontarget hazard analysis, particularly since DvSnf7 dsRNA is intended for control of corn rootworm, which is a soil-dwelling corn pest. More specifically, the panel stated that it would have been more appropriate to test soil dwelling pest nematode species and the microbial community. They also

concluded that additional measurements on reproductive endpoints should have been included for all coleopteran species tested (it appears that the insidious flower bug was erroneously included in the list of suggested species). The SAP also reiterated in this section recommendations from previous questions.

The SAP report noted discussion and disagreement over soil microbiota testing and omics-based testing, and a point was made that the requirement of additional data was discovery-driven in nature and not necessarily appropriate for regulatory risk assessment needs. Ultimately, the panel agreed that testing of nematode species may be appropriate, since several species are known to be sensitive to environmental dsRNA. However, plant pest species of nematodes are not considered as non-target organisms by definition and typically would not be part of a FIFRA-based risk assessment.

EPA Response: As discussed above, exposure in soil is not expected to reach levels that would cause adverse effects in nontarget organisms. Additionally, data on soil dwelling invertebrates were included in EPA's risk assessment, showing no adverse effects. Additional data on soil microorganisms are also available from Monsanto Company, which indicated no adverse effects on soil microorganism population size and function (Bachman et al. 2016). Additionally, testing with pest nematode species as surrogate organisms runs counter to the advice of the 2014 SAP, recommending testing of nontarget species specifically exposed in corn environments. The nematode species of concern would be free-living beneficial species that would not be necessarily as closely associated with the corn plants as the pest species and would have different levels of exposure. With regard to other testing requirements additional discussion is presented below.

Charge Question 4b

Charge Question 4b requested comment from the SAP about the applicability of biological barriers known to limit dsRNA uptake in mammals to other vertebrates, and inclusion of these barriers as an additional line of evidence supporting EPA's conclusion of minimal risk to vertebrate nontarget organisms.

SAP Comment: The SAP commented that it found EPA's human health risk assessment to be appropriate, and referred EPA to the response to Charge Question 1.

EPA Response: It is unclear whether the comment, as written, answers the question posed. The 2014 SAP indicated that barriers to uptake would likely limit human exposure to dsRNAs. However, EPA specifically posed this question to confirm the appropriateness of EPA's assumption that such barriers also exist in other terrestrial and aquatic vertebrates. The SAP commented elsewhere that adverse effects are not expected in nontarget vertebrates.

Charge Question 4c

EPA concluded that off-target and other unintended effects related to dsRNA exposure are unlikely in nontarget organisms, based on lack of effects observed in nontarget testing. Charge Question 4c requested comment from the panel regarding these conclusions.

SAP Comment: The SAP indicated concern for horizontal transfer of the transgenic gene cassettes from MON 87411 corn, which could potentially lead to expression at higher concentrations, leading to potentially greater impacts, including unintended effects. This comment was countered by another panel member in that this concern would apply to all transgenic plants made in this manner and is not specific to DvSnf7 dsRNA. The remainder of the comment concerned the 28-day rodent study.

EPA Response: EPA has previously covered the issue of potential horizontal transfer, and concluded that it is unlikely (USEPA 2010a). As noted by the associate panel member, these concerns would not be specific to DvSnf7 dsRNA. The SAP's comment does not change EPA's conclusions regarding this issue.

C. Charge Question 5

Charge Question 5 requested comment from the SAP regarding EPA's conclusions about synergism studies submitted to support the risk assessment for the combined trait product, MON 89034 x TC1507 x MON 87411 x DAS-59122-7. Specifically, EPA reviewed five studies on synergism of DvSnf7 dsRNA with Cry proteins expressed in MON 89034 x TC1507 x MON 87411 x DAS-59122-7, and asked the SAP to comment on EPA's analyses of these data and their scientific value to the risk assessment.

SAP Comment: The SAP agreed overall with EPA's analysis of these data, and stated that the data had high scientific value. The SAP stated that it was uncertain whether the Fixed Lethal or Concentration Addition models represented the most rigorous approaches for determining synergism. However, the SAP determined that models used in the studies were adequate, given their previous application to synergism studies with PIPs. One panel member noted that the endpoints used may have been limited in that they did not include reproduction.

EPA Response: EPA noted issues with the Fixed Concentration model used in certain synergism studies; however, other studies using a more robust approach were also submitted, and the set of data were determined to be sufficient to show that synergism between DvSnf7 dsRNA and the Cry proteins expressed in MON 89034 x TC1507 x MON 87411 x DAS-59122-7 does not occur. It is assumed that the SAP suggested testing reproduction because this endpoint can be affected at low concentrations. However, growth inhibition was used in several studies, and it is understood to be reliable as an indicator of toxicity at low concentrations in insects. Additionally, it can be observed within a shorter time frame compared to reproduction, reducing the potential for variation in response that might occur over a longer observation period. It is also unclear whether DvSnf7 dsRNA has effects on reproduction of the target insects, since it does not target specifically a gene solely involved in reproduction. Most likely it causes mortality outright or indirectly through reduction in growth.

II. Updated Endangered Species Assessment

In the 2016 ecological risk assessment for MON 89034 x TC1507 x MON 87411 x DAS-59122-7, EPA made "no effect" determinations for DvSnf7 dsRNA for direct and indirect effects to all

federally listed threatened and endangered ("listed") species and their designated critical habitats. The SAP's comments do not change the risk conclusions for DvSnf7 dsRNA, so this determination still applies.

For the Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, and Cry34/35Ab1 proteins also expressed in MON 89034 x TC1507 x MON 87411 x DAS-59122-7, EPA concluded in the 2016 ecological risk assessment that because these proteins are selective for either coleopteran or lepidopteran species, any adverse effects to listed species other than insects within those orders was unlikely. Additionally, loss of the target lepidopteran or coleopteran insect pests is not expected to cause indirect effects, such as loss of food resources. Therefore, "no effect" determinations were made for direct and indirect effects to all other listed species, and the listed species assessment for these Cry proteins would thus be focused on potential direct effects to listed coleopteran and lepidopteran species. EPA stated in the 2016 assessment that due to additions to the list of species that were recent at the time, an updated assessment for Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, and Cry34/35Ab1 proteins would be conducted prior to making a registration decision.

