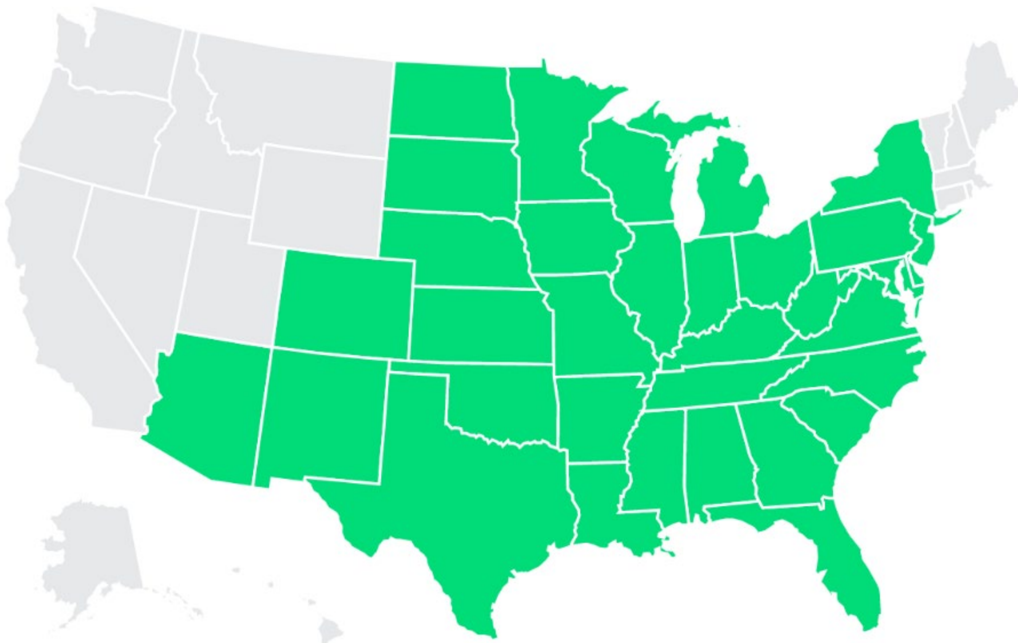
DRAFT

Draft Biological Opinion on the Registration of Enlist One and Enlist Duo Pursuant to the Federal Insecticide, Fungicide, and Rodenticide Act



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INTRODUCTION

This document represents the U. S. Fish and Wildlife Service's (Service) Biological Opinion (Opinion) based on our review of the Environmental Protection Agency's (EPA) proposed national registration of Enlist One and Enlist Duo and their effects on endangered and threatened species and designated critical habitat in accordance with section 7 of the Endangered Species Act (ESA) of 1973, as amended (16 U.S.C. 1531 et seq.). On January 10, 2022, EPA submitted a section 7 consultation initiation package, which requested initiation of formal consultation.

This Opinion is based on information provided in the final Biological Evaluation (BE) for Enlist One and Enlist Duo and its subsequent addendums, many interagency meetings, and other sources of information as described herein.

Due to the complexity and duration of consultation and the Action, and ongoing consideration of listing decisions anticipated during and immediately following the consultation period, EPA and the Service (the Agencies) agreed to evaluate effects to proposed species and critical habitat and candidate species via conferencing, using similar methods for their analyses of listed species and designated critical habitats in both the BE and Opinion.

CONSULTATION BACKGROUND

The ESA section 7(a)(2) consultation process regarding the registration of pesticides pursuant to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) has a long history as discussed below. For more than a decade, the Agencies struggled unsuccessfully to reach consensus on the approaches for assessing the risks of pesticides on endangered and threatened species and their critical habitat. This led to stalled discussions between EPA and the Service and bouts of inactivity on pesticide consultations. The lack of progress resulted in litigation by various non-governmental organizations. Subsequently, the Agencies asked the National Research Council of the National Academies of Science (hereafter, NAS) to evaluate scientific and technical aspects of determining the risks to endangered and threatened species. This section provides a short summary of pesticide litigation related to ESA compliance for FIFRA registration, and the NAS report that led to a path forward for the consultation process.

NAS Report and Path Forward

In September 2010, the Agencies, the National Marine Fisheries Service (NMFS) and the U.S. Department of Agriculture (USDA) jointly requested the NAS to examine scientific and technical issues associated with determining the risk of pesticide registration and use to endangered and threatened species protected under the ESA. The Agencies asked the NAS to provide advice on a range of subjects related to risk assessment and the consultation process, including:

(1) identifying best available scientific data and information;

(2) considering sublethal, indirect and cumulative effects;

(3) assessing the effects of chemical mixtures and inert ingredients;

(4) using models to assist in analyzing the effects of pesticide use;

(5) incorporating uncertainties into the evaluations effectively; and

(6) using geospatial information and datasets in the course of the assessments.

The NAS released its report, entitled "Assessing Risks to Endangered and Threatened Species from Pesticides," on April 30, 2013[1]. It had recommendations on scientific and technical issues related to pesticide consultations under the ESA and FIFRA. Since then, the Agencies worked to implement the recommendations. Joint efforts to date include collaborative relationship building between the Agencies; clarified roles and responsibilities for the Agencies; agency processes designed to improve stakeholder engagement and transparency during the review and consultation processes; multiple joint agency workshops and meetings resulting in interim approaches to assessing risks to endangered and threatened species from pesticides; a plan and schedule for applying the interim approaches to a set of pesticide compounds; and multiple workshops and meetings with stakeholders to improve transparency as the pesticide consultation process evolves. While the Agencies continue their efforts to improve the consultation process, this consultation has incorporated the report's overarching recommendation to implement a three-step risk assessment and consultation approach. This fundamental approach includes the following steps:

1. In Step 1, EPA makes the no effect/may affect determination. If EPA finds that the action will have no effect on *any* listed species or critical habitat, it may proceed with its action without further consultation with the Service. If EPA also finds that the action may affect other listed species or critical habitat designations and seeks the Service's concurrence or requests formal consultation with the Service, the Service will determine whether to adopt EPA's no-effect finding(s) as part of its concurrence or biological opinion.

2. In Step 2, if EPA determines that a pesticide may affect a listed species or it's designated critical habitat, the potential impact is assessed to determine whether species or their designated critical habitats are likely to be adversely affected. The EPA initiates formal consultation for species or their designated critical habitats that are likely to be adversely affected and seeks concurrence from the Service on its "not likely to adversely affect" determinations.

3. In Step 3, using the information provided by EPA in its Step 2 analysis, the Service make jeopardy and destruction or adverse modification determinations for the species and designated critical habitats that EPA determined are likely to be adversely affected.

For Enlist One and Enlist Duo, EPA expanded upon their activities defined in Step 2 of this process by conducting an initial evaluation of the likelihood of jeopardy and destruction or adverse modification for species and critical habitats for which adverse effects were reasonably

[1] The NAS report with recommendations is available on the National Academy of Sciences website using the following hyperlink: http://www.nap.edu/catalog.php?record_id=18344.

certain to occur but for its action. For those species and critical habitat that their analyses suggested were likely to result in a jeopardy or adverse modification call by the Service in formal consultation, EPA developed mitigation measures to reduce exposure to listed species that were incorporated into the action. This is the first BE in which EPA has comprehensively incorporated measures to protect listed species in this manner prior to Step 3 of the process.

Consultation History

The following timeline describes coordination and informal consultation between the EPA and the Service and identifies key points in the consultation process for the proposed national registration of Enlist One and Enlist Duo. While many of the events related to the NAS report and subsequent activities discussed in the paragraphs above form the consultation history for this biological opinion, the listing below is focused on the more recent activities.

Coordination on EPA's Biological Evaluation:

January 2022	EPA transmitted the Enlist One and Enlist Duo biological evaluation (BE) (USEPA 2022a) and the January 10, 2022, addendum (Addendum 1, which included evaluations of agreed upon mitigations between EPA and the registrants) (USEPA 2022b) to the Service.
March 2022	During review of the BE and Addendum 1, the Service determined it was necessary to discuss EPA's proposed new approach and initiated regular meetings between EPA and Service staff to facilitate interagency discussions. Regular staff meetings continued into October 2022.
April 2022	The Service sent a 7(b) letter to EPA and the registrants requesting additional time to continue to work with EPA to improve the informational bases upon which the biological opinion will be issued and, as appropriate, to incorporate that data into our effects determinations. EPA and the registrants agreed to the extension, which anticipates issuance of a final biological opinion by November 18, 2023.
April 2022	EPA transmitted the March 24, 2022, addendum (Addendum 2) to the Service (USEPA 2022c), which evaluated the effects to listed species and designated critical habitat from Enlist One and Enlist Duo use in 128 counties not previously requested by the product registrant for registration, including counties in Minnesota where prohibitions were no longer deemed necessary to protect listed species.
April 19, 2022	The Service held an initial meeting with the registrants (Corteva Agroscience).
June 27, 2022	EPA transmitted the June 16, 2022, Addendum (Addendum 3) to the Service, which re-evaluated the potential effects to 93 listed species and 60 designated critical habitats that EPA previously made "no effect" determinations for the on- and/or off-field exposure zones (USEPA 2022d).
December 02, 2022	EPA transmitted a draft memorandum containing Tier 3 refinements of 2,4-D runoff exposure to wetland plants, as well as revised effects

	determinations for listed species occurring on corn, cotton, and soybean fields (USEPA 2022e).
February 21, 2023	The Service and the EPA met with the technical registrants to discuss additional conservation measures necessary to protect certain species.

CONCURRENCE

In their BE for Enlist One and Enlist Duo, EPA provided determinations of "no effect" for 230 listed species and 27 critical habitats (See Appendix A); the Service adopted EPA's no-effect determinations, and these species were not required to be further evaluated in our biological opinion. The EPA also made "may affect, not likely to adversely affect" determinations for 224 species and 53 critical habitats. We describe our concurrence with EPA's "not likely to adversely affect" determinations in Appendix A.

BIOLOGICAL OPINION

DESCRIPTION OF THE ACTION

The proposed Federal action addressed in this Opinion (hereafter, the Action) is the registration renewal of Enlist One and Enlist Duo under FIFRA. Enlist One contains the active ingredient (AI) 2,4-D choline salt (hereafter, 2,4-D) and Enlist Duo contains the active ingredients 2,4-D and glyphosate dimethylammonium salt (hereafter, glyphosate). Pursuant to FIFRA, before a pesticide product may be sold or distributed in the U.S., it must be exempted or registered with a label identifying approved uses by EPA's Office of Pesticide Programs. Once registered, a pesticide may not legally be used unless the use is consistent with directions on its approved label(s). The EPA authorization of pesticide uses are categorized as FIFRA section 3 (new product registrations), section 18 (emergency use), or 24(c) Special Local Needs. FIFRA requires these chemicals to be reregistered every 15 years according to the Section 3 and Section 24(c) registration. Thus, the Service considers the duration of the Action to be 15 years. The following chemical-specific descriptions are taken largely from EPA's BE for Enlist One and Enlist Duo.

For these herbicides, the Action includes registration of the uses, as described by product labels for Enlist One and Enlist Duo, including the active ingredients, their metabolites and degradates, other ingredients within the formulations (such as adjuvants and inert ingredients), approved tank mixtures, application methods, and required mitigation measures. The Action also includes all authorizations for use of pesticide products, including the use of existing stocks, where applicable. This consultation includes additional proposed changes that are not currently included on product labels, such as changes in county-level use prohibitions and additional conservation measures.

In their BE, EPA considered the authorized use of the chemical over the duration of the Action. If new uses, rate increases, or an application method that increases exposure beyond what was addressed in the BE and this Opinion are approved or proposed, re-initiation of consultation may be required.

Labeled Uses

Current registrations and product labels for Enlist One and Enlist Duo allow application to genetically modified corn, cotton, and soybean crops (i.e., crops containing Enlist traits) for the control of annual and perennial broadleaf weeds as preplant, pre-emergence, and post-emergence (over-the-top) sprays. All of these crops contain genetic traits that make them tolerant to the Enlist pesticide AIs 2,4-D and glyphosate. The genetic traits of the tolerant crops allow for application of 2,4-D and glyphosate to these herbicide-tolerant cotton, corn, and soybeans later in the growing season (later vegetative growth stages; and during reproductive growth stages of soybean and cotton) than to conventional varieties of these crops, thus adding greater flexibility in weed management. The labels also provide use directions for conventional/non-GMO (genetically modified organism) corn, cotton, and soybean as well as for maintenance of fallow acres to be planted with these crops. Ground boom application[2] of these products is the only allowable application method for these crops, and the label mandates the use of specific spray drift mitigation measures including selected spray nozzles and an in-field downwind setback of 30 feet from sensitive vegetation.

In order to lawfully use Enlist One and Enlist Duo, individuals are required to adhere to EPA's registered uses described on the label of products containing Enlist One and Enlist Duo. Pesticide labels are legally enforceable, with all labels containing the following statement: "It is a violation of Federal law to use this product in a manner inconsistent with its labeling." Therefore, because only Enlist One and Enlist Duo products registered under FIFRA may be lawfully used and registered and Enlist One and Enlist Duo products may only be legally used in the manner specified on EPA's label, any effects on the landscape from Enlist One and Enlist Duo application would not occur but for EPA's registration.

Table 1 shows the maximum application rates for corn, cotton, and soybean uses of 2,4-D and glyphosate, as described on the submitted Enlist Duo and Enlist One labels (EPA Reg. Nos. 42719-649 and 42719-695).

Table 1. Label Rates and Application Information for Enlist One and Enlist Duo

<table>
<tr><th>Label (EPA Reg. #)</th><th>Active Ingredient(s) (%)</th><th>Application Method Crop Growth Stage and Maximum Application Rate[1], Application Interval</th></tr>
<tr><td>Enlist Duo®
(42719-649)</td><td>2,4-D choline salt (24.4)[2]

Glyphosate dimethylammonium salt (22.1)[3]</td><td rowspan="2">Ground Broadcast Spray

Pre-plant (Burndown)[6]:
Single application at 0.95 lb a.e./A for 2,4-D choline and 1.009 lb a.e./A for glyphosate (12 days preemergence)[4]

Post-emergence[6]:
Corn: up to V8 stage – 48 inches
Soybean: R2 (full flowering stage)
Cotton: Mid-bloom stage

Maximum single application at 0.95 lb a.e./A for 2,4-D Choline and 1.009 lb a.e./A for glyphosate[4]

Maximum of 2 post-emergence applications</td></tr>
<tr><td>Enlist One®
(42719-695)</td><td>2,4-D choline salt (55.7)[5]</td></tr>
</table>

[2] A method of broadcast pesticide application where an apparatus mounted with spray nozzles systematically applies pesticides to agricultural fields.

		Minimum of 12 days between applications An annual maximum of 3 lb a.e./A[4,6]
[1] Maximum single application rate lb a.e/A (pounds acid equivalent per acre) based on percent a.e. in product and the labeled maximum 4.75 pints/A for Enlist Duo and 2 pints/A rates for Enlist One. [2] Enlist Duo -2,4-dichlorophenoxyacetic acid equivalent: 16.62% - 1.6 lb/gal [3] Enlist Duo -Glyphosate acid equivalent: 17.48% - 1.7 lb/gal [4] Application rates were rounded to nearest 1.0 lb a.e./A for all exposure and risk modeling [5] Enlist One -2,4-dichlorophenoxyacetic acid equivalent: 38% - 3.8 lb/gal [6] Fallow uses are restricted to those acres that will be planted in corn, cotton or soybean. The maximum single application rate and annual maximum rate per acre apply to the fallow use as well.		

Enlist One and Enlist Duo are approved for use in the following 34 states: Alabama, Arizona, Arkansas, Colorado, Delaware, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland, Michigan, Minnesota, Mississippi, Missouri, Nebraska, New Jersey, New Mexico, New York, North Carolina, North Dakota, Ohio, Oklahoma, Pennsylvania, South Carolina, South Dakota, Tennessee, Texas, Virginia, West Virginia, and Wisconsin. For some of these states, there are specific counties that either have sub-county geographic restrictions or completely prohibit the use of these products. See Table 3 in the *Action Area* section of the Opinion.

In addition to these labeled geographic restrictions, there are several mandatory conservation measures included on Enlist and Enlist Duo labels designed to reduce pesticide residues leaving fields through drift and runoff, and to minimize exposure in habitats of listed species and critical habitat. These label requirements are described below in *Conservation Measures* section.

Multi-Active Ingredient Products/Tank Mixes

It is common for these products to be tank mixed with other pesticide products and non-pesticidal agricultural chemicals. To address any concerns with tank mixes that could adversely affect the spray drift properties of these two products, the product labels require that applicators use only approved tank-mix partners from a list maintained by the registrants: https://www.enlist.com/en/herbicides/approved-tank-mix.html.

For Enlist One, there are currently over 1,700 products available to use as a tank mix. These include herbicides, including glyphosate and glufosinate products, insecticides, fungicides, plant growth regulators, fertilizers/nutrients, and other products (e.g., horticultural oils). Approved Enlist Duo tank mixes generally include the same types of products as Enlist One with the exclusion of glyphosate and glufosinate products.

In addition to suitable tank mixes for Enlist One and Enlist Duo, the registrants provide a list of preferred herbicide partners on their website: https://www.enlist.com/en/herbicides/herbicide-partners.html#anchor_1, Table 2.

Table 2. Preferred herbicide partners for Enlist herbicide tank mixtures

Enlist Product	Herbicide Partner	Herbicide Partner active ingredient
Enlist One	Durango DMA	Glyphosate (50.2%)
Enlist One	Liberty 280 SL	Glufosinate-ammonium (24.5%)
Enlist One, Enlist Duo	Kyber	Flumioxazin (5.29%), Metribuzin (15.86%), Pyroxasulfone (6.76%)
Enlist One	EverpreX	S-metolachlor (87.3%)

Inert Ingredients

An inert ingredient or other ingredient, as commonly referred to on product labels, is any substance (or group of structurally similar substances if designated by EPA), other than an "active" ingredient, which is intentionally included in a pesticide product. It is important to note, the term "inert" does not imply that the chemical is nontoxic.

Inert ingredients play a key role in the effectiveness of a pesticidal product. Pesticide products may contain more than one inert ingredient; however, Federal law does not require that these ingredients be identified by name or percentage on the label. All inert ingredients in pesticide products, including those in an inert mixture, must be approved for use by EPA. For those inert ingredients applied to food crops, a tolerance or tolerance exemption is required. Impurities are not included in the definition of inert ingredient. As part of the review process for all new ingredients, a screening-level ecological effects hazard assessment is conducted, in which available data on the toxicity of the inert ingredient to non-target organisms is considered.

For the most current list of inert ingredients approved for food use pesticide products, see the Electronic Code of Federal Regulations (e-CFR)[3]. The majority of inert ingredients can be found in "40 CFR 180.910-180.960." Several sections in "40 CFR Part 180" also include tolerances

[3] https://www.ecfr.gov

and tolerance exemptions[4] for specific inert ingredients where their use is usually significantly limited. The listing of nonfood use inert ingredients, including those that also have food uses, can be found in InertFinder[5].

For Enlist and Enlist Duo, studies that include testing of the formulated product will be inclusive of any inert products in that formulation. Therefore, any effects of inert ingredients will be captured by formulated product testing.

Conservation Measures

The Action also includes conservation measures related to use patterns and label language, including:

- From the BE January 2022 addendum "Mitigation to Avoid Likely Jeopardy for species that use Corn, Cotton and/or Soybean Fields": "Labels submitted for the 2016 assessment were revised to prohibit the use of Enlist Duo in counties where [species] were known to occur, the same prohibitions were placed on the Enlist One product label when it was registered." The list of restricted counties can be found in Table 2 of the BE January 2022 addendum and in the "Description of the Action," above. From the BE "Characterization of the Proposed Use of Enlist One and Enlist Duo" (pg. 22-24): "In addition to these labeled geographic restrictions, there is a mandatory in-field downwind spray drift buffer required for all applications adjacent to 'sensitive areas.'" Sensitive areas are defined as any landscape that is not:
    - Roads and paved or gravel surfaces.
    - Planted agricultural fields (with exception of those planted with "susceptible plants" identified on the labels).
    - Areas covered by the footprint of a building, shade house, silo, feed crib, or other man-made structure with walls and/or roof.
- From the BE January 2022 addendum "Label Changes to Mitigate Runoff Exposures" (pg. 16-17): "Corteva revised the Enlist One and Enlist Duo labels (January 2022) to include updated restrictions to address EPA's concerns about runoff exposure. These updates include a statement about soil moisture and rainfall as well as irrigation":
    - "Do not apply this product when soil is saturated or at field capacity, or when a storm event likely to produce runoff from the treated area is forecasted (by NOAA/National Weather Service, or other similar forecasting service) to occur within 48 hours following application"
    - "Do not irrigate treated fields within 48 hours of application"

[4] See https://www.epa.gov/pesticide-tolerance for details on what tolerances and tolerance exemptions are.
[5] InertFinder is an online database for searching substances used as inert ingredients in pesticide products. It can be found at: https://iaspub.epa.gov/apex/pesticides/f?p=INERTFINDER:1:0::NO:1

- "To reduce the potential for runoff and avoid off field impact from treated fields to maximum extent practicable, applicator must plan/schedule applications to maximize time between an application of this product and anticipated rainfall (or planned irrigation). Application must take place no less than 48 hours prior to irrigation or predicted rainfall (by NOAA/National Weather Service, or other similar forecasting service)"

- From the BE January 2022 addendum "Label Changes to Mitigate Runoff Exposures" (pg. 16-17): "In addition, to minimize runoff, two label statements, consistent with EPA's targeted level of reduction, indicating the requirement of a subset of the runoff mitigation measures listed in Table 3 have been added":

  - "For land with Hydrologic Soil Groups A & B [i.e., soils with low runoff capacity, as determined by the U.S. Department of Agriculture Natural Resource Conservation Service]: The land manager/applicator must effectively implement measures in the following tables to equal a minimum of 4 credits," which are outlined in more detail in Table 3 below.

  - "For land with Hydrologic Soil Groups C & D [i.e., soils with high runoff capacity, as determined by the U.S. Department of Agriculture Natural Resource Conservation Service]: The land manager/applicator must effectively implement the measures in the following tables to equal a minimum of 6 credits"

A copy of Table 3 from the BE January 2022 addendum has been provided below (Table 3).

Table 3. Proposed conservation measures to mitigate runoff exposures

R**unoff Mitigation Measure Name**	**Credits Earned for Effectiveness of Mitigation Measures**[6] [7]
Reducing Chemical Loading Reduced number of applications of the products per year. Applications may be made at any time during crop development, but must maintain a minimum 12-day retreatment interval	3 applications = 0 2 applications = 2 1 application = 4

[6] EPA's runoff concentration reduction effectiveness ration (credit system) is defined as follows: 1 = low, 2 = medium, 3 = high, 4 = very high. Credits assigned to each measures are based on modeling and/or available literature. EPA's numerical effectiveness ratings are approximations of the general amount of reduction and should not be seen as exact values.
[7] The credits assigned to these mitigation practices and the associated reduction of 2,4-D concentrations in surface water runoff should not be regarded as precise measures, but are provided as the approximate level of protection of non-target organisms achievable for each mitigation practice.

Runoff Mitigation Measure Name	**Credits Earned for Effectiveness of Mitigation Measures**[6] [7]
Residue and Tillage Management: no-till, strip-till, ridge-till, and mulch-till	4
Vegetative Filter Strips • 30 foot off-field vegetative buffer on down slope, or • 100 foot off-field vegetative buffer on down slope	30ft: Hydrological Group A or B soils = 2 100ft: Hydrological Group A or B soils = 4 Hydrological Group C or D soils = 1
Cover Crop	2
Contour Buffer Strips or Terracing or Vegetative Barrier	2
Field Border or Grassed Waterways	2
Water and Sediment Basins	1
Contour Farming or Contour Strip cropping	1

In addition to existing conservation measures included on current product labels, the Action includes additional species-specific conservation measures that were added after coordination efforts with the EPA and technical registrants. These measures are in the form of pesticide use limitation areas (PULAs) that are communicated to applicators through the EPA's *Bulletins Live! Two* online platform, which all users are required to access to determine if additional measures apply within their application area. The species-specific measures are as follows:

- For the Attwater's greater prairie-chicken: "Do not make more than 2 applications of Enlist system herbicides per year within the use limitation area."
- For the Spring Creek bladderpod: "Do not apply Enlist system herbicides within the use limitation area from Sept. 30 to May 1."

- For the whorled sunflower: “Do not apply Enlist system herbicides within the use limitation area.”

Details regarding the species-specific conservation measures (as well as the associated letter of commitment from the technical registrant) can be found in Appendix D.

ACTION AREA

The action area is defined as all areas to be affected directly or indirectly by the Federal action, and not merely the immediate area involved in the Action (50 CFR 402.02). Consistent with the ESA Section 7 implementing regulations, in delineating the action area for Enlist One and Enlist Duo, we evaluated the physical, chemical, and biotic effects of the Action on the environment that would not occur but for the action and are reasonably certain to occur. For the reasons mentioned below, the action area for this consultation, as delineated by these effects to the environment, is contained within the following 34 states and illustrated in Figure 1 (USEPA 2022a, 2022b, draft proposed labels dated 5/14/21, 1/11/22):

> Alabama, Arizona, Arkansas, Colorado, Delaware, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland, Michigan, Minnesota, Mississippi, Missouri, Nebraska, New Jersey, New Mexico, New York, North Carolina, North Dakota, Ohio, Oklahoma, Pennsylvania, South Carolina, South Dakota, Tennessee, Texas, Virginia, West Virginia, and Wisconsin.

Figure 1. Map of states that authorized for use of Enlist One and Enlist Duo, as reflected in product labels. States in black are not authorized for Enlist One or Enlist Duo use and are not part of the action area.

Current product labels include prohibitions within a number of counties (and in some cases, subcounty prohibitions) for Enlist One, Enlist Duo, or both for the protection of listed species. After coordination with the EPA and technical registrants, including additional analyses of anticipated risks to listed species within these areas, the Action includes the removal of most of these county level restrictions (Table 4).

Table 4. Summary of county and subcounty restrictions for Enlist herbicides on current labels and whether the proposed Action considers removing county restrictions.

State	Current list of counties where Enlist herbicide use is restricted	Proposed changes to prohibitions
Alabama	Only Enlist Duo restrictions: Covington	Replace with sub-county restriction
Arizona	Prohibited in Yuma, Pinal and Pima counties in areas south of Interstate Highway 8 and west of US Highway 85. In Yuma, Pinal, Maricopa, Pima, La Paz, and Santa Cruz counties, do not use on land administered by the US Fish and Wildlife Service or National Park Service	Removal of restrictions
Colorado	Weld	Removal of restrictions
Florida	Enlist One and Enlist Duo restrictions: Brevard, Broward, Charlotte, Collier, DeSoto, Glades, Hardee, Hendry, Highlands, Hillsborough, Indian River, Lee, Manatee, Martin, Miami--Dade, Okeechobee, Orange, Osceola, Palm Beach, Polk, Sarasota, and St. Lucie Only Enlist Duo restrictions: Jackson, Santa Rosa	No change to restrictions
Georgia	Only Enlist Duo restrictions: Baker, Berrien, Brooks, Burke, Calhoun, Early, Irwin, Lee, Miller, Screven, Worth	Replace with sub-county level restriction
Louisiana	Only Enlist Duo restrictions: Natchitoches	Removal of restrictions

State	Current list of counties where Enlist herbicide use is restricted	Proposed changes to prohibitions
New York	Only Enlist Duo restrictions: Genesee, Seneca, Wayne	Removal of restrictions
Pennsylvania	Adams, Berks, Chester, Cumberland, Lancaster, Lebanon, York	Removal of restrictions
South Carolina	Orangeburg	Removal of restrictions
Tennessee	Wilson	Replace with sub-county level restrictions
Texas	Enlist One and Enlist Duo restrictions: Bell, Cameron, Hidalgo, Hill, McLennan, Nueces, San Patricio, Willacy, Williamson Only Enlist Duo restrictions: Bastrop, Burleson, Colorado, Milam, Refugio, Robertson, Victoria	Replace with sub-county level restrictions

EPA determined the action area using the labeling, including any mandatory control measures for use of the product. Considering existing mitigations required to reduce spray drift, we anticipate the primary route of transport away from application sites is through runoff (see the *Effects of the Action* section for more details). Thus, in addition to the application sites, we expect the action area extends from pesticide use sites (as described above in the *Description of the Action*) to the geographic extent of all of the physical, chemical, and biotic alterations to the environment caused by the stressors produced by the action, which the EPA estimates is 30 meters. The EPA expects typical environmental conditions would limit the extent of runoff to areas close to treatment sites as runoff would be intercepted by physical features like vegetation or other physical obstacles, redirected by local topography, and lost through penetration into the soil column and sorption onto sediment. While runoff may reach further than 30 meters through channelized flow, the EPA expects this runoff will similarly dissipate, degrade, or dilute with distance from treatment sites such that concentrations of Enlist pesticides will be below levels expected to cause adverse effects to the environment. Thus, we agree with EPA's assessment that 30 meters is a sufficient estimate of the extent of off-field exposure.

The product labels for Enlist One and Enlist Duo contain discrete geographic restrictions listed above. Furthermore, local environmental conditions (e.g., weather or topography) is expected to result in varying amounts of transport of Enlist One and Enlist Duo over and/or into terrestrial and aquatic habitats, as well as transport downstream via water bodies, such as wetlands, rivers, and lakes through runoff. Therefore, based on the labeled uses, transport from application sites, and absence of geographic restrictions, it is reasonable to assume one or more labeled uses could occur in any one of the 34 states of the United States (except in the prohibited counties mentioned previously) throughout the duration of the Action. We recognize there may be some areas within the defined action area where applications would generally not occur, and we incorporate that information on a species-by-species basis when relevant.

Analytical Framework for Jeopardy and Destruction or Adverse Modification Determinations

Jeopardy Determination

Section 7(a)(2) of the ESA requires that Federal agencies ensure that any action they authorize, fund, or carry out is not likely to jeopardize the continued existence of listed species. "Jeopardize the continued existence of" means to engage in an action that reasonably would be expected, directly or indirectly, to reduce appreciably the likelihood of both the survival and recovery of a listed species in the wild by reducing the reproduction, numbers, or distribution of that species (50 CFR § 402.02).

The jeopardy analysis in this Opinion considers the effects of the Action and any cumulative effects on the rangewide survival and recovery of the listed species. It relies on four components: (1) the *Status of the Species*, which describes the rangewide condition of the species, the factors responsible for that condition, and its survival and recovery needs; (2) the *Environmental Baseline*, which analyzes the condition of the listed species in the action area, without the consequences to the listed species caused by the Action; (3) the *Effects of the Action*, which includes all consequences to listed species that are caused by the Action, including the consequences of other activities that are caused by the Action; and (4) the *Cumulative Effects*, which evaluates the effects of future, non-Federal activities in the action area on the species.

For purposes of making the jeopardy determination, the Service: (1) reviews all the relevant information, (2) evaluates the current status of the species and environmental baseline, (3) evaluates the effects of the Action and cumulative effects, (4) add the effects of the action and cumulative effects to the environmental baseline, and, in light of the status of the species, determines if the Action is likely to jeopardize listed species.

Destruction or Adverse Modification Determination

Section 7(a)(2) of the ESA requires that Federal agencies ensure that any action they authorize, fund, or carry out is not likely to destroy or to adversely modify designated critical habitat.

> *"Destruction or adverse modification means a direct or indirect alteration that appreciably diminishes the value of critical habitat as a whole for the conservation of a listed species."* (50 CFR 402.02)

The destruction or adverse modification analysis in this Opinion relies on four components: (1) the *Status of Critical Habitat*, which describes the range-wide condition of the critical habitat in terms of the key components (i.e., essential habitat features, physical and biological features, or primary constituent elements) that provide for the conservation of the listed species, the factors responsible for that condition, and the intended value of the critical habitat as a whole for the conservation/recovery of the listed species; (2) the *Environmental Baseline*, which analyzes the condition of the designated critical habitat in the action area, without the consequences to the designated critical habitat caused by the Action; (3) the *Effects of the Action*, which includes all consequences to the critical habitat that are caused by the Action, including the consequences of other activities that are caused by the Action; and (4) *Cumulative Effects*, which evaluate the effects of future non-Federal activities that are reasonably certain to occur in the action area on the key components of critical habitat that provide for the conservation of the listed species and how those impacts are likely to influence the conservation value of the affected critical habitat.

For purposes of making the destruction or adverse modification determination, the Service: (1) reviews all relevant information, (2) evaluates the current status of the critical habitat and environmental baseline, (3) evaluates the effects of the Action and cumulative effects, (4) add the effects of the action and cumulative effects to the environmental baseline, and, in light of the status of the critical habitat, determines if the Action is likely to result in the destruction or adverse modification of critical habitat.

STATUS OF THE SPECIES AND CRITICAL HABITAT

In their BE, EPA identified numerous listed species and designated critical habitats that may be affected by the Action. Species addressed in this Opinion are listed in Table 5. The detailed status of each listed, proposed and candidate species and their proposed or designated critical habitat is provided in Appendix C.

Table 5. Listed species and designated critical habitats addressed in this Opinion for Enlist One and Enlist Duo.[8]

Entity ID	Species	Scientific name	Status	Effect determination
82	Attwater's greater prairie-chicken	*Tympanuchus cupido attwateri*	Endangered	LAA
182	Bog turtle	*Clemmys muhlenbergii*	Threatened	LAA
208	Dusky gopher frog	*Rana sevosa*	Endangered	LAA
558	Pecos (=puzzle =paradox) sunflower	*Helianthus paradoxus*	Threatened	LAA
558	Pecos sunflower	*Helianthus paradoxus*	Designated critical habitat	LAA
568	Spring Creek bladderpod	*Lesquerella perforata*	Endangered	LAA

[8] For calls and conclusions: LAA = "may affect, likely to adversely affect;" NLAA = "may affect, not likely to adversely affect;" NE = "no effect;" NA = Not Applicable (e.g., critical habitat has not been designated for a species).

Entity ID	Species	Scientific name	Status	Effect determination
653	Brooksville bellflower	*Campanula robinsiae*	Endangered	LAA
764	Mohr's Barbara's buttons	*Marshallia mohrii*	Threatened	LAA
819	Green pitcher-plant	*Sarracenia oreophila*	Endangered	LAA
852	Cooley's meadowrue	*Thalictrum cooleyi*	Endangered	LAA
875	Sensitive joint-vetch	*Aeschynomene virginica*	Threatened	LAA
891	Decurrent false aster	*Boltonia decurrens*	Threatened	LAA
960	Pondberry	*Lindera melissifolia*	Endangered	LAA
967	Rough-leaved loosestrife	*Lysimachia asperulaefolia*	Endangered	LAA
976	Canby's dropwort	*Oxypolis canbyi*	Endangered	LAA
982	Godfrey's butterwort	*Pinguicula ionantha*	Threatened	LAA
994	Alabama canebrake pitcher-plant	*Sarracenia rubra ssp. Alabamensis*	Endangered	LAA
996	American chaffseed	*Schwalbea americana*	Endangered	LAA
1028	Virginia sneezeweed	*Helenium virginicum*	Threatened	LAA
1881	Whorled sunflower	*Helianthus verticillatus*	Endangered	LAA
1881	Whorled sunflower	*Helianthus verticillatus*	Designated critical habitat	LAA
3412	Dakota skipper	*Hesperia dacotae*	Threatened	LAA
3412	Dakota skipper	*Hesperia dacotae*	Designated critical habitat	LAA
6617	Neches River rose-mallow	*Hibiscus dasycalyx*	Threatened	LAA
9386	Panama City crayfish	*Procambarus econfinae*	Threatened	LAA
9386	Panama City crayfish	*Procambarus econfinae*	Designated critical habitat	LAA
10147	Poweshiek skipperling	*Oarisma poweshiek*	Endangered	LAA

Entity ID	Species	Scientific name	Status	Effect determination
10147	Poweshiek skipperling	*Oarisma poweshiek*	Designated critical habitat	LAA

ENVIRONMENTAL BASELINE

The environmental baseline is defined as "the condition of the listed species or its designated critical habitat in the action area, without the consequences to the listed species or designated critical habitat caused by the Action. The environmental baseline includes the past and present impacts of all Federal, State, or private actions and other human activities in the action area, the anticipated impacts of all proposed Federal projects in the action area that have already undergone formal or early section 7 consultation, and the impact of State or private actions which are contemporaneous with the consultation in process. The consequences to listed species or designated critical habitat from ongoing agency activities or existing agency facilities that are not within the agency's discretion to modify are part of the environmental baseline." (50 CFR 402. 02).

This Opinion relies on a general discussion of major categories of stressors to listed species and critical habitat that could occur anywhere in the action area. In addition to past and ongoing use of Enlist One and Enlist Duo and other registered pesticides, we explore factors that affect the environmental baseline for listed species and designated critical habitats including, among others, habitat degradation, invasive species, pollution, harvesting, water-related issues, and climate change.

Pesticides

Pesticides are used to kill or manage unwanted plants, animals and other pests (e.g., fungi, microbes). Pesticide use benefits forestry and public health, as well as agriculture. For example, benefits of pesticide use in agriculture are increased food production, increased profits for farmers, and the prevention of diseases. Pesticides benefit human health by killing pests such as mosquitos that that carry and transmit diseases (e.g., malaria, West Nile virus, and Zika). Pesticides are also used in non-agriculture sites for forestry and land management. For example, herbicides are used to control unwanted or invasive non-native plants in natural environments or to aid in the restoration of native habitat.

The use of pesticides and pesticide mixtures as part of past Federal and non-Federal actions have resulted in impacts to listed species, their habitats, and other species on which they depend. When pesticides are applied, they are often mobile in the environment and can enter air, water, and soil. They can have adverse effects to the health of wildlife. Pesticides are stressors that have contributed to the current status of some listed species and designated critical habitats. We further discuss the current and past use of pesticides below.

Enlist One and Enlist Duo Overview

The active ingredient for Enlist One is 2,4-D, a plant growth regulator. The active ingredients for Enlist Duo are 2,4-D and glyphosate. Glyphosate is a systemic herbicide that disrupts key enzymatic pathways in plants. Enlist One and Enlist Duo are only authorized for use on corn, cotton, and soybeans (as well as the maintenance of fallow acres to be planted with these crops) and can only be applied by ground application methods. Enlist pesticide products were initially submitted for consideration of registration in 2013. EPA completed several risk assessments and effects determinations from 2014 through 2016 as a result of additional crops and geographic regions being requested for use. Since 2016, the Enlist One and Enlist Duo products have been registered for use on Enlist corn, Enlist cotton, and Enlist soybeans in 34 states.

While the Enlist system of pesticides are a relatively recently developed formulation of herbicides, the active ingredients 2,4-D and glyphosate have a longer history of use within the United States. 2,4-D has been registered in the United States since 1948 as a post-emergence selective control of broadleaf weeds. While Enlist pesticides, specifically, are only authorized for use on corn, cotton, and soybean, other 2,4-D products are used a variety of other use sites, including other crops, turf, lawns, rights-of-way, and aquatic and forestry applications. While there are various forms of 2,4-D in use, data indicates that 2,4-D generally degrades rapidly in soils, aerobic aquatic environments, is relatively persistent in anaerobic aquatic environments, and has a low binding affinity in mineral soils and sediment (USEPA, 2,4-D RED Facts, 2005).

EPA issued a Registration Standard for glyphosate in 1986. Similar to 2,4-D, there are multiple forms of glyphosate registered aside from the form used in Enlist Duo (i.e., glyphosate dimethylammonium salt). In addition to corn, cotton, and soybean fields authorized on Enlist labels, other glyphosate product labels authorize a variety of other glyphosate uses, such as residential uses, greenhouse, forestry, industrial rights-of-ways, and many additional crop types. Glyphosate absorbs strongly to soil and is generally not expected to move vertically below the six-inch soil layer and are considered immobile in soil. Glyphosate forms are readily degraded by soil microbes to AMPA, which can be further degraded to carbon dioxide. Degradation by hydrolysis or photolysis is generally not expected to be a major source of removal from the environment (USEPA, 2020; USEPA, 1993).

Habitat Degradation

One of the primary factors negatively affecting imperiled species are impacts or changes to their habitat. Human activities have significant and sometimes devastating effects on species and habitats, such as through the introduction of physical and chemical pollutants, or alternation of the environment and the complex ecological systems on which many species depend. There are many kinds of habitat modification activities that have occurred in the United States throughout human history. The earliest modifications likely included the use of fire to encourage or discourage the growth of certain plant communities. The types and extent of habitat changes have increased through time, with much of the land in the United States now used for agriculture, forestry, urban and industrial development, and mining. Each of these land-uses affects species and habitats somewhat differently. The following paragraphs discuss some of the general types of habitat impacts that have been caused by land use conversion and development. Subsequent sections will discuss impacts from various categories of land-use activities.

Data from the USDA (2013) suggest that more than 398,000 acres of grasslands, forests, and other lands were converted to cropland between 2011 and 2012 (Figure 2). Conversion of natural lands also occurs from urbanization, as population centers expand, or to meet demand for various products or resources. For example, beginning in the 1600s and continuing into the early twentieth century, forests of the United States were harvested at a high rate (Masek, et al., 2011). Over the last 100 years, the area of forest cover in the United States has been relatively stable (Masek, et al., 2011), though reforested areas may not provide the same quality of habitat as unharvested, old-growth forests for ESA-listed species.

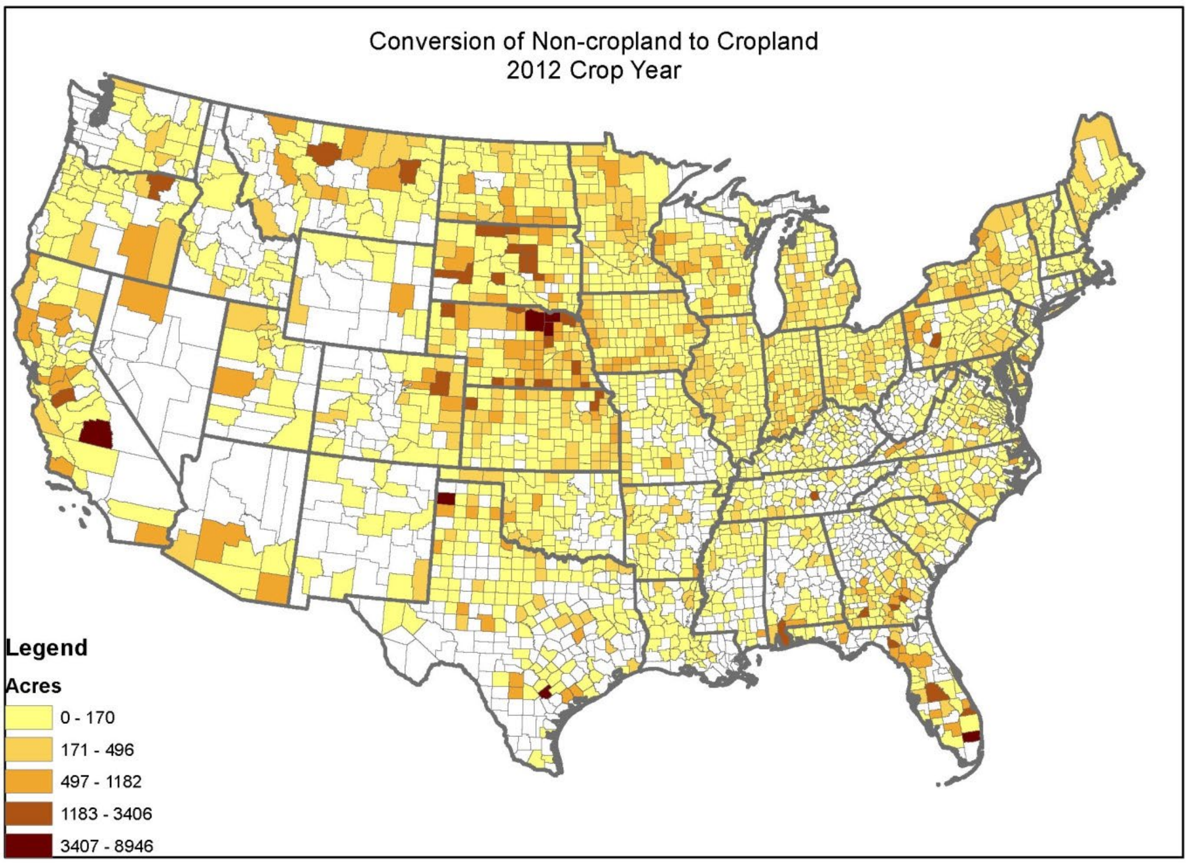

Figure 2. The conversion of land to cropland in 2012 (USDA, 2013)

Through an analysis of threat data compiled from Federal Register documents, Czech et al. (Czech, Krausman, & Devers, 2000) identified urbanization and agriculture as the second and third most common causes of species endangerment in the United States, behind non-native species interactions. Table 6 identifies the causes of endangerment to 877 ESA-listed species identified through Federal Register documents (Czech, Krausman, & Devers, 2000). Species may also be affected by multiple stressors at the same time.

Table 6. Causes of endangerment for ESA-listed species. Modified from Czech et al. 2000.

Cause	Number of species endangered by cause (% of species endangered by cause)
Non-native species	305 (35)
Urbanization	275 (31)
Agriculture	224 (26)
Recreation	186 (21)
Ranching	182 (21)
Reservoir and water diversions	161 (18)
Fire suppression	144 (16)
Pollution	144 (16)
Mining/oil & gas	140 (16)
Industry/military activities	131 (15)
Harvest	120 (14)
Logging	109 (12)
Roads	94 (11)
Loss of genetics viability	92 (10)
Aquifer depletion/wetland filling	77 (9)
Native species competition	77 (9)
Disease	19 (2)
Vandalism	12 (1)

ESA-listed species requiring ephemeral habitats, such as those maintained by fire or flooding, have experienced range reductions because the stochastic events that maintain their habitat are often incompatible with human infrastructure and other development. For example, suppression of wildfires and natural flood events that would occasionally disturb climax ecological communities and create early successional and transitory habitat have reduced habitat available for many species.

While human-induced impacts have occurred throughout history, some activities have also included strategies and actions to reduce these impacts such as the establishment of protected areas and reserves, and implementation of restoration or conservation activities to benefit listed species.

Loss and Degradation of Freshwater Habitats

Freshwater habitats are among the most threatened ecosystems in the world (Leidy & Moyle, 1998). Reviews of aquatic species' conservation status over the past three decades have documented the cumulative effect of anthropogenic and natural stressors on freshwater aquatic ecosystems, resulting in a significant decline in the biodiversity and condition of indigenous fish, mussel, and crayfish communities (Taylor, et al., 2007) (Jelks, et al., 2008). Anthropogenic stressors, the result of many different impacts, are present to some degree in all waterbodies of the United States. These stressors often lead to long-term environmental degradation associated with lowered biodiversity, reduced primary and secondary production, and a lowered capacity or resiliency of the ecosystem to recover to its original state in response to natural perturbations (Rapport & Whitford, 1999).

Rivers and Streams

Many of our nation's rivers and streams have been affected by anthropogenic factors. Degradation of water quality, changes in water quantity (e.g., flows and/or timing), and habitat changes, such as impacts to riparian zones and in-stream features, often reduce habitat quality for listed species. Other changes have included the construction and operation of dams, stream channelization, and dredging to stabilize water levels or depths in rivers or lakes or for other purposes. When examining the impacts of large dams alone, for instance, it is estimated that 75,000 large dams have modified at least 600,000 miles of rivers across the country (IWSRCC, 2011). Habitat loss coupled with other stressors has led to impacts on fish communities as well. By the early 1980s, Judy et al (Judy, Jr., et al., 1984) estimated that approximately 81% of the native fish communities in the United States had been impacted by human activities.

Wetlands

Wetlands provide habitat and perform functions that contribute to the health of ecosystems used by many species. There are many kinds of wetlands (e. g., bogs, fens, estuaries, marshes, etc.), each of which has different characteristics and functions. Wetlands are found in diverse landscapes, including forests, prairies, deserts, and within floodplains of streams (WDNR, 2000). They help maintain cool water temperatures, retain sediments, store and desynchronize flood flows, maintain base flows, and provide food and cover for fish and other aquatic organisms (Beechie, Beamer, & Wasserman, 1994) (Mitsch & Gosselink, 1993) (WDNR, 1998). Wetlands also can improve water quality through nutrient and toxic-chemical removal and/or transformation (Hammer, 1989) (Mitsch & Gosselink, 1993).

The United States originally contained almost 392 million acres of wetlands. During the period between the 1780s and the 1980s, 118 million acres of wetlands were lost. Arkansas, Illinois, Indiana, Iowa, Kentucky, Maryland, Missouri and Ohio lost 70% or more of their original wetland acreage. Florida lost approximately 9. 3 million acres or 46% of its 1780s total (Dahl, 1990). Additionally, the functions of existing wetlands have been reduced. Various factors have contributed to wetland loss and wetland function reduction including agricultural development, urbanization, timber harvest, road construction, and other land-management activities. Efforts to create and restore wetlands and other aquatic habitats by agencies of Federal, state, and local governments, non-governmental organizations, and private individuals have dramatically reduced the rate at which these ecosystems have been destroyed or degraded, but many aquatic habitats continue to be lost each year. Between 2006 and 2009, approximately 13,800 acres of wetlands were lost per year (Dahl, 2011). While this is significantly less than losses experienced in the previous decades (Figure 3), an estimated 72% of U. S. wetlands have already been lost when compared to historical estimates (Dahl, 2011).

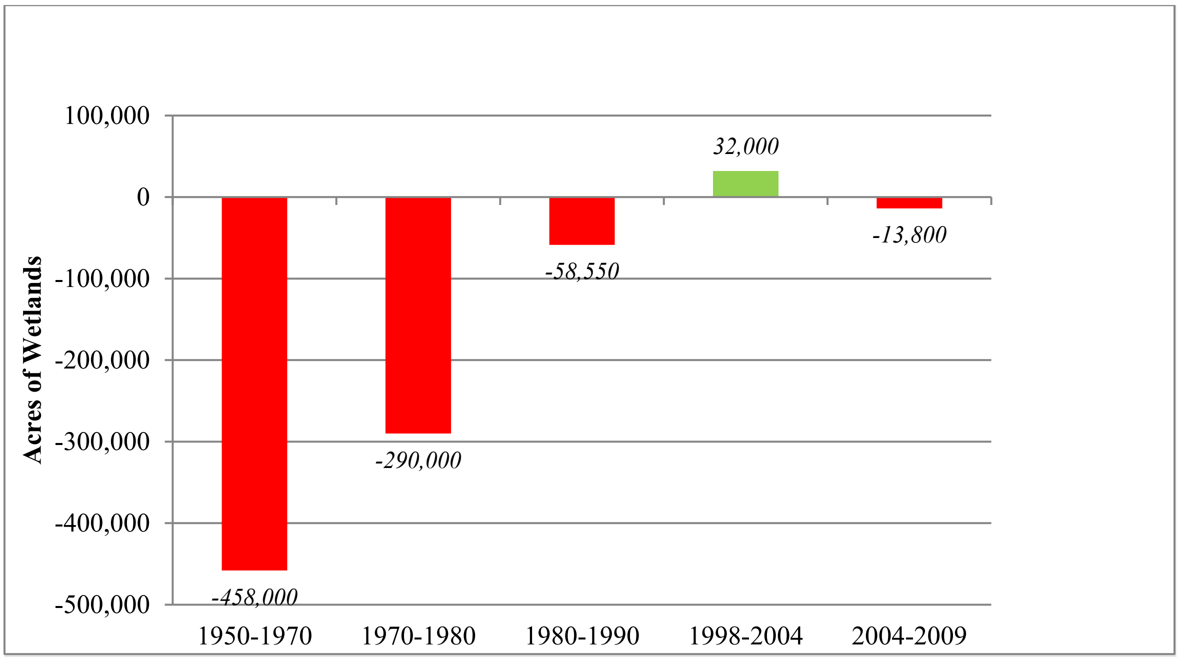

Figure 3. Average annual net wetland acreage loss and gain estimates for the conterminous United States (Dahl, 2011)

Estuaries

Estuaries are some of the most productive ecosystems in the world. Thousands of species of birds, mammals, fish, and other wildlife depend on estuarine habitats as places to live, feed, and reproduce. Many marine organisms, including most commercially important species of fish, depend on estuaries at some point during their development. Estuaries are important nursery and rearing habitat for fishes such as salmon and sturgeon, sea turtles, and many other species. For example, in estuaries that support salmon, changes in habitat and food-web dynamics have altered their capacity to support juvenile salmon (Bottom, Jones, Cornwell, Gray, & Simenstad,

2005) (Fresh, Casillas, Johnson, & Bottom, 2005) (Allen, Pondella, & Horn, 2006) (LCFRB, 2010). Diking and filling activities have reduced the tidal prism, reduced freshwater inflows, reduced sediment inputs, and eliminated emergent and forested wetlands and floodplain habitats. Similarly, dredging activities in shallow coastal estuaries can increase the tidal prism, increase salinities, increase turbidity, release contaminants, lower dissolved oxygen, and reduce nutrient outflow from marshes resulting in a host of negative consequences to these ecosystems. These changes have: reduced fishery productivity; contributed to land losses (e.g., Louisiana, Florida); contributed to fish kills; reduced avian habitats and use; and reduced the resiliency of these areas to stochastic events (e.g., hurricanes). Restoration of estuarine habitats, particularly diked emergent and forested wetlands, reduction of avian predation by terns, and flow manipulations to restore historical flow patterns, may have begun to enhance estuarine productive capacity for salmon, although historical changes in population structure and salmon life histories may prevent salmon from making full use of the productive capacity of estuarine habitats. Mitigation of losses of estuarine marsh in the mid-Atlantic and Gulf of Mexico may roughly keep pace with the losses of the last two decades, but they have not reversed the large losses of the mid-twentieth century (Dahl, 2011).

Shorelines

Significant development and urbanization along shorelines have also occurred in many areas throughout the action area. Impacts have been to mainstem river channels, estuarine, and nearshore marine habitats, and sub-basins in the lower part of major watersheds have been altered as well. Impacts have also occurred in key areas that are important to fish and wildlife, such as coastal and inland avian habitats and salmonid spawning and rearing areas, which may be well upstream of the lowlands.

Portions of nearshore and shoreline habitats in estuarine areas and certain freshwater lakes have been altered with vertical or steeply sloping bulkheads and revetments to protect various developments and structures (e.g., railroads, piers) from wave-induced erosion, stabilize banks and bluffs, retain fill, and create moorage for vessels (BMSL (Battelle Marine Sciences Laboratory), Pentec Environmental, Striplin Environmental Associates, Shapiro Associates, Inc., & King County Department of Natural Resources, 2001). Habitats at risk from direct human alteration include riparian buffers, freshwater habitats (e.g., streams, lakes), and shallow subtidal, intertidal, and shoreline habitats known collectively as the “marine nearshore.” Depending on placement in relationship to drift cells, and other shoreline characteristics, armoring of the shoreline can interrupt the natural inputs of sand from landward bluffs, resulting in sediment deficits within the landscape.

Shoreline development has affected many sensitive habitats. One such sensitive habitat type is submerged aquatic vegetation such as seagrasses. For example, eelgrass beds on the Atlantic coasts grow in the intertidal zone and in mud and sand in the shallow sub-tidal zone and support numerous aquatic species, from geese and dabbling ducks to spawning forage fish. Similarly, turtle grass, shoal grass, manatee grass, and wigeon grass occupy similar ecological niches in the estuaries of the northern Gulf of Mexico. Losses of these sensitive and highly productive habitats are estimated at 20% to 100% in northern Gulf of Mexico estuaries (Duke & Krucynski, 1992). Significant areas containing aquatic beds have been impacted due to harbor development, dock building, dredging, and bottom trawling. Shipping, docks, bulkheads, and other shoreline developments likely contribute to the reduction in submerged aquatic vegetation and other spawning and rearing areas for forage fish.

Agriculture and Grazing

Agriculture is one of the principal industries in many states. Agriculture operations include farming and animal operations and vary in size.

Many animal husbandry operations exist across the country. Large operations include cattle (beef and dairy) and poultry. Other smaller operations raise horses, pigs, sheep, geese and ducks, dairy goats, rabbits, and exotic animals (e.g., llamas, emus, alpacas, ostriches). In 2019, the cattle inventory in the United States was approximately 95 million head. Texas is the state with the most cattle (13%) in the United States, followed by Nebraska and Kansas. Thirty-one states have over 1 million, fourteen have over 2 million and nine have over 3 million head of cattle (based on USDA NASS data as cited in (Cook, 2019)).

Past and present grazing activities have also occurred in a large portion of the action area. (Oliver, Irwin, & Knapp, 1994)(Oliver, Irwin, & Knapp, 1994). In the early 1900s, livestock grazing was authorized on National Forest lands (Oliver, Irwin, & Knapp, 1994). Grazing fees and regulations were implemented in 1906, with grazing allotments initiated the following year, although enforcement efforts were not substantial enough to prevent trespass by unregulated livestock. Grazing resulted in a number of effects, including a general decline in range conditions; excessive use of available forage and resulting conflicts between livestock owners; removal of highly flammable fuels and reduction in ground fires; purposeful setting of fires (by livestock owners) leading to uncontrolled fires; establishment of invasive, non-native vegetation; and increase in siltation of water bodies (Oliver, Irwin, & Knapp, 1994).

As a result, the Bureau of Land Management began regulating grazing on public rangelands in the 1930s. Asian grasses were introduced as stabilizing vegetation for the erosion caused by overgrazing and other practices. The reduction in the number of sheep and localized declines in grazing pressure by cattle in some areas allowed recovery of some of the rangelands (which included forestlands (Oliver, Irwin, & Knapp, 1994)). By the 1960s and 1970s, legislation allowed for monitoring, improvements, and better stewardship of rangeland (including those in National Forests).

Grassland, rangeland, pastureland and cropland forage resources of the conterminous United States include intensively managed pasturelands and croplands throughout the country, and the extensive management of arid and semi-arid regions in central and western United States. Rangelands, pasturelands, and meadows collectively comprise about 55% of the land surface of the United States (approximately 405 million hectares). Privately owned lands constitute about 45% of this total (approximately 260 million hectares). These lands represent the largest and most diverse land resources in the United States. Rangelands and pasturelands include areas such as the semi-arid cold deserts of the Great Basin, the prairies of the Great Plains, the humid native grasslands of the South and East, and the pastures and meadows (natural or semi-natural grasslands often associated with the conservation of hay or silage) within all 50 states.

Effects to Natural Resources

Agricultural lands also provide some benefits for fish and wildlife species. For example, there is generally less impervious surfaces associated with agricultural lands than in urbanized or industrial areas. However, there are several other types of impacts to listed species habitats that are sometimes associated with farms and animal operations. Agricultural practices have contributed to the loss of side-channel areas and riparian vegetation in the floodplain in some areas. The effects of livestock grazing, dairy operations, and crop production often extend many miles upstream or downstream of these activities.

Agricultural operations may also result in the degradation of water quality due to contaminants, such as through introduction or runoff of excess nutrients, fertilizers, pesticides, and other chemicals. For example, livestock production often degrades water quality with the addition of excess nutrients, while pesticides applied to crops can leach into the water table and enter streams from surface water runoff (Rao & Hornsby, 2001) (Spence, Lomnicky, Hughes, & Novitzki, 1996). In periodic reconnaissance studies of streams in nine Midwestern states, the U. S. Geological Survey has documented that large amounts of herbicides and their degradate products are flushed into streams during post-application run-off (Scribner, Battaglin, Goolsby, & Thurman, 2003). In addition, elevated nutrient concentrations from animal manures and agricultural fertilizer application can contribute to excessive growth of aquatic plants and reduced levels of dissolved oxygen, which can adversely affect fish (Embrey & Inkpen, 1998) and other aquatic organisms.

Water quality can also be affected by increases in temperature and sediment loading from agricultural operations. Irrigation systems often result in warmer water temperatures in canals and streams. Warmer temperatures can result from the clearing of shade-providing riparian areas along streams or other waterways, and from solar heating of water flowing across fields or in shallow waterways.

Effects from livestock grazing can alter the nature of the habitat in several ways if management practices are not sufficient to protect habitat functions (WDNR, 1998) (Wissmar, et al., 1994) (Belsky, Matzke, & Uselman, 1999). Community composition can be affected depending on which plants are eaten or trampled by livestock. Trampling can also damage the fragile moss and lichen layer that protects the soil against erosion and non-native invasive vegetation colonization (e.g., cheatgrass) and provides nutrients to the soil. Additional impacts to water quality may result from other practices such as improper spreading of manure and increased surface runoff from overgrazed pasture and/or other areas in which large numbers of animals are confined (Green, Hashim, & Roberts, 2000).

Other impacts result from the maintenance of grazing lands. Fencing can provide environmental benefits such as keeping cattle out of sensitive areas, although there can be periodic impacts from construction, reconstruction, and maintenance activities that require transport and staging of materials, digging of holes, and stringing or re-stringing wires or fences. Chemically treated-wood posts are often used at corners with braces, with interspersed metal posts, wooden posts, or live trees. On flat terrain, power equipment may be used to auger holes and construct fence. On steep terrain, hand tools and chain saws become more common. Rock cribs are often used when crossing areas of bedrock.

Attempts have been made to begin correcting some of the past impacts on the country's ecosystems from agricultural operations. In 1988, EPA began implementing the Federal Insecticide, Fungicide, and Rodenticide Act to regulate the registration and use of chemical pesticides, although some authors note challenges associated with its implementation (Edge, 2001). Additionally, State and Federal landowner-assistance programs have been organized to aid landowners in voluntarily managing their properties to improve water and habitat quality (Edge, 2001).

Forestry

In 1630, at the beginning of European settlement, it is estimated that 46%, or 423 million hectares, of what would become the United States was forest lands. In 2012, forests comprised 309 million hectares (USDA, 2014). From 1850 to 1997, forest land remained relatively stable across the country. According to the U.S. Forest Service, the most acreage of forest lands occurs in the western United States, followed by large areas in the southern and northern parts of the country. Forest lands have been converted to other uses such as agricultural and urban uses. Reserved forest land has doubled since 1953 and now stands at 7% of all forest land in the United States. This reserved forest area includes State and Federal parks and wilderness areas, but does not include conservation easements, areas protected by nongovernmental organizations, and most urban and community parks and reserves. Significant additions to Federal forest reserves occurred after the passage of the Wilderness Act in 1964 (USFS, 2001).

Forested areas that were considered unsuitable for agriculture were frequently managed for timber harvest. Pioneers used river systems to transport logs and other goods. Trees were felled directly into streams, rivers, and saltwater and floated to their destinations, or pulled to streams and trapped behind splash dams, which were dynamited or pulled away, causing logs to sluice downstream. Roads for oxen, then railroads, followed transportation by water. In railroad logging, powerful steam-powered "donkey" engines pulled logs across great distances on the ground, crossing streams and anything else in the way. Following World War II, truck road systems replaced railroads, but smaller streams continued to be used as transportation corridors (CH2M Hill, 2000). After 1930, the introduction of motorized trucks and chainsaws allowed for substantial increases in harvest. Fueled by the demand for new housing and development after World War II, harvest increased dramatically. Initially, harvest focused on large-diameter trees; smaller trees were then harvested, ultimately reducing the number of large-diameter trees. Harvest of uneven-aged trees was practiced until 1940; by the 1950s, even-aged management was practiced.

Much of the lowlands initially harvested for timber were subsequently cleared for agriculture and residential development. While timber harvest continues to occur across the country, conversion of forest lands to other uses have become more common as the human population has grown. Comprehensive tracking of forest conversion rates began in the late 1970s, with the Forest Service Forest Inventory and Analysis data (Bolsinger, McKay, Gedney, & Alerich, 1997).

These data, combined with limited data from the 1930s to the 1970s, indicate general trends in forest conversion..

Effects to Forests

Forestlands have experienced effects related to many different changes, which often vary by area. These changes, which disrupt natural processes that influence forest health, are produced by direct and/or indirect human activities that have occurred in the past and present. These activities include timber harvest, grazing, fire suppression, road construction, and management practices and other influences that have resulted in increases in disease and pests. The impacts of grazing have been discussed previously and will not be addressed in this section.

Intensive forest management generally results in adverse effects such as loss of older forest habitats and habitat structures, increased fragmentation of forest age classes, loss of large contiguous and interior forest habitats, decreased water quality, degradation of riparian and aquatic habitats, and increased displacement of individual species members.

Intensive forest management on most private lands generally maintain these lands in an early seral stage (e.g., 40 to 50 years of age) with relatively few structures such as snags, down logs, large trees, variable vertical layers, and endemic levels of forest “pests” and “diseases,” when compared to what was historically present prior to intensive management.

Timber Harvest

Timber harvest occurs across the nation. Patterns of timber harvesting are influenced by natural events (fire, ice, insects, and disease), management practices, public policies, and market conditions. The average size of harvest units depends on harvesting methods.

There are many kinds of activities associated with timber harvest, with varying degrees or types of impacts associated with each activity. Timber harvest and associated activities, such as road construction and skidding, can increase sediment delivery to streams, clogging substrate interstices, and decreasing stream channel stability and formation. Harvest in riparian areas decreases woody debris recruitment and negatively affects the stream’s response to runoff patterns. Stream temperatures may rise with decreases in the forest canopy and riparian zone shading. Runoff timing and magnitude can also change delivering more water to streams in a shorter period, which causes increased stream energy and scour and reduces base flows during summer months.

Impacts from timber-harvest management have included the removal of large trees that support in-stream habitat structure (“large woody debris”), reduction in riparian areas, increases in water temperatures, increases in erosion and simplification of stream channels (Quigley & Arbelbide, 1997). Past timber harvest practices include the use of heavy equipment in channels, skidding logs across hill slopes, splash damming to transport logs downstream to mills, and road construction (USFS, 2002). Improvements in methodologies have reduced some of the effects from these practices (Oliver, Irwin, & Knapp, 1994). In some areas harvest units have been restricted in size, and greater consideration has been given to the health and appearance of forest landscapes and the biotic communities that depend on them. In some cases, equipment is used and/or engineered in ways to minimize soil disturbance and other habitat impacts. In other cases, however, the methods used may result in increased soil disturbance and extreme fire hazards (e.g., machine piling and burning, accumulation of dead slash from thinning activities, etc.) (Oliver, Irwin, & Knapp, 1994)).

Fire Suppression

Under historical fire regimes, natural disturbance to streams from forest fires resulted in a mosaic of diverse habitats. However, forest management and fire suppression over the past century have increased the likelihood of large, intense forest fires in some areas.

From 1930 to 1960, forest management began in earnest on National Forest lands, and many rural settlers moved to urban areas. Grazing occurred in previously burned areas, while other areas developed into dense stands. Fire-suppression efforts were intensified, with additional funding and crews made available to respond effectively to fight fires. The buildup of fuels likely led to larger, more-destructive fires. From the 1960s to the 1990s, fire prevention allowed the development of dense, closed stands of trees, which varies significantly from pre-management times. Oliver et al. (Oliver, Irwin, & Knapp, 1994) reported that this growth pattern makes stands increasing susceptible to disease and pests. In the 1960s, attitudes toward burning began to change, and the beneficial role of fire was recognized. The use of prescribed fire in certain environments was also encouraged, with certain precautionary measures.

Although scientists have recognized the value of prescribed burning as one of many tools to help return landscapes to natural conditions, some managers have been slow to embrace prescribed burning partially due to the issues surrounding liability. There are also other constraints upon prescribed burning including short-term expenses and air-quality regulations.

Disease and Pests

Pests and disease were present in forestlands prior to European settlement. Several kinds of defoliating insects have been documented, including, but not limited to: Tussock moths, pine butterflies, and bark beetles in Washington State (Oliver, Irwin, & Knapp, 1994). Starting in the 1930s, pest surveys and control were used to combat these pests. Pest control included selective harvesting/or salvage harvest to remove infested trees, the spraying of pesticides (e.g., ethylene dibromide, DDT, and other insecticides), and removal of host plants (e.g., currant [Ribes spp.], host of white pine blister rust).

Since the 1960s, integrated pest management (IPM) has been used to control insect outbreaks. With IPM, several different management and pest-control alternatives are rated against cost/benefit analyses, alternative strategies, ecological considerations, and other concerns to determine the best recourse against the target pest(s). Examples of IPM alternatives include favoring resistant stand structures and/or species in thinning and planting activities, fire prescription, selective use of pesticides, and salvage logging (Oliver, Irwin, & Knapp, 1994).