As discussed in the 2016 ecological risk assessment, the action area for consideration in the listed species assessment is limited to fields in which MON 89034 x TC1507 x MON 87411 x DAS-59122-7 corn is grown, since exposure to insects in these two orders is expected to be limited to direct consumption of corn tissue. Therefore, for any listed species potentially susceptible to the Cry protein toxins expressed in MON 89034 x TC1507 x MON 87411 x DAS-59122-7 corn (coleopterans and lepidopterans) that does not utilize corn plants or corn fields as part of its habitat, a "no effect" determination can be made based on a conclusion of no exposure. EPA has previously determined that indirect effects to listed species are unlikely to result from cultivation of corn expressing Cry proteins specific for coleopteran and lepidopteran pests (USEPA 2010b).

EPA has determined that listed coleopterans and lepidopterans in certain states are not expected to be present on corn fields (see USEPA 2016b, 2016c, 2016d, and 2017). One coleopteran species, the American burying beetle (*Nicrophorus americanus*), may be found in corn fields; however, EPA also previously determined that this species would not be exposed to Cry proteins due to their specific food requirements (USEPA 2010a, 2010b).

Since these analyses did not include all corn growing areas throughout the entire U.S. and its territories, this update expands on/off-field determinations to complete the analysis for lepidopteran and coleopteran species wherever corn may be grown in the U.S. and its territories. A proximity analysis was performed for additional states and territories not included in the assessments cited above, and habitat requirements were investigated for an additional 30 coleopteran and lepidopteran species (see Appendix A below). Based on habitat descriptions provided in U.S. Fish and Wildlife Service documents, as cited in Appendix A, EPA determined that habitat for these coleopteran and lepidopteran species does not include corn fields. Therefore, EPA makes "no effect" determinations for these listed coleopteran and lepidopteran species. Since EPA has determined that no adverse effects will occur to any nontarget organism as a result of the Cry proteins expressed in MON 89034 x TC1507 x MON 87411 x DAS-59122-7 corn, effects to listed species and their designated critical habitats are also not expected. Therefore, a 'No Effect' determination is made for direct and indirect effects to listed species and

their designated critical habitats resulting from the uses of MON 89034 x TC1507 x MON 87411 x DAS-59122-7.

III. Conclusions

The 2016 SAP generally concluded that the data reviewed to support the ecological risk assessment for DvSnf7 dsRNA expressed by event MON 87411 in MON 89034 x TC1507 x MON 87411 x DAS-59122-7 combined trait corn were adequate. The SAP indicated some specific concerns; however, none of the issues raised by the SAP changes EPA's initial risk assessment conclusions for DvSnf7 dsRNA expressed alone or in combination with Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, and Cry34/35Ab1 proteins in MON 89034 x TC1507 x MON 87411 x DAS-59122-7 corn. Therefore, EPA's previous conclusions that DvSnf7 dsRNA is not expected to cause adverse effects to nontarget organisms are still applicable, including conclusions for listed species. EPA also updated the listed species assessment for Cry proteins expressed in MON 89034 x TC1507 x MON 87411 x DAS-59122-7 combined trait corn, and made "no effect" determinations for all listed species and concluded no modification to any designated critical habitats.

To address uncertainties raised by the SAP, additional data would be helpful to confirm environmental fate assumptions used in the risk assessment. These data include:

1) DvSnf7 dsRNA concentrations in soils collected during the growing season and after harvest from fields planted with MON 89034 x TC1507 x MON 87411 x DAS-59122-7 corn; these data would provide *in situ* concentrations of DvSnf7 dsRNA, the lack of which the SAP indicated was an uncertainty
2) Data showing degradation of DvSnf7 dsRNA in MON 89034 x TC1507 x MON 87411 x DAS-59122-7 corn plant tissue in aquatic environments; these data would address uncertainties regarding environmental fate of DvSnf7 dsRNA in corn plant debris deposited in aquatic environments

The above data are not expected to alter EPA's conclusions about nontarget risks, but will address uncertainties raised by the SAP.

IV. References

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Appendix A. No effect determinations for listed lepidopteran and coleopteran species not expected to be in the action area due to habitat requirements that exclude them from corn fields.

Common Name	Scientific Name	Status	States	Habitat Description	References
Lepidopterans					
Bay checkerspot butterfly	*Euphydryas editha bayensis*	T	California	Native grasslands on serpentine soils or similar soils that support larval host plants and nectar sources for adults. The primary larval host plant is a native plantain; larvae use other secondary host plants later in the season. Adults feed on nectar of plants associated with serpentine grasslands. Life cycle is closely associated with host plant biology; host plants germinate from early October to late December and senesce from early April to mid May. Flight season is late February to early May.	Recovery Plan for Serpentine Soil Species in the San Francisco Bay Area (1998), http://ecos.fws.gov/docs/recovery_plan/980930c_v2.pdf
Behren's silverspot butterfly	*Speyeria zerene behrensii*	E	California	Coastal terrace prairie, associated with proximity to the ocean and factors (soil and climatic conditions, disturbance regimes) that maintain prairie habitat. Grazing appears to provide sufficient disturbance at some sites to maintain required habitat. May also be supported by coastal dune systems with similar characteristics and larval host plants. Occupied sites must have larval host plants (Western early blue violet [Viola adunca] and other violets), adult nectar sources, and adult sheltering areas. Adult nectar plants not well known but thought to be reasonably similar to those used by other closely related coastal subspecies (Oregon and Myrtle silverspot). Those nectar sources include several plants from Asteraceae as well as other plant families (see list page 5 of 2012 5-Year Review).	Recovery Plan for the Behren's Silverspot Butterfly (*Speyeria zerene behrensii*) (2015), http://ecos.fws.gov/docs/recovery_plan/20160314_Final%20Behren's%20RP_signed.pdf; Behren's Silverspot Butterfly (*Speyeria zerene behrensii*) 5-Year Review: Summary and Evaluation (2012), http://ecos.fws.gov/docs/five_year_review/doc4007.pdf
Blackburn's sphinx moth	*Manduca blackburni*	E	Hawaii	Mixed species mesic and dry forest communities with both native and introduced plants. Life span is long, and adults are highly mobile. Larvae feed on plants in the nightshade family, including four native tree species within the Nothocestrum genus (two of which are federally listed as endangered: N. breviflorum and N. peltatum), as well as introduced species - Nicotiana tabacum (commercial tobacco), Nicotiana glauca (tree tobacco), Solanum melongena (eggplant), Lycopersicon esculentum (tomato), and possibly Datura stramonium (Jimson weed). Adults have been known to feed on native morning glory (Ipomea indica), halepepe plant (Pleomele auwahiensis), and native Hawaiian species of caper, Capparis sandwichiana and Plumbago zeylanica. I. indica, C. sandiwichiana, and P. zeylanica display characteristics of moth-pollinated plants.	Recovery Plan for Blackburn's Sphinx Moth (*Manduca blackburni*) (2005), http://ecos.fws.gov/docs/recovery_plan/050926.pdf
Callippe silverspot butterfly	*Speyeria callippe callippe*	E	California	Found exclusively within grasslands on hills surrounding San Francisco Bay. Habitat must have sufficient numbers of larval host-plant, Viola pedunculata, on which larvae feed exclusively, and adequate nectar sources for adults. Adults appear to prefer	Callippe silverspot butterfly (*Speyeria callippe callippe*) 5-year review (2009), http://ecos.fws.gov/docs/five_year_review/doc2518.pdf

Common Name	Scientific Name	Status	States	Habitat Description	References
				several species of thistle and mint plants for nectaring, but will utilize other native and non-native plants (see page 8 of 2009 5-year review). Females oviposit near (within 0.9 m) of dried remnants of larval host plant.	
Carson wandering skipper	*Pseudocopaeodes eunus obscurus*	E	California, Nevada	Little is known about the specific habitat requirements of the CWS beyond the similarities recognized among known locations of this subspecies. Habitat is generally characterized as lowland grassland on alkaline substrates with presence of larval host plant and nectaring sources that bloom during flight period of May-July. Larval host plant is Distichlis spicata. Several nectaring sources identified that are tolerant of alkaline soils. Alkaline-intolerant species also used if located in wet areas near larval host plant.	Carson Wandering Skipper (Pseudocopaeodes eunus obscurus) 5-Year Review (2012), http://ecos.fws.gov/docs/five_year_review/doc4039.pdf
El Segundo blue butterfly	*Euphilotes battoides allyni*	E	California	Known only from the El Segundo sand dunes. Distribution is dependent on its food plant, the coast buckwheat (Eriogonum parvifolium), and appears further limited to habitats with high sand content (unclear whether the butterfly could live in habitats containing its food plant but without loose sand). Onset of flight is closely synchronized to the beginning of the flowering cycle of coast buckwheat, and all stages of life cycle depend on this plant. Upon emerging from their pupae, the female El Segundo blue butterflies fly to the flower heads of the food plant to mate and lay eggs. Larvae remain concealed within the flowerhead and pupate underground or in the leaf litter at the base of the food plants. Adult El Segundo blue butterflies are sedentary animals that spend the bulk of their time perching and searching for mating opportunities (males) and ovipositing and feeding (females). From mark-release-recapture work, a few individuals moved distances equivalent to the farthest reaches of the habitat. Other researchers set out mature potted plants at sites up to 0.3 mile (0.5 kilometer) outside the normal distribution area with the objective of finding the offspring of dispersing females. The results were negative. All the flowerheads of two isolated plants in the disturbed foredune area were sampled with no El Segundo blue butterfly early stages found on 184 flowerheads. These data, along with the observation of one adult male at Ballona Wetlands in 1987, indicate dispersal, and/or distant food plant locating ability across distances does occur, but is not a common event.	Recovery Plan for the El Segundo Blue Butterfly (*Euphilotes battoides allyni*) (1998), http://ecos.fws.gov/docs/recovery_plan/980928d.pdf El Segundo Blue Butterfly (*Euphilotes battoides allyni*) 5-Year Review: Summary and Evaluation (2008), http://ecos.fws.gov/docs/five_year_review/doc1896.pdf
Fender's blue butterfly	*Icaricia icarioides fenderi*	E	Oregon	Occurs on upland prairies historically characterized by native bunch grasses (Festuca spp.); association with this habitat mainly results from its dependence on certain lupines as larval host plants, but also uses wet prairies for nectaring and dispersal habitat (according to critical habitat final rule, Fender's blue butterflies use wet prairies that occur near larval host plant	Recovery Plan for the Prairie Species of Western Oregon and Southwestern Washington (2010), https://ecos.fws.gov/docs/recovery_plan/100629.pdf 71 FR 63862-63977 - final rule for designation of critical habitat for Fender's blue butterfly