Urban and Industrial Development

In the United States, urban land acreage quadrupled from 1945 to 2007, with an estimated 61 million acres in urban areas in 2007 (Nickerson, Ebel, Borchers, & Carriazo, 2011). The Census Bureau estimated that urban area increased almost 8 million acres in the 1990s (Lubowski, Vesterby, Bucholtz, Baez, & Roberts, 2006), but despite similar increases for the last several decades, this still represents just 3% of the land area of the U.S. (Bigelow & Borchers, 2017). Figure 4 depicts the 2010 human population density by county and serves as a coarse representation of urbanization. In general, urbanization (including impervious land uses, manufacturing and waste, housing densities, and contributions to greenhouse gas emissions) concentrates effects of water, land, and mineral use, increases loads of pollutants in waters and on the land, increases the likelihood of noise and air pollution, contributes to degradation of ecosystems and habitat for fish, wildlife and plants, lessens biodiversity, and contributes to changes in climate at varying scales.

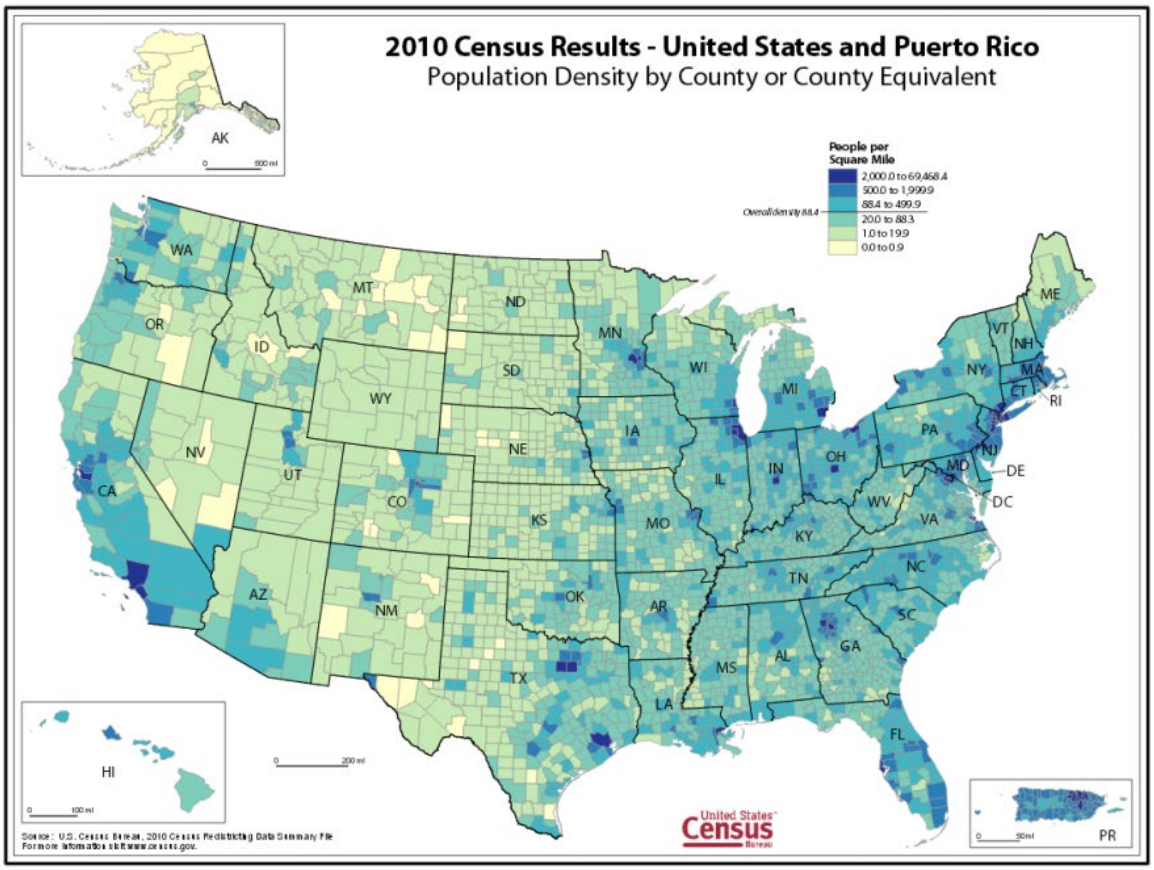

Figure 4. U. S. population density by county (USCB 2010)

Impervious Surfaces

Scientific studies indicate there is a strong relationship between the amount of forest cover, levels of impervious and compacted surfaces in a basin, and the degradation of aquatic systems (Klein, 1979) (Booth, Hartley, & Jackson, 2002). Impervious surfaces associated with residential development and urbanization create one of the most-lasting impacts to stream systems. Changes to hydrology (increased peak flows, increased flow duration, reduced base flows) as a result of loss of forest cover and increases in impervious surfaces are typically the most-common outcomes of intensive development in watersheds (May, Horner, Karr, Mar, & Welch, 1997) (Booth, Hartley, & Jackson, 2002). Increased peak flows and flow duration often lead to the need to engineer channels to address flooding, erosion, and sediment-transport concerns.

Stormwater runoff continues to be a significant contributor of non-point source water pollution in core spawning and rearing areas and foraging, migration, and overwintering habitat areas for salmonids (WSCC, 1999a) (WSCC, 1999b) (KCDNR and WSCC, 2000). Although not typically a direct measure of the influence of development, basin imperviousness is commonly used as an indicator of basin degradation (Booth, Hartley, & Jackson, 2002). Reduction in forest cover and conversion to impervious surfaces can change the hydrological regime of a basin by altering the duration and frequency of runoff, and by decreasing evapotranspiration and groundwater infiltration (May, Horner, Karr, Mar, & Welch, 1997) (Booth, Hartley, & Jackson, 2002). Such changes can be detected when the total percentage of impervious surface in the watershed is as low as 5 to 10% (Booth, Hartley, & Jackson, 2002). Watershed degradation, however, likely occurs with incremental increases in impervious surfaces below these levels, and it is exacerbated by other factors such as reduced riparian cover and pollution (Booth, 2000) (Karr & Chu, 2000) (Booth, Hartley, & Jackson, 2002). Booth et al. (Booth, Hartley, & Jackson, 2002) state, “[t]he most commonly chosen thresholds, maximum 10% effective impervious area and minimum 65% forest cover, mark an observed transition in the downstream channels from minimally to severely degraded stream conditions.” They further assert, “Development that minimizes the damage to aquatic resources cannot rely on structural best management practices (BMP) because there is no evidence that they can mitigate anything but the most egregious consequences of urbanization. Instead, control of watershed land cover changes, including limits to both imperviousness and clearing, must be incorporated.”

The amount of new impervious surfaces has increased significantly in recent history, and this trend will likely continue this trend in the future. Nonetheless, several entities have implemented actions to begin to counter the effects of impervious surface water and stormwater runoff on natural resources. Projects using low-impact development technologies have been planned or constructed. Projects in various areas have included the construction of swales, rain gardens, and narrower roads, and the installation of permeable pavement, among other technologies. Land use planning, zoning, and parks and natural area acquisitions are being used in many communities to incorporate Green Infrastructure into developed landscapes that can help to maintain functional floodplains, stream flows, water quality, fish and wildlife habitat, and other ecosystem functions and public benefits.

Loss of Riparian Buffers

The riparian zone along a stream is a transitional area between the stream and uplands. These areas perform a variety of functions in the ecosystem (WDNR, 2000). Trees and shrubs along the bank provide shade and cover for fish and other aquatic biota, while their roots provide bank stabilization and help to control erosion and sedimentation into the stream. The riparian zone also contributes nutrients, detritus, and fallout insects into a stream, which supports aquatic life.

Vegetation and soils in the riparian zone protect the stream against excess sediments and can sequester pollutants. The riparian zone contributes to the reduction of peak stream flows during floods, and acts as a holding area for water, which is released back into the stream during times of low flow. The trees in the riparian zone serve the ecosystem even after they fall, many of them altering flow and creating habitat features (e.g., pools, riffles, slack areas and off-channel habitats) which benefit fish and other aquatic biota at various life stages.

Many kinds of human activities have impacted riparian zones along streams across the country. These activities include, but are not limited to, urbanization, agriculture, grazing, mining, channelization and damming of streams, logging, and recreational activities (Bolton & Shellberg, 2001). It is estimated that 70% of the original area of riparian ecosystems have been cleared in the United States (Swift, 1984).

While human-related activities conducted within the riparian zone can damage the integrity of a riparian system, activities that occur outside the riparian zone can also create impacts (Kauffman, Mahrt, Mahrt, & Edge, 2001). Riparian zones are often relatively flat and/or are situated at low elevations when compared to adjacent upland topography within a watershed; as a result, sediment and soils, nutrients, water, and substances carried by these vectors from upslope or upstream activities are often deposited by gravity within riparian zones. While the riparian zone helps to buffer streams against these materials, too large a volume can impact the riparian zone's ability to properly function in either the short or long term. The buffering ability of a riparian zone can be affected by landslides, erosion, altered flow regimes, degraded water quality, contaminant inputs, or other sources. Logging, agriculture and grazing, road construction, or other activities can generate these impacts, if appropriate safeguards are not in place.

Although recent changes have been made to many regional and local development regulations to provide protection (i.e., buffers or conservation zones) for riparian areas and streams, the integrity of these areas is frequently compromised by encroachment (May, Horner, Karr, Mar, & Welch, 1997). There is no prescribed corridor size to protect a stream or other water body from all potential impacts. Different riparian widths are required depending on the characteristics of each potential pollutant and the integrity and/or quality of a particular riparian zone; therefore, unless riparian zone widths are carefully evaluated based on adjacent land use and threats, the success of the riparian zone in adequately buffering streams from pollutants is uncertain at best. For many small stream systems, riparian areas are highly degraded or no longer exist, and their restoration is precluded by existing development. Although functional riparian areas have the capacity to mitigate for some of the adverse impacts of development (Morley & Karr, 2002), they cannot effectively address significant impacts from changes to stream hydrology resulting from significant losses of forest cover (May, Horner, Karr, Mar, & Welch, 1997) (Booth, Hartley, & Jackson, 2002).

Roads and Rights-of-Ways

Road (e.g., street and rail) and right-of-way (ROW; e.g., cleared surface and below grade utility lines, pipelines, transmission lines) construction in watersheds can promote simplification and channelization of streams, which reduce the connectivity of surface water and groundwater. Activities associated with road/ROW construction, maintenance, and use can also result in loss or degradation of riparian areas, loss, degradation and fragmentation of terrestrial plant and animal habitats, sedimentation, erosion and slope hazards, reduction of passage, dispersal, or migration (e. g, invertebrate, fish, amphibian, reptile, and mammalian) and increased strike hazards to many classes of animals to name but a few.

Historical methods of road construction were destructive to stream habitats (Palmisano, Ellis, & Kaczynski, 2003). Stream materials (e.g., sand, gravel and cobbles) were often used as fill, and excess excavation materials were pushed over the side of the road bank, where it frequently entered streams. Riparian vegetation and stream banks were damaged using heavy equipment adjacent to and in streams. Side channels were often cutoff or eliminated, and stream channels were confined, resulting in increased bank erosion in certain areas. Lack of adequate drainage led to saturation of roadside soils. In many parts of the action area, road and ROW siting, construction and maintenance practices have not changed significantly through time with regard to conservation of fish, wildlife, and ecosystems. Constriction of floodplains resulted in increased flooding, which continues today in certain areas.

Little specific information is available on the historical origins and use of roads in forested areas outside of the Forest Service lands. Within the Forest Service lands, most forest roads were originally constructed by harvesters for access to forested areas, who then deducted the costs of road construction from final payments to the Forest Service (Oliver, Irwin, & Knapp, 1994). Beginning in the 1950s, the Forest Service began to assert more direct control over the road network on Forest Service lands, and the network increased.

Mining and Mineral Extraction

The U. S. has a history of mining that dates to the early 17th century when iron, lead, silver copper and coal were discovered and mined by the early colonial settlers of New England and the Mid-Atlantic states. Today, every state (and Puerto Rico) produces mined materials or extracts minerals from below the surface (e.g., fuels - coal, oil and gas, building materials – sand, gravel, clay; rare Earth minerals; and those used for industry – aluminum and copper). From the surface loss of habitats (land and water) associated with mining to the effects on (surface and ground) water quality and chemistry, air quality, and effects related to mining waste disposal, few human endeavors have such large scale and consequential effects on the environment as mining and mineral extraction. There are no readily available summary data to illustrate the scale of the various forms of mining; however, a 1975 Corps of Engineers study on strip mining estimated 4.4 million acres and approximately 13,000 miles of rivers and tributaries had been disturbed or adversely impacted by surface coal mining (USACE, 1979). There are surely additional millions of acres, collectively, of surface impacts to land and water given the many other forms of mineral mining and extraction.

Invasive Species

Invasive species are non-native species capable of causing great economic or ecological impacts in areas where they become established. Ecological impacts from biological invasion include predation, disease transmission, competition (for food, light, space), and hybridization. The rate of species invasion has increased over the past 40 or more years due to human population growth, alterations of the environment, and technological advances that allow for the rapid movement of people and products (Pimentel, Zuniga, & Morrison, 2004). Invasive species are considered a contributing factor in the decline of 49% of the imperiled species in the United States (Wilcove, Rothstein, Dubnow, Phillips , & Losos, 1998). Based on factors affecting species associated with island ecology (e.g., small populations, small ranges, high rates of endemism), the impact is often even greater. It is estimated that 75% of the world's threatened birds confined to islands face severe threats from introduced species (BirdLife International, 2008).

There are an estimated 50,000 or more non-native terrestrial and aquatic plant species established in the United States, many of which are outcompeting native plants for habitat (Pimentel, Zuniga, & Morrison, 2004). About half of these species are plants. In some cases, non-native plants are capable of completely dominating new habitats, forming dense monocultures, and completely excluding other native plants. Approximately 97 non-native birds exist in the United States. Many of these non-native birds compete with or displace native birds, and they are vectors for avian diseases. Approximately 53 species of reptiles and amphibians have been introduced to the United States, which often prey upon native species (Pimentel, Zuniga, & Morrison, 2004). More than 4,600 non-native invertebrate species inhabit the United States, some of which are well known for vast ecological impacts, including the decline or extirpation of native species (Pimentel, Zuniga, & Morrison, 2004).

Pollinator Decline

Insects have been experiencing a worldwide decline with potentially negative implications for plant pollination. The drastic declines in insect biomass, abundance and diversity reported in the literature have raised concerns. Extrapolated across the world, insect biomass losses of approximately 25% per decade project a potential little noticed catastrophe. The critical environmental functions of insects mean that consequences of their declines could impact ecosystems by reducing such services as pollination and seed dispersal (Dornelas & Daskalova, 2020). The scope of global and national pollinator decline has been evaluated in numerous studies, a few of which are summarized here.

A study in Illinois used historic data sets to determine the degree of change over 120 years in a temperate forest understory community. The results showed that 50% of bee species in the study area were extirpated and 46% of the original forb-bee interactions were lost (246 of 532) even though all 26 forbs remained present. Specialist pollinators were lost more than generalists even though their host plants were still present. Bees that were specialists, parasites, cavity-nesters, and/or those that participated in weak historic interactions were more likely to be extirpated. The richness of bee species visiting forb C. virginica did not change between 1891 and 1971 but declined by over half in the following 40 years. This decline appeared to be the result of changes in forested habitat (Burkle, 2013).

A second study in Illinois compared a survey of wild bees from 1970–1972 with a survey from 75 years earlier. The more recent survey found 140 bee species, implying a 32% reduction in biodiversity compared to historical records from the same location. Only 59 of the 73 prairie-inhabiting bees and 15 of the 27 forest-dwelling bees were found (Marlin & LaBerge, 2001).

Bumblebee surveys performed in 2004–2006 were compared to surveys from 1971 to 1973 at the same sites, and they were used to evaluate changes in community composition. This study showed quantitative evidence that a bumblebee diverse region of Eastern North America has undergone declines in bumblebee species richness, diversity and relative abundance. During the period ending in 1973, 14 bumblebee species were found, during the period ending in 2006, 11 species were found. No new species were identified. The rusty patched bumblebee (*B. affinis*) was previously widespread and common but has undergone drastic decline and has likely been extirpated throughout much of its range. Of 14 species collected in the first survey, 7 were found to be either absent or decreasing in relative abundance in the second survey, while 4 species exhibited increases in relative abundance (Colla & Packer, 2008).

Another study evaluated changes in the distribution of six bumblebee species by comparing historical records with intensive surveys across 382 locations in the USA. Half of the species declined in abundance by as much as 96% of their initial populations in the last 30 years, and their geographical range was reduced between 23 and 87% (Lozier, Strange, Stewart, & Cameron, 2011).

In Oklahoma, a study determined that only 5 of the 10 species of bumblebees that were present in 1949 were found in 2013 after extensive surveys in 21 counties. Additionally, the species B. variabilis was presumed extinct (Figueroa & Bergey, 2015).

Long term surveys in North America and Europe show terrestrial insects declined in abundance by an average of 9% per decade, whereas freshwater insects increased by 11%. The decline of terrestrial insects was estimated to be 0.92% per year while the increase of freshwater insects was estimated at 1.08% per year. The most compelling evidence for declines in terrestrial insect assemblages was found in North America. Strong evidence exists for both directional trends in temperate zone, Mediterranean and desert climates. The declines appear to be associated with changes in land use. Moderate evidence exists for a negative relationship between terrestrial insect abundance trends and landscape urbanization and may be explained by habitat loss and light and/or chemical pollution (van Klink, et al., 2020).

There is evidence of recent declines in both wild and domesticated pollinators, and parallel declines in the plants that rely upon them. In 54 studies covering 89 plant species, the most frequent proximate cause of reproductive impairment of wild plant populations in fragmented habitats was pollination limitation (Potts, et al., 2010).

Pollution

In addition to direct loss and alteration of aquatic habitat, various contaminants and pollutants have impacted many aquatic ecosystems. In 2008, the Heinz Center for Science, Economics and the Environment (Heinz Center) (Heinz, 2008) published a comprehensive report on the condition of our nation's ecosystems. In their report, the Heinz Center noted the following:

(1) From 1992 to 2001, benchmarks for the protection of aquatic life were exceeded in 50% of streams tested nationwide – 83% of streams in urbanized areas – and 94% of streambed sediments.

(2) Contaminants were detected in approximately 80% of sampled freshwater fish and most of these detected contaminants exceeded wildlife benchmarks (1992 to 2001 data) (Gilliom, et al., 2006). Nearly all saltwater fish tested had at least five contaminants at detectable levels, and concentrations exceeded benchmarks for the protection of human health in one-third of fish tissue samples—most commonly DDT, PCBs, PAHs, and mercury (USEPA, 2007).

(3) Toxic contaminants, as noted above have, been documented in the Lower Columbia River and its tributaries (LCREP, 2007). More than 41,000 bodies of water are listed as impaired by pollutants that include mercury, pathogens, sediment, other metals, nutrient, and oxygen depletion, and other causes (USEPA, 2013a). Pennsylvania reported the greatest number of impaired waters (6,957), followed by Washington (2,420), Michigan (2,352), and Florida (2,292). These figures likely underestimate the true number of impaired water bodies in the United States. For example, EPA's National Aquatic Resource Surveys (NARS) is a probability-based survey that provides a national assessment of the nation's waters and is used to track changes in water quality over time. Through this method, EPA estimates that 50% of the nation's streams (approximately 300,000 miles) and 45% of the nation's lakes (approximately seven million acres) are in fair to poor condition for nitrogen or phosphorus levels relative to reference condition waters (USEPA, 2013b). However, data submitted by the States indicates that only about half of the NARS estimate (155,000 miles of rivers and streams and about four million acres of lakes) have been identified on EPA's 303(d) impaired waters list for nutrient related causes (USEPA, 2013b).

Water quality problems, particularly the problem of non-point sources of pollution, have resulted from changes that humans have imposed onto the landscapes of the United States over the past 100 to 200 years. The mosaic or land uses associated with urban and suburban centers are cited as the primary cause of declining environmental conditions in the United States (Flather, Knowles, & Kendall, 1998) and other areas of the world (Houghton, 1994). Most land areas covered by natural vegetation are highly porous and have very little sheet flow; precipitation falling on these landscapes infiltrates the soil, is transpired by the vegetative cover, or evaporates. The increased transformation of the landscapes of the United States into a mosaic of urban and suburban land uses has increased the area of impervious surfaces such as roads, rooftops, parking lots, driveways, sidewalks, and others. Precipitation that would normally infiltrate soils in forests, grasslands and wetlands falls on and flows over impervious surfaces.

That runoff is then channeled into storm sewers and released directly into surface waters (rivers and streams), which changes the magnitude and variability of water velocity and volume in those receiving waters.

Increases in polluted runoff have been linked to a loss of aquatic species diversity and abundance, which include many important commercial and recreational fish species. Nonpoint source pollution has also contributed to coral reef degradation, fish kills, seagrass bed declines and algal blooms (including toxic algae; (NOAA, 2013)). In addition, many shellfish bed and swimming beach closures can be attributed to polluted runoff. As discussed in EPA's latest National Coastal Condition Report (NCCR), nonpoint sources have been identified as one of the stressors contributing to coastal water pollution (USEPA, 2012a). Since 2001, EPA has periodically released these reports detailing condition of the nation's costal bays and estuaries and assessing trends in water quality in coastal areas. The latest NCR report indicates that coastal water conditions have remained "fair" and the trend assessment demonstrates no significant change in the water quality of U. S. coastal waters since the publication of the NCCR II in 2004 (USEPA, 2012a).

In many estuaries, agricultural activities are major source of nutrients to the estuary and a contributor to the harmful algal blooms in summer, although according to McMahon and Woodside 1997 (USEPA, 2006b) nearly one-third of the total nitrogen inputs and one-fourth of the total phosphorus input to the estuary are from atmospheric sources. The National Estuary Program Condition Report found that nationally, 37% of national estuary program estuaries are in poor condition.

Throughout the twentieth century, mining, agriculture, paper and pulp mills, and municipalities contributed large quantities of pollutants to many estuaries. For example, the Roanoke River and the Albemarle-Pamlico Estuarine Complex which receives water from 43 counties in North Carolina and 38 counties and cities in Virginia. This estuarine system supports an array of ecological and economic functions that are of regional and national importance. Both the lands and waters of the estuarine system support rich natural resources that are intertwined with regional industries including forestry, agriculture, commercial and recreational fishing, tourism, mining, energy development, and others. The critical importance of sustaining the estuarine system was reflected in its Congressional designation as an estuary of national significance in 1987. Even so, today the Albemarle-Pamlico Estuarine Complex is rated in good to fair condition in the National Estuary Program Coastal Condition Report despite that over the past 40-year period data indicate some noticeable changes in the estuary, including increased dissolved oxygen levels, increased pH, decreased levels of suspended solids, and increased chlorophyll a levels (USEPA, 2006b).

Since 1993, EPA has compiled information on locally issued fish advisories and safe eating guidelines. This information is provided to the public to limit or avoid eating certain fish due to contamination of chemical pollutants. The EPA's 2010 National Listing of Fish Advisories database indicates that 98% of the advisories are due (in order of importance) to: mercury, PCBs, chlordane, dioxins, and DDT (USEPA, 2010). Fish advisories have been issued for 36% of the total river miles (approximately 1. 3 million river miles) and 100% of the Great Lakes and connecting waterways (USEPA, 2010). Fish advisories have been steadily increasing over the

National Listing of Fish Advisories period of record (1993 to 2010), but EPA interprets these increases to reflect the increase in the number of water bodies being monitored by States and advances in analytical methods rather than an increase in levels of problematic chemicals (USEPA, 2010).

Water-quality concerns related to urban development include adequate sewage treatment and disposal, transport of contaminants to streams by storm runoff, and preservation of stream corridors. Water availability has been and will continue to be a major, long-term issue in many areas. It is now widely recognized that ground-water withdrawals can deplete stream flows (Morgan & Jones, 1999), and one of the increasing demands for surface water is the need to maintain instream flows for fish and other aquatic biota.

Harvesting

Some ESA-listed species, such as salmonids and freshwater mussels, are economically important species harvested as food. Harvesting and exploitation, often associated with the pearl industry, is identified as a contributing factor to18% of the imperiled freshwater mussels of the United States (Strayer, et al., 2004). After species are listed as threatened or endangered under the ESA, they receive protection from overharvesting since this action would require a permit issued by the Service, with permits generally limited to certain categories of activities that would benefit the conservation and recovery of the species. Although harvest is a historical threat to many ESA-listed species and illegal harvests still likely occur to some degree, it, now, rarely affects species substantially, and it is not expected to greatly affect currently listed species in the action area in the future.

Water-Related Issues

As noted above in the sections related to rivers and streams, wetlands, and estuaries, impacts to species and their habitat have occurred in these habitats due to various human activities. Stream channels in many areas have been significantly altered by dredging, channelization, and the construction of dikes and revetments for flood control and bank protection. These activities have simplified once complex stream channels. More specifically these changes are degrading and eliminating important foraging and migration, as well as overwintering habitats for salmonids and other biota. Such changes can also result in the removal of riparian vegetation, thus precluding recruitment of large woody debris. Developments such as these can also reduce or preclude options for restoration of floodplain areas important for reestablishing off-channel habitats and maintaining groundwater recharge.

The following subsections briefly describe different impacts to features or characteristics of aquatic habitats.

Water Diversion

Dikes, levees, dams, and other diversions have reduced the level of watershed connectivity in several areas of the country. Diversion projects have been implemented for several human needs, including but not limited to, flood control, conversion of wetlands to agricultural lands, bank protection, water supply, road construction, or a combination of these objectives.

Many streams have been channelized, diverted, and confined through the construction of dikes, levees, berms, revetments, embankments, and other structures. The shapes and configurations of the structures vary based on their purpose; however, the construction of each kind of structure results in physical and biological impacts to the stream morphology and community (Bolton & Shellberg, 2001). The construction of flood-control structures, tide gates, and water-diversion structures have contributed to the degradation and fragmentation of migratory corridors, and elimination of historical foraging, migration, and overwintering habitats within the region. Channelization (and often its associated bank armoring) results in simplification of the stream, and has resulted in changes in flow, velocity, and movement of water in many streams. These changes are often at least a portion of the goal of a project, which may be designed to reduce flood damage to property, exclude water, or store water for future use. While these changes may be favorable to property owners or project proponents, such actions often result in substantial changes to aquatic and terrestrial habitats and their use by biota.

Dikes and levees result in several impacts to aquatic species and habitat. Aside from loss of estuarine habitat from construction, dikes reduce tidal flushing, sometimes resulting in increased sedimentation; dikes also may have marked effects on tidal channel biota on the seaward side of the structure (Hood, 2004). The construction of dikes may result in decreased sinuosity and complexity in certain channels and prevent energy dissipation during flood events.

Florida has two large restoration projects underway to address environmental problems caused by dikes. In 1992, the Kissimmee River Restoration Program was authorized by Congress. In 1999, the U. S. Army Corps of Engineers (USACE) and the South Florida Water Management District began construction in central Florida. Upon its completion in 2020, the project will restore 20,000 acres of wetlands and 44 miles of historic river channel (USACE, 2019).

The greater Everglades ecosystem historically encompassed 18,000 sq. miles from central Florida to the Florida Keys. Water flowed south into Lake Okeechobee and then spilled over its banks into the sawgrass plains, open water sloughs, rocky glades, and marl prairies and finally into the Gulf of Mexico and Florida and Biscayne Bays. The USACE installed a massive network of canals, levees, and water conservation areas that blocked sheet flow to urban areas and provided water for dry season use. The Comprehensive Everglades Restoration Plan was authorized by Congress in 2000. The plan will “restore, preserve, and protect the south Florida ecosystem while providing for other water –related needs of the region, including water supply and flood protection” (SFNRC, 2016).

Recent restoration efforts have focused on the benefits of restoring ecosystem functions affected by diversion structures. In 2002, the Nisqually Tribe removed a portion of a dike in Red Salmon Slough, reconnecting 31 acres of former pastureland to the Nisqually River Estuary (SPSSEG, 2002) (Carlson, 2005). This action was undertaken to benefit juvenile salmonids, other fish species, and migratory birds. At Spencer Island in Snohomish County, two 250-foot-long breaches were made in an estuary dike to reconnect approximately 250 acres of estuarine marsh (Carlson, 2005).

Culverts and Other Fish-passage Barriers

Improperly installed, sized, or failed culverts have been identified as barriers for fish movement and migration. Although historically placed, fish-passage barriers continue to impede fish passage in many streams. Several groups have made efforts to inventory and remove fish barriers under their jurisdiction, often either removing barrier culverts or replacing them with a more-suitable structure (Peck, 2005). Removal of fish barriers may be achieved through several different kinds of activities (Peck, 2005). Removal of a barrier culvert is often undertaken when a crossing is no longer needed. If a crossing is necessary, other options include bridges or other specific methodologies: stream simulation, roughened-channel design, no-slope methodology, or hydraulic design.

Dams

Dams are built for many purposes, including power generation, irrigation, flood control, recreation, and water supply (WDNR, 2000). These facilities have far-reaching effects on both aquatic and terrestrial habitat and biota. The controlled flow from a dam facility often slows the movement of the rivers, and changes the natural cycle of river flows, resulting in areas that are either drier than normal (because the water is being held behind the reservoir) or flooded by much higher levels of water. Changing the depth and flow of rivers also affects the water's temperature, either increasing or decreasing temperatures from the normal state. Dams affect the flow of many different materials (e.g., sediments, nutrients, and other materials such as large woody debris) carried in the river waters. Free-flowing rivers regularly flood and recede, collecting and depositing these materials both laterally and downstream. For example, rivers carry a great deal of sediment and nutrients down river, eventually depositing it in the deltas and estuaries where freshwater enters saltwater. Dams arrest this process; consequently, reservoirs eventually fill with sediments and inadequate amounts of sediment reach the downstream deltas and estuaries. Coastal beaches in turn lose the source of sand normally deposited on them by coastal currents that would ordinarily redistribute the sediments.

Dams often delay or block passage of anadromous fish to upstream reaches of the stream; such an obstacle can increase predation rates on these fish, cause injury or mortality as fish are trapped in unscreened canals or attempt to travel through turbines. In many cases, dams have likely been constructed at or near historical natural barriers to anadromous fish passage (USFWS 2004). The ability of anadromous fish to access areas above man-made barriers is important not only for the survival of individuals and populations of the species, but also for the integrity of the ecosystems they support (Cederholm, et al., 2000). Anadromous fish provide organic matter and nutrients to both aquatic and terrestrial habitats via their carcasses, eggs, milt, excrement, and fry. Staging and spawning adults are also consumed as prey by aquatic and terrestrial predators. The organic matter and nutrients contributed by anadromous fish enrich macroinvertebrate and terrestrial communities, which in turn provide food for other organisms, including anadromous salmonid fry and juveniles. Scavenging and predatory fish, birds, mammals, and other animals also consume fry, juvenile, and adult salmon, their eggs, and their carcasses, often leaving remnants of carcasses in a more-accessible form for smaller scavenging fauna. Rich marine-derived nutrients from anadromous fish are transported to the reach of stream in which they die, into the lower reaches of the stream and estuary through downstream drift, and across habitat or ecosystem boundaries by mobile mammals, birds, and fish.

Certain facilities have implemented fish-passage structures or transport systems to allow upstream movement of anadromous fish; however, the risk of disease, stress, and other interference with migration and reproduction may occur as a result of these systems.

Water Quantity and Use

The diversion, storage, and use of water is based on increasing demand, fueled by population and economic growth. Water availability varies based on annual weather patterns and may change in the future as climate change affects weather patterns and water supply. Year-round water withdrawals are no longer available from many lakes and streams, to protect aquatic species and existing water rights in many western states.

A significant amount of water is used for irrigation of agricultural lands, which can affect ecosystems. Irrigation is used to maintain urban irrigated lands, forest nurseries, seed orchards, and recreational areas. Water withdrawal also occurs as a source for rural domestic use, stock watering, municipal and light industrial water supply, and for industrial use; however, the dominant off-channel water use is for irrigation (Wissmar, et al., 1994).

Effects associated with irrigation-water withdrawal includes effects from water storage and drainage, increased water temperatures (which can become thermal barriers for salmonids and other aquatic species), pollutants (such as runoff containing pesticides and fertilizers), high sediment levels, and lower stream flows (Wissmar, et al., 1994) (Krupka, 2005). Lower flows and associated stream dewatering affect aquatic habitat and biota (Wissmar, et al., 1994). Diversions and fish ladders associated with irrigation also have a variety of effects since not all are screened or pass all life stages of fish; irrigation systems may also divert a substantial amount of stream flow. The effects of these structures in aggregate to anadromous fish and other aquatic biota can be severe. However, through permitting and the Federal Energy Regulatory Commission relicensing processes, several efforts have been initiated to reduce existing effects. These efforts include but are not limited to: proper screening of existing diversions and other structures; reduction of temperature, sediment, and pesticide effects to waterways; reduction of the quantity of water diverted to provide access; and reduction of fish-passage barriers.

There have been several attempts to reduce impacts from dams, irrigation-water withdrawal, and other water-diversion activities. Some of the efforts to minimize effects to anadromous fish were undertaken relatively early (Palmisano, Ellis, & Kaczynski, 2003). For example, irrigation diversions were screened in the 1930s, although the screens did not protect all life stages, nor were they adequately maintained. More recently, watershed-planning units have been organized in some areas in response to the Watershed Planning Act, to address issues regarding water availability and quality, instream flow, and habitat protection (WDNR, 2000).

Water Quality

Good water quality is essential to the health of habitats and the biotic communities that depend on them. Poor water quality affects both aquatic terrestrial species and communities through the food chain. There are many kinds of pollutants or contaminants that affect water quality in waterways, many of which are direct results of the activities described elsewhere in the baseline discussion. In addition to contaminants, such as metals or fecal coliform, water quality is also determined by abiotic (temperature, dissolved oxygen levels, pH, turbidity, etc.), and biotic (invertebrates, fish, etc.) indicators.

This analysis will look at several contaminants in aquatic habitats, and then examine water quality from the perspective of abiotic and biotic indicators associated with marine and freshwater environments. It should be noted that analyses of many pollutants that "exceed recommended levels" are based on statistics for human exposure and health. While effects to animals (e.g., fish) are often used in acute and chronic tests, such tests generally are limited to observations of mortality or relatively short-term growth and development; they are not commonly performed on listed species. Sublethal effects, such as behavior and long-term survival, are also not generally analyzed.

Contaminants

Contaminants enter waterways through a variety of pathways. Contaminants in stormwater runoff, for example, may include oil, grease, and heavy metals from roadways and other paved areas, and pesticides from residential developments. Other sources of toxic contaminants are discharges of municipal and industrial wastewater, leaching contaminants from treated wood (e.g., creosote) and other components of shoreline structures, and channel dredging, which can result in resuspension of contaminated sediments. Discharges from sewage-treatment plants may be treated prior to discharge into receiving waters. However, according to the literature, the treatment likely does not adequately remove potentially harmful compounds that are considered persistent, bio-accumulative, and toxic, or those that may have endocrine-disrupting properties (Bennie, 1999) (CSTEE, 1999) (Daughton & Ternes, 1999) (Servos, 1999).