Common Name	Scientific Name	Status	States	Habitat Description	References
				habitat). Habitat requirements include lupine host plants (Lupinus sulphureus ssp. kincaidii or L. arbustus, and occasionally L. albicaulis) for larval food and oviposition sites and native wildflowers for adult nectar food sources (Allium amplectens, Calochortus tolmiei, Sidalcea malviflora ssp. virgata, Eriophyllum lanatum and Geranium oreganum). Non-native vetches (Vicia sativa and V. hirsuta) are also frequently used as nectar sources, although they are inferior to the native nectar sources. Limited in dispersal ability. Adult butterflies may remain within 2 kilometers (1.2 miles) of their natal lupine patch; anecdotal evidence exists of adult Fender's blues dispersing as far as 5 to 6 kilometers (3.1 to 3.7 miles), but not likely to occur anymore because of habitat fragmentation. At large patches, most are found within 10 meters (33 feet) of lupine patches. The primary larval host plant, Lupinus sulphureus ssp. kincaidii, is federally listed (threatened).	(2006), https://www.gpo.gov/fdsys/pkg/FR-2006-10-31/pdf/06-8809.pdf#page=2
Hermes copper butterfly	*Lycaena hermes*	C	California	Inhabits coastal sage scrub and southern mixed chaparral, and use only spiny redberry (Rhamnus crocea) as a host plant. Researchers report adults are rarely found far from spiny redberry, and take nectar almost exclusively from Eriogonum fasciculatum (California buckwheat). Woody canopy openings with a northern exposure in stands of spiny redberry and adjacent stands of California buckwheat appear to be components of suitable habitat for Hermes copper butterfly. Females deposit single eggs on spiny redberry in the early summer. Eggs overwinter, with larvae reported from mid-April to mid-May followed by pupation on the host plant. Little is known regarding larval biology, as this life stage is little-studied and extremely difficult to find in the field. Adults are typically relatively sedentary – more information is needed, but studies infer that most individuals move less than 656 ft. (200 m), and one study recorded no adult movement across non-habitat areas. Females may disperse longer distances than males, which are represented more in sampling techniques for these studies; however, dispersal is likely inhibited by lack of available habitat in many areas.	Species Assessment Form for *Lycaena hermes (2014)* https://ecos.fws.gov/docs/candidate/assessments/2015/r8/I05C_I01.pdf
Island marble Butterfly	*Euchloe ausonides insulanus*	C	Washington	Previously occurred exclusively in grassland habitat that historically was dominated by the grasses Festuca roemeri (native bunchgrass), Elymus glaucus (blue wildrye), Danthonia californica (California oat-grass), and native forbs. It is now only found on San Juan Island in a single population centered on American Camp, a unit of the San Juan Island National Historical Park that is managed by the National Park Service. According to the latest FR notice regarding status (2016), three known plants serve as larval host plants for the island marble	81 FR 87246-87272 (12-month finding on *petitions to list island marble butterfly*, etc., Dec. 2, 2016), https://www.gpo.gov/fdsys/pkg/FR-2016-04-05/pdf/2016-07809.pdf 71 FR 66292-66298 (12-month finding on *petition to list island marble butterfly*, Nov. 14, 2006), https://www.gpo.gov/fdsys/pkg/FR-2006-11-14/pdf/E6-19064.pdf#page=1

Common Name	Scientific Name	Status	States	Habitat Description	References
				butterfly, all in the mustard family (Brassicaceae): Lepidium virginicum var. menziesii (Menzies' pepperweed), a native species; Brassica rapa (field mustard), a nonnative species; and Sisymbrium altissimum L. (tumble mustard), a nonnative species. Each larval host plant is associated with a specific habitat type: Menzies' pepperweed grows in coastal, nearshore habitat; tumble mustard grows primarily in higher elevation sand-dune habitat; field mustard grows in upland habitat. The island marble butterfly primarily nectars on its larval host plants, but also nectars on a wide variety of additional native and nonnative species. Use of nonnative species may have resulted in a shift in dominance to pasture grasses and other sod-forming grasses associated with agricultural practices, which reduce the establishment and maintenance of native forb species, though it is unclear whether this was brought on by changing preference or availability.	
Kern primrose sphinx moth	*Euproserpinus euterpe*	T	California	Currently known to exist at Walker Basin, Carrizo Plain in San Luis Obispo County, and in the Cuyama Valley. At Walker Basin, habitat includes sandy washes consisting of coarse to fine textured, decomposed granite soil, and dominant vegetation that includes red-stemmed stork's beak (Erodium cicutarium), baby blue-eyes (Nemophila menziesii), rabbit brush (Chyysothamnus nausseosus), gold fields (Lasthenia chrysostoma), and brome grass (Bromus arenarius). At this site the presence of its primary food plant, sun cup or evening primrose Camissonia contorta, is essential. At the Carrizo Plain and Cuyama Valley, habitat includes sandy washes with open soil for morning basking, young alluvial sandy soils that support the food plant, field primrose (Camissonia campestris), soil that is loose enough to allow larvae to burrow and construct shallow pupal chambers, and sufficiently dense stands of C. campestris that allow Kern primrose sphinx moth larvae to travel from stand to stand as they consume their host plants. The flight season of this species was observed to occurs late January through late February at Carrizo Plain, and from mid-March through early April at Walker Basin. Adult nectaring sources were not well known at the time of listing, and further study was included as part of the recovery plan (pages 24-25). Little is further stated about this in the 5-year review. NatureServe species profile states that adults apparently do not feed often, but sometimes take nectar as available from native or exotic flowers.	Kern primrose sphinx moth (*Euproserpinus euterpe*) 5-Year Review (2007), https://ecos.fws.gov/docs/five_year_review/doc1157.pdf Recovery Plan: Kern Primrose Sphinx Moth (*1984*), https://ecos.fws.gov/docs/recovery_plan/840208.pdf NatureServe Species Profile Kern Primrose Sphinx Moth http://explorer.natureserve.org/servlet/NatureServe?searchName=Euproserpinus+euterpe (accessed 3/7/2017)
Laguna Mountains skipper	*Pyrgus ruralis lagunae*	E	California	The proposed rule for designation of critical habitat (2005) states that the Laguna Mountains skipper has specialized habitat requirements within a narrow geographic distribution. It occurs in a matrix of pine and mixed conifer/oak forests, meadows,	Draft Recovery Plan for Laguna Mountains *Skipper* (*Pyrgus ruralis lagunae*) (2015), https://ecos.fws.gov/docs/recovery_plan/20151216_Draft_LMS_RP.pdf