Many of the contaminants are associated with sediments, and they are taken up by bottom-dwelling biota and many of the organisms at the base of the food chain. Many sediment contaminants do not break down very quickly. Animals that live in contaminated sediments can accumulate high levels of these substances, with concentrations in biota sometimes thousands of times higher than background levels in the surrounding habitat. As these animals move into other areas, or are preyed upon by more-mobile animals, the contaminants are transmitted up the food chain and may biomagnify. Consequently, predators can have very high contaminant levels, even if they have spent little or no time within the contaminated areas.

Contaminants (and their concentrations in the environment) vary by region and habitat type, and include inorganic (e.g., metals) and organic chemicals (e.g., certain pesticides, phthalates). Some chemicals, such as chlorinated organic compounds and their breakdown products, persist in the environment because bacteria and chemical reactions break them down slowly (PSWQAT, 2000). Although the effects from many of these chemicals have been at least partially analyzed, little is known about the synergistic effects of the chemicals; in many areas, multiple substances are present in the habitat and/or biota.

Inorganic Chemicals

Inorganic chemicals include, among other substances, metals and certain pesticides. Sources of mercury, lead, and other metals in water bodies include hazardous material spills, pipes, vehicle emissions, discarded batteries, paints, dyes, and stormwater runoff and can cause neurological or reproductive damage in humans and other animals (Hinman, 2005). The presence of certain metals in marine waters have triggered fish and shellfish consumption advisories in many areas. Overall, however, levels of arsenic, copper, lead, and mercury have either declined or remained steady (as opposed to increasing) in sediments and shellfish tissues during the past decade (Hinman, 2005).

Organic chemicals

A variety of organic chemicals have been detected in waterways, including, but not limited to, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), poly-bromated diphenyl ethers (PBDEs), chlorinated pesticides (e.g., DDT [dichloro diphenyl trichloroethane]), dioxins, certain pharmaceuticals and other emerging compounds.

PAHs are present in fossil fuels and other sources; certain types of PAHs are formed when fossil fuels and other organic materials are burned. Other sources include coal, oil spills, leaking underground fuel tanks, creosote, and asphalt. PAHs are found in urban and industrial areas and are associated with liver lesions in English sole in small, concentrated areas of sediment or “hot spots” (Hinman, 2005). Fish and shellfish consumption advisories have been issued in some areas due to the presence of this chemical. Exposure is linked to increased risks of cancer and to impaired immune function, reproduction, and development. Concentrations of PAHs in the Sound are often quite high compared to concentrations measured elsewhere around the United States.

Chlorinated organic compounds, such as PCBs, dioxins, and DDT are found in solvents, electrical coolants and lubricants, pesticides, herbicides, and treated wood (Hinman, 2005). These compounds and their breakdown products persist in the environment because bacteria and chemical reactions break them down slowly (PSWQAT, 2000). The use of PCBs was common until the 1970s when they were phased out in the United States and Canada. These chemicals are now banned in the United States; however, they continue to leach from landfills, other disposal sites, and contaminated sediments. PCBs enter natural environments and biota from these sources and from airborne fallout deposited after circulating across the globe from continuing sources in Asia (WDNR, 2000). PCBs are slow to degrade, float in air and water, permeate soil, and accumulate in animal fat. Generally speaking, the higher an animal is on the food chain, and the longer lived, the greater the concentrations of these toxins.

Chemicals, such as dioxins and furans, are generated as industrial process byproducts, and they are linked to cancer, liver disease, and skin lesions in humans. Chlorinated pesticides, such as DDT, are linked to liver disease, cancer, hormone disruption, the thinning of bird eggshells, and reproductive and developmental damage. Fry (Fry, 1995) identified organochlorine compounds as a prevalent non-oil pollution threat within the range of the murrelet. Specifically, polychlorinated dibenzo-dioxins (PCDD) and polychlorinated dibenzo-furans (PCDF) which are contained in pulp-mill discharges, cause significant injury to fish, birds, and estuarine environments. PCDDs and PCDFs bio-accumulate in marine sediments, fish, and fish-eating birds and impair bird health and production. There has been no record of bio-accumulated residues or breeding impairment in marbled murrelets to date, although murrelets that feed in areas of historical or current discharge from bleached-paper mills could be at risk from eating fish with bio-accumulated organochlorine compounds.

Other chemicals include phthalates, which come from plastics, certain soaps, and other products. Much of the exposure from these chemicals to biota occurs via wastewater from treatment plants. The effects from these chemicals are not well known, but they may affect growth and development in fish (Hinman, 2005). Pharmaceuticals and personal-care products, such as oral contraceptives, antibiotics, and other prescription drugs, as well as soaps, fragrances, and other compounds, enter the aquatic environment through sewage and wastewater-treatment plants. Effects and risks to aquatic biota from these substances have not been fully analyzed; however, Daughton and Ternes (1999) note that even substances that are not persistent but are frequently or continually released may impact aquatic species, which may have exposure throughout entire lifecycles and multiple generations. They also note that many of these products are being released worldwide in volumes comparable to chemicals associated with agriculture.

Fecal Coliform

The presence of fecal coliform bacteria is a significant water-quality issue in some areas. Fecal waste enters waters from sources such as poorly managed septic systems, wastewater treatment facilities, stormwater (which washes fecal matter in upland areas into waterways), and animal operations, and contains bacteria and viruses that can result in the contamination of shellfish beds and other resources (WDOE, 2000) (Hinman, 2005).

Levels of fecal coliform in streams and rivers are measured along with other water-quality parameters. The (WDOE, 2000) reports that 52 freshwater monitoring stations have been consistently surveyed since 1995 for fecal coliform, and that, with one exception, the stations are indicating that stream conditions regarding this parameter are either improving or there has been no change (i.e., no significant deterioration) in stream conditions.

Members of two bacteria groups, coliforms and fecal streptococci, are used as indicators of possible sewage contamination because they are commonly found in human and animal feces. Although they are generally not harmful, they indicate the possible presence of pathogenic (disease-causing) bacteria, viruses, and protozoans that also live in human and animal digestive systems. Therefore, their presence in streams suggests that pathogenic microorganisms might also be present; swimming in water and eating shellfish are possible risks to the human and animal health. Since it is difficult, time-consuming, and expensive to test directly for the presence of a large variety of pathogens, water is usually tested for coliforms and fecal streptococci instead. Sources of fecal contamination to surface waters include wastewater treatment plants, on-site septic systems, domestic and wild animal manure, and storm runoff. In addition to this possible health risk, these pathogenic organisms can cause the occurrence of cloudy water, unpleasant odors, and an increased oxygen demand." (USEPA, 2012b).

Excess Nutrients

Excessive amounts of nutrients can come from many sources, including lawn fertilizers applied to yards and other areas, agricultural chemicals applied to fields, and fecal matter from septic fields and failing septic systems. Excess nutrients can affect both surface water and groundwater. For example, (WDOE, 2005) reports that 7% of public-water-supply wells have high nitrate-nitrogen levels, with many of the affected sites clustered in highly populated and rural farming areas. As a result of the input of excess nutrients, aquatic systems and the biota that depend on them have experienced several effects (WDNR, 2000). Excessive nutrients in water cause algae and phytoplankton to grow prolifically. This prolific growth results not only in increased photosynthesis, but also in increased respiration by algae, phytoplankton, and other aquatic plants, which depletes the oxygen necessary for aquatic fauna survival. An increase in numbers of algae and phytoplankton decreases light penetration, reducing the depth to which freshwater and marine aquatic plants (e.g., eelgrass) can grow, especially in lacustrine and marine environments. In turn, there are fewer aquatic plants to provide oxygen and high volumes of decomposing organic matter further consumes valuable oxygen.

Toxic algae blooms are another result of excess nutrient input into aquatic systems. Certain types of algae cause Paralytic Shellfish Poisoning, also known as red tide, which affects organisms (including humans) that consume shellfish, although they seem to be harmless to the shellfish themselves.

Other Pollutants

In addition to the pollutants listed above, other contaminants have impacted aquatic (and terrestrial) habitats around the country. Hazardous waste is generated by a variety of sources. Large industries, which generate most of the hazardous waste, include (in order of decreasing contributions) equipment manufacturing, primary and fabricated metals, chemicals and petroleum, lumber and wood products, and other sources. Smaller businesses, such as dry cleaners, printers, and auto repair shops, also generate hazardous waste, which can pollute aquatic and terrestrial habitats if the waste is not handled properly.

Solid waste (i.e., trash) is generated in almost all aspects of society. As populations have grown, the amount of solid waste generation has also increased. Solid waste is generated primarily from municipal sources, and to a lesser degree from industrial and commercial waste and other sources. Leakages from landfills as well as unauthorized dumping of garbage and waste chemicals can be a problem whether they occur directly into waters or on land with the potential to impact aquatic and terrestrial habitats and the species that inhabit them.

Abiotic Indicators

In addition to the presence of contaminants, other parameters are also indicative of water quality. These indicators include (but are not limited to) temperature, dissolved oxygen, pH, turbidity, and instream flow. Many of the activities discussed elsewhere in the Environmental Baseline section can have effects on these indicators. For example, sediment erosion may transport substances such as pesticides or fertilizers into a stream. The addition of excess nutrients from fertilizers often results in a decrease in the levels of dissolved oxygen as described above, potentially resulting in impaired function in the stream. The excess amount of sediments introduced during an acute or chronic erosion event may also result in suspended sediment and turbidity impacts to aquatic biota, which would further stress fauna experiencing low impact levels. An increase in temperature (as a result of removal of shading riparian vegetation, for example) is another type of stressor on aquatic biota, and when such an increase occurs in concert with other impacts, the result can be devastating to aquatic biota. If conditions do not result in lethal or sublethal effects to biota, they may influence the amount of time a mobile organism spends in the affected reach of a stream.

Biotic Indicators

Certain types of organisms have been used to indicate the health of aquatic systems. The species evaluated may focus on specific concerns, such as the effects of fisheries on certain fish populations, or they may provide general information regarding water-quality trends. Aquatic invertebrates can also provide site-specific information on the health of aquatic systems such as streams, lakes, or estuaries. For example, protocols have been designed to assess water quality and habitats by sampling benthic invertebrates in streams (Barbour, Gerritsen, Snyder, & Stribling, 1999) and in estuarine environments (Simenstad, Tanner, Thom, & Conquest, 1991). Biological monitoring provides better information for aquatic biota because degradation of sensitive ecosystem processes is more often detected. This type of monitoring directly measures the most sensitive at-risk resources and looks at human influence on stream characteristics over time. Of the 31 sites, data on 24 reaches were reported (Butkus, 2004). The results of this monitoring indicated that 50% of the sites were not meeting the conditions necessary for supporting the aquatic community; it was recorded that only 21% of the sites were designated as fully supportive.

Climate Change

All species discussed in this Opinion are or may be threatened by the effects of global climatic change. The Intergovernmental Panel on Climate Change (IPCC) estimated that observed global mean surface temperature for the decade 2006-2015 was 0. 87 °C (likely between 0. 75°C and 0. 99°C) higher than the average over the 1850-1900 period (IPCC, 2018). This temperature increase is greater than what would be expected given the range of natural climatic variability recorded over the past 1,000 years (Crowley & Berner, 2001). The IPCC estimates that the last 30 years were likely the warmest 30-year period of the last 1,400 years, and that global mean surface temperature change will likely increase in the range of 0. 3 to 0. 7 degrees Celsius over the next 20 years.

Warming water temperatures attributed to climate change can have significant effects on survival, reproduction, and growth rates of aquatic organisms (Staudinger, et al., 2012). For example, warmer water temperatures have been identified as a factor in the decline and disappearance of mussel and barnacle beds in the Northwest (Harley, 2011). Shifts in migration timing of pink salmon (*Oncorhynchus gorbuscha*), which may lead to high pre-spawning mortality, have also been tied to warmer water temperatures (Taylor J. A., 2008).(McMenamin, Hadly, & Wright, 2008). Increasing atmospheric temperatures have already contributed to changes in the quality of freshwater, coastal, and marine ecosystems. Also, they have contributed to the decline of populations of endangered and threatened species (Karl, Melillo, & Peterson, 2009) (Littell, Elsner, Whitely-Binder, & Snover, 2009) (Mantua, Hare, Zhang, Wallace, & Francis, 1997).

Climate change is also expected to impact the timing and intensity of stream seasonal flows (Staudinger, et al., 2012). Warmer temperatures are expected to reduce snow accumulation and increase stream flows during the winter, cause spring snowmelt to occur earlier in the year, and reduced summer stream flows in rivers that depend on snow melt. As a result, seasonal stream flow timing will likely shift significantly in sensitive watersheds (Littell, Elsner, Whitely-Binder, & Snover, 2009). Warmer temperatures may also have the effect of increasing water use in agriculture, both for existing fields and the establishment of new ones in once unprofitable areas (ISAB, 2007). This means that streams, rivers, and lakes will experience additional withdrawal of water for irrigation and increasing contaminant loads from returning effluent. Changes in stream flow due to use changes and seasonal run-off patterns may alter predator-prey interactions and change species assemblages in aquatic habitats. For example, a study conducted in an Arizona stream documented the complete loss of some macroinvertebrate species as the duration of low stream flows increased (Sponseller, Grimm, Boulton, & Sabo, 2010). As it is likely that intensity and frequency of droughts will increase across the southwest (Karl, Melillo, & Peterson, 2009), similar changes in aquatic species composition in the region are likely to occur.

Warmer water also stimulates biological processes which can lead to environmental hypoxia. Oxygen depletion in aquatic ecosystems can result in anaerobic metabolism increasing, thus leading to an increase in metals and other pollutants being released into the water column (Staudinger, et al., 2012). In addition to these changes, climate change may affect agriculture and other land development as rainfall and temperature patterns shift. Aquatic nuisance species invasions are also likely to change over time, as ecosystems become less resilient to disturbances (USEPA, 2008). Invasive species that are better adapted to warmer water temperatures would outcompete native species that are physiologically geared toward lower water temperatures(Lockwood & Somero, 2011).

In summary, effects of climate change include increases in atmospheric temperatures, decreases in sea ice, and changes in sea surface temperatures, patterns of precipitation, and sea level. Other effects of climate change include altered reproductive seasons/locations, shifts in migration patterns, reduced distribution and abundance of prey, and changes in the abundance of competitors and/or predators. Climate change is most likely to have its most pronounced effects on species whose populations are already in tenuous positions (Isaac, 2009).

EFFECTS OF THE ACTION

The ESA regulations define "Effects of the Action" as "all consequences to listed species or critical habitat that are caused by the Action, including the consequences of other activities that are caused by the Action. A consequence is caused by the Action if it would not occur but for the Action, and it is reasonably certain to occur. Effects of the action may occur later in time and may include consequences occurring outside the immediate area involved in the Action." (50 CFR 402.02). Action means "all activities or programs of any kind authorized, funded, or carried out, in whole or in part, by Federal agencies in the United States or upon the high seas". (50 CFR 402.02).

For this Opinion, our analysis of the effects of the proposed registration of Enlist One and Enlist Duo on listed resources under the Service's purview is presented using the Approach to the Analysis described previously and further defined below in this Opinion. The Effects of the Action section of this Opinion is divided into several sections and subsections. First, in the General Effects section, we briefly summarize the anticipated toxicological effects related to the Action, which we have divided into broad categories of organisms (i.e., terrestrial animals, aquatic animals, terrestrial plants, aquatic plants). In the Exposure section, we discuss the extent and general likelihood of exposure, including the anticipated general pathways of exposure to listed species taxa groups and their designated critical habitat. We outline how we use information regarding the anticipated effects and likely routes of exposure in our assessment of the effects of the action to each species in the Approach to the Effects Analysis section. The Risk Characterization further contextualizes which taxa of listed species are at higher risks of adverse effects by considering exposure and effects together. We follow this analysis with a review of any cumulative effects identified for the Action. Finally, we summarize the analysis of the effects of the Action and in the Integration and Synthesis section (Appendix B) in the context of the status of the species and critical habitat, environmental baseline, and cumulative effects.

General Effects

The risk of Enlist One and Enlist Duo use to listed species is evaluated below. To determine risk, we estimated exposure and effects after carefully examining factors that may influence those parameters. In the sections "Effects" and "Exposure" below, we describe those factors and how we chose to incorporate them into our analysis. Theses sections are broadly broken into sections for Terrestrial Animals, Aquatic Animals, and Plants due to fundamental differences in how these groups of species may be exposed, and in turn, respond to Enlist One and Enlist Duo use. Taxa-specific information that brought meaningful information to the analysis was included wherever possible. In "Effects by Taxa" section, we describe the methodology used to integrate exposure and effects information to determine and report risk for Terrestrial Animals, Aquatic Animals, and Plants. We made the approaches parallel across these groups to the extent possible, recognizing the inherent differences in exposure and effect pathways. The final section, "Risk Characterization," summarizes the general findings for each taxonomic group, which also incorporates the species' response in light of the general and species-specific label changes described in the conservation measures.

Toxicological Effects

As described in the BE, and above, Enlist One contains dichlorophenoxyacetic acid choline salt (2,4-D) and Enlist Duo contains both 2,4-D and glyphosate dimethylammonium salt (glyphosate) as active ingredients. 2,4-D is a plant growth regulator (synthetic auxin herbicide) that is commonly used for selective control of broadleaf weeds post-emergence. 2,4-D causes disruption of multiple growth processes in susceptible plants, leading to growth and reproduction abnormalities on new growth, resulting in deformities such as stem and petiole twisting, leaf malformations, undifferentiated cell masses and adventitious root formation on stems, and stunted root growth. Disrupted reproductive processes can result in sterile or multiple florets and nonviable seed production. Severe deformities or uncontrolled growth can ultimately lead to mortality. Glyphosate is a phosphono amino acid, non-selective, systemic herbicide that is used to control weeds in agricultural crops and non-agricultural sites. Glyphosate inhibits enzyme 5-enolpyruvaylshikimate 3-phosphate (EPSP) synthase, which is a critical enzyme in the shikimate pathway and is essential for the biosynthesis of aromatic amino acids and other aromatic compounds. This disruption of EPSP synthase leads to plant cell death. The shikimate pathway is absent in animals.

Enlist Duo represents a pesticide mixture in the form of a formulated product containing two active ingredients. Species and habitats exposed to pesticide mixtures may be at greater risk of adverse effects than when exposed to single pesticides. Literature reviewing studies of pesticide mixtures indicate that additivity, which refers to a response to a mixture that is based on the expected responses to mixture components in the absence of any toxic interactions, is the appropriate default assumption when considering mixture toxicity. In light of this, the National Academy of Sciences recommended in its 2013 *Report Assessing Risks to Endangered and Threatened and Species to Pesticides* that in the absence of data showing a synergistic (i.e., more than additive) response between a pesticide active ingredient and another mixture component, the analysis of effects should proceed on the assumption of additivity. In a review encompassing responses to 194 binary pesticide mixtures, synergistic effects occurred in only 7% of the mixtures tested (Cedergreen, 2014). The review showed that for pesticides, the combinations causing synergy were not random but included either cholinesterase inhibitors or azole fungicides in 95% of the described cases. Furthermore, the review also cautioned that these interactive responses were frequently recorded at concentrations above those expected in the environment and suggested that even for those pesticides showing interactions, there may be a threshold which concentrations have to exceed before resulting in a synergistic response. Therefore, in the absence of data demonstrating synergistic effects for particular species and taxa, we assume that effects of the Enlist Duo mixture will be additive based on the recommendation of the National Academy of Sciences, and data from the open literature revealing this to be the outcome in the vast majority of mixtures tested.

Effects by Taxa

The effects of 2,4-D and glyphosate have been studied extensively in many taxa, particularly in the target organisms, plants. In animals, available data include acute and chronic laboratory data from both registrant-submitted studies and the open literature. Many studies examine the effects of the technical pesticide, which is the pure form of a pesticide as it is manufactured prior to being formulated into an end-use product. Where available, we consider effects beyond that of single active ingredients, including effects from exposure to formulated products.

Laboratory tests are extrapolated to responses we expect to occur in organisms exposed in the field, with the recognition that these types of studies are limited in their ability to recreate natural settings and exposure routes. Most toxicity studies, including those required under FIFRA, are single stressor/single species toxicity tests that are designed to rule out the effects of all other stressors: food is accessible, mates are proximate, predators and competitors are absent, no migration is required, etc. Thus, acute sensitivity of species is determined under conditions that are largely artificial. In addition, these tests are generally not designed to capture and illustrate the consequences of sublethal responses to individual fitness. Sublethal responses, such as decreased olfactory ability, altered schooling behavior for fish, etc., may affect behaviors that cannot adequately be measured in these tests (e.g., feeding, selecting a mate, escaping predation, migrating, etc.) that would otherwise be deleterious to an individual's survival and reproduction (Golden, Noguchi, Paul, & Buford, 2012). In this sense, laboratory toxicity tests designed to be conservative in one manner (constant exposures to chemicals) do not consider many other factors when extrapolated to natural settings. It is not uncommon when reviewing field-based or mesocosm studies to see effects that are not measurable in standard toxicity testing (e.g., changes in community composition due to increased or decreased competition) or effects at concentrations below which have been identified in lab studies that attributable to the presence of other stressors (e.g., increased or decreased predation).

For population-level analysis, the magnitude of response of individuals to pesticide exposure is an integral piece of toxicological information. The magnitude of response or dose-response relationship describes the range of effects an organism may exhibit at different concentrations of a given chemical. This relationship can be used to assess the responses of individuals within a species, to explore differences among taxonomic levels within a given group to determine sensitivities (e.g., among fish, are Perciformes more sensitive to a given stressor than Salmoniformes or Cypriniformes?), or to explore differences across taxonomic groups (e.g., is a fish more sensitive to a specific stressor than a bird or an insect?). The toxicity data used in Steps 1 and 2 (to inform EPA's BE) as well as other sources of relevant literature considered acceptable for the BE may be used to determine the magnitude of response in Step 3. Steps 1-3 are previously described in the Section *NAS Report and Path Forward* within this opinion.

Toxicity data were divided into eight taxonomic groups (i.e., mammals, birds, fish, reptiles, amphibians, aquatic invertebrates, terrestrial invertebrates, and plants) similar to those assessed in the BE. Depending on availability, we identified quantitative or qualitative information to assess the expected biological response for multiple endpoints (i.e., direct and indirect effects[9],

[9] While our Opinion considers all consequences of the proposed action (per the definition of effects of the action at 50 CFR Part 402.02), the terms "direct" and "indirect" effects used in EPA's BE and in environmental risk assessment, in general, do not have the same meaning as these terms have been used in the context of the ESA Section 7 regulatory definition of "effects of the action" that previously existed prior to the 2019 regulations. For purposes of the effects analysis in this biological opinion, direct and indirect effects to species are those caused by the pesticide itself through dietary, dermal, or inhalation routes of exposure. Indirect effects occur when the pesticide acts on elements of the ecosystem that are required by the species, such as alterations to prey or shelter

including mortality, growth, reproduction and others) at predicted exposures. Where these analyses have already been performed in the BE, they have been directly carried over.

For each taxonomic group, endpoints for mortality, growth, or other sublethal effects were selected with the goal of ensuring the sensitivity of the species being assessed was captured. Mortality endpoints include the LD_{50} ("Lethal Dose" that causes 50% mortality of test subjects), LC_{50} ("Lethal Concentration" that causes 50% mortality of test subjects), and HC values ("Hazardous Concentration" extrapolated from species sensitivity distribution (SSD) curves). For LD_{50} and LC_{50} data, the most sensitive endpoint was generally chosen. For taxa with SSDs, HC_{05} values (representing the LD_{50} or the LC_{50} of the 5th percentile most sensitive species of the SSD) are generally chosen. Slopes for dose-response curves were derived from information in the BE and were either contained in the studies that generated the toxicity endpoint, contained in one of studies near the HC_{05} in the case of SSDs, simulated using numerical models, or using EPA's default slope of 4.5. Data were also examined to determine if species-specific data were available or if sufficient information existed to group into finer taxonomic categories (e.g., Order or Family level) that may be more or less sensitive to toxicological effects, and therefore more or less susceptible to the impacts of the pesticide. Within the finer taxonomic groups, factors considered included the number of species, how representative they may be of listed species within the taxa, and the variability of response. The data were also examined for information related to specific life-stages. A similar process was conducted for each sublethal response endpoint (e.g., growth or reproduction). For these response endpoints, toxicity data are generally derived from hypotheses-based testing (i.e., effects observed at a limited number of doses). For this reason, rather than constructing dose-response curves, information about the magnitude of response was generally gathered from effects described at different pesticide exposure concentrations. For some taxonomic groups, many studies were available for one or more response endpoints. For other taxonomic groups, few studies were available to describe effects for one or more response endpoints, and the magnitude of response was wholly based on those data. In other cases, no data were available to describe a particular response endpoint. In these cases, effects were either extrapolated from data from another taxonomic group, or that response was not carried forward in the analysis, as applicable.

A description and analyses of the data available for taxonomic groups are presented below. All data referenced below are from EPA's BE unless otherwise noted. Citations in descriptions below that begin with "MRID" (Master Record Identifier) are studies submitted by registrants, and those that begin with "E" are from EPA's ecotoxicology knowledgebase (ECOTOX). Full citations for these references can be found in EPA's BE.

General Effects to Terrestrial Animals

Terrestrial species may be exposed to pesticides such as Enlist One and Enlist Duo through one or more routes of exposure, including ingestion, dermal absorption, or inhalation. Effects from each type of exposure can be predicted by extrapolating the results of laboratory studies. However, the difficulty in recreating natural settings and exposure routes in the laboratory limits the relevance of these studies when assessing affects to species in their natural environment. Some of these limitations, especially for terrestrial vertebrates, are discussed below, followed by a description of the available data for each taxonomic group.

Mortality

For terrestrial vertebrates, most laboratory studies measure effects of toxicity from the ingestion route of exposure. This is accomplished either by providing subjects with contaminated food (concentration based, for derivation of LC_{50}'s) or by administering a single dose such as oral gavage or injection (dose-based, for derivation of LD_{50}s). Generally, only orally administered routes are considered to be environmentally relevant and directly comparable to estimated environmental concentrations (EECs), as the route of transport in the body is equivalent to how individuals would be exposed to these concentrations in the wild. However, the intraperitoneal exposure route has been demonstrated to have an absorption route with a similar circulatory pathway (initial absorption into portal system) as ingested substances for organic compounds and may be selected toxicity testing (for derivation of LD_{50}s) to avoid potential regurgitation of the administered dose in certain cases (Lukas, Brindle, & Greengard, 1971). Both dietary endpoints (LC_{50}'s) and dose-based endpoints (e.g., LD_{50}s) produced from these tests are derived in a manner that is reflective of certain aspects of how species are likely to be exposed in the wild. Both assess the sensitivity of species to potentially toxic food sources only, but not other routes of exposure (i.e., dermal or inhalation) nor other methods of ingestion such as drinking water. (We discuss our assessment of these routes of exposure below.) The LC_{50} studies provide an estimate of toxicity based on constant exposure to a set concentration of pesticide in food over a series of days, while the LD_{50} studies provide an estimate of toxicity based on a single potentially lethal exposure. Both of these methods capture a subset of conditions in which terrestrial species may be exposed to pesticides. Species in some feeding guilds such as granivores or insectivores are likely to feed and ingest pesticide throughout the day if confined to a contaminated area, while predatory or scavenging species may be exposed to a dose of a pesticide from an exposed carcass and not feed again for one or more days. However, listed species may undertake a large variety of feeding styles beyond those emulated in toxicity testing. Species with high mobility may receive intermittent doses of pesticides from feeding at different locations with varying levels of contamination. Secondary predators may get a large dose of pesticide that is neither biologically incorporated nor on the surface of prey, but in the gastrointestinal tract in its parent form (i.e., unmetabolized) (Hill & Mendenhall, 1980). Frequency or types of dietary items vary throughout the year, depending on availability, needs for migration, or reproduction. Long-distance migrators such as the red knot may gorge feed at stopover locations, then travel long distances on food stores from these events.

We recognize that it is not possible to emulate all exposure regimes or recreate all stressors in a laboratory setting. We acknowledge that current toxicity testing can provide some estimate of the sensitivity of species for a given exposure route and source. For the assessment of acute toxicity, where both dose-based and concentration-based data exist, while we consider all data, we often rely on the results of dose-based exposures (i.e., LD_{50}s) to produce an estimate of mortality for birds and mammals. In many cases, data exist for a greater number of species within these taxonomic groups for dose-based toxicity testing than for concentration-based testing, increasing the likelihood of including data from species with a greater range of sensitivities. This helps to reduce the uncertainty that we have captured the sensitivity of listed species, as often data exist for only a small number of species (e.g., as few as six for FIFRA-required studies) that must be extrapolated across all listed species representing varying taxonomic groups and ecological guilds. In many cases, these data vary widely, even within taxonomic groups and for individuals of the same species, suggesting that sensitivity is not easily captured by a small number of species. Dose-based studies are also coupled with taxa-specific conversion factors that have been generated from available data to convert acute mortality values across species based on body weight and food ingestion rate, increasing their accuracy when extrapolating to species with different physiological characteristics. Dose-based studies often, but not always, result in effects at lower concentrations for these taxa. This is likely attributable to a number of factors, including the greater number of species available as surrogates. This helps to account for some of the conservatism that is lost when extrapolating to field conditions, and thus provide a more accurate representation of the breadth of effects to species being assessed in the Opinion.

For reptiles and amphibians, we often have greater uncertainty in predicting their responses than other taxonomic groups as there is no testing requirement under FIFRA for these taxa, data from the open literature are often lacking, and taxa-specific conversion factors are generally derived from a smaller breadth of species than for birds and mammals. Where taxa-specific data are lacking to predict species response, we use toxicity data from birds to predict effects, as we consider amphibians and reptiles to be more closely related to birds than other broad taxa groups (such as mammals, arthropods, etc.). While there is notable uncertainty in this approach, we rely on the conservative nature of endpoint selection (e.g., most sensitive species, lowest endpoint, use of dose-based studies) to adequately capture the sensitivity of these taxa.

For all taxonomic groups, we generally assess mortality using a toxicity endpoint and its corresponding slope based on either 1) the most sensitive LD_{50} or LC_{50}, or 2) the HC_{05}, where an SSD is available. While we acknowledge that data do not exist to show that listed species are generally more inherently sensitive to pesticides than non-listed species, in most cases we lack the information to ascertain what that sensitivity may be. By choosing toxicity values that represent the most sensitive of those tested, we are more likely to ensure that we have captured the sensitivity of the species being assessed and not missed potential impacts. The likelihood that we have, in fact, captured the sensitivity of any species is influenced by the number of species tested and the breadth of responses among those species.

For sublethal endpoints, while all data are considered, analyses often rely on concentration-based studies. Most studies that are designed to examine sublethal effects such as growth, behavior, and reproduction are chronic dietary studies. Many endpoints carried over into our analysis are derived from registrant-submitted studies that examine these endpoints as part of long-term reproduction studies (e.g., 20 weeks for birds). Since these studies incorporate many aspects of the reproductive cycle (e.g., litter size, copulation, egg formation, parental care, growth of young), one or more responses to pesticide exposure may be incorporated into ultimate effects to reproduction. In this way, many parts of the reproductive cycle are examined, but it is often difficult to tease out specific impacts or which aspect of the reproductive process was compromised. For these types of studies, we consider the nature and magnitude of impacts at test concentrations as well as in the No Observed Adverse Effect Concentration (NOAEC). In some cases, there may be considerable impacts within the NOAEC range that are not distinguishable from controls due to test design and sensitivity. In all cases, it is important to consider impacts that could occur in the span of concentrations between the NOAEC range and the Lowest Observed Adverse Effect Concentration (LOAEC) range, especially when there are high impacts at the LOAEC.

Effects to Birds

2-4, D

For birds, 2-4, D toxicity data were available for three species, the northern bobwhite (*Colinus virginianus*), mallard (*Anas platyrhynchos*), and canary (*Serinus canaria*) and summarized in Table 7 below. Too few species were tested to construct an SSD. All data referenced below is from the Ecological Effects Characterization, section 2.3 of the BE.

Mortality: For dose-based mortality, toxicity data from the northern bobwhite resulted in an LD_{50} of 218.7 mg a.e./kg-bw. For concentration-based mortality, no mortalities were observed for the northern bobwhite or mallard at concentrations up to 3,035 mg a.e./kg-diet, and no mortalities were observed for the canary at concentrations up to 4,790 mg a.e./kg-diet.

Reproduction, growth, and other sublethal effects: The chronic toxicity of 2-4, D was evaluated in a laboratory-based avian reproduction study using the bobwhite quail; these studies are designed to estimate the quantity of toxicant required to adversely affect the reproductive capabilities of a test population of birds. For these studies, the test substance is administered by incorporating it into the mixture of the breeding birds' diets throughout their breeding cycle. Test birds approach their first breeding season at 18 to 23 weeks old. The onset of the exposure period is at least 10 weeks prior to egg laying. Exposure period during egg laying is generally 10 weeks with a withdrawal period of three additional weeks if reduced egg laying is noted. No sublethal effects were observed at all test levels, resulting in a NOAEC of 962 mg a.e./kg-diet and a LOAEC > 962 mg a.e./kg-diet. These concentrations are above the amount that birds are expected to encounter following use of Enlist and Enlist Duo.

Incident Reports*:* We are unaware of any incident data (i.e., adverse non-target effect resulting from pesticide usage) related to birds for Enlist One or Enlist Duo.

Table 7. Toxic effects of 2,4-D on birds

Test	**Species**	**2,4-D form tested**	**Endpoint**	**MRID**
Acute oral (dose-based)	Northern bobwhite	Triisopropanol amine salt of 2,4-D	LD_{50} = 218.7 mg a.e./kg-bw	41644401
Acute dietary (concentration-based)	Canary	2,4-D acid	LC_{50} > 4,790 mg a.e./kg-diet	49472501

Test	Species	2,4-D form tested	Endpoint	MRID
Acute dietary (concentration-based)	Northern bobwhite	Triisopropanol amine salt of 2,4-D	LD_{50} >3,035 mg a.e./kg-diet	41644402
Acute dietary (concentration-based)	Mallard	Triisopropanol amine salt of 2,4-D	LC_{50} >3,035 mg a.e./kg-diet	41644403
Chronic reproduction	Northern bobwhite	2,4-D acid	NOAEC = 962 mg a.e./kg-diet LOAEC > 962 mg a.e./kg-diet (No observed effects)	45336401

Glyphosate

For birds, glyphosate toxicity data were available for four species, the northern bobwhite (*Colinus virginianus*), mallard (*Anas platyrhynchos*), canary (*Serinus canaria*) and chicken (*Gallus gallus domesticus*), and summarized in Table 8 and Table 9 below. Too few species were tested to construct an SSD. All data referenced below is from the Ecological Effects Characterization, section 3.3 of the BE.

Mortality: For dose-based mortality, no mortalities were observed at concentrations up to 4,570 mg a.e./kg bw for the northern bobwhite (Table 8). For the canary study, there were no mortalities at 2,000 mg a.e./kg-bw and regurgitation was observed at 3,300 mg a.e./kg-bw. EPA calculated an effective dose (ED_{50}) of 2,819 mg ae/kg-bw for acute avian toxicity, using regurgitation as an endpoint. For concentration-based mortality, no mortalities were observed for the northern bobwhite or mallard at concentrations up to 4,971 a.e./kg-diet.

Table 8. Acute toxicity testing of glyphosate in birds

Test	Species	% glyphosate	endpoint	MRID
Acute oral (dose-based)	Northern bobwhite	83	LD_{50} >3196.3 mg a.e./kg bw	00108204

Test	Species	% glyphosate	endpoint	MRID
Acute oral (dose-based)	Northern bobwhite	98.5	LD_{50} >4570 mg a.e./kg bw	00076492
Acute oral (dose-based)	Canary	96	LD_{50} >2,000 mg a.e./kg bw	48934206
Acute dietary (concentration-based)	Mallard	98.5	LD_{50} >4,570.4 mg a.e./kg diet	108107, 37765
Acute dietary (concentration-based)	Northern bobwhite	95.6	LD_{50} >1,912 mg a.e./kg diet	44320626
Acute dietary (concentration-based)	Mallard	95.6	LD_{50} >4,971.2 mg a.e./kg diet	44320627
Acute dietary (concentration-based)	Northern bobwhite	95.6	LD_{50} >4,971.2 mg a.e./kg diet	44320628

Reproduction, growth, and other sublethal effects: The chronic toxicity of glyphosate was evaluated in laboratory-based avian reproduction studies involving the northern bobwhite, mallard, and chicken that were both registrant-submitted and from the open literature (Table 9). Two registrant-submitted studies conducted with technical glyphosate found no effects on growth or reproduction following exposure to either mallards or bobwhite quail up to concentrations of 830 mg a.e./kg-diet. In the mallard study, one mortality was reported at 830 mg a.e./kg-diet and body weight gain decreased 25% compared to the control, though this difference was not statistically significant. An additional study on mallards reported no reproductive or body weight effects at 30 mg-kg diet. This study has several limitations, including low sample sizes and use of outdoor pens, but provides some limited information on the potential for effects at low dietary levels. A qualitative study from the open literature reports noted a 50% reduction in male and female body weights for domesticated chickens at glyphosate concentrations of 4,505 mg a.e./kg-diet (Kubena, 1981). One additional registrant-submitted study for the mallard (MRID 48876602) found significant decreases of 99%, 59%, and 127% in male body weight gain at the low-, mid-, and high- test concentration, respectively. However, further examination of this study shows that all adult birds in all treatment and control groups were losing weight during the first 8 weeks of the study, with negligible differences across treatments and no dose response pattern. At test termination, an increase in body weights for both the control and the 987 mg a.e./kg-diet treatment group was reported for all birds. While the terminal weights appear to be significantly different across treatments and controls, this difference was only observable in the final week and likely only reflects the poor study performance within the first 8 weeks. Based on these factors, the Service agrees with EPA's conclusion (EPA 2022e) that this does not represent a reliable endpoint, and that the 830 mg a.i./kg-diet endpoint represents a more reliable NOAEC that is supported by the body of avian chronic toxicity data.

Table 9. Reproductive, growth, and other sub-lethal effects of glyphosate to birds.

Species	**% glyphosate**	**NOAEC**	**LOAEC**	**Effect**	**MRID**
Mallard	96	<501 mg a.e./kg diet	501 mg a.e./kg diet	Effects to hatchling and 14-day body weights, including 99% decrease in male weight gain	48876602
Mallard	83	830 mg a.e./kg diet	-	25% reduction in weight gain at 830 mg a.e./kg-diet, not statistically significant	00111953
Northern bobwhite	83	830 mg a.e./kg diet	-	No effects reported	00108207
Mallard	90.4	30 mg a.e./kg diet	-	No effects reported	00113457
Chicken	Not reported	608 mg a.e./kg diet	6,080 mg a.e./kg diet	50% reduction in male and female body weight by day 7	Kubena et al. 1981

Effects to Reptiles

2-4, D

No toxicity data are available for reptiles exposed to 2,4-D to extrapolate to listed species within this class. Therefore, the toxicity data for birds will be used as a surrogate for reptiles since reptiles are more closely related to birds than other broad taxa groups (such as mammals, arthropods, etc.). Please refer to Table 7, above describing effects to birds for an illustration of assumed effects of 2,4-D to reptiles. There is a notable uncertainty in this approach as the relative sensitivities between birds and reptiles are unknown. We are unaware of any incident data related to reptiles.

Glyphosate

No toxicity data are available for reptiles exposed to glyphosate to extrapolate to listed species within this class. Therefore, the toxicity data for birds will be used as a surrogate for reptiles since reptiles are more closely related to birds than other broad taxa groups (such as mammals, arthropods, etc.). Please refer to Table 8 and Table 9 above describing effects to birds for an illustration of assumed effects of glyphosate to reptiles. There is a notable uncertainty in this approach as the relative sensitivities between birds and reptiles are unknown. We are unaware of any incident data related to reptiles.

Effects to Terrestrial Amphibians

2,4-D

No toxicity data are available for terrestrial amphibians exposed to 2,4-D to extrapolate to listed species within this class. Therefore, the available toxicity data for birds will be used as a surrogate for terrestrial amphibians as we believe effects to birds are more representative for amphibians than effects observed in other broad taxa groups (such as mammals, arthropods, etc.). Please refer to Table 7 above describing effects to birds for an illustration of assumed effects of 2,4-D to terrestrial amphibians. There is notable uncertainty in this approach as the relative sensitivities between birds and amphibians are unknown. We are unaware of any incident data related to terrestrial amphibians.

Glyphosate

There are insufficient data available for terrestrial amphibians exposed to glyphosate to extrapolate to listed species within this class. Therefore, the available toxicity data for birds will be used as a surrogate for terrestrial amphibians as we believe effects to birds are more representative for amphibians than effects observed in other broad taxa groups (such as mammals, arthropods, etc.). Please refer to Table 8 and Table 9 above describing effects to birds for an illustration of assumed effects of glyphosate to terrestrial amphibians. While there is notable uncertainty in this approach as the relative sensitivities between birds and amphibians are unknown, one study from the open literature determined that the 96-hr LD_{50} value for field-collected rough-skinned newts (*Taricha granulosa*) exposed to technical glyphosate was greater than 2,600 mg/kg-bw (McComb, Curtis, Chambers, Newton, & Bentson, 2008). This study was considered for qualitative use only because of limitations in study reporting, but results are consistent with the endpoints for birds from studies conducted with technical glyphosate. We are unaware of any incident data related to terrestrial amphibians.

Effects to Mammals

2,4-D

For mammals, 2-4, D toxicity studies were limited to a single species, the Norway rat (*Rattus norvegicus*), and summarized in Table 10 below. Too few species were tested to construct an SSD. All data referenced below is from the Ecological Effects Characterization, section 2.3 of the BE.

Mortality: A single oral gavage study in Norway rats was available to assess dose-based mortality, resulting in an LD_{50} of 441 mg a.e./kg bw.

Reproduction, growth, and other sublethal effects: The chronic toxicity of 2-4, D was evaluated in laboratory-based reproduction studies using Norway rats. Based the renal elimination rate of 2,4-D, toxicological effects do not begin to appear until the intake rate exceeds the elimination rate, which occurs at concentrations greater than 55 mg a.e./kg/day. Thus, 55 mg/kg/day is an estimate of the threshold for chronic effects that incorporates this pharmacokinetics information and serves as the endpoint for assessment of mammalian reproduction risks.

Incident Reports*:* We are unaware of any incident data related to mammals for Enlist One or Enlist Duo.

Table 10. Toxic effects of 2,4-D to mammals.

Test	**Species**	**2,4-D form tested**	**Endpoint**	**MRID**
Acute oral (dose-based)	Norway rat	Triisopropanol amine salt of 2,4-D	LD_{50} = 441 mg a.e./kg bw	41413501
Chronic reproduction/ developmental	Norway rat	2,4-D acid	NOAEL = 55 mg a.e./kg bw/day LOAEL > 55 mg a.e./kg bw/day	00150557, 00130407, 47417902, 47417901

Glyphosate

For mammals, glyphosate toxicity was evaluated based on studies with the Norway rat (*Rattus norvegicus*) and summarized in Table 11 and Table 12 below. All data referenced below is from the Ecological Effects Characterization, section 3.3 of the BE.

Mortality: No mortalities were observed in any of the eight acute dose-based rat studies ranging up to 4,860 mg a.e./kg-bw glyphosate (Table 11).

Table 11. Acute toxicity testing of glyphosate in mammals

Test	Species	% glyphosate	Endpoint	MRID
Acute oral (dose-based)	Norway rat (*Rattus norvegicus*)	96	LD_{50} > 4800 mg a.e./kg bw (no mortalities)	43728003
Acute oral (dose-based)	Norway rat (*Rattus norvegicus*)	95	LD_{50} > 4750 mg a.e./kg bw (no mortalities)	45058306
Acute oral (dose-based)	Norway rat (*Rattus norvegicus*)	97.2	LD_{50} > 4860 mg a.e./kg bw (no mortalities)	46760505
Acute oral (dose-based)	Norway rat (*Rattus norvegicus*)	88	LD_{50}> 4400 mg a.e./kg bw (no mortalities)	44320604
Acute oral (dose-based)	Norway rat (*Rattus norvegicus*)	95	LD_{50} > 4750 mg a.e./kg bw (no mortalities)	46998805
Acute oral (dose-based)	Norway rat (*Rattus norvegicus*)	76	LD_{50} > 3800 mg a.e./kg bw (no mortalities)	41400601

Test	Species	% glyphosate	Endpoint	MRID
Acute oral (dose-based)	Norway rat (*Rattus norvegicus*)	96	LD_{50}>1920 mg a.e./kg bw (no mortalities)	44142104
Acute oral (dose-based)	Norway rat (*Rattus norvegicus*)	95.4	LD_{50} > 4770 mg a.e./kg bw (no mortalities)	46816107

Reproduction, growth, and other sublethal effects: The chronic toxicity of glyphosate was evaluated in laboratory-based reproduction studies using Norway rats (Table 12). In the first study, the NOAEL is 500 mg/kg/day for both parents and offspring, and the LOAEL is 1,500 mg/kg/day based on decreased body weight gain in both parents and offspring (MRID 41621501, 1990). These results are used as the mammalian endpoint for risk analysis. The reproductive NOAEL is 1,500 mg/kg/day in both sexes.

In the second 2-generation reproduction study using the rat, the NOAEL is 5,000 mg/kg-diet (equivalent to 408/423 mg/kg/day in males/females, respectively) with a LOAEL of 15,000 mg/kg-diet based on delayed age at sexual maturation. For parents, no effects were observed at any test level (NOAEL =15,000 mg/kg-diet; equivalent to 1,234/1,273 mg/kg/day in males/females, respectively).

Table 12. Reproductive, growth, and other sublethal effects of glyphosate to mammals

Species	% glyphosate	NOAEL	LOAEL	Effect	MRID
Norway rat (*Rattus norvegicus*)	97.67	NOAEL: 500 mg/kg/day Reproductive NOAEL: 1500 mg/kg/day	LOAEL: 1500 mg/kg/day	Decreased body weight	41621501
Norway rat (*Rattus norvegicus*)		NOAEL: 408/422 mg/kg mg/kg bw/day	LOAEL: 1234/1273	Delayed age and increased weight at	48865101-48865105

Species	% glyphosate	NOAEL	LOAEL	Effect	MRID
		(males/females)	(males/female) mg/kg bw/day LOAEL > 1234/1273 mg/kg bw/day for parental or reproductive toxicity (no observed effects)	male sexual development in offspring	

Effects to Terrestrial Invertebrates

2,4-D

Toxicity of 2,4-D to terrestrial invertebrates is assessed by analyzing effects to adult and larval honey bee (*Apis mellifera*) and summarized in Table 13 below. Too few species were tested to construct an SSD. All data referenced below is from section 2.3 (Ecological Effects Characterization) and Appendix B (Animal and Plant Toxicity Data) of the BE.

Mortality: Acute mortality was assessed via contact and oral toxicity studies of 2,4-D salts in adult honey bees. Endpoints for these studies were non-definitive as <50% mortality occurred at the highest dose tested (66 µg/a.e./bee for contact, and 62.2 µg/a.e./bee for oral).

Chronic toxicity: Chronic toxicity in adult honey bees resulted in NOAEL of 5.3 µg a.e./bee/day (276 mg a.e./kg-diet) and a LOAEL of 8.2 µg a.e./bee/day (668 mg a.e./kg-diet), which was associated with 71% mortality. Accounting for the weight of adult bees of 0.0881 µg/bee (in the control), the NOAEL and LOAEL yield endpoints expressed as mass of pesticide per unit weight of 60 and 93 mg a.e./kg-bee, respectively.

In honey bee larvae, the most sensitive endpoint was mortality, with a LOAEL of 0.459 µg a.e./bee/day (11.9 mg a.e./kg-diet), associated with 24% mortality. Because the researchers were unable to calculate a NOAEL, EPA calculated a threshold for the discernable effects level by considering the control and treatment variance of the binomial survival data from the study to derive a minimum statistically detectible difference (MSDD). Results indicate 15% mortality was the approximate MSDD at 0.039 µg a.e./bee (LC_{15} of 2.91 mg a.e./kg-diet).

A chronic dietary feeding study from the open literature (Gupta and Bhattacharya, 2008) with Jute Hairy caterpillar (*Spilarctia obliqua*) reported a dose response in larval survival with 10, 14, 17, 17, 21, and 35% mortality observed at 0.55, 1.1, 2.2, 4.4, 8.9 and 18 mg a.e./kg-diet. Because this study used a formulation not registered in the U.S., results were not used quantitatively. However, its findings are generally consistent with the honey bee study and adds to the weight of evidence supporting the selected honey bee-based endpoint for evaluating the toxicity of 2,4-D to non-Apis terrestrial invertebrates, including Lepidopterans.

Incident Reports*:* We are unaware of any incident data related to terrestrial invertebrates for Enlist One or Enlist Duo.

Table 13. Toxic effects of 2,4-D to terrestrial invertebrates

Test	**Species**	**2,4-D form tested**	**Endpoint**	**MRID**
Acute contact (adult)	Honey bee (*Apis mellifera* L.)	2,4-D DMA (67.3%)	LD_{50} > 83.3 μg a.e./bee	44517304
Acute contact (adult)	Honey bee (*Apis mellifera* L.)	2,4-D EHE TGAI 97% a.i.	LD_{50} > 43.7 μg a.e./bee	44517301
Acute oral (adult)	Honey bee (*Apis mellifera* L.)	2,4-D choline TGAI 99% a.i.	LD_{50} > 42.3 μg a.e./bee	48892404
Acute oral (adult)	Honey bee (*Apis mellifera* L.)	2,4-D DMA (67.3%)	LD_{50} > 83.3 μg a.e./bee	44517303
Acute oral (adult)	Honey bee (*Apis mellifera* L.)	2,4-D EHE TGAI 97% a.i.	LD_{50} > 66.2 μg a.e./bee	44517302
Acute oral (larval)	Honey bee (*Apis mellifera* L.)	2,4-D acid TGAI 98.8% a.i.	LD_{50} > 63 μg a.e./larva LC_{50}= 156 mg a.e./kg-diet	50282701

Test	**Species**	**2,4-D form tested**	**Endpoint**	**MRID**
Chronic oral (adult)	Honey bee (*Apis mellifera* L.)	2,4-D acid TGAI 97.5% a.i.	NOAEL = 5.3 µg a.e./bee LOAEL = 8.2 µg a.e./bee NOAEC = 276 mg a.e./kg-diet LOAEC = 668 mg a.e./kg-diet	50751201
Chronic oral (larval)	Honey bee (*Apis mellifera* L.)	2,4-D acid TGAI 97.5% a.i.	NOAEL <0.459 µg a.e./larva LOAEL = 0.459 µg a.e./larva NOAEC < 11.9 mg a.e./kg-diet LOAEC = 11.9 mg a.e./kg-diet Day 22 LD_{15} = 0.039 µg a.e./larva (2.91 mg a.e./kg-diet)	50751301

<u>Glyphosate</u>

Toxicity of glyphosate to terrestrial invertebrates is assessed by analyzing effects to adult and larval honey bees (*Apis mellifer*a) and summarized in Table 14 below. Too few species were tested to construct an SSD. All data referenced below is from the Ecological Effects Characterization, section 3.3 of the BE.

Mortality: Acute mortality was assessed via contact and oral toxicity studies of 2,4-D salts in adult honey bees. Endpoints for these studies were non-definitive as <50% mortality occurred at the highest dose tested with technical glyphosate (contact), technical glyphosate with an adjuvant (contact), and glyphosate formulation (oral; LD_{50}'s >100 µg/bee, >103 µg a.e./bee, and 182 µg /a.e./bee, respectively). Oral and contact studies with other terrestrial invertebrate species (i.e., bumblebees, predatory mites, earthworms, parasitic wasps) also resulted in similar findings, with

<50% mortality up to the highest dose tested, and often no effects reported. Similar results were found for soil exposure tests involving annelids and several arthropod species.

Chronic toxicity: Chronic toxicity in adult honey bees was not available, though chronic toxicity using a formulation resulted in no significant mortality up to 179.9 µg/a.e./bee/day. A NOAEC of 234 mg a.e./kg-diet was based on effects to food consumption, with a 57% effect at the LOAEC of 595 mg a.e./kg-diet (9.5 µg/a.e./bee/day).

A semi-field residue and colony-feeding study found no significant effect on survival of eggs, young or old larvae or on larvae weight at concentrations up to 255 mg a.e./L, meant to simulate exposures twice as high as the expected exposure from applications at 1.92 lb a.e./A. As acute and chronic laboratory-based toxicity data (i.e., mortality and emergence data) for honey bee larvae are not available, this study is used to evaluate larval mortality for relevant exposure pathways and application rates.

AMPA toxicity: Reproductive effects of a major glyphosate degradate, AMPA, have been reported for earthworms in two studies. Von Merey et al., 2016 (E179154; MRID 50603804) reported no mortality on adult *Eisenia fetida* survival up to 1000 mg/kg soil for earthworms, soil mites, and springtails. A clear dose response was reported for a reduced number of juvenile earthworms, with an EC_{50} of 654.7 mg/kg soil (56 days). The NOAEL and LOAEL were 198.1 and 297.1 mg/kg soil, respectively (28 days). Domínguez et al. 2016 (E179126) also reported reduced fecundity in another earthworm species (*Eisenia andrei*) with a NOAEL and LOAEL of 0.75 and 1.0 mg/kg soil, respectively. This study showed reduced fecundity (fewer cocoons) at 14 days, then an increased number of juveniles and cocoons, but with lower biomass per cocoon/juvenile, at 56 days. No mortality effects were reported.

Incident Reports*:* We are unaware of any incident data related to terrestrial invertebrates for Enlist One or Enlist Duo.

Table 14. Toxic effects of glyphosate to terrestrial invertebrates

Test	**Species**	**% a.i.**	**Endpoint**	**MRID**
Acute oral (adult)	Honey bee (*Apis mellifera* L.)	98.5%	LD_{50} > 100 µg/bee NOAEL: not reported	00026489
Acute contact (adult)	Honey bee (*Apis mellifera* L.)	98.5%	LD_{50} > 100 µg/bee NOAEL: not reported	00026489

Test	Species	% a.i.	Endpoint	MRID
Acute contact (adult)	Honey bee (*Apis mellifera* L.)	97.6%	LD_{50} > 103 µg/bee NOAEL = 103 µg/bee	48876603
Acute oral (adult)	Honey bee (*Apis mellifera* L.)	97.6%	LD_{50} >182 µg/bee NOAEL= 182 µg/bee	48876603
Chronic oral (adult)	Honey bee (*Apis mellifera* L.)	Glyphosate monoammonium salt, 65.6%	LD_{50} > 170 µg/a.e./bee NOAEL = 170 µg/a.e./bee	50603803

General Effects to Aquatic Animals

Listed aquatic species that may be affected by Enlist One or Enlist Duo include fish, aquatic phase amphibians, and various taxa of aquatic invertebrates (i.e., aquatic insects, crustaceans, and mollusks). For those species that are exclusively aquatic, all life stages may be affected by exposure to Enlist pesticides in water. Some species of aquatic insects (e.g., dragonflies, damselflies, and stoneflies) and amphibians (e.g., frogs, toads, and some salamanders) have both aquatic and terrestrial life stages and may, therefore, be affected by exposures in either aquatic or terrestrial habitats, or both. Certain species also have obligate relationships with other species. For example, early life stages of freshwater mussels (glochidia) are parasitic and require a host fish to complete their development. Consequently, we also assess the potential effects of Enlist pesticides on host fish in the effects analyses for mussels. Similarly, effects to a listed species from impacts to their food items (such as aquatic vegetation) were included in our analyses. There are no studies from the BE that assess the effects of the specific forms of 2,4-D and glyphosate used in Enlist pesticides together on aquatic species. Thus, our analysis of effects to aquatic species considers the effects of 2,4-D and glyphosate separately, assuming only additive effects (rather than synergistic effects).

Most of the available toxicity data provided in the BE for aquatic species are from laboratory tests, conducted under controlled conditions where organisms are exposed in water (typically over a range of concentrations) for set durations (e.g., 1 hour, 1 day, 4 days, 21 days, or full life cycle) and the desired measurement endpoints (e.g., mortality, growth, behavioral response, fecundity, spawning/hatching success) are reported. These types of tests are valuable for establishing causal relationships between exposure to the pesticide and response of the organism to that exposure, while recognizing the limits of clinical exposure being representative of field exposure and associated response.

In order to address the uncertainty with whether the studied taxa constitute adequate surrogates for listed species, our approach to applying toxicity data for assessing effects to listed species relies on the lowest (most sensitive) LC_{50} for acute data and the lowest NOAEC for sublethal or chronic data, as sufficient data were not available to construct an SSD. While we acknowledge that data do not exist to show that listed species are generally more inherently sensitive to pesticides than non-listed species, in most cases, we lack the information to ascertain what that sensitivity may be. By choosing these values, we are more likely to ensure that we have captured the sensitivity of a species and not missed any corresponding response. The likelihood that we have, in fact, captured the sensitivity of any species is influenced by the number of species tested and the breadth of responses among those species.

Effects to Fish and Aquatic-Phase Amphibians

We primarily rely on toxicity data carried forward from the BE for our effects analysis to fish and aquatic phase amphibians. Overall, there were no reports on the acute lethality or sublethal effects of 2,4-D to fish, but there are several studies that address sublethal effects on growth and behavior. Relatively few studies report effects on reproduction, and there was only one study from the BE that tested effects on sensory function. For aquatic-phase amphibians, there were limited numbers of studies, and few species tested. For glyphosate, there were several studies available for both freshwater and marine fishes, but there were no studies focused on effects to aquatic phase amphibians. In cases where no data were available, we used fish data as a surrogate for aquatic phase amphibians.

Consequently, we will generally be using the fish toxicity endpoints as surrogates for aquatic and aquatic-phase amphibians where there are no data for amphibians and will discuss both taxa groups together in this section. The toxicity data utilized to assess the effects of Enlist One and Enlist Duo are provided below and in Table 15 and Table 16. There are no records of incident reports for Enlist One and Enlist Duo for these species. All data referenced in the following sections are from the Effects Characterization chapter of the BE.

<u>Mortality</u>

<u>2,4-D</u>

In Appendix B of their BE, EPA provides a list of 2,4-D studies that EPA evaluated when selecting the most sensitive 2,4-D endpoints for their ESA risk assessment (Table 15). Many of the studies submitted to EPA were for the 2,4-D ester form and were not deemed appropriate by EPA to include in its ecological risk assessment of the water-soluble salt form of 2, 4-D. The remaining number of toxicological studies for 2,4-D choline salt or 2, 4-D acid were considered in EPA’s BE. A study in rainbow trout (*Oncorhynchus mykiss*) exposed to 2,4-D choline salt observed no mortality or sublethal effects. Similarly, a study in tidewater silverside (*Menidia beryllina*) found no mortality or sublethal effects either. The NOAECs for the two studies was determined to be 23,300 and 14,200 µg a.e./L, which is orders of magnitude greater than the highest EECs for 2,4-D expected to result from Enlist pesticide usage. An acute freshwater amphibian study in leopard frogs determined that the LC_{50} was 349,000 µg a.e./L, which is a concentration unlikely to occur in the environment as a result of Enlist pesticide usage. Therefore, we do not expect 2,4-D from Enlist pesticide formulations will cause mortality in fish and aquatic-phase amphibians.

Table 15. Toxicity Values for 2,4-D for Fish and aquatic-phase Amphibians (Table 2-7 from the BE)

Taxon	**Species**	**Endpoint (µg a.e./L)**	**Effects**	**MRID**
Acute freshwater fish	Rainbow trout (*Oncorhynchus mykiss*)	96-h LC_{50} > 48,000 NOAEC = 23,300 LOAEC = 45,850	None observed	48892401
Chronic freshwater fish (early life cycle)	Fathead minnow (*Pimephales promelas*)	NOAEC = 14,200 LOAEC = 37,600 based on length	Reduction in growth	41767701
Acute estuarine/marine fish	Tidewater silverside (*Menidia berylina*)	96-h LC_{50} > 80,240	None observed	42018301
Acute freshwater amphibians	Leopard frog tadpoles (*Rana pipiens*)	96-h LC_{50} = 337,000	mortality	44517306

Glyphosate

Similarly for glyphosate, in Table B-8 of Appendix B in the BE, EPA provides a list of studies evaluated to select the most sensitive endpoint for the ESA risk assessment. The studies provided incorporated glyphosate as the technical product and/or the glyphosate dimethyl ammonium salt as the ingredient tested.

The endpoint determined to be the most sensitive for freshwater fish mortality for technical glyphosate is based on the most sensitive acute 96-h LC_{50} value of 43 mg a.e./L for bluegill sunfish (MRID 44320630). Additional studies in other fish species, such as rainbow trout (*Oncorhynchus mykiss*), fathead minnow (*Pimephales promelas*), and channel catfish (*Ictalurus punctatus*) demonstrate a range of toxicity, with 96-hour LC_{50} values ranging from 69.4-128.1 mg a.e./L. Similarly, an acute study in Australian tree frogs (*Litoria moorei*) observed mortality with exposure to glyphosate and determined the LC_{50} was 150 mg a.e./L. Another acute toxicity study in Australian tree frogs found similar results and determined a 96-hour LC_{50} of 103.2 mg a.e/L. Given that the most sensitive endpoint in fish is an order of magnitude below the most sensitive amphibian endpoint, fish toxicity endpoints appear to be protective of aquatic-phase amphibians. Below in Table 16 are the remaining toxicity values for glyphosate for chronic toxicity to fish. In addition, data were available for both acute and chronic toxicity to aquatic phase amphibians and are also presented below in Table 16.

Table 16. Toxicity values for glyphosate for fish and aquatic-phase amphibians (Table 3-7 from the BE)

Taxon	Species	Endpoint (µg a.e./L)*	Effects	MRID
Acute freshwater fish	Bluegill sunfish (*Lepomis macrochirus*)	96-hr LC_{50} = 43,000	mortality	44320630
Chronic freshwater fish	Fathead minnow (*Pimephales promelas*)	NOAEC = 25,700	NA; highest concentration tested	00108171
Acute aquatic-phase amphibian	Australian tree frog *(Litoria moorei*)	96-hr LC_{50} = 103,200	mortality	43839601 (1995) Supplemental
Chronic aquatic-phase amphibian	Leopard frog (*Rana pipiens*)	NOAEC = 1,800	NA; highest concentration tested	46650501 (2004) Supplemental

NA = not applicable; *a.e. = acid equivalents (defined as the portion of a formulation that theoretically could be converted back to the corresponding or parent acid and thus represents the active form).

<u>Sublethal effects</u>

<u>2,4-D</u>

There were no studies available on the sublethal effects of 2,4-D choline salt available, however, results from a number of studies using different forms of 2,4-D were available in the BE and in the open literature. In a chronic toxicity study, early life stage fathead minnow (*Pimephales promelas*) exposed to 2,4-D dimethylamine salt experienced growth effects and were significantly shorter and gained less weight than control fish. The studied determined a NOAEC of 11,833 µg a.e/L and a LOAEC of 31,333 µg a.e/L based on length (MRID 41767701). Similarly, a study in African clawed frogs (*Xenopus laevis*) tadpoles exposed to 2,4-D acid showed no signs of either advanced or delayed development, asynchronous development, or significant histopathological effects of the thyroid gland. The study authors determined the NOAEC to be 113 mg a.e./L (Coady et al., 2013). Based on the low observed acute toxicity comparable to freshwater fish and the non-definitive endpoints, EPA's risk assessment in their BE uses the freshwater fish chronic effect level as a surrogate endpoint.

Other types of sublethal effects include impacts to reproduction or behavior. A study using fathead minnows exposed to 2,4-D acid found no significant differences in fertility, wet weight, length, gonado-somatic indices, tubercle scores, or measures of endocrine disruption. The study authors determined the no observable effect concentration was 96.5 mg a.e./L for fathead minnow reproduction (Coady et al., 2013). One study found significant reductions in prey capture ability in larval zebrafish from 0.74-4 ppm of pure 2,4-D and 4-8 ppm of 2,4-D DMA (a different form from the one used in Enlist formulations). Similarly, the same study found significant decreases in prey capture ability in larval yellow perch (*Perca flavescens*) at exposures of 4-8 ppm 2,4-D DMA (Dehnert, Karasov, & Wolman, 2019).

<u>Glyphosate</u>

A chronic fish life cycle study in fathead minnows exposed to technical glyphosate found no sublethal effects up the highest concentration tested (25.7 mg acid equivalent/L; MRID 00108171). Similarly, a study exposing threespine stickleback larvae (*Gasterosteus aculeatus*) found no differences in wet weight, sex ratio, or condition of juvenile fish exposed to 0.1 mg/L glyphosate. A chronic study of glyphosate exposure in leopard frogs (*Rana pipiens*) found no sublethal effects up to the highest level tested (1.3 mg/L; MRID 46650501).

Numerous studies available in the open literature observed various behavioral effects in fish from glyphosate exposure. A study in pacu (*Piaractus mesopotamicus*) found exposures to 0.6 and 0.6 ppm glyphosate temporarily decreased food intake, but exposures at 1.8 ppm cause dramatic decreases in food intake that did not recover by the end of the study (15 days) (Giaquinto et al., 2017). A study in zebrafish larvae found significant decreases in locomotory behaviors (e.g., distance traveled, mean speed, line crossings) at 0.5 mg/L glyphosate. Furthermore, exposures to 0.5 mg/L Roundup ® (a common glyphosate formulation) led to significant memory impairment in adult zebrafish (Bridi et al., 2017).

<u>Effects to Dietary Items</u>

Additionally, we consider impacts to fish and aquatic-phase amphibian dietary items as part of our effects analysis. These include effects to aquatic vegetation, phytoplankton, and zooplankton. 2,4-D and glyphosate can affect growth and yield in both vascular and non-vascular aquatic plants. See the *General Effects to Plants* section below for a more detailed description of anticipated effects to aquatic plants. Similarly, mesocosm studies have found that glyphosate can significantly alter zooplankton community biomass and structure, but only at concentrations that are much higher than what we anticipate will result from Enlist pesticide usage. Furthermore, zooplankton species showed a wide range of sensitivity to glyphosate, which could lead to biomass compensation when sensitive species experience effects from glyphosate exposure (Barbosa da Costa, et al., 2021; Hebert, et al., 2021). Given that neither aquatic vegetation nor zooplankton communities are expected to experience significant declines in biomass with Enlist pesticide use, we do not expect any declines in food availability for aquatic species will occur.

Incident Reports: We are unaware of any incident data related to fish or amphibians for Enlist One or Enlist Duo

Effects to Aquatic Invertebrates

The effects of the active ingredients in Enlist One and Enlist Duo (2,4-D and 2,4-D plus glyphosate, respectively) on aquatic invertebrate species has been well-documented in the literature. As a group, aquatic invertebrates include species that occur in aquatic habitats during all or a portion of their life cycle, and can include certain insects (such as dragonflies, damselflies, stoneflies, aquatic beetles, etc.), aquatic or semi-aquatic snails and limpets, and aquatic crustaceans (crayfish, isopods, amphipods). Studies on the effects of 2,4-D and glyphosate on estuarine/marine species of aquatic invertebrates are also available such as mussels and clams. There are registrant-submitted studies involving aquatic invertebrates, including acute and chronic laboratory studies with either 2,4-D or glyphosate. From Tables 2-8 and 3-8 in the BE, Table 17 and Table 18 below describe the concentrations and effects observed for studies involving both freshwater and estuarine/marine aquatic invertebrates for 2,4-D and glyphosate, respectively.