Common Name	Scientific Name	Status	States	Habitat Description	References
				small forest openings, and forest edges that support larval host plants between 3,800 and 6,000 feet (ft) (1,158 and 2,000 meters (m)) in elevation. According to the draft recovery plan (2015), it currently inhabits large wet mountain meadows and associated forest openings at elevations above 3,900 feet (ft) (1,189 meters (m)). Its primary larval host plant, Horkelia clevelandii (Cleveland's Horkelia) is a key component of its habitat. Females deposit eggs on the leaves of the host plant. Larvae then occupy silken shelters constructed on host plants and feed on the host plant during development. They will also use Potentilla glandulosa (common cinquefoil) as a host in the wild, though this plant is not believed to independently support any populations, and may not be used independently of Horkelia clevelandii. Adults use diverse nectar sources in spring, but in summer months, the larval host plant is the main available nectar source.	70 FR 73699-73717 (Designation of Critical Habitat for Laguna Mountains Skipper: Proposed Rule), https://ecos.fws.gov/docs/federal_register/fr4490.pdf
Lange's metalmark butterfly	*Apodemia mormo langei*	E	California	Endemic to the Antioch Dunes of Contra Costa County, California, and the only known extant populations inhabit the Antioch Dunes National Wildlife Refuge. Host plant is the perennial naked stemmed buckwheat (Eriogonum nudum var. auriculum), which occupies areas with open ground and is a sole food source for larvae. Adults use the host plant for perching and also as one of several nectar sources (host plant is preferred nectar source. Females use a greater variety of nectar sources than males. Males have greater tendency to perch or aggregate than females, and move more locally (within 30 m); females may move up to 400 m. Movements of just over one mile have been recorded. Neither sex tends to move far from buckwheat plants – in surveys, adults are typically found closely associated with mature buckwheat stands. Species is univoltine; adults emerge in early August and are observed through September. Egg laying occurs throughout adult flight period; eggs are placed on host plant and are dormant until the rainy season. Larvae overwinter at the base of the host plant, and feed on new plant growth in late fall or early winter. Pupation occurs in mid-summer at the base of the host plant.	Recovery Plan for Three Endangered Species Endemic to Antioch Dunes, California (1984), https://ecos.fws.gov/docs/recovery_plan/Antioch%20Dunes%20Species%20(1).pdf Lange's metalmark butterfly (*Apodemia mormo langei*)…5-year review (2008) https://ecos.fws.gov/docs/five_year_review/doc1927.pdf
Lotis blue butterfly	*Lycaeides argyrognamon lotis*	E	California	Little is known about the biology and life history of this species; putative life history is based on what is known about other subspecies of the northern blue butterfly (of which the lotis blue is also a subspecies). Historically it was recorded from coastal locations in Mendocino and northern Sonoma counties, California. The lotis blue butterfly likely inhabits wet meadows and sphagnum willow bogs; other subspecies of the northern blue butterfly typically occur in wet meadows, bogs, seeps or springs, or in streamside areas. The last known site for the	Lotis Blue Butterfly (*Lycaeides argyrognomon lotis*) 5-Year Review (2011), https://ecos.fws.gov/docs/five_year_review/doc3960.pdf

Common Name	Scientific Name	Status	States	Habitat Description	References
				species was located in a sphagnum bog; however, such habitats may not be typical for this species as they may not support its putative host plant. The lotis blue probably has a single generation per year, with a relatively long adult flight period, extending from mid-April to early July. Eggs are likely laid during the adult flight season. Newly hatched larvae begin to feed immediately, then overwinter in dormancy as small larvae, then resume feeding the next spring. The larvae probably feed for about 4-6 weeks in the spring before pupating. Lotis blue larvae have apparently not been observed; therefore, the larval host plants are not known. Based on closely related species, native plants in the pea family (Fabaceae) are likely candidates. The coast trefoil (Lotus formosissimus) is thought to be a larval food plant. This plant generally occurs in damp areas in meadows, roadside ditches, and forest edges and clearings. Other possible food plants include herbaceous species of lupine.	
Mariana eight-spot butterfly	*Hypolimnas octocula mariannensis*	E	Guam	Mariana eight-spot butterfly is dependent upon two relatively rare host plant species, Procris pedunculata (no common name) and Elatostema calcareum (common name: tapun ayuyu). Both of these forest herbs are found only on karst substrate within the forest ecosystem, draped over boulders and small cliffs. When adult butterflies have been observed, they were always in proximity to the host plants. The two host plants have been recorded on the islands of Guam, Rota, Saipan, and Tinian; however, despite recent surveys (2011–2013) the butterfly is currently known only from the island of Guam.	80 FR 59423-59497 (Endangered Status for 16 Species and Threatened Status for 7 Species in *Micronesia; Final Rule, 2015*), https://www.gpo.gov/fdsys/pkg/FR-2015-10-01/pdf/2015-24443.pdf
Mariana wandering butterfly	*Vagrans egistina*	E	Guam, N. Mariana Islands	Mariana wandering butterfly (Vagrans egistina) is endemic to the islands of Guam and Rota in the Mariana archipelago, in the forest ecosystem. It is thought to be extirpated in Guam and its presence on Rota is not currently known. It may exist in other islands where its host plant is present, but where it has not previously been recorded. The larvae of this butterfly feed on the plant species Maytenus thompsonii (luluhut) in the Celastraceae family, which is endemic to the Mariana Islands.	80 FR 59423-59497 (Endangered Status for 16 Species and Threatened Status for 7 Species in *Micronesia; Final Rule, 2015*), https://www.gpo.gov/fdsys/pkg/FR-2015-10-01/pdf/2015-24443.pdf
Mission blue butterfly	*Icaricia icarioides missionensis*	E	California	Typical habitat is coastal scrubland and grassland vegetation that contains at least one of three larval host plants. The coastal prairie grasslands occupied by this species are disclimax communities (maintenance and regeneration of tbe plants characteristic of these ecosystems are dependent upon irregular perturbation processes tbat preclude normal succession), so presence of colonies is relatively short-lived. Tbe three known larval host plants - Lupinus albifrons (silver lupine), L. varicolor (manycolored lupine), and L. formosus (summer lupine) - are dependent upon natural disturbance processes to establish	San Bruno Elfin Butterfly (*Callophrys mossii bayensis*) and Mission Blue Butterfly (*Icaricia icarioides missionensis*) 5-Year Review (2010), https://ecos.fws.gov/docs/five_year_review/doc3216.pdf