Table 17**.** Effects of 2,4-D on freshwater and estuarine/marine aquatic invertebrates (Table 2-8 from the BE)

Taxon	**Species**	**Endpoint (µg a.e./L)**	**Effects**	**MRID**
Freshwater aquatic invertebrate (acute)	Water flea (*Daphnia magna*)	48-hr EC_{50} = 25,000	NA; NOAEC at highest concentration tested	41158301
Freshwater aquatic invertebrate (chronic)	Water flea (*Daphnia magna*)	NOAEC = 16,050 LOAEC = 25,473	Survival and reproduction (i.e., number of neonates, number of broods, and brood size)	42018303
Estuarine/marine aquatic invertebrate (acute)	Eastern oyster (*Crassostrea virginica*)	96-hr EC_{50} = 62,800		41429003
Estuarine/marine aquatic invertebrate (chronic)	Eastern oyster (*Crassostrea virginica*)	Calculated using ACR NOAEC = 31,800		

Table 18**.** Effects of glyphosate on freshwater and estuarine/marine aquatic invertebrates (Table 3-7 from the BE)

Taxon	**Species**	**Endpoint (µg a.e./L)**	**Effects**	**MRID**
Freshwater aquatic invertebrate (acute)	Early 4th instar midge larvae (*Chironomus plumosus*)	EC_{50} = 53,200	Mortality	00162296
Freshwater aquatic invertebrate (chronic)	Using data from Water flea (*Daphnia magna*) and Early 4th instar midge larvae (*Chironomus plumosus*) (see BE section 3.3.1)	Calculated midge NOAEC using ACR from daphnia and midge data = 9,220	Mortality	-
Estuarine/marine aquatic invertebrate (acute)	Pacific oyster embryos (*Crassostrea gigas*)	48-hr EC_{50} = 40,000	Mortality and abnormal development	44320634
Estuarine/marine aquatic invertebrate (chronic)	Using data from amphipod (*Acartia tonsa*) and Water flea (*Daphnia magna*; see BE section 3.3.1)	Calculated NOAEC = 6,110	Mortality	-

Mortality

2,4-D

An invertebrate study exposed water flea (*Daphnia magna*) to 2,4-D choline salt did not observe any mortality in a 48-hour period at any doses tested. They determined that the 48-hour LC_{50} was greater than 40.7 mg a.e./L (MRID 48892402). Similarly, another study in *Daphnia* exposed to 2,4-D acid determined that the LC_{50} was 25 mg a.e./L (MRID 41158301). A study in eastern oyster (*Crassostrea virginica*) observed mortality of a single individual at the highest treatment level tested (115 mg a.e./L). Additional studies in other aquatic invertebrates exposed to other forms of 2,4-D have comparable results. The 96-hour LC_{50} in fiddler crabs (*Uca pugilator*) exposed to 2,4-D DMA is 83.3 mg a.e./L (MRID 25389), in grass shrimp (*Palaemonetes pugio*) exposed to 2,4-D 2-EHE is greater than 1.26 mg a.e./L (MRID 41835206), and pink shrimp (*Penaeus duorarum*) exposed to various forms of 2,4-D range from 67.3-467 mg a.e./L (MRID 41975107, 41737306).

Glyphosate

A study in fourth instar *Chironomus* midge larvae determined the LC_{50} was 53.2 mg acid equivalent (a.e.)/L (MRID 0016296). Studies in water flea (*Daphnia magna*) show a range of toxicities, with the LC_{50} ranging from 128.1-647.4 mg a.e./L (MRID 4320631, 00108172). Available studies from the open literature showed that copepods (*Acartia tonsa*) had a 48-hour acute LC_{50} of 35.5 mg a.e./L (Tsui and Chu, 2003). Similarly, an acute toxicity study in mysid shrimp (*Americamysis bahia*) determined the 96-hour LC_{50} was 76 mg a.e./L (MRID44320633).

Sublethal Effects

2,4-D

Sublethal effects resulting from chronic exposure can include effects to growth, reproduction, or behavior. A chronic *Daphnia* study observed effects to survival and reproduction (i.e., number of neonates produced, number of broods produced, and brood size). The study authors determined the no observable adverse effect concentration was 16.05 mg a.e./L (MRID 42018303). Eastern oyster exposed to the isopropylamine salt of 2,4-D showed significant reductions in feeding behavior as well as reduced shell growth. The chronic EC_{50} based on growth for the eastern oyster was determined to be 49.6 mg a.e./L.

Glyphosate

A chronic toxicity test in *Daphnia magna* reported a NOAEC of 49.9 mg a.e./L and a lowest observed adverse effect concentration (LOAEC) of 95.7 mg a.e./L. A developmental study in Pacific oyster (*Crassostrea gigas*) embryos determined the EC50 for normal development was 40 mg a.e./L. Given the dearth of chronic toxicity studies available in both freshwater and marine invertebrates, EPA analytically determined a NOAEC for chronic fresh and estuarine/marine invertebrates using an acute to chronic ratio. The calculated chronic NOAEC for freshwater and marine/estuarine invertebrates was 9.22 mg a.e./L and 6.11 mg a.e./L, respectively.

Incident Reports for Aquatic Invertebrates: We are unaware of any incident data related to aquatic invertebrates for Enlist One or Enlist Duo.

General Effects to Plants

Exposure to plants occurs through contact can occur either through foliar spray application or through runoff. Toxicity data provided in the BE are primarily from greenhouse experiments or fields studies using planted crops, which are conducted under conditions that mimic those occurring on agricultural fields. These studies use spray application designed to expose plants to predetermined concentrations of active ingredients and are carried out for a set duration (e.g., 14 days, 28 days) with a desired endpoint in mind (e.g., plant height, seedling emergence, yield). These types of tests are valuable for establishing causal relationships between exposure to the pesticide and response of the organism to that exposure. At the same time, such tests are limited in being representative of field exposure and associated species response. While greenhouse and field crop studies are designed to mimic exposures occurring on agricultural fields, there are fewer studies that mimic off-site exposure.

2,4-D and glyphosate have been shown to cause a wide variety of effects in plants. Primary effects include growth inhibition, which, if severe enough, can lead to mortality. Other possible effects include effects to seedling emergence and reproduction (e.g., reduced yield). Whenever available, studies exposing plants to Enlist Duo (2,4-D and glyphosate in combination) are used as references to address any interactive effects that the two active ingredients may have.

Effects to Aquatic Plants

Most of the available 2,4-D and glyphosate toxicity studies for aquatic plants focus on growth endpoints. The available toxicity data are provided below for aquatic plants, and this section also includes a discussion of available incident reports, which describe any exposure or effect from a pesticide's use that is not expected or intended. Pesticide incidents may involve humans, wildlife, plants, domestic animals (e.g., pets) and bees. Pesticide spills can also be a type of incident. The discussion of the following data is specifically related to growth. These data are used to help assess the potential for indirect effects (i.e., impacts to food or habitat resources) for any listed species or critical habitat that relies on plants.

Growth

Numerous studies have tested the toxicity of 2,4-D and glyphosate on non-vascular and vascular aquatic plants; however, there are no studies on the effects of Enlist Duo on aquatic plants. The toxicity values for aquatic plants are based on experimentally determined endpoints for 2,4-D and glyphosate based on varying durations, exposure routes, and study designs. Toxicity values in this assessment are based on endpoints expressed in, or readily converted to, environmentally relevant exposure concentrations (i.e., µg a.i./L). Toxicity data for 2,4-D and glyphosate are available across four orders of non-vascular plants (i.e., Nostocales, Naviculales, Sphaerocleales, and Thalassiosirales), represented by 4 families (i.e., Nostocaceae, Naviculaceae, Selenastraceae, and Skeletonemataceae), and five genera (Anabaena, Navicula, Pseudokirchneriella, Selenastrum, and Skeletonema). Vascular plants are represented by one dicot (duckweed, *Lemna minor*) and one monocot (*Myriophyllum aquaticum*).

2,4-D

Because of the relatively few species of aquatic vascular and nonvascular plants that have been tested, it is not possible to derive an SSD specific to aquatic plant growth effects. Therefore, the aquatic plant toxicity values are based on the lowest values available for the taxon and are discussion below (Table 19). Toxicity values are provided in exposure units of 'µg a.e./L' and are provided for post-emergence (e.g., vegetative vigor studies) exposures. A non-vascular aquatic plant study for 2,4-D choline salt (the form of 2,4-D present in Enlist formulations) was available using green alga, which found that yield and growth rates were not affected at doses below 23,300 µg a.i./L. Studies have noted more sensitive responses in aquatic plants to other forms of 2,4-D, such as 2,4-D dimethylamine (DMA), which caused 50% growth inhibition at 3,880 µg a.i./L. Similarly, 2,4-D diethanolamine (DEA) caused 50% growth inhibition in duckweed at 297 µg a.i./L. The lowest observed adverse effect concentration (88 µg a.i./L) caused a 40% reduction in frond number. Higher concentrations of 2,4-D DEA led to colony breakup, root destruction, and an increase in frond chlorosis were observed Table 19 below.

While there is data that suggests the various forms of 2,4-D can negatively affect aquatic vegetation, the specific form of 2,4-D in Enlist pesticide formulations are expected to cause relatively lower levels of effect on aquatic plants.

Table 19**.** Toxicity Values for 2,4-D and Aquatic Plant Species (Table 2-9 from the BE)

Taxon	**Species**	**Endpoint (µg a.i./L)**	**Effects**	**MRID**
Non-vascular aquatic plant	Green algae (*Pseudokirchneriella subcapitata*)	IC_{50} > 45,850 NOAEC = 23,300 LOAEC = 45,850	Reduction in yield	48892405
Non-vascular aquatic plant	Freshwater diatom (*Navicula pelliculosa*)	EC_{50} = 3,880 NOAEC = 1,410 LOAEC = 1,925	Reduction in growth	41505903
Vascular aquatic plant	Duckweed (*Lemna gibba*)	EC_{50} = 297 NOAEC = 47 LOAEC = 88	Reduced frond number (i.e., growth)	42712204

<u>Glyphosate</u>

Toxicity tests in non-vascular aquatic plants (e.g., bluegreen algae) exposed to glyphosate resulted in a four-day EC_{50} of 11.4 mg a.i./L. A study using aquatic vascular plants (duckweed) gave similar results with a 14-day EC_{50} of 11.9 mg a.i./L. An SSD for glyphosate is available in EPA's draft glyphosate biological evaluation, which indicates that 95% of species are expected to show less than 25% growth inhibition response (i.e., HC_{05}) at 5 mg a.i./L. These results suggest that aquatic plants are quite tolerant of glyphosate exposure. Toxicity values for all aquatic plants exposed to glyphosate are provided in Table 20 below.

Table 20. Toxicity Values for glyphosate and Aquatic Plant Species (adapted from Table 3-7 from the BE)

Taxon	Species	Endpoint (µg a.i./L)	Effects	MRID
Non-vascular aquatic plant	Bluegreen algae (*Anabaena flos-aquae*)	4-day EC_{50} = 11,400 NOAEC = NR	Reduced growth	40236904
Vascular aquatic plant	Duckweed (*Lemna gibba*)	14-day EC_{50} = 11,900 NOAEC = 1,300	Reduced growth	44320638

Other effects: No other toxic endpoints were reported for glyphosate in aquatic plants.

Incident data*:* We are unaware of any incident data on 2,4-D, glyphosate, or Enlist Duo involving aquatic plants.

Effects to Terrestrial Plants

Most of the available 2,4-D and glyphosate toxicity studies for terrestrial plants focus on seedling emergence and growth endpoints (i.e., vegetative vigor), however survival and reproduction data are also reported in the BE and summarized here and in Table 21 below. The available toxicity data are provided below for terrestrial plants along with a discussion of available incident reports, which describe any exposure or effect from a pesticide's use that is not expected or intended. Pesticide incidents may involve humans, wildlife, plants, domestic animals (e.g., pets) and bees. Pesticide spills can also be a type of incident.

The discussion of the following data is formatted to broadly follow the effects endpoints, specifically those related to seedling emergence and growth. These data are used to help assess the potential for direct effects (i.e., mortality and sublethal impacts) to listed terrestrial plants and their designated critical habitats (if applicable) for any listed species or critical habitat that relies on listed plants.

There is available data on the effects of Enlist Duo on terrestrial plants for growth, seedling emergence, and mortality. Additional information regarding effects to reproduction are available for 2,4-D and glyphosate separately. Data are available across eight orders of plants, including six dicots (i.e., Caryophyllales, Brassicales, Cucurbitales, Fabales, Asterales, and Solanales) and two monocots (i.e., Poales and Asparagales), seven families of dicots (i.e., Polygonaceae, Brassicaceae, Curcubitaceae, Amaranthaceae, Fabaceae, Asteraceae, and Solanaceae) and two families of monocots (i.e., Poaceae and Amaryllidaceae), and 10 species of dicots (i.e., buckwheat, cabbage, cucumber, mustard, rapeseed, radish, soybean, sugarbeet, sunflower, and tomato) and five species of monocots (i.e., corn, oat, onion, sorghum, and wheat). Additional studies conducted using one monocot weed species (quackgrass, *Agropyron repens*) and two dicot weed species (horseweek, *Conzya canadensis* and lambsquarter, *Chenopodium album*), which are target species of Enlist pesticides.

Growth

Crop species seedlings exposed to a single application of Enlist Duo exhibited significant effects to growth (vegetative vigor), including height and dry weight metrics. Growth responses varied across species and were generally less severe in monocot (i.e., wheat, onion, corn, sorghum) than dicot crops. Tomato was the most sensitive species tested, showing 69, 82, and 88% inhibitions in plant height at 0.044, 0.088, and 0.176 lb 2,4-D choline salt/acre treatments, respectively (glyphosate was assumed to be present in amounts proportional to the original formulation concentration). Tomato plants had significant inhibitions in dry weight, with a 12 and 96% reduction at 0.011 and 0.176 lb 2,4-D choline salt/acre treatment, respectively. Wheat was the most sensitive monocot species tests, and had 36, 44, and 42% reductions in plant height and 52, 47, and 58% reduction in dry weight at 0.088, 0.176. and 0.35 lb 2,4-D choline salt/acre treatment, respectively. These results suggest that dry weight is more sensitive of an indicator than plant height (although the two responses are similar in magnitude). The calculated dry weight NOEC and IC_{25} for tomato is 0.0145 and 0.011 lb ai/acre, respectively. The calculated dry weight NOEC and IC_{25} for wheat is 0.071 and 0.0873 lb ai/acre, respectively.

Similarly, common weed species also show significant reductions in growth (i.e., plant height and dry weight) with a single post-emergence exposure to Enlist Duo. Horseweed (*Conyza canadensis*), lambsquarter (*Chenopodium album*), and quackgrass (*Agropyron repens*) height was reduced up to 59, 77, and 27% at 0.179, 0.7, and 0.35 lb 2,4-D choline salt/acre, respectively. Reductions in dry weight were slightly more severe than effects to plant height, and were up to 88, 71, and 70% less than control for horseweed, lambsquarter, and quackgrass at 0.083, 0.7, and 0.35 lb 2,4-D choline salt/acre. Quackgrass was the only monocot weed tested and had a dry weight NOEC and IC_{25} of 0.083 and 0.113 lb 2,4-D choline salt/acre. Horseweed was the more sensitive dicot weed and had a dry weight NOEC and IC_{25} of 0.011 and 0.0275 lb 2,4-D choline salt/acre.

Lambsquarter seeds exposed to a single pre-emergence application of Enlist Duo similarly exhibited significant reductions in plant height and dry weight. Seedling height was 39, 38, 49, and 52% of control plant height when treated with 0.176, 0.36, 0.72, and 1.42 lb 2,4-D choline salt/acre. Seedling dry weight was reduced relative to control seedlings by 61, 62, 84, and 67% at 0.176, 0.36, 0.72, and 1.42 lb 2,4-D choline salt/acre treatments. The calculated NOEC and IC_{25} for lambsquarter seedling dry weight is 0.088 and 0.101 lb 2,4-D choline salt/acre, respectively.

Table 21. Species toxicity for growth endpoints (in lb 2,4-D choline salt/acre) stage plants exposed to Enlist Duo (from Table 2-13 in BE)

Taxa	**Species**	**Endpoint**	**NOEC**	**IC_{25}**
Dicot	Buckwheat	Dry weight	0.011	0.0291

Taxa	**Species**	**Endpoint**	**NOEC**	**IC_{25}**
Dicot	Cabbage	Dry weight	0.0027	0.044
Dicot	Cucumber	Dry weight	0.0055	0.052
Dicot	Horseweed	Dry weight	0.011	0.0112
Dicot	Lambsquarter	Height	0.044	0.0352
Dicot	Mustard	Height	0.0055	0.0157
Dicot	Rapeseed	Dry weight	0.0221	0.056
Dicot	Radish	Dry weight	0.011	0.0234
Dicot	Soybean	Dry weight	0.0221	0.141
Dicot	Sugarbeet	Dry weight	0.0221	0.141
Dicot	Sunflower	Dry weight	0.011	0.0329
Dicot	Tomato	Dry weight	0.011	0.0145
Monocot	Corn	Dry weight	0.176	0.161
Monocot	Oat	Dry weight	0.088	0.301
Monocot	Onion	Dry weight	0.37	0.304
Monocot	Quackgrass	Dry weight	0.083	0.113

Taxa	**Species**	**Endpoint**	**NOEC**	**IC_{25}**
Monocot	Sorghum	Height	0.176	0.314
Monocot	Wheat	Dry weight	0.044	0.0711

Mortality

Crop species seedlings exposed to a single application of Enlist Duo, post-emergence, exhibited significant effects to survival. Similar to growth effects, there is a range of mortality observed in greenhouse studies across multiple species, with monocot species generally showing less sensitivity than dicot species. Complete mortality was observed in all tested crop species except corn and onion. Tomato was the most sensitive plant species tested, showing 33, 97, and 100% mortality at 0.088, 0.176, and 0.37 lb 2,4-D choline salt/acre, respectively. Wheat was the most sensitive monocot species tested, showing 60, 77, and 100% mortality at 0.176, 0.35, and 0.71 lb 2,4-D choline salt/acre. The calculated mortality NOEC and LC_{25} for tomato is 0.0145 and 0.011 lb ai/acre, respectively. The calculated dry weight NOEC and LC_{25} for wheat is 0.071 and 0.0873 lb ai/acre, respectively.

Similarly, common weed species showed a range of effects to survival in response to post-emergence exposure to Enlist Duo. Quackgrass seedling survival was reduced by 24% at 0.35 lb 2,4-D choline salt/acre, but in contrast, horseweed and lambsquarter seedlings showed to significant inhibitions to survival. Similarly, lambsquarter seeds exposed to a single application of Enlist Duo pre-seedling emergence showed no effect on survival.

Seedling Emergence

Weed species seeds exposed to a single, pre-emergence application of Enlist Duo showed significant inhibition of seedling emergence. Lambsquarter seeds showed significant inhibition in seedling emergence over 28 days at 1.41 lb 2,4-D choline salt/acre (glyphosate was presumed to be present in amounts proportional to 2,4-D reflective of the formulation concentrations). The calculated IC_{50} for seedling emergence is 0.786 lb 2,4-D choline salt/acre, and the calculated NOEC is 0.71 lb 2,4-D choline salt/acre.

Reproduction and Yield

2,4-D

Studies have shown that 2,4-D exposure can decrease plant yield, indicating reproductive effects are likely. A 28-day field-based toxicity study with soybean showed that a treatment of 0.026 lb 2.4-D/acre reduced yield by 10% (Robinson, Davis, Simpson, & Johnson, 2013). Similarly, Andersen et al. found that 0.05 lb 2,4-D/acre exposures led to a 7% yield reduction in V3 soybeans, and 25-32% yield loss at 0.1 lb 2,4-D/acre (Anderson, Clay, Wrage, & Matthees, 2004). Studies using field cotton show similar results, with observations of 10% yield loss occurring at 0.0025 lb 2,4-D/acre and 15-97% yield reductions occurring at 0.025-0.25 lb 2,4-D/acre (Everitt & Keeling, 2009; Marple, Al-Khatib, & Peterson, 2008). Furthermore, yield effects have been shown to be time dependent, as exposures occurring in younger plants resulting in higher yield losses (Everitt & Keeling, 2009). Similarly, additional exposure events can exacerbate reproductive effects. Marple et al. (2008), found that 2-3 exposures at 0.00625 lb 2,4-D/acre caused approximately 40% reductions in yield, indicating that frequent exposures at lower concentrations can cause effects of similar magnitudes as single exposures at higher concentration.

While 2,4-D exposure can cause adverse effects to plant yield and reproduction, these types of effects either only begin to occur at exposure levels higher than those where growth or morphological impacts occur or show a smaller impact than growth effects at the same concentration. Everitt and Keeling only observed substantial visible signs of injury (VSI; a morphological effect related to growth) in field cotton at all test concentrations but only observed significant yield loss at the two highest concentrations (Everitt & Keeling, 2009). Robinson et al., observed a larger magnitude of VSI effects than yield loss in field soybean crops, where a 0.026 lb 2,4-D/acre treatment resulted in a 10% seed-yield loss but a 35% VSI (Robinson, Davis, Simpson, & Johnson, 2013). Other field soybean studies show that reductions in plant weight were greater compared to reductions in yield across multiple test concentrations and time points. In the same study, VSI was found to be generally two times greater than the impacts to yield (Anderson, Clay, Wrage, & Matthees, 2004). Thus, we consider reproductive effects as less sensitive than effects to growth or survival as they either occur at concentrations higher than those that cause growth or morphological effects or result in smaller impacts than growth or morphological effects. Thus, in this consultation, we primarily focus on effects to plant growth as these effects likely occur at lower concentrations and are more severe than sublethal effects to reproduction or yield. We expect that focusing on growth will be protective of any sublethal effects to reproduction.

Glyphosate

Recent research has demonstrated that glyphosate can affect plant reproduction and seed yield. Greenhouse and field studies of rice varieties found that yield decreases with increasing glyphosate exposure, however, the magnitude of yield reduction can vary drastically across different varieties of rice, with modeled 50% yield reductions ranging from 0.05-0.3 lb glyphosate/acre (Koger, et al., 2005). Studies in weed species have also found reduction in seed production related to late season glyphosate exposure. Barnyard grass, Palmer amaranth, pitted morning glory, prickly side, and sicklepod all exhibited significant reductions in seed production when exposed at flowering stages to repeated doses of 0.32 lb glyphosate/acre every 10 days or with a single exposure at 0.76 lb glyphosate/acre (Walker & Oliver, 2008). Additional greenhouse studies using *Xanthium strumarium*, *Sesbania exaltata*, and *Senna obtusifolia,* showed substantial reduction in the number of seeds produced as well as seed weight when exposed to 0.76 lb glyphosate/acre, but only when exposed at seed or fruit set (Clay & Griffin, 2000).

Species sensitivity distributions (SSDs)

Species sensitivity distributions of plant growth effects help inform our analyses of indirect effects to listed species that are dependent on non-listed plant species (i.e., for food or habitat). EPA developed SSDs models of 25% growth inhibition (IC_{25} values) for vegetative vigor endpoints for plants exposed to 2,4-D choline salt (other forms of 2,4-D tested were not included as they do not match the chemical form of 2,4-D used in Enlist products). SSDs were generated using height and weight in order to estimate hazard criterion (HC) values, which are used to characterize the potential effects to non-listed plant species that provide food or shelter for listed species (i.e., plant-based resources). HC values for dry weight were lower, and thus more sensitive, than plant height, however, the two plant growth SSD metrics were overall very similar to each other. HC values for vegetative vigor are summarized below in Table 22. The HC_{10} and HC_{25} values, which are commonly used thresholds for characterizing potential broader impacts across species, habitats, and communities, for plant height are 0.014 and 0.023 lb a.i/acre, respectively. The HC_{10} and HC_{25} values for weight are 0.017 and 0.027, respectively.

Table 22. HC value estimates (lb a.i./acre) from SSDs derived from the IC_{25} of plant heigh and weight from vegetative vigor growth studies (Table 2-15 from BE)

Endpoint	**HC_{05}**	**HC_{10}**	**HC_{25}**	**HC_{50}**	**HC_{75}**	**HC_{90}**	**HC_{95}**
Height	0.01	0.014	0.023	0.045	0.11	0.3	0.61
Weight	0.013	0.017	0.027	0.052	0.12	0.31	0.62

Incident reports

There are, as of publication of EPA's final BE in 2022, there were 16 terrestrial plant incident reports linked to Enlist One and 12 terrestrial plant incident reports linked to Enlist Duo with a certainty index of 'possible' or 'probable' in the Incident Data System. All incident reports were related to drift and only affected cotton crops.

Exposure

Enlist One and Enlist Duo enter the environment via direct application to use sites and may be sprayed directly onto soil or foliage. In general, spray drift and runoff are considered the primary routes of offsite transport of pesticides. Current product labels require a downwind 30-foot in-field buffer for application areas adjacent to sensitive areas (as defined on the label), which we expect will retain almost all spray drift within the field (see the *Description of the Action* section for more details). EPA's spray drift models indicate that the residual fraction of spray drift that will leave application sites is unlikely to cause any toxic effects to any organisms (discussed further below). Thus, we consider runoff as the primary route of offsite transport.

In general, EPA derived exposure estimates for listed species using fate and transport models. The methodology used to derive these geographically specific EECs are described and presented in EPA's BE. EPA used combinations of several transport models including the Pesticide Root Zone Model (PRZM), the Variable Volume Water Model (VVWM), the Plant Assessment Tool (PAT), Terrestrial Residue Exposure (T-REX), and AgDrift (version 2.2.1) to generate EECs in aquatic and terrestrial habitats used by listed species, assuming pesticides were applied according to label specifications.

Rate, Frequency, and Number of Applications

Environmental concentrations are influenced, in part, by the allowable manner of pesticide use as described by the label, including the application rate, frequency of application, and the maximum number of applications per season or year. For our analyses, we assumed applicators would use the maximum application rate and number of applications and the minimum re-treatment interval allowed on the label. We recognize that Enlist pesticides may not always be used in a manner that produces maximum concentrations in the environment, but we have no usage data to estimate the specific manner that these pesticides will be used on the landscape. Thus, we rely on an approach that is conservative and that allows us to evaluate whether EPA has ensured that its action is not likely to jeopardize listed species or result in adversely modifying critical habitat.

Volatilization and Atmospheric Drift

Based on a relatively low vapor pressure (1.4×10^{-7} mm Hg), low Henry's Law Constant ($7.16*10^{-11}$ atm-m^3/mol) and moderate soil/water partitioning, 2,4-D has low volatilization potential from moist and dry soil surfaces. Results from field volatility studies suggest that volatility flux rates of 2,4-D choline salt are much lower than other forms of 2,4-D. A field study conducted on a small field (~5 acres) with 2,4-D choline salt found no signs of injury in grape and cotton plants placed near air monitoring stations approximately one hour after herbicide application (5 and 15 meters beyond the field's edge) for three days (MRID 48912102). Additionally, EPA's Probability Exposure and Risk Model for Fumigants (PERFUM) determined that estimated environmental concentrations off-field resulting from volatilization will not cause adverse damage to plants for applications of less than 80 acres. While larger application areas can have volatilization flux rates high enough to cause adverse effects to plants, EPA modeling indicates that these adverse effects will be limited to areas near application sites and are only expected to occur for a short period of time after application. Estimated environmental concentrations are expected to drastically decrease beyond one hour after application and are not expected to cause any adverse effects beyond 24 hours after application. Based on submitted studies and model results covering a wide range of field sizes, we expect volatilization of 2,4-D will cause, at most, a low magnitude of adverse effects that would occur with a low frequency, which we do not expect will cause measurable changes to survival, reproduction, or otherwise adversely affect individual fitness. As such, we do not further consider the effects of volatilization exposure in this Opinion.

Similarly, glyphosate, which has low vapor pressure and a low Henry's constant (2.1×10^{-14} atm-m^{-3} $mole^{-1}$), is expected to have a low potential of volatilization. We expect results from the 2,4-D volatilization flux rate models described above are applicable to glyphosate as well considering that glyphosate is less volatile than 2,4-D. Thus, we anticipate glyphosate also has a low potential for adverse effects as a result of volatilization and do not further consider this route of exposure in this Opinion.

Exposure Assessment for Exposure via Spray Drift

Enlist pesticide use labels require a downwind 30-foot in-field spray drift setback (buffer) for all application sites adjacent to sensitive vegetation. Combined with the requirement for all applications to be made using ground spray equipment, we expect this buffer will contain almost all spray application within agricultural fields. EPA spray drift modeling results indicate that a small fraction of spray drift (up to 0.167% of the application rate) will occur beyond the 30-foot in-field buffer. This fraction of spray drift would result in exposures to 2,4-D and glyphosate that are well below levels described above that are anticipated to cause adverse effects for all taxa. Therefore, we believe no adverse effects are expected to occur from residual spray drift that leave application sites.

Chemical Persistence

The predominant form of 2,4-D that occurs off-field is 2,4-D acid, which is the main degradate of 2,4-D choline salt. Degradation of all forms of 2,4-D to 2,4-D acid ranges from rapid to moderately rapid under aerobic terrestrial and aquatic environments, with half-life (DT_{50}) values ranging from 12-15 days. Degradation in anaerobic aquatic conditions is much slower, with DT_{50} values ranging from 29 to 333 days. We generally do not expect exposure to listed species will occur in anaerobic conditions because degradation of 2,4-D is rapid, occurring on the order of days to a few weeks. However, 2,4-D is moderately soluble in water (569 mg/L) and has a low average soil sorption coefficient (Kd) of 0.52 mg/g, and an average organic carbon-water partition co-efficient (K_{OC}) of 72 ml/g, which suggests it has the potential to move into surface water via runoff and erosion and to groundwater via leaching. Thus, the major route of transportation to locations outside of the application sites is through runoff. Bioaccumulation potential of 2,4-D is low as the octanol/water partition coefficient (K_{OW}) is low at neutral pH (K_{OW}=0.18).

Degradation of glyphosate in aerobic conditions is primarily through microbial transformation. Aerobic soil metabolism half-life ranges from 1.8 (at 25°C) to 109 (at 20°C) days and aerobic aquatic metabolism half-life ranges from 14.1 (at 25°C) to 518 (at 20°C) days. Glyphosate has a relatively high solubility (12,000 mg/L) and low K_{OC} (<0.001). While solubility is high, glyphosate salts are expected to dissociate rapidly into glyphosate acid and its counter ion, which allows it to form various metal complexes. This facilitates the formation of glyphosate-metal complexes (particularly with iron and aluminum) in soil, sediment, and aquatic environments, resulting in a high sorption affinity and reduced capacity for transport through runoff.

These properties were incorporated into EPA's environmental fate modeling to determine the range of EECs of 2,4-D and glyphosate that are likely to occur in the ranges of listed species. More details regarding the parameterization of EPA's fate modeling can be found in Chapters 2 and 3 of the Enlist One and Enlist Duo BE.

Routes of Exposure

Exposure of listed species can occur through a variety of means, including through diet, direct contact (with spray or runoff), preening, drinking water, and inhalation at different life stages. Various factors influence the likelihood and extent of exposure at both the individual and population level including both properties of the pesticide (e.g., number of applications, persistence) and life history factors of the species (e.g., dietary preference, feeding habits, species distribution, and local and long-distance movement). As described below, we consider dietary and dermal routes of exposure in animals and contact exposure for plants.

<u>Ingestion - dietary exposure</u>

A primary route of exposure to pesticides for terrestrial organisms is from ingestion of food items that have been contaminated after a pesticide application. For contaminated food items, exposure may be from pesticide residues that have been biologically incorporated into plant or animals or deposited on the surface or the plant or animal. Secondary predators may also be exposed to pesticide within prey that has not yet been biologically incorporated but resides within the gastrointestinal tract of prey (Hill & Mendenhall, 1980). The frequency of food ingestion can vary by species. Some species may hunt or graze on dietary items daily, either at certain times (e.g., dawn and dusk), or throughout the day. Other species, such as predators and scavengers (e.g., California condor, snakes) may ingest a prey item or carcass and not feed again for one or more days. Life stage may also affect the frequency of feeding, as young of altricial species may be reliant on parents to bring food back to the nest site one or more times per day. Long-distance migrators such as the red knot may gorge feed at stopover locations, then travel long distances on food stores from these events.

For terrestrial species, EPA's BE provides EECs based on output from the T-REX model on and in food items of terrestrial vertebrates as both concentration-based and dose-based values (as described in Attachment 1-7) for exposure on use sites. Pesticide concentrations vary by dietary item. Therefore, individual species may be exposed to multiple EECs based on the number of food items consumed.

For many species, dietary preferences are unknown, or the information is not readily available. For these species, we assume that individuals are only consuming the food item that produces the highest dosage of pesticide possible in order to generate conservative estimates of dietary exposure. In these cases where dietary preferences are known, we have increased confidence in the likelihood of exposure to the pesticide concentration associated with preferred dietary items. However, even if a dietary item is less preferred, it should be considered whether it may be consumed at a high enough rate to cause effects even once over the course of the entire year. In some cases, prey exposed to pesticides could be taken preferentially, as such exposure may make it more susceptible to predation (e.g., (Hunt, Bird, Mineau, & Schutt, 1992)).

<u>Contact exposure – direct spray or contact with contaminated media</u>

Terrestrial species may be exposed to pesticides through direct contact with a pesticide followed by dermal absorption. Exposure may occur from pesticides directly deposited on an individual during a spray or individuals contacting contaminated media after a spray, such as walking on a treated field or brushing against treated foliage. Studies involving cholinesterase-inhibiting pesticides in particular have shown this can be a significant route of pesticide exposure for terrestrial vertebrates, especially for birds (Henderson, Yamamoto, Fry, Seiber, & Wilson, 1994; Vyas , et al., 2006; Schafer, Brunton, Lockyer, & De Grazio, 1973; Hudson, Haegele, & Tucker, 1979). However, for Enlist One and Enlist Duo, contact exposure is not expected to be of concern. While data are lacking for contact toxicity of 2,4-D in other terrestrial vertebrates, acute studies in mammals showed dermal exposure to be a less sensitive route of exposure than oral toxicity, with no mortalities and only mild effects at concentrations nearly twice the oral acute LD_{50} (MRID 414135-02). For honey bees, EPA estimated contact exposure using the Bee-REX model (version 1.0), which calculates high end but reasonably conservative exposure concentrations. Models indicate that on-field contact with 2,4-D residues will result in environmental concentrations well below levels used in reference toxicity tests where acute effects were observed. For glyphosate, we expect only low levels of adverse effects for terrestrial vertebrates, which show little sensitivity to this pesticide through from oral exposure. Similarly, reference toxicity studies of glyphosate in adult honey bees all showed low mortality at even the highest doses tested. Given that Bee-REX model results indicate dermal contact exposure to glyphosate is expected to be well below highest test concentrations, we expect only low levels of adverse effects to terrestrial invertebrates resulting from contact exposure.

Overall, based on the data available for contact exposure in terrestrial animals, we do not anticipate this type of exposure will result in adverse effects to listed animal species. In addition, given the conservative nature of the dietary assessment (i.e., diets consisting of only forage/prey items exposed at maximum concentrations) and the comparative data between the two routes of exposure, we do not expect that contact exposure will result in adverse effects to terrestrial animals.