Common Name	Scientific Name	Status	States	Habitat Description	References
				seedlings. All reproductive activities are carried out among patches of the three known larval host plants. Females oviposit on and first and second instar larvae feed on the host plants. Second instar larvae undergo an obligate diapause; most diapause in the leaf litter at the base of the food plants. The following spring, the larvae break diapause and resume feeding. The last instar larvae pupate on or near the base of the Lupinus spp. food plant. Adults feed on a variety of nectar flowers, but do not tend to wander far from areas containing the larval host plants.	
Mount Charleston blue butterfly	*Icaricia (Plebejus) shasta charlestonensis*	E	Nevada	Known to occur only in the high elevations of the Spring Mountains, approx. 40 km west of Las Vegas, Nevada, and centered on lands managed by the Forest Service in the Spring Mountains National Recreation Area of the Humboldt-Toiyabe National Forest within Upper Kyle and Lee Canyons. Natural habitat is relatively flat ridgelines above 2,500 m; isolated individuals have been observed as low as 2,000 m. Areas occupied have exposed soil and rock substrates with limited or no canopy cover or shading. Adults have been documented feeding on nectar from a number of different flowering plants, most frequently Erigeron clokeyi (Clokey's fleabane), Eriogonum umbellatum var. versicolor (sulphurflower buckwheat), Hymenoxys cooperi (Cooper rubberweed), and Hymenoxys lemmonii (Lemmon bitterweed). Nectar plants typically occur within 10 m of larval host plants. Several species appear to be important food plants for larvae, including Astragalus calycosus var. calycosus, Oxytropis oreophila var. oreophila, and Astragalus platytropis. Pupation most likely occurs in the ground litter near the larval host plant. After pupation, adults feed and mate in the same areas where larvae diapause and pupation occurs.	79 FR 41225-41245 (Designation of Critical Habitat for Mount Charleston Blue Butterfly (*Plebejus shasta charlestonensis*, 2014); https://www.gpo.gov/fdsys/pkg/FR-2014-07-15/pdf/2014-16355.pdf
Myrtle's silverspot butterfly	*Speyeria zerene myrtleae*	E	California	Typical habitat for the butterfly and its host plant are coastal dunes, coastal scrub, or coastal prairie, particularly those areas protected from winds. The only known larval host plant for the Myrtle's silverspot butterfly is Viola adunca (western dog violet), though it is unknown if the larvae will feed off other Viola species – other related butterflies will feed from several closely related violet species. The presence of the host plant is a critical factor to the presence of the butterfly, as is availability of nectar sources. Females oviposit on the dried leaves and stems of the host plant. Newly hatched larvae migrate a short distance and enter diapause. In spring, larvae feed on fresh leaves of the host plant. A variety of other flowering plants serve as nectar sources for the adult. Based on a survey Monardella undulata (western pennyroyal) was the most used nectar plant, followed by	Myrtle's silverspot butterfly (Speyeria zerene *myrtleae) 5-Year Review* (2009), https://ecos.fws.gov/docs/five_year_review/doc2394.pdf

Common Name	Scientific Name	Status	States	Habitat Description	References
				Grindelia spp., Erigeron glaucus (seaside daisy), and Abronia latifolia (yellow sand verbena). Other sources include several other broadleaf plants (see list page 7 of 2009 5-year review cited).	
Oregon silverspot butterfly	*Speyeria zerene hippolyta*	T	California, Oregon, Washington	Occupies early successional, coastally-influenced grassland habitat. Presence of the larval host plant, early blue violet (Viola adunca), and adult nectar sources are key factors determining suitable habitat. Females oviposit within or adjacent to areas that contain early blue violets; a field study showed that they select areas with high violet densities for egg-laying. Little is known about the biology of larvae or pupae. Newly hatched first-instar larvae immediately enter diapause, remaining until host plants send up new growth in spring. While the early blue violet is the primary host plant, larvae are also known to feed on yellow stream violets (V. glabella) and Aleutian violets (V. langsdorfii). Pupation occurs in the summer; adults emerge July - September. Adults feed on a variety of nectar sources, and were found to use species in close proximity to violets. Nectar plants most frequently used are native members of the aster (composite) family. They will also nectar on two common introduced species, tansy ragwort (Senecio jacohaea) and false dandelion (Hypochaeris radicata). Adults may travel relatively long distances for nectar.	Oregon silverspot butterfly (Speyeria zerene hippolyta) 5-Year Review Summary and Evaluation (2011), https://ecos.fws.gov/docs/five_year_review/doc3967.pdf Revised Recovery Plan for the Oregon Silverspot Butterfly (*Speyeria zerene hippolyta*) (2001), https://ecos.fws.gov/docs/recovery_plan/010822.pdf
Palos Verdes blue butterfly	*Glaucopsyche lygdamus palosverdesensis*	E	California	Endemic to the Palos Verdes Peninsula in Los Angeles County, California; inhabits coastal sage scrub, which occurs on sandy marine terraces and dry rocky slopes along the Southern California coastline. Requires suitable numbers of larval hostplants and nectar resources to successfully use a habitat patch for an extended period. Coast locoweed (Astragalus trichopodus lonchus) once thought to be the exclusive larval hostplant; however, larvae also are now known to feed on deerweed (Acmispon glaber). The adult flight period is tied to hostplant flowering and generally occurs between late January and early May, and oviposition occurs throughout the flight period. This butterfly is univoltine. Females oviposit on leaves or flowers of the host plant. Larvae feed on the host plant, and pupate in leaf litter beneath host plant. Adults are thought to be relatively poor dispersers. Silvery blue butterflies, of which the Palos Verdes blue butterfly is a subspecies, use a variety of flowers as nectar sources, primarily Asteracae.	Palos Verdes Blue Butterfly (*Glaucopsyche lygdamus palosverdesensis*) 5-Year Review: *Summary and Evaluation* (2014), https://ecos.fws.gov/docs/five_year_review/doc4334.pdf Palos Verdes Blue Butterfly Recovery Plan (1984), https://ecos.fws.gov/docs/recovery_plan/840119.pdf
Puerto Rico harlequin butterfly	*Atlantea tulita*	C	Puerto Rico	Endemic to Puerto Rico; occurs in subtropical moist forest life zone on limestone-derived soil in the northern karst region and in the subtropical wet forest on serpentine derived soil in the Maricao Commonwealth Forest. These areas cover	Species Assessment for Puerto Rico harlequin *butterfly* (2015), https://ecos.fws.gov/docs/candidate/assessments/2016/r4/I0VK_I01.pdf

Common Name	Scientific Name	Status	States	Habitat Description	References
				approximately 1.19% of the total area of Puerto Rico. Has only been observed utilizing the Oplonia spinosa (prickly bush) as its host plant, and only lays eggs in the vegetative stems of the apical zone of the plant. No other stage of host plant is used for ovoposition. Species dispersion is limited by the monophagus habit of the larvae. Chrysalises have been observed attached to dried twigs of the host plant. Adult butterflies feed from the nectar of the flowers available in the areas they occur, including flowers of sea grape, palo de vaca, and cariaquillo. It has also been suggested that this butterfly is relatively sedentary.	
Quino checkerspot butterfly	*Euphydryas editha quino* (=*E. e. wrighti*)	E	California	Habitat characterized by patchy scrub. Adult butterflies will only oviposit on plants recognized as host plants, which include Plantago erecta (erect or dwarf plantain), P. patagonica (Patagonian plantain), Anterrhinum coulterianum (white snapdragon), and Collinsia concolor (Chinese houses). Egg clusters and pre-diapause larval clusters have also been documented on Cordylanthus rigidus (thread-leaved bird's beak) and Castilleja exserta (purple owl's-clover), though use of these plants is rare. Newly hatched larvae remain on the host plant during the first two instars, afterward wandering in search of secondary host plant, which may be the same or a different species. When host plants senesce, larvae may enter diapause. Diapause location is unknown, but thought to be near dense grass and shrub cover. Univoltine; adult flight period occurs late January through early May, though second generation may emerge with sufficient late summer and autumn rainfall. Adults use a variety of nectar sources; physical structure of flowers is the primary factor that determines nectar source use; adult Euphydryas checkerspot butterflies cannot feed on flowers with deep corolla tubes or flowers evolved to be opened by bees. Adults are relatively sedentary; nectar sources greater than 200 meters from larval host plants are not likely used. However, when larval host plants are in short supply, adults will disperse to other areas with suitable habitat. Habitat patch suitability is determined primarily by larval host plant density, topographic diversity, nectar resource availability, and climatic conditions.	Quino Checkerspot Butterfly (*Euphydryas editha quino*) 5-Year Review (2009), https://ecos.fws.gov/docs/five_year_review/doc4341.pdf Recovery Plan for the Quino Checkerspot Butterfly (*Euphydryas editha quino*) (2003), https://ecos.fws.gov/docs/recovery_plan/030917.pdf
San Bruno elfin butterfly	*Callophrys mossii bayensis*	E	California	Habitat is coastal chaparral. Found on steep north facing slopes in the fog-belt of the mountains near San Francisco Bay. Closely associated with its only known larval host plant, Sedum spathulifolium, which occurs in coastal scrub and grassland vegetation, and readily invades road cuts and old quarry faces. The species is univoltine; adult flight season extends from late-February to mid-April. Courtship, mating and reproduction are carried out in the immediate space around the larval host plant. Adults feed on nearby flowering plants with small	San Bruno Elfin Butterfly (*Callophrys mossii bayensis*) and Mission Blue Butterfly (*Icaricia icarioides missionensis*) 5-Year Review (2010), https://ecos.fws.gov/docs/five_year_review/doc3216.pdf

Common Name	Scientific Name	Status	States	Habitat Description	References
				inflorescences, particularly plants in the Apiaceae (carrot) and Asteraceae (sunflower) families. Adults are highly sedentary, typically moving less than 100 meters, with a maximum recorded movement of 800 meters. Eggs are laid on the larval hostplant throughout the flight season. First instar larvae feed on the host plant until they mature, after which they descend to the ground and enter pupal diapause in loose soil and leaf litter.	
Smith's blue butterfly	*Euphilotes enoptes smithi*	E	California	Range is split into two locations along the California coast, in which the butterfly uses different habitats: 1) the northern portion where the butterfly uses dune habitats along Monterey Bay, and 2) scrub, chaparral, and grasslands along the coast of Monterey and northern San Luis Obispo Counties. Vegetation in both habitats is dependent on disturbance. Smith's blue butterflies are univoltine. Adults emerge at peak flowering with host plants, and flight season extends from mid-June to early September. All life stages are dependent on their host buckwheat plants (coast buckwheat (Eriogonum latifolium) and seacliff buckwheat (E. parvifolium), with adults feeding on the nectar and depositing eggs on the flowers and larvae feeding on the flowers and seeds and pupating on or beneath the plants. Adults may also take nectar from naked buckwheat (E. nudum), hut use of this species by larvae has not been observed. The butterflies overwinter as pupae and emerge the following flight season.	Smith's Blue Butterfly (Euphilotes enoptes smithi) 5-year Review: Summary and Evaluation (2006), https://ecos.fws.gov/docs/five_year_review/doc777.pdf
Taylor's (=whulge) Checkerspot	*Euphydryas editha taylori*	E	Oregon, Washington	Occupies open grassland habitat found on prairies, shallow-soil balds (small openings on slopes in a treeless area, dominated by herbaceous vegetation), grassland bluffs, and grassland openings within a forested matrix in south Vancouver Island, British Columbia; the north Olympic Peninsula and the south Puget Sound, Washington; and the Willamette Valley, Oregon. The population on Denman Island in Canada occupies an area that is dominated by grass and forb vegetation. The butterfly is univoltine; adult flight period is late April through early July. Larvae overwinter in the fourth or fifth instar. Females and their larvae utilize plants that contain defensive chemicals known as iridoid glycosides, which have been recognized to influence the selection of oviposition sites by adult nymphalid butterflies. These larval host plants include members of the Broomrape family (Orobanchaceae), such as Castilleja (paintbrushes) and Orthocarpus, which is now known as Triphysaria (owl's clover), and native and nonnative Plantago species. Additional food plants, Veronica serpyllifolia (thymeleaf speedwell) and V. beccabunga ssp. americana (American speedwell), are also used. Remaining populations in Oregon depend on Plantago lanceolata.	78 FR 61451-61503 (Determination of Endangered Status for the Taylor's Checkerspot Butterfly and Threatened Status for the Streaked Horned Lark; Final Rule; 2014), https://www.gpo.gov/fdsys/pkg/FR-2013-10-03/pdf/2013-23567.pdf