Determining Exposure to Enlist Pesticides

Percent Overlap

We determined the exposure of species to pesticides by considering the overlap of pesticide use sites and associated off-site transport with individuals within the landscape, as determined by the range of the species and the anticipated distribution of individuals within the range. We derive the estimate of exposure for each species, in part, by determining the extent that the range of a species overlaps with use sites for which the pesticide is registered, combined with anticipated off-site transport. The process for establishing the use site footprint is generally described in Section 4.1.2 of EPA's BE. Briefly, EPA conducted a review of the proposed labels for Enlist One and Enlist Duo to determine the use sites, application requirements and restrictions, and any required geographical restrictions on the proposed labels, identified potential use sites of Enlist products within the states and counties included on the proposed labels, determined how far off application sites' effects are reasonably certain to occur, and established a geographical information (GIS) data layer that combines use sites with the extent of off-site areas. For the overlap with species range, the BE considers the aggregate of the six years of available Cropland Data Layers (CDL) data for corn, cotton, and soybeans to ensure the full footprint is captured for each use.

This exposure data layer was overlaid on species and critical habitat ranges to determine the “percent overlap”, which we use as a metric of the extent of possible exposure. Pesticide concentrations are expected to vary greatly between on- and off-site exposure and pose different levels of risk to listed species. Thus, we determine the percent overlap with use sites and the percent overlap with offsite areas (i.e., runoff zones) and consider them separately to describe the anticipated exposure to listed species more accurately.

When mapping use sites, EPA found redundancies among various use sites. That is, mapped use sites are not mutually exclusive of one another. For instance, crop rotation practices can lead to soybeans and corn grown in the same fields in different years. As USDA CDL layers aggregate crop presence over many years, the same fields will be included in both corn and soybean layers. For this reason, combining the percent overlap for use sites may overestimate the total amount of a species’ range that is overlapping with use sites.

Distribution of Individuals and Presence Within the Action Area

Our default assumption is that individuals are uniformly distributed throughout the range and that the percent overlap indicates the percent of the population potentially exposed. The assumption of a uniform distribution can either increase potential exposure by artificially expanding the area of exposure to the whole range or decrease the potential exposure by failing to identify high density areas that overlap with pesticide use sites. We modify this assumption for the probability of exposure with relevant species-specific information whenever available. Factors such as habitat preference, behaviors like colonial nesting or flocking, and known areas of high or low density of individuals are considered in determining the extent of exposure for the entire species. For instance, where information exists that precludes the use of agricultural areas by individuals of a species, we consider that exposure occurring on application sites is likely to be low, regardless their extent of overlap with the species range. We used input from species experts within the Service and any available Service documents (e.g., recovery plans, five-year reviews, previous biological opinions) to inform species-specific assessments of exposure.

Timing of Exposure with Key Life History Events

The timing of important life history events (e.g., migration, hibernation/estivation, germination) can also modulate the likelihood of exposure and can either increase or decrease the risk of adverse effects to listed species. Given that Enlist One and Enlist Duo have a narrow range of authorized application periods that are associated with specific stages of crop development (e.g., post-emergence applications can only be made up to a certain developmental stage in corn, soybean, and cotton), the timing of important life history events can provide important information regarding the likelihood of exposure.

Approach to the Effects Analysis

Where the BE indicated likely effects to an individual of a listed species, we carried forward with a population level assessment. We assessed the following responses for each listed species, where applicable, by considering all effects observed in toxicity studies, including:

1. Mortality to portions of the population(s) of a listed species from direct, acute exposure from the use of Enlist One and Enlist Duo according to registered labels

2. Altered growth among portions of the population(s) (potential for decreased survival) from the use of Enlist One and Enlist Duo according to registered labels

3. Indirect effects to species, including declines in other organisms on which the species depends to complete its life history (e.g., prey/food of a listed species, host fish for mussel glochidia, pollinators and seed dispersers) and impacts to suitability and quality of habitat on which the listed species depends

To assess each effect endpoint, we determined what percentage of the individuals were anticipated to be exposed to Enlist One and Enlist Duo at concentrations that may cause adverse effects, the expected magnitude of those effects, and, when applicable, the frequency that exposures would cause significant effects. To determine the proportion of individuals exposed, we considered the overlap of the species range with pesticide use sites, incorporating life history information when available and relevant, as it pertains to the likelihood of exposure occurring. To determine the magnitude of effect (e.g., anticipated percent growth reduction or probability of mortality), we used the most applicable taxonomic endpoint for each species to assess direct mortality or sublethal effects to listed species, and indirect mortality via loss of food resources, habitat, host species, or pollinators and seed dispersers. To further contextualize the likelihood of adverse effects occurring, we evaluated the proportion of modeled exposure scenarios that are expected to cause significant effects to the species. Given that existing product labels already include spray drift control measures that we expect are effective in keeping the majority of product applied on field (i.e., within the area of application on the crop), we expect the subsequent risk of adverse effects to listed species will vary depending on whether exposure occurs on or off agricultural fields where Enlist pesticides are applied. We separate our analyses of effects to listed species based on whether we expect on- or off-field (or both types) exposure is likely to occur. We summarize our approach in Table 23.

Table 23. Summary of Approach to Effects Analysis

Key Questions	Information	Risk Metrics
What is the evidence supporting risk to individual fitness?	Anticipated exposures and concentration-response relationships	Overlap, expected magnitude of effect, and risk modifiers
What is the anticipated magnitude of the risk to individuals?	Species-specific demographic and life history information when available	Magnitude of effect in exposed individuals, percent of population affected
What proportion of the population is likely to be affected?	Overlay exposure and species distribution in space and time	Percent overlap with use sites and runoff areas

Key Questions	Information	Risk Metrics
How often are toxic effects expected to occur?	Spatially refined runoff exposure estimates	Proportion of runoff events likely to cause adverse effects

Methodology

To carry out these analyses for the species included in this consultation, we characterized the extent of exposure, the magnitude of effect, and the expected likelihood of any exposure event to cause adverse effects (based on spatially refined exposure estimates specific to each species). The general approach is provided below.

<u>Extent of Exposure</u>

To approximate the extent of exposure listed species are likely to experience, we use the overlap between the species' range and Enlist herbicide application sites (i.e., corn, cotton, and soybean fields) and their respective runoff zones. We use Assuming that species has a uniform distribution, we expect the percent overlap represents the percent of individuals that are likely to experience exposure.

We adjust this extent of exposure when available species-specific information suggests this uniform distribution assumption is inappropriate (e.g., occurrence data, known habitat preference, specific life history traits) and that the percent overlap over- or underestimates the likely extent of exposure. We consider the likelihood of exposure in context of the species' life history and vulnerability. We also reviewed available information (e.g., species range maps, agricultural use maps) to determine whether any areas of particular importance to the species (e.g., mating grounds, migration stopovers, spawning grounds) are likely to experience exposure that could result in a disproportionate adverse impact to the species.

On-field Exposure

We expect that listed species will experience toxic effects (i.e., reductions in growth or mortality) from direct contact with pesticide residues via spray application or from consuming food items exposed in this manner. The majority of spray applications are likely restricted to directly on agricultural fields (referred to as on-field exposure) as current label instructions include a mandatory 30-foot in field buffer during application, which effectively keeps spray drift within fields (see the *Conservation Measures* section for more details). While some amount of spray drift could leave the field and cause off-field exposure to listed species, EPA's spray drift deposition models indicate that only a very small fraction of applied pesticide is expected to move beyond the in-field buffer (i.e., the fraction of applied pesticide depositing beyond 30 feet is less than 0.167%). This level of spray deposition will lead to exposures well below toxic thresholds for even the most sensitive species. Thus, we consider the effects of spray application as an on-field occurrence only.

We use the percent of a species' range that overlaps with corn, cotton, or soybean fields to represent the extent of on-field exposure. Application sites will have the highest environmental concentrations of Enlist pesticides. We modify the expected extent of on-field exposure, when appropriate, based on relevant information indicating a listed species may have an increased or decreased tendency to occur on-field. Examples include timing of active and dormant periods that may not overlap with pesticide application periods, or habitat preferences that include highly modified or disturbed areas such as row crop fields. We qualitatively adjust the expected extent of on-field exposure when relevant information is available.

Off-field Exposure

As discussed above, we consider off-field exposure through spray drift as negligible and runoff as the only source of potential exposure occurring off-field. We anticipate that runoff will contain the highest off-field estimated environmental concentrations (EECs) in areas adjacent to agricultural fields. To estimate the extent of possible runoff exposure for listed species, we used the overlap between the species range and application sites buffered out to 30 meters. We anticipate that the likelihood of runoff exposure will decrease with increasing distance from application sites as runoff is likely to be intercepted by vegetation, redirected through local topography, and lost through penetration into the soil column. Thus, we consider 30 meters a sufficient estimate of the extent of runoff exposure in field-adjacent areas. While it is possible for runoff to reach wetland habitats located further than 30 meters from agricultural sites through channelized flow, we expect this runoff will similarly dissipate, degrade, or dilute with distance from crop fields. Thus, we consider 30 meters a sufficient estimate of the extent of runoff exposure in field-adjacent areas.

We anticipate that runoff exposures will be greatest in areas immediately adjacent to agricultural fields. We use the overlap between the range of the species and application sites buffered out to 30 meters to estimate the likely extent of runoff exposure for listed species. We anticipate that most runoff will decrease with increasing distance from application sites as sheet flow from agricultural fields is likely to be intercepted by vegetation or other features, redirected through local topography, and lost through penetration into the soil column and sorption onto soil material. It is possible for runoff to reach wetland habitats located further than 30 meters from agricultural sites through channelized flow, though we expect this runoff will similarly dissipate, degrade, or dilute with distance from crop fields. Thus, we consider 30 meters a sufficient estimate of the extent of runoff exposure in field-adjacent areas.

We adjust the expected extent of runoff exposure based on relevant life history traits whenever that information is available. We incorporate species-specific factors such as habitat preference, life history traits, behaviors like colonial nesting or flocking, habitat characteristics (e.g., hydrology, climate, topography), and known areas of high or low density of individuals of the species into our analyses to ensure we are appropriately assessing the likelihood of off-field exposure.

<u>Magnitude of Effect</u>

We consider the effects of 2,4-D and glyphosate exposure together whenever possible to address any potential interactive effects that may occur with co-exposure to the two AIs. Toxicity data on Enlist Duo is available for terrestrial plants but is not available for animals or aquatic plant species. In cases where 2,4-D and glyphosate mixture data are not available, we assess the effects of 2,4-D and glyphosate separately and assume that toxic effects resulting from co-exposure to the two AIs follow an additive relationship, rather than a synergistic one (see *Toxicological Effects* section above).

To determine the magnitude of direct and indirect effects to listed species, we compare EPA's exposure estimates for each species to dose-response curves corresponding to the relevant toxic endpoint to determine the likely magnitude of effect that will occur as a result of exposure. Given that different taxa will have different sensitivities to herbicides like Enlist One and Enlist Duo, we break down our approach for analyzing direct and indirect effects to plants and animals separately.

Effects to Growth and Mortality

Listed Plant Species

We expect that exposure (whether through spray application or runoff) will cause growth effects in plants, which, if severe enough, could lead to mortality or reduced long-term viability. Greenhouse studies indicate that effects to growth (i.e., reductions in plant height and weight) are the most sensitive plant responses to 2,4-D and glyphosate exposure as they occur at much lower concentrations than effects to reproduction or yield (see the *Effects to Terrestrial Plants* section for more details). While sublethal effects like impacts to reproduction or indirect effects (e.g., effects to other plant species, effects to pollinators, effects to seed dispersers) may be possible, we do not expect these impacts will result in measurable effects to the species compared to the impact of direct toxic effects to growth and survival. Thus, we only consider growth effects and mortality when analyzing effects to terrestrial plant species.

On-field exposure will likely cause mortality of plants, as Enlist pesticides are designed specifically to kill non-genetically modified plant species that occur on agricultural fields. Thus, we assume all listed plant species individuals occurring on-field will experience mortality.

To determine the expected magnitude of off-field effects resulting from runoff exposure, we constructed dose-response curves for growth effects and mortality based on the most sensitive data reported in EPA's BE (i.e., growth and mortality effects to tomato). We use these dose-response curves to determine the likely percent reduction in growth an exposed individual may experience and the percent of exposed individuals likely to die associated with the 95th percentile runoff EEC predicted to occur within the species range. We use the 95th percentile runoff EEC as a representative exposure for our analyses as it roughly corresponds to a one-in-ten-year value, which we expect is likely to occur at least once within the 15-year duration of the Action. Thus, we consider it an appropriately conservative exposure estimate that is still reasonably certain to occur.

We consider responses of 50-99% growth inhibition or 1% or greater mortality as a high magnitude of adverse effect. We categorize growth effects ranging from 25-50% as a moderate magnitude of adverse effect. While direct mortality is unlikely at this exposure, we expect adverse impacts to long-term survival of individuals are still likely as this level of reduced growth will likely reduce an individual plant's capacity to recover from herbivory, pest pressure, or other environmental stressors (e.g., drought). Exposures causing less than 25% growth inhibition are considered low in magnitude as these effects are likely temporary, recoverable within a growing season, and not likely to impede recovery from other stressors.

Listed Animal Species

EPA's environmental fate modeling indicates that off-field EECs of both 2,4-D and glyphosate are well below toxic thresholds for even the most sensitive species. Thus, we expect that direct effects to growth and mortality for animals will only occur on-field. On-field exposures to vertebrates will primarily occur through ingestion of contaminated food items. EPA estimated exposures based on species-specific factors such as body mass, assimilation efficiency, and dietary items, among other considerations. On-field exposures to invertebrates will occur primarily through direct contact with spray application and ingestion of pesticide residues through food sources such as pollen and nectar, which are modeled based on honey bee contact and oral exposure models. We compare expected body burdens in on-field animals to reference dose-response curves to determine the likely magnitude of adverse effects resulting from on-field exposures (i.e., the percent of the population that may experience growth effects or mortality).

Our analyses of direct effects to animals assumes that species are foraging on-fields after the maximum number of applications are made, are only consuming contaminated dietary items, and are only consuming the dietary item that results in the highest dietary exposure possible. There are not enough data to create SSDs for animals in response to 2,4-D or glyphosate exposure, so the most sensitive endpoint observed in the scientific data was used for each taxa to determine the magnitude of effect. We qualitatively modify the expected extent of on-field exposure based on any species life history information available regarding their preference for and tendency to enter agricultural sites. We focus on growth and mortality effects in animals, as discussed in the *Toxicological Effects* section.

Effects to Plant-based Resources

While runoff EECs will not likely ever be high enough to cause direct effects to growth or mortality in listed animal species, we expect indirect effects resulting from effects to plants that listed animal species rely on for food or habitat may still occur. We compared the 95th percentile runoff EECs, which we consider to be the highest EEC that is reasonably certain to occur within the duration of the action, to a plant growth SSD to estimate the proportion of plant species occurring in runoff zones that are likely to experience moderate growth effects (i.e., at least 25% growth inhibition). We assumed that the proportion of sensitive plant species experiencing moderate growth effects reflects an equivalent loss of plant-based resources for animals (e.g., if 27% of plant species experience moderate growth effects, that represents a 27% loss in plant-based resources for animals).

We consider EECs that result in moderate effects to 50% or more plant species a high magnitude of effect. While most plant species will likely only experience moderate adverse growth effects at this exposure, more sensitive species may experience high levels of reduction in growth and may even experience some level of acute mortality, which could result in immediate impacts to the availability of plant-based resources. We consider EECs that result in moderate effects to 25-50% of plant species a moderate magnitude of effect to plant-based resources as we do not expect acute mortality of plant species is likely to occur at these exposure levels (even in the most sensitive plant species). However, growth effects may be severe enough to impact the long-term survival of exposed plants, which could reduce long-term availability of plant-based resources for listed animals. We consider EECs that result in moderate effects to less than 25% of plant species a low magnitude of effect as we expect no mortality is likely and only the most sensitive plant species are likely to experience measurable impacts to growth, suggesting only minimal effects to plant-based resources are likely to occur at these exposures.

Spatially Refined Exposure Evaluation

We can further refine the risk of adverse effects expected to occur from runoff exposure by assessing individual runoff scenarios that are likely to occur within a species' range. The EPA modeled location-specific runoff scenarios within the range of each species to predict how often runoff EECs are likely to cause more than low levels of adverse effects (described in greater detail in USEPA 2022e). Each runoff scenario is associated with a specific location within the species range and incorporates locally specific information, such as soil type, crop type, and local climatic records, to generate a site-specific distribution of EECs. Any given species range can contain hundreds to thousands of scenarios within their range, each with their own distribution of EECs. Because EPA's model does not identify which of these scenarios occur in areas of the species' range that overlap with Enlist runoff zones, we assume all scenarios modeled will occur within the areas of overlap between the species' range and the 30-m runoff zones.

We compare the 95th percentile runoff EEC from each scenario (i.e., the 1 in 10-year runoff EEC for that location) to the relevant toxic reference (i.e., growth and mortality dose response curves for plants) to determine how many locations within the species' range are not likely to ever experience runoff exposures that will exceed relevant toxic thresholds for the species. We use this information to further contextualize the likelihood that runoff exposure will cause an adverse effect to listed species. For example, if 100% of modeled scenarios are likely to exceed toxic thresholds within the duration of the action, then we expect all areas of overlap between the species' range and the runoff zone are at risk of adverse toxic effects. As the percent of scenarios likely to exceed toxic thresholds decrease, we can qualitatively reduce the expected risk of adverse effects to the species in the runoff zone.

This analysis is accompanied by a visual inspection of both the species' range as well as areas of expected high runoff EECs. As needed, Service biologists visually inspect individual species ranges using maps that delineate relevant features such as USDA cropland maps, tree cover estimation, hydrologic soil groups, elevation and topography, state and federally protected land, and areas of known importance to specific species (e.g., preferred nesting habitat, foraging grounds, slope and aspect). We compare these features directly to maps that illustrate locations where EPA's Tier 3 geographic distribution models anticipate will experience high levels of runoff EECs. Using these visual tools, we can further assess the likelihood of exposure to Enlist pesticide runoff and further modify the expected risk to the species overall. An example of such visual checks is illustrated in Figure 5.

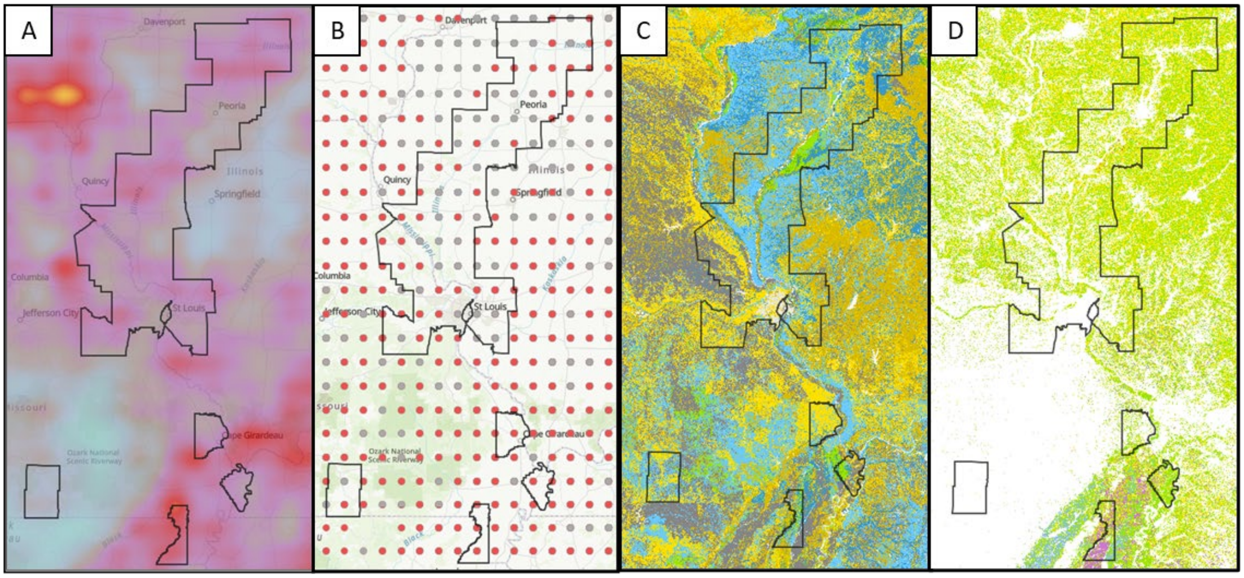

Figure 5. Taken from USEPA 2022e: Comparisons of corn scenario EECs (A; areas of deeper red-orange are higher concentrations than areas in blue), site exceedances (B; red exceed, grey do not exceed), landscape soils (C; different colors represent different hydrologic soil types) and agriculture (D; different colors represent different crop data layers) against species range (black outlined areas within maps)

<u>Species Determinations</u>

We reviewed each individual species, considering all the information described above, to provide a determination of whether the Action is likely to jeopardize the continued existence of the species. Species at low risk of adverse effects from the Action (e.g., only low levels of effects to a few individuals) that had no additional factors or considerations that could increase the extent of exposure or magnitude of effect were given “not likely to jeopardize” determinations. The EPA and technical registrants proposed species-specific mitigation measures to further reduce the risk of jeopardy for any species that our analysis deemed were at higher risk of adverse effects (e.g., high overlap and high magnitude of effects). Our analysis for each species considered in this consultation can be found in Appendix B.

Critical Habitat Effects Analyses

We assessed whether the registration of Enlist One and Enlist Duo is likely to reduce the conservation value of designated or proposed critical habitat. Critical habitat designation rules have included a variety of terms, such as “physical or biological features” (PBFs), “primary constituent elements” (PCEs), or “essential features” to characterize the key components of critical habitat needed for the conservation of the listed species. The 2016 critical habitat regulations (81 FR 7413) discontinue use of the terms PCEs and essential features and rely exclusively on the term PBFs originally used in the ESA 1986 amended regulations (50 CFR §402.02). However, the shift in terminology does not change the approach used in conducting a “destruction or adverse modification” analysis, which is the same regardless of whether the original critical habitat designation identified PCEs, PBFs or essential features. For those reasons, in this Opinion, we broadly use the term PBFs when referring to the key components of critical habitat that are described as essential for the conservation of the listed species in critical habitat designations as a standardized way to cover all features described by these terms.

When designating critical habitat, the Service assesses whether the areas within the geographical area occupied by the species at the time of listing contain the PBFs that are essential to the conservation of the species, and which may require special management considerations or protection. Specific areas outside the geographical area occupied by the species at the time it is listed may also be designated if determined to be essential for the conservation of the species. Our analysis of effects to critical habitat are separate from our analysis of effects to the species and do not consider whether the species is known to currently occupy critical habitat units. To determine the effects to critical habitat, we focus our analysis on the effects to relevant PBFs, and whether adverse effects to one or more of the PBFs appreciably diminish the conservation value of critical habitat as a whole for the listed species.

General PBFs include but are not limited to: (1) space for individual and population growth and for normal behavior; (2) food, water, air, light, minerals, or other nutritional or physiological requirements; (3) cover or shelter; (4) sites for breeding, reproduction, or rearing (or development) of offspring; and (5) habitats that are protected from disturbance or are representative of the historical, geographic, and ecological distributions of a species. Specific PBFs are also often included in critical habitat rules to describe habitat elements that are essential for the species based on the best scientific data available about the species' habitat, ecology, and life history. A feature may be a single habitat characteristic, or a more complex combination of habitat characteristics and functions.

For purposes of assessing whether or not a destruction or adverse modification determination is appropriate, the effects of the Action, together with the status of critical habitat, the environmental baseline, and any cumulative effects, are evaluated to determine if the critical habitat range-wide would remain functional or retain the current ability for the PBFs to be functionally re-established in areas of currently unsuitable but restorable habitat, to serve its intended conservation and recovery role for the species. Destruction or adverse modification means a direct or indirect alteration that appreciably diminishes the value of critical habitat as a whole for the conservation of a listed species. Such alterations may include, but are not limited to, those that alter the PBFs essential to the conservation of a species. We analyze effects to critical habitat separately from effects to the species. The effects to PBFs are related to but are not always the same as effects to the species, and the species does not have to be present for adverse effects to the critical habitat to occur.

We identified the PBFs that are susceptible to effects from herbicides like Enlist One and Enlist Duo, which fell into four categories: (1) habitat or vegetative structure, (2) food availability, (3) reproduction and recruitment resources, and (4) a lack of chemical contaminants. We describe the pertinent PBFs and outline our process for determining effects to critical habitat in greater detail below.

Given that nearly all of the critical habitats analyzed for this consultation are located completely within their respective species range, we expect the status, environmental baseline, and cumulative effects of critical habitat to be similar, if not identical to the status and baseline of the species. Even in cases where critical habitat does not fully fall within the boundaries of the species range, critical habitat areas are typically immediately adjacent to or within a small distance from the species range, indicating that status and baseline are likely to be very similar.

Critical Habitat PBFs Susceptible to Enlist One and Enlist Duo Applications

Rules designating critical habitat often provide PBFs that are specific to the species, but the degree of specificity varies. For example, habitat quality parameters may describe specific chemicals or conditions, or a general underlying requirement, such as that habitat quality be sufficient to support the species. Proposed and final rules for the designation of critical habitat outline the details of species-specific PBFs when identified. Table 24 lists general PBFs and identifies some of the typical components that may be specified for plants and animals considered in this Opinion.

Table 24. General Physical and Biological Features with examples of the types of elements that may be specified for plants and animals

PBF	Plant	Animal
Space for individual and population growth and for normal behavior	Sufficient space and soil for root growth, recruitment and adequate numbers of individuals for viable populations	Foraging areas, breeding areas, overwintering sites, home ranges and movement corridors
Food, water, air, light, minerals, or other nutritional or physiological requirements	Sufficient precipitation or groundwater to support tissue growth, soil nutrients and minerals, adequate light to support photosynthesis, and adequate climate to support plant survival and reproduction	Sufficient prey base or forage material, sufficient quantity and quality of water, air of sufficient quality to support species survival, and climate conditions that support survival and growth of individuals and populations
Cover or shelter	Vegetative canopy, riparian habitat, forest habitat	Vegetation, canopy cover, geologic formations, cavity trees, burrows, moisture, riparian habitat, woody debris, stream geomorphological features

PBF	Plant	Animal
Sites for breeding, reproduction, or rearing (or development) of offspring	Locations that support pollinator communities, soil seed banks, sufficient habitat space and structure to support reproduction	Vegetative communities, food resources, geologic formations, cavity trees, temporary or permanent water sources, substrate, habitat structure, elevation, aspect
Habitats that are protected from disturbance or are representative of the historical, geographic, and ecological distributions of a species	Natural fire or flooding regimes, dispersal pathways, lack of human disturbance	Natural fire or flooding regimes, hydrology, migration corridors, habitat connections, natural vegetative communities, lack of human disturbance

Not all PBFs are susceptible to pesticides, and some PBFs may be susceptible to some types of pesticides, such as herbicides, but not others. As an herbicide with low toxicity to animals and aquatic plants, we anticipate the effects of Enlist One and Enlist Duo to critical habitat PBFs will primarily manifest through effects to plants that support the listed species. The expected response of a particular critical habitat PBF to Enlist pesticide exposure is thus dependent on how important plants are to each relevant PBF, as well as the specific plants that are required to maintain PBF function (e.g., herbaceous forbs versus trees, woody shrubs, grasses, or aquatic vegetation).

Habitat or Physical Structure

Plants serve important roles as biotic features of habitat. In many cases, the mere presence of specific plant species or a particular community of plants is a key feature of critical habitat as vegetation provides shelter or refuge for listed animals or physical structure supporting conditions that facilitate the growth of listed plant species. Vegetation provides spaces for rest and refuge from environmental stressors and can influence microclimates that suit different species' needs. For instance, canopy structure provided by forest communities can be an important component of habitat for some listed plant species as the density of overstory can influence light conditions, which can influence growing conditions in the understory. Canopy structure can also influence temperature profiles and microclimate conditions, providing shade and cooler conditions that may be necessary for animals and plants that are more prone to desiccation or heat stress. Additionally, the physical presence and structure of vegetative communities can provide refuge from predation by providing spaces for animals to hide, or, alternatively, provide cover for ambush predators. Many listed plant species require the presence of other plants as they provide physical structures on which to grow (e.g., epiphytic plants), or modify the physical environment in ways that facilitate listed plant species (e.g., bank or dune stabilization, changes in soil pH, addition of organic matter through detritus, etc.). Thus, critical habitat PBFs that mention plant species, plant communities, canopy structure, or other such features as necessary features of habitat are considered sensitive to Enlist herbicide usage.

We expect non-listed plants will express a variety of sensitivities to Enlist pesticide exposure, with some species exhibiting high magnitudes of adverse effects (e.g., herbaceous forbs) while other species exhibiting low magnitudes (e.g., grasses) or even no measurable adverse effects from exposure (e.g., large woody plants). We expect listed species that are only dependent on a general vegetative community (rather than a single critical plant resource) will be resilient to any reductions of a few, sensitive, plant species within the broader plant community. As such, we expect a very high level of exposure is required in order to cause sufficient magnitudes of adverse effects to an entire community of plants resulting in decreased conservation value of critical habitat as a whole for the listed species. Thus, we expect a low risk of adverse effects in critical habitats where only a general vegetative community is listed as a component of PBFs. In contrast, critical habitat designations that specifically require vulnerable types of vegetation may be more vulnerable to Enlist pesticide exposure as adverse effects to habitat/physical structure PBFs are likely to occur at lower concentrations than community-wide effects.

Food Availability

The food availability PBF may be adversely affected by Enlist One and Enlist Duo through effects to plant-based food resources. Enlist pesticide usage may disproportionately impact food availability PBFs for critical habitats supporting herbivore specialists that only consume certain types or species of plants as restrictive food requirements may make the PBF less resilient to adverse effects from pesticide exposure. We expect food availability PBFs for generalist herbivores are likely more robust to herbicide exposure as we anticipate some plant types will be less sensitive to Enlist pesticides. Thus, while there may be some reduction in food availability, we expect there will likely still be food resources available to support the PBF. Omnivore or carnivore species' critical habitats are not sensitive to herbicide use as we expect animal prey will not experience any reductions from Enlist pesticide use. While a robust plant community is required to support herbivore prey species that listed carnivore and omnivore species rely on, we anticipate very high environmental concentrations of Enlist pesticides would be required to cause such trophic cascades. EPA's environmental fate modeling indicates that, even in worst case scenarios, environmental concentrations of Enlist pesticide AIs will not be high enough to cause severe effects to non-listed plants as to appreciably affect omnivore or carnivore prey availability. Conversely, we expect that food availability PBFs for obligate herbivores' critical habitats are at greater risk of adverse effects from Enlist exposure because adverse effects are likely to occur at lower concentrations of Enlist pesticides. In contrast, we do not expect that food availability PBFs for omnivores' and carnivores' critical habitats will experience any adverse effects from Enlist pesticide exposure because predicted environmental concentrations of Enlist pesticides are not expected to cause more than low levels of adverse effects to vegetative communities, indicating no more than low levels of effects to the prey species that depend on vegetative communities and that support listed carnivore and omnivore species.

Reproduction and Recruitment Resources

For some species, resources needed for reproduction or recruitment are an important critical habitat PBF that may be adversely affected by pesticides. Features that are required for reproduction can include specific areas like spawning grounds or leks, as well as specific features like appropriate substrate for egg deposition or areas for metamorphoses or emergence. Like other PBFs, plants can contribute in varying degrees to the function of these reproduction and recruitment PBFs, depending on the species. For example, a specific vegetative community may be required for critical habitat to serve as a lek or suitable spawning ground. While small alterations to vegetative communities may occur with herbicide exposure, we do not expect Enlist pesticide use will be high enough to result in widespread, broad changes to vegetative communities, given that different plant groups within that community will likely experience different levels of adverse effects. While groups like herbaceous forbs will potentially experience adverse effects from Enlist pesticide exposure, other groups like trees, woody shrubs, grasses, and aquatic vegetation are likely much more tolerant to Enlist pesticide exposure and less likely to experience adverse effects overall. Thus, while some impacts to certain parts of the vegetative community may occur, we do not expect that these effects will prevent reproductive function of the critical habitat for most species. Conversely, some species have obligate relationships with plants for reproduction and recruitment, such as specific host plants for larvae to pupate, as is the case with many listed insect species. Critical habitat for these species may be more susceptible to herbicide use as impacts to only one or a few plant species can severely impact recruitment-related PBFs, potentially reducing the conservation value of critical habitat as a whole for the listed species. We consider critical habitat PBFs that list specific plant types or species as a component of reproduction or recruitment resources as sensitive to Enlist herbicide usage. However, risk of adverse effects to the PBF and the conservation value of the critical habitat varies based on individual species needs.

Habitat Quality - Lack of Chemical Contaminants

High quality, uncontaminated habitat is specifically singled out as a key feature of critical habitat for a number of listed species. In cases where species are highly sensitive to a particular class of compounds, such as listed plant species to herbicides like Enlist One or Enlist Duo, even a small residual amount of pesticide can prevent the use of critical habitat by the species, reducing the conservation value of the critical habitat where exposed. Conversely, in situations where the species is less sensitive to a certain pesticide, such as animal species to herbicides, a lack of chemical contaminants may not be as critical for that particular pesticide. For example, we do not expect any adverse effects will occur to listed aquatic animal species as concentrations of Enlist pesticide AIs will not likely ever be high enough to cause measurable effects to growth or mortality for those species. Thus, we expect that the lack of contaminants requirement (hereafter referred to as the habitat quality PBF) for the critical habitats of these species are not sensitive to Enlist pesticides. In contrast, plant species are more sensitive to Enlist pesticide AIs and may experience adverse effects at predicted environmental concentrations. Thus, the presence of Enlist pesticides in critical habitat may adversely affect the habitat quality PBF for certain species and the function of critical habitat.

Exposure of Critical Habitat to Enlist One and Enlist Duo

The registration of Enlist One and Enlist Duo covers 34 states. The spatial footprint of the action area includes all pesticide use sites based on labeled uses for the chemical and the offsite transport footprint due to runoff (i.e., 30 meters from use sites). Similar to the approach used for analyzing effects to the species, we consider critical habitat areas that occur on agricultural areas ("on-field") separately from critical habitat areas that occur adjacent to agricultural areas ("off-field") as the risks to critical habitat PBFs are different between these two areas. We anticipate only minimal adverse effects will occur off-field as a result of spray drift because we expect the spray drift control measures required on product labels will result in extremely low concentrations of Enlist pesticide AIs leaving treatment sites through drift.

Agricultural areas within critical habitat represent highly modified areas that, typically, no longer contain the necessary PBFs to support the conservation of listed species. We individually review each critical habitat and their PBFs to confirm whether any application sites that may occur in critical habitat are likely to still function as critical habitat. In cases where agricultural land use precludes the presence of critical habitat PBFs, we consider these areas non-functional and do not further analyze the effects of direct application of Enlist pesticides to these areas. In these instances, we assume runoff is the only route of exposure to critical habitat PBFs. In cases where application sites may still function as critical habitat, we expect direct application of Enlist pesticides in these on-field areas will result in the greatest adverse effects to critical habitat.