Common Name	Scientific Name	Status	States	Habitat Description	References
Coleopterans					
Casey's June Beetle	*Dinacoma caseyi*	E	California	"Knowledge of Casey's June beetle habitat characteristics is primarily based on correlation of known, mapped environmental features with species occupancy. Historically associated with native Sonoran (Coloradan) desert vegetation located on desert alluvial fans and bajadas (compound alluvial fans) at the base of the San Jacinto Mountains, including areas of sandy dry washes with ephemeral flow, and dry upland areas associated with soil deposition from extreme flood events. Most commonly associated with Carsitas series soil (CdC), described by the U.S. Department of Agriculture (USDA) as gravelly sand on 0 to 9 percent slopes, Riverwash (RA) soils, and also Carsitas cobbly sand (ChC) soils. Its burrowing habit would suggest the Casey's June beetle needs soils that are not too rocky or compacted and difficult to burrow in. Occupied habitats such as unprotected vacant lots and wash areas are often characterized by an intermediate level of disturbance, and may include a relatively high cover of nonnative plant species. The species is also present within a gated community adjacent to Palm Canyon Wash, and the survival of the species is thought to be related to low soil disturbance and irrigation that mimics soil moisture levels found in the wash. Larval food plants not well known. "	Recovery Outline for Casey's June Beetle—*March 2013*, http://ecos.fws.gov/docs/recovery_plan/CJB_Recovery_Outline_FINAL.pdf; Endangered and Threatened Wildlife and Plants; Listing Casey's June Beetle (*Dinacoma caseyi*) as Endangered and Designation of Critical Habitat https://www.gpo.gov/fdsys/pkg/FR-2009-07-09/pdf/E9-16282.pdf#page=1
Delta green ground beetle	*Elaphrus viridis*	E	California	Lives in areas of grassland interspersed with vernal pools. Much about life cycle and habitat affinities remains unknown. Both larvae and adults are thought to be generalized predators able to eat many different kinds of prey, though springtails appear to be an important food source. It is believed that adults emerge from diapause and females lay their eggs in early winter, and then the species disappears from view until active adults reappear the following winter. It is also believed that, as vernal pool habitats become dry, the beetle larvae crawl into cracks in the soil, and survive the hot, dry summer and fall as diapausing pupae. The beetle is typically found along the margins of vernal pools and in bare areas along trails and roadsides, where individuals often hide in cracks in the mud and under low-growing vegetation. Adults usually have been found around margins of vernal pools and in bare areas along trails and roadsides, where individuals often hide in cracks in the mud and under low-growing vegetation such as Erodium sp. and Navarretia leucocephala ssp. bakerii. Extent of use of surrounding grasslands is unknown (appears to be affected by rainfall and fullness of vernal pools), but observations of individuals along trails far from water suggests that they may range into the grassland. Based on the 5-year review (page 8), the beetle was found to be closely	Recovery Plan for Vernal Pool Ecosystems of *California and Southern Oregon (2005)*, http://ecos.fws.gov/docs/recovery_plan/060614.pdf; Delta Green Ground Beetle (*Elaphrus viridis*) 5-Year Review: Summary and Evaluation *(2009)*, http://ecos.fws.gov/docs/five_year_review/doc2384.pdf

Common Name	Scientific Name	Status	States	Habitat Description	References
				associated with Pescadero Clay (which forms the clay base to vernal pools and lakes) without excessive build-up of invasive plants.	
Mount Hermon June beetle	*Polyphylla barbata*	E	California	Known only from the Zayante sandhills of Santa Cruz County, California, primarily distributed over an area that is likely less than 10.0 mi2. The Zayante sandhills are comprised of outcrops of sandy soils of the Zayante series, which are endemic to this county. These soils create a microclimate that supports flora distinctly different from the surrounding forest and chaparral communities. Loose, sandy soil is required for burrowing by hoth sexes and all life stages. Habitat conversion to soils with higher organic matter and more advanced successional characteristics does not support populations of this beetle. Majority of life cycle is spent underground. Larvae of this species are believed to be generalists, foraging on roots and subterranean stem material, and fungal mycorrhizae. It is likely that adult males may not feed (life span is thought to be very short); foraging information regarding adult females is unknown.	Zayante band-winged grasshopper and Mount *Hermon June Beetle 5-Year Review* (2009), https://ecos.fws.gov/docs/five_year_review/doc2572.pdf Recovery Plan for Insect and Plant Taxa from *the Santa Cruz Mountains in California* (1998), https://ecos.fws.gov/docs/recovery_plan/980928a.pdf
Ohlone tiger beetle	*Cicindela ohlone*	E	California	Endemic to Santa Cruz County, California; known only from coastal terraces with native grassland habitat. Habitat is associated with specific soil types characterized by shallow, pale, poorly drained clay or sandy clay soil that bakes to a hard crust by summer. The area of habitat currently occupied by active Ohlone tiger beetle larval burrows was estimated to be less than 10 acres as of 2009, though suitable habitat covered an area of 200-300 acres. Both adult and larval Ohlone tiger beetles are found where grasses are low and sparse enough to leave hare ground; open areas are required for construction of larval burrows, thermoregulation, and foraging. Female beetles oviposit in the soil, where, upon hatching, the larvae excavate a burrow. Burrows are found in same habitat occupied by adults. Both adults and larvae are predatory, feeding on small arthropods. Adults are active from late January to early April. The 5-year review says of tiger beetles in general, "Tiger beetles are a well-studied taxonomic group with a large body of scientific literature…Individual species of tiger beetle are generally highly habitat-specific because of oviposition and larval sensitivity to soil moisture, composition, and temperature."	Ohlone tiger beetle (Cicindela ohlone) 5-Year *Review (2009)*, https://ecos.fws.gov/docs/five_year_review/doc3220.pdf
Valley elderberry longhorn beetle	*Desmocerus californicus dimorphus*	T	California	Endemic to the Central Valley of California; dependent on and found only in association with its host plant, elderberry (Sambucus spp.). The elderberry is a common shrub component of riparian forests and adjacent upland vegetation along river corridors of the Central Valley. Adult beetles feed on elderberry nectar, flowers, and foliage. Females lay eggs on the leaves or	77 FR 60237-60276 (Proposed Rule; Removal of the Valley Elderberry Longhorn Beetle From the Federal List of Endangered and Threatened *Wildlife*, 2012), https://www.gpo.gov/fdsys/pkg/FR-2012-10-02/pdf/2012-23843.pdf

Common Name	Scientific Name	Status	States	Habitat Description	References
				stems of living elderberry shrubs. After hatching, larvae bore into living stems where they remain, feeding on pith. Pupation occurs within the stem. Adults live from a few days to a few weeks after emerging between mid-March and mid-June.	

[1] E = Endangered; T = Threatened; C = Candidate