Runoff from application sites is expected to result in substantially lower concentrations of Enlist pesticide concentrations and is generally expected to result in lower risks of adverse effects to critical habitat. That said, runoff exposure in critical habitat may still result in adverse effects to critical habitat PBFs if there is extensive overlap with runoff areas, or if local environmental conditions result in higher concentrations of Enlist pesticide AIs occurring in runoff.

Approach to Critical Habitat Analysis

Similar to the approach used in the effects analyses for the species, for the critical habitat analysis, we characterized the extent of exposure, the magnitude of effect, and the expected likelihood of any exposure event to cause adverse effects (based on spatially refined exposure estimates specific to each critical habitat).

<u>PBF categorization</u>

We reviewed each critical habitat designation to determine which critical habitats have PBFs that may be vulnerable to adverse effects from Enlist One and Enlist Duo exposure (i.e., plants as habitat or vegetative structure, plants as food, plants needed for reproduction or recruitment, and low levels of contaminants, as discussed above). We did not conduct further analyses for critical habitats that had no relevant PBFs as no level of exposure would result in adverse effects to those critical habitats.

For critical habitats with relevant PBFs, we determined the expected sensitivity of each PBF to Enlist pesticides. We assigned a high, medium or low concern ranking to each PBF based on the types of plants that are listed as integral to each critical habitat. All critical habitats that only specified plant groups that are known to be sensitive to Enlist pesticides (i.e., herbaceous forbs) are likely sensitive to Enlist herbicides and were assigned a high concern ranking. Critical habitat PBFs that listed sensitive plant groups along with non-sensitive plant groups (e.g., trees, woody shrubs, aquatic plants) were assigned a medium concern ranking as not all plant groups listed in the PBF are sensitive to Enlist herbicides. Critical habitats that only specified plant groups known to be tolerant to Enlist pesticides (e.g., woody plants, phytoplankton, algae) or only listed general plant communities (e.g., longleaf pine ecosystems, native grassland prairies) were assigned a low concern ranking as we do not expect Enlist herbicides will cause substantial adverse effects to these PBFs.

Extent of Exposure

We anticipate areas of critical habitat may overlap with Enlist herbicide use sites to various degrees. While we expect on-field areas most likely do not function as critical habitat for most species, there are some species that can still use areas degraded by agricultural practices and may still have designated critical habitat on agricultural fields. In cases where PBFs indicate critical habitat may still occur on agricultural areas, we anticipate that activities taken to convert land use and maintain agricultural practices (e.g., clearing of tree canopy, changes to surface water availability, fire suppression, tillage) would result in much greater impacts to critical habitat than the occasional use of Enlist herbicides. Thus, we do not anticipate use of Enlist herbicides will further affect on-field PBF quality or function beyond baseline conditions.

However, we anticipate exposure off-field through runoff is still likely to occur. Existing product labels require applicators to use a 30-foot in-field spray buffer, which we expect will contain the majority of spray drift to on-field areas (see the Approach to the Effects Analysis in the Opinion for more details). While some amount of spray drift could leave the field and expose critical habitat in areas adjacent to use sites, EPA's spray drift deposition models indicate that only a very small fraction of applied pesticide is expected to move beyond the in-field buffer (i.e., only 0.167% of pesticide applied on-field is expected to drift beyond the 30-foot buffer). We do not expect this level of exposure will result in measurable adverse impacts to critical habitat PBFs. Thus, we consider off-field exposure through spray drift as negligible and runoff as the only source of exposure occurring off-field.

We anticipate that runoff exposures will contain the highest off-field EECs in areas adjacent to agricultural fields. To estimate the extent of possible runoff exposure for critical habitats, we used the overlap between the critical habitat and application sites buffered out to 30-meters. We anticipate that the likelihood of runoff exposure will decrease with increasing distance from application sites as runoff is likely to be intercepted by vegetation, redirected through local topography, and lost through penetration into the soil column (we do not expect any adverse effects will result from groundwater contamination as residues will likely be sufficiently diluted to levels that will not cause any adverse effects to listed species). Thus, we consider 30 meters a sufficient estimate of the extent of runoff exposure in field-adjacent areas. While it is possible for runoff to reach wetland habitats located further than 30 meters from agricultural sites through channelized flow, we expect this runoff will similarly dissipate, degrade, or dilute with distance from crop fields. Thus, we consider 30 meters a sufficient estimate of the extent of runoff exposure in field-adjacent areas. Similar to on-field exposure, the criteria used for the species analysis, we consider overlaps greater than 10% as a high, overlaps between 5-10% as a medium, and overlaps less than 5% as a low extent of exposure.

Magnitude of Effects

We expect adverse effects to critical habitat due the associated adverse effects of Enlist herbicide use on non-listed plant species that are necessary for PBF quality and function. To estimate the magnitude of effects to PBFs, we followed a similar procedure as the one previously described above for estimating effects to plant-based resources for listed animal species. Briefly, we compared the 95th percentile runoff EEC, which we consider to be the highest EEC that is reasonably certain to occur within the duration of the action, to a plant growth species sensitivity distribution (SSD) to estimate the proportion of plant species occurring in runoff zones that are likely to experience moderate growth effects (i.e., at least 25% growth inhibition). We assumed that a proportion of sensitive plant species experiencing moderate growth effects will result in an equivalent effect to critical habitat PBFs (e.g., if 27% of plant species experience moderate adverse growth effects, that represents a 27% effect to the PBF).

We consider EECs that result in moderate effects to 50% or more plant species as a high magnitude of effect. While most plant species will likely only experience moderate adverse growth effects at this exposure, more sensitive species may experience high levels of reduction in growth and may even experience some level of acute mortality, which could result in immediate decreases in the availability of plant-based resources. We consider EECs that result in moderate effects to 25-50% of plant species as a moderate magnitude of effect to plant-based resources because we do not expect that acute mortality of plant species is likely to occur at these exposure levels (even in the most sensitive plant species). However, growth effects may be severe enough to impact the long-term survival of exposed plants, which could reduce long-term availability of plant-based resources for listed animals. We consider EECs that result in moderate effects to less than 25% of plant species a low magnitude of effect as we expect no mortality is likely and only the most sensitive plant species are likely to experience measurable impacts to growth, suggesting only minimal adverse effects to plant-based resources are likely to occur at these exposures.

Spatially Refined Exposure Evaluation

We further characterize the likelihood of adverse effects occurring to critical habitat PBFs using EPA's Tier 3 assessment. As described in the *Approach to the Effects Analysis* section above, we determined the proportion of locations within the runoff zone that are not likely to ever experience runoff exposure that will cause more than low levels of adverse effects to non-listed plant species that make up the PBFs. Given that most critical habitat units are too small to contain a sufficient sample size of runoff scenarios to be adequately predictive of future runoff events, we applied the runoff scenarios from the species range to critical habitat. As the percent of scenarios that are not likely to cause adverse effects to critical habitat PBFs increases, we further reduce the likelihood of adverse effects occurring to critical habitat as a whole.

In addition to examining spatially refined runoff model results, we reviewed each critical habitat to confirm if any other relevant factors that might influence the extent of exposure or magnitude of effect could be qualitatively considered in our analysis. Risk modifiers may include factors such as existing conservation agreements that prevent pesticide applications, or if there are specific timing windows when only some PBFs are required (e.g., reproductive resource PBFs are only required during specific times of the year). These factors may influence the risk to critical habitat in any number of ways and may result in high concern critical habitats changing to low concern, or vice versa if the risk modifiers amplify the extent of exposure or magnitude of effects.

Critical Habitat Determinations

We reviewed each individual critical habitat, considering all the information described above, to determine whether the Action is likely to destroy or adversely modify critical habitat. Critical habitats at low risk of adverse effects from the Action (either through a low likelihood of exposure or a low magnitude of adverse effects expected) that had no additional factors or considerations that could increase the extent of exposure or magnitude of effect to PBFs were given “not likely to destroy or adversely modify” determinations. For any critical habitats that our analysis deemed were at higher risk of adverse effects (e.g., high overlap and high magnitude of effects), the EPA and technical registrants proposed critical habitat-specific mitigation measures to reduce the risk of destruction or adverse modification and protect critical habitat. Our analysis for each critical habitat considered in this consultation can be found in Appendix B.

Risk Characterization

As noted in the exposure section above, we expect listed species will be exposed to much higher concentrations of Enlist pesticides from on-field areas than from runoff in off-field areas. As not all species are expected to occur on-field (even if a substantial portion of the species range overlaps with application sites), we consider on- and off-field risk separately to better capture the risk to individual species. Furthermore, we expect significant differences in the risk profiles between listed plant and animal species, as plants are more highly susceptible to Enlist pesticides than animals. We characterize the expected risk of adverse effects to each of these groups of listed species below.

On-field Exposure

We do not anticipate effects to most listed species in this consultation from on-field exposure because 1) the species’ range does not overlap with corn, soybean, or cotton fields (i.e., Dakota skipper, range only overlaps with runoff zones); 2) the species is not expected to forage in these fields (i.e., Poweshiek skipperling, dusky gopher frog, Panama City crayfish), or; 3) corn, cotton, and soybean fields represent unsuitable habitat (e.g., American chaffseed, whorled sunflower, Neches River rose-mallow).

As noted in the *Exposure* section above, we generally anticipate contact and inhalation exposure will result in negligible on-field exposure to terrestrial vertebrate species. Thus, we are primarily concerned with exposure through consumption of contaminated dietary items. EPA calculated potential dietary exposures through consumption of 2,4-D and glyphosate residues on food items following foliar spray applications using the T-REX v.1.5.2 model. Expected 2,4-D and glyphosate dosage for different dietary items are summarized in Table 25 below. Consumption of grasses, leaves, and small mammals are expected to result in the highest dosages of both 2,4-D and glyphosate. In contrast, food items like fruit, seeds, and large mammals are expected to result in lower dosages of 2,4-D and glyphosate, while amphibians, arthropods, birds, reptiles, and soil invertebrates are expected to result in moderate dosages.

Table 25. Exposure doses resulting from consumption of contaminated dietary items on Enlist pesticide use sites.

Diet	**2,4-D (mg a.i./kg-food)**	**Glyphosate (mg a.i./kg-food)**
Amphibians	164.83	187.35
Arthropods	144.7	164.5
Birds	164.83	187.35
Fruit	23.1	26.25
Grass	369.5	420
Leaves (surrogate for fungi)	207.8	236.25
Mammals (large)	56.5	64.17
Mammals (small)	352.30	400.44
Reptiles	164.83	187.35
Seeds	23.1	26.25
Soil Invertebrates	144.7	164.5

EPA further adjusted dosage estimates based on individual species factors, such as listed species mass, assimilation efficiency, metabolic rate, to determine what the highest potential dietary exposure may occur as a result of the Action. In general, body size and metabolism adjustments reduce the level of dietary exposure for larger vertebrates with slower metabolisms in contrast to small, high metabolism vertebrates. Thus, dietary dosage is typically greater for smaller vertebrate species.

In addition to physiological parameters, we qualitatively consider relevant information regarding life history traits to further contextualize the risk of on-field effects to listed species. Terrestrial animals are mobile and may preferentially spend time on- or off- application sites depending on their nutritional requirements or behaviors. Species that preferentially forage in agricultural areas may have increased risk of toxic effects as they have more opportunities for consuming contaminated food items, whereas species that are unlikely to enter row crop fields are less likely to consume contaminated food items. Similarly, most terrestrial vertebrates consume a variety of different food sources, which can reduce the overall body burden of 2,4-D and glyphosate accumulated through consumption of contaminated dietary items. The timing of key life history events, such as migration, hibernation/dormancy, and emergence, can also influence the likelihood of on-field exposure and either increase or decrease the risk of toxic effects. We individually assessed each species expected to occur on-field to determine if any relevant life history traits, behaviors, or the timing of key life cycle events to determine whether EPA's T-REX model results are accurate representations of risks.

Among terrestrial vertebrates, we expect on-field exposure to Enlist for the bog turtle and Attwater's prairie chicken. For the bog turtle, we do not expect any direct adverse effects will occur as they are unlikely to consume any contaminated food while dispersing through Enlist application areas. While runoff exposure may occur, we do not expect this exposure will cause any direct adverse effects either as the turtle's main food items are not likely to accumulate significant levels of Enlist herbicide AIs that would result in accumulation and toxic effect in the turtle. The bog turtle is not reliant on any specific plant species for food or habitat, indicating no more than low levels of indirect effects are likely to occur either.

In contrast, the Attwater's prairie-chicken is likely to forage on Enlist application sites, which, in the absence of additional species-specific conservation measures, would result in high levels of adverse effects. Given that vegetation contains some of the highest concentrations of Enlist herbicide AIs compared to other food sources (Table 25), and due to the Attwater's greater prairie-chicken's propensity for consuming vegetation on agricultural fields, we expect individuals may accumulate sufficient levels of 2,4-D to cause mortality. We do not expect any adverse effects to the prairie-chicken will result from glyphosate exposure as predicted body burdens of glyphosate are not likely to reach levels where adverse effects have been previously observed in other bird species. However, with the implementation of a species-specific conservation measure, which limits the number of applications of Enlist herbicides growers can apply per year, we anticipate, at most, a low risk of mortality.

For plants, we expect only the spring creek bladderpod (*Lesquerella perforata*), in the absence of additional conservation measures, will experience on-field exposure to Enlist pesticides. While agricultural fields represent unsuitable habitat for most listed plant species, we expect the spring creek bladderpod to occur on corn, soybean, or cotton fields. Given that Enlist pesticides are designed to target non-GMO plants, we expect direct exposure to spray applications will result in mortality of individuals on-field. However, with the implementation of a species-specific conservation measure, which prohibits applicators from using Enlist system herbicides from September 30 to June 1 in areas where the bladderpod occurs, we anticipate no more than low levels of adverse effects on-field are likely to occur.

Off-field Exposure

<u>Plants</u>

The primary route of transport of Enlist pesticides from application sites is through runoff (as described in the *Exposure* section above). Results from EPA's PWC models indicate that runoff EECs are substantially lower than on-field concentrations. However, given that Enlist pesticides specifically target non-GMO crop plants, runoff exposure is still expected to result in adverse effects to terrestrial plants. While mortality is possible in extreme scenarios, we expect the predominant response to runoff exposure will be reduced growth rates.

Listed plant species that occur solely within terrestrial habitats (e.g., areas that are not regularly inundated for prolonged periods of time) adjacent to Enlist use sites are generally at less risk of adverse effects from Enlist pesticide runoff than plant species that occur in wetland-type habitats adjacent to Enlist use sites. Regional-level PWC modeling shows that, with the implementation of required mitigation measures, runoff EECs in terrestrial habitats are on average two to three times lower than those expected to occur in wetland habitats. Even in worst-case runoff scenarios, terrestrial habitat EECs are estimated to reach as high as 0.014 lbs AI/acre, which would correspond to a 25% reduction in growth with no acute mortality likely nor any expected effects to an exposed individual's long-term survival. Thus, plant species that can occupy these terrestrial habitats, such as the American chaffseed or the Mohr's Barbara's buttons (which occur at least partially on terrestrial habitats in addition to wetland-like habitats), are at lower risk of adverse effects.

To further assess the risk of runoff effects to wetland plant species, the EPA conducted spatially refined Tier 3 exposure assessments. In contrast to broad, regional-level PWC models, this assessment generates runoff EEC predictions based on a large number of scenarios for each species. Each runoff scenario incorporates specific information gathered from individual locations within a species' range, including local features like hydrographic soil type and climate data (e.g., 30 years of rainfall data from local weather stations), to develop a series of runoff scenarios specific to a single species. After evaluating EEC predictions for each wetland plant species of concern, such as for the Godfrey's butterwort or the whorled sunflower, we expect EECs, with the implementation of required mitigation measures, may reach as high as 0.014-0.038 lbs AI/acre, which corresponds to a 24-78% reduction in growth and up to a 0.05% chance of mortality if exposed to these concentrations (i.e., 1 in 2000 exposed individuals).

While EECs may occasionally reach high levels, we do not expect all runoff events will cause moderate or high levels of growth effects throughout the duration of the Action. Thus, in addition to characterizing risk to wetland plants using predicted EEC values, we can further contextualize the likelihood of adverse effects based on the proportion of runoff scenarios that are expected to cause only low levels of effects. For example, while runoff EECs in the Cooley's meadowrue's range may reach as high as 0.024 lbs AI/acre (causing up to 50% growth effect in exposed individuals), 56-66% of runoff scenarios within the species range are not expected to cause more than low level effects throughout the duration of the action. Using this additional information, we do not expect high risk of adverse effects for any wetland plant species occurring off-field.

Many listed plant species are reliant on animal species for pollination and seed dispersal, indicating that indirect effects to plants may occur through the loss of animal pollinators and seed dispersers. However, given that most animal taxa are not sensitive to either 2,4-D nor glyphosate (as discussed above), we do not expect pollinator and seed disperser animal species will likely experience more than low levels of adverse effects. As such, we primarily focus our analysis of effects to listed plant species on direct effects to growth and survival as these effects are more likely to occur.

Terrestrial Animals

As noted in the *Exposure* section above, we anticipate the main form of off-site transport of Enlist pesticides is through runoff. EPA's PWC modeling results indicate that runoff EECs are expected to be much lower than on-field exposures and at levels below which no adverse effect levels for all animal taxa have been observed, indicating that the Action poses no risk of direct toxic effects to terrestrial animals off-field. However, as noted above, runoff is still expected to result in varying levels of adverse effects to plants off-field, which could adversely affect listed animal species that depend on plants for food, habitat, or in any other manner. Tier 3 assessment runoff EECs are compared to a plant growth species sensitivity distribution (SSD) to determine the proportion of non-listed plant species that are likely to experience at least moderate growth effects. We use the percent of plant species experiencing at least 25% growth inhibition (i.e., the HC_{25}) as a proxy measurement for the percent reduction in plant-based resources that are available for listed animal species.

Tier 3 assessment runoff EECs for listed animal species that occupy wetland-like habitats range from 0.014-0.033 lbs AI/acre, which corresponds to a moderate adverse growth effect in 7-39% of sensitive plant species. We consider this a small to moderate effect to potential plant-based resources, indicating a low to moderate risk of adverse effects to listed animal species reliant on plants for food or habitat.

While listed animals in wetland runoff zones that depend on plant-based resources could potentially experience moderate reductions in resource availability, the impact of these reductions to the species will vary depending on how reliant the species is on plant-based resources and which specific plants are required by the species. We do not expect all plants in runoff zones will be susceptible to Enlist pesticide AIs. Plants such as trees, woody shrubs, grasses and other monocots, and aquatic vegetation are not expected to experience effects from Enlist runoff exposure (see the *Assumptions and Uncertainties* section for more details). Thus, despite potentially high runoff exposures, listed animal species that primarily depend on non-sensitive plant types (i.e., the Dakota skipper and Poweshiek skipperling, which rely on grasses and monocots) or those that can utilize a wide variety of plants (i.e., the dusky gopher frog, bog turtle, and Attwater's prairie chicken) are not likely to experience significant reductions in resource availability. In contrast, listed species with very narrow resources requirements, such as specific types or species of plants to feed on or use as reproductive structures, may experience a high degree of resource loss resulting from Enlist pesticide runoff. In general, we expect listed animal species that have broad, generalist requirements for plant-based resources are at low risk of off-field adverse effects resulting from the Action. In contrast, species that are highly or disproportionately dependent on sensitive vegetation types or have a very narrow requirement for plant-based resources may be at high risk of resource loss as a result of the Action.

Aquatic Species

In general, aquatic species, including both plants and animals, are not expected to experience adverse effects from the Action, as EECs in aquatic habitats are not expected to exceed the level in which no effects are anticipated for plants and animals in those habitats (see Table 26 and Tables 15-18). Furthermore, we expect non-listed aquatic plants that listed aquatic animals use for food, such as algae and periphyton, or habitat or reproductive resources, such as aquatic emergent vegetation, are relatively tolerant to Enlist pesticide AIs at high concentration levels, as described in the *General Effects to Plants* section. Thus, we anticipate few, if any, impacts to food, habitat, or reproductive resources that listed aquatic species rely on.

Table 26. Highest estimated environmental concentrations (EECs) expected to occur in aquatic habitats based on results from EPA's Pesticides in Water Calculator (PWC) model (USEPA 2022a).

Crop	2,4-D (µg/L)	Glyphosate (µg/L)
Corn	35.6	14.6
Cotton	30.4	13.5
Soybean	26.5	12.6

There was one exception for aquatic species, where EPA's environmental fate modeling predicted that EECs following Enlist applications were sometimes expected to reach thresholds in which adverse effects have been observed for plants – this exception was the critical habitat of the Panama City crayfish. While no direct effects to the crayfish are expected, the Panama City crayfish is specifically reliant on herbaceous vegetation for food and shelter, which are among the plant species expected to be sensitive to Enlist pesticides. Thus, we anticipate a reduction in food and habitat resources for this species.

Risk Characterization Summary

We expect toxic effects to plants are likely to occur both on Enlist application sites (where exposure to direct spray application is likely) and in areas adjacent to application sites through runoff transport. While plants occurring on-field are expected to experience mortality, we expect only one listed plant species to be at risk of on-field effects – the spring creek bladderpod. Plants occurring off-field are expected to primarily experience adverse growth effects through runoff exposure. Environmental fate modeling indicates that plants located in wetland-type habitats in areas immediately adjacent to agricultural fields are at the greatest risk of off-field adverse effects as these spaces will receive the highest levels of runoff EECs; however, EPA's spatially refined Tier 3 exposure assessments provide species-specific information regarding EECs as well as the likelihood of adverse effects occurring for each individual species, indicating that nearly all species were at low risk of adverse effects from off-field exposure.

Direct effects to listed animal species are limited to on-field exposure related to the consumption of contaminated food items; runoff exposure off-field is not expected to cause any direct toxic effects to animals. Only two animals are expected to be exposed to Enlist pesticides from foraging on-field: the bog turtle and the Attwater's prairie-chicken. Of those, EECs in prey or forage items are only expected to reach levels that will cause adverse effects in the Attwater's prairie-chicken. While Enlist runoff is not expected to cause direct toxic effects to animals, it may still adversely affect animals by reducing the availability of plants needed for food, habitat, or reproduction. However, despite potentially high runoff exposures, listed animal species, such as the Dakota skipper and Poweshiek skipperling, are not likely to experience significant reductions in resource availability due to their dependence on non-sensitive plant types. In addition, some listed animal species, including the dusky gopher frog, bog turtle, and Attwater's prairie chicken, are not likely to experience significant reductions in resource availability because they tend to utilize a wide variety of plants.

Lastly, while most aquatic species are not at risk of both direct or indirect adverse effects, we expect EECs to reach thresholds for adverse effects to plants in the habitat of the Panama City crayfish, making this species susceptible to a reduction in food and habitat resources.

Assumptions and Uncertainties

There are many uncertainties and assumptions that accompany an analysis of this size and scope. The manner in which chemicals can move through the environment and interact with other biotic and non-biotic stressors is highly complex and necessitates that we focus our analysis on those factors that are identifiable, reasonably predictable, likely to influence whether species are exposed and affected, and for which we have data to characterize those effects. As such, we have made assumptions about certain elements of the analysis for which we are limited in our abilities to address directly due to lack of relevant data or appropriate models. Below we identify several assumptions and uncertainties we have considered in our analysis for the overall approach as well as specific to the effects analysis. In some instances, we are aware that certain assumptions, when taken alone, may under-predict effects to listed species. However, by using conservative assumptions in other areas that may overestimate effects in some instances, we believe that we capture the overall breadth of effects to species and critical habitat in evaluating whether EPA's action is likely to jeopardize listed species or adversely modify critical habitat. For example, we lack data to quantitatively assess the effects of Enlist One and Enlist Duo to individual species in combination with other stressors in the environment (e.g., temperature, pH, other chemicals; exposure to multiple stressors, below). However, by making conservative assumptions about exposure to Enlist One and Enlist Duo at maximum environmental concentrations and looking at the full extent of lethal and sublethal effects, we believe that we capture the breadth of effects to species, including those that may manifest at sub-maximal concentrations, but in combination with other environmental stressors. In some cases, we are unable to predict whether individual assumptions will under- or over-predict effects to listed species and critical habitats. Overall, we believe that when taken together, the assumptions we have made are based upon the best scientific and commercial data available, capture the magnitude and extent of the effects of the action, and are otherwise consistent with the ESA and its implementing regulations. Runoff exposure assumptions

We assume that exposures resulting from runoff will result in toxic effects comparable to exposure conditions in laboratory studies employing foliar spray application. This assumption is likely to result in over-estimating toxic effects that are likely to occur to plants. Laboratory and greenhouse studies use spray application to expose all parts of the plant (e.g., the stem, the leaves, the soil), which is expected to maximize the effect of herbicides – especially non-systemic active ingredients like 2,4-D, which cause toxic effects only in parts of the plants that receive exposure. We expect runoff exposure will not likely result in complete coverage of plants but mainly expose the roots, stems, and low hanging leaves, which could have important implications for determining the magnitude of toxic effects as well as the distribution of effects throughout the exposed plant.

Furthermore, we expect that differential dilution of different formulation chemicals and additives will also change the efficacy of dissolved active ingredients, affecting the magnitude of effects to off-target organisms exposed by runoff. Adjuvants, surfactants, and other inert ingredients in pesticide formulations are important components that influence uptake and distribution within an organism. These non-active ingredients will dilute differentially in runoff based on their individual physical/chemical properties (e.g., water solubility, sorption potential, etc.), which is expected to reduce the efficacy of 2,4-D and glyphosate exposure in organisms exposed through runoff. Due to the lack of available data that simulates runoff exposure, we are unable to determine the relative difference in magnitude of toxic effect and thus assume effects resulting from runoff exposure are equivalent to laboratory studies employing spray application, which will over-estimate effects likely to occur in reality.

Surrogate Data

In the General Effects by Taxa section, we briefly discuss how we used toxicity data to analyze effects to listed species. Very few listed species have toxicity data specifically addressing effects from Enlist One and Enlist Duo. We therefore discuss toxicity data that are available for the taxa groups and the decision process we employed to arrive at the toxicity values we used for our effects analyses. Where toxicity data are lacking, such as for reptiles and amphibians, we discuss the use of toxicity data from other taxonomic groups in the General Effects for Reptiles and General Effects for Amphibians sections. More specifically, we used fish and bird data for amphibians and bird data for reptiles. For amphibians and reptiles, data are also lacking to convert doses and dose-based endpoints across individuals, as discussed above. For aquatic plants, toxicity data are reported as mg AI/L, which are differing units from how terrestrial plant toxicity data are provided (lb AI/ acre). Aquatic plant toxicity data are most often based on studies on non-vascular algae which may or may not be applicable to listed aquatic vascular plants to assess effects. For many plants, often the only correlation between tested species and the listed species is that they share a seed growth mechanism, such as if both the listed and test species are dicots. However, there are several listed ferns and other allies, conifers/cycads, and some lichens that would not be comparable to any tested species, and we use available toxicity data from dicot species for these non-flowering plants.

In addition, there are several data gaps for basic biology for plant and animal species covered under this consultation that add additional complexity to this analysis. There are often little to no available data regarding effects of pesticides on species that are rare, highly specialized, and occur in specialized habitats. The toxicity data we have chosen to use and have discussed in depth in the general effects to taxa sections, is the best available information we have regarding the impacts of these pesticides to listed species.

Other Considerations for Plants

In addition to the assumptions made regarding surrogate data noted above, we made additional assumptions regarding the potential effects to non-listed plants that listed animal species depend on, given the nature of Enlist pesticides as herbicides. Available toxicity data on 2,4-D and glyphosate are available for only select species of crop and weed plants, which represent a relatively small portion of the diversity of plant types and physiologies. Furthermore, these test species are typically exposed as seedlings, which is a particularly vulnerable developmental stage. Given the known mechanisms of action of 2,4-D and glyphosate, as well as differences in physiology and life history across the diversity of plant, we do not expect other types of plants, such as trees, woody shrubs, perennials, aquatic plants, and larger, longer-lived plant species, will exhibit effects from Enlist pesticide exposure. These plants have larger biomass, requiring a larger dose of pesticide to exhibit the same level of effects as those seen in herbaceous sapling greenhouse studies. Additionally, many of these plants have extensive energy stores, which can facilitate faster recovery after injury or toxic effects. Older, established plants with established root systems or features that are not actively growing are less susceptible to sublethal growth effects than young saplings that are used in greenhouse studies. Given the range of expected toxicity to different types of plants, we assess the effects to non-herbaceous plants qualitatively on a case-by-case basis to best account for these differences in toxicity.

Impacts to soil microbial communities and mycorrhizae have been noted for pesticides. However, there is little to no information available regarding the degree of impact to the soil microbial community or mycorrhizae after pesticides are applied. Additionally, for many species where we may know or assume there is a mycorrhizal associate (i.e., orchids), the identity and basic biology of that associate species is often unknown.

Mixtures

Pesticide mixtures can be divided into three categories: formulated products, tank mixes, and environmental mixtures. Formulated products are produced and sold as one product containing multiple active ingredients. We have the most confidence in species being exposed to these types of mixtures, as application of these products ensures that both active ingredients enter the environment at the same time. Enlist One and Enlist Duo are themselves formulated products. Tank mixes refer to a situation where the pesticide applicator applies multiple pesticides simultaneously at the use site. Though we have less certainty in these types of mixtures occurring, specific tank mixes are described on Enlist product labels and can only be made with one of the pre-approved pesticides on the listed on the label (see the *Description of the Action* section for more details). Environmental mixtures result from unrelated pesticide use over the landscape and are typically detected in ambient water quality monitoring efforts. From monitoring efforts, we have high confidence that these types of mixtures occur. Monitoring data from state and Federal agencies described in the BE and elsewhere have indicated that multiple pesticides often co-occur in aquatic habitats located throughout the U.S. Studies conducted by the U.S. Geological Survey, under the National Water Quality Assessment program, have routinely detected the presence of multiple chemicals in surface water and groundwater samples.

Species and their habitats exposed to pesticide mixtures may be at greater risk of adverse effects than when exposed to single pesticides. For formulated product mixtures, as in the case of Enlist Duo, we assume that exposure to species of both active ingredients will result in a response that is additive of the effects of the two pesticides analyzed. While there is some uncertainty in this approach, we believe it is protective of species based on the best scientific and commercial data available, as described in the *Toxicological Effects* section above. For other types of mixtures, we consider these stressors in the environmental baseline of the species.

Estimated Environmental Concentrations

We assume that individuals will be exposed to modeled annual maximum pesticide concentrations, although we acknowledge this assumption may overestimate exposure to listed species. In addition, exposures are based on pesticide scenarios that generate the highest EECs, which also may overestimate effects. However, effects are limited to a single exposure of Enlist One and Enlist Duo, when, in reality, individuals may be exposed more than one time to concentrations that could cause effects; thus, this assumption may also underestimate effects.

Species-specific Information

Where more life history information was available for a species (e.g., preferred habitat, foraging behavior, occurrence data), it allowed us to make fewer assumptions about how species may be exposed to Enlist One and Enlist Duo. However, projecting the likelihood of exposure varied across species. This lack of information could result in an overestimation or underestimation.

An individual is assumed to occur at a single location and cannot be exposed to pesticides at other locations or at other times. Exceptions to this include migratory birds, migratory fish, or migratory mammals where additional exposure could be realized along a migratory path (e.g., whooping crane, Gulf sturgeon, some bat species). This may overestimate exposure for mobile species that may not be present during application or underestimate exposure for mobile species that forage on more than one treated field or are exposed during different stages of migration.

Effects to Critical Habitat

For aquatic and terrestrial animal species that have critical habitat, where physical and biological features (PBF, or other features as defined in Critical Habitat Approach to the Assessment) are discussed, our analyses assume that if a pesticide will impact these features now or preclude their development in the future (e.g., plants as food resources, plants as habitat features), then the critical habitat would be negatively affected. If no specific PBFs that would be likely to be affected by exposure to pesticides have been identified in the critical habitat rule, then the critical habitat would not be impacted (e.g., if PBFs pertain to features that are not susceptible to pesticides, such as geological features such as talus slopes, sandy areas in pine rockland, moist, well-drained moss mats growing on rocks and boulders, or plant structures such as nesting trees).

Species Range Maps

One of the main uncertainties within the analysis for this consultation is the reliance on current ranges for each species that may not accurately reflect the species' actual distribution within those mapped ranges. Though many species range maps have been recently refined, some remain defined as entire counties or smaller subunits within which the species is known to occur but do not identify actual areas of suitable habitat where the species is likely to be found. Without detailed information on where a species can be found, our assumption for this assessment is that each species analyzed is uniformly distributed within its range. This may overestimate or underestimate our understanding of where a species is found. Exceptions to this assumption were for species where information is known based on specific data from the Service's Recovery Plans or 5-Year Reviews. Some species will have information where specific segments of the range have been identified for recovery, for critical habitat, or for other specified uses, and the locations of populations of the species are known within these areas.

Cumulative Effects

Cumulative effects are defined in ESA Section 7 implementing regulations as "those effects of future State or private activities, not involving Federal activities, that are reasonably certain to occur within the action area of the Federal action subject to consultation." (50 CFR 402.02). Future Federal actions that are unrelated to the Action are not considered in this section because they require separate consultation pursuant to section 7 of the ESA.

Declines in the abundance or range of many threatened, endangered, and other special status species are attributable to various human activities on state or private lands. We anticipate human population expansion and associated infrastructure, commercial, and private development will occur in the action area via various State, tribal, local and private actions. Such activities will likely include, but are not limited to:

- water use and withdrawals (e.g., water retention, diversion, or dewatering of springs, wetlands, natural and artificial impoundments, and streams);
- land and water development including excavation, dredging, construction of roads, housing, and commercial and industrial activities;
- mining and mineral extraction activities;
- recreational activities;
- expansion, or changes in land use for agricultural or grazing activities, and other land uses including alteration or clearing of native habitats for domestic animals or crops; and
- inadvertent introductions of non-native plant, wildlife, or fish or other aquatic species, which can alter native habitats or out-compete or prey upon native species.

All manner of development and competing use projects and activities (as above) are likely to continue in many areas, resulting in clearing, addition of impervious surfaces, and introductions of non-native species. These activities are expected to result in various impacts to water quality, habitat quality, and other negative effects to listed species and their critical habitats. In some cases, increased pesticide use, including those in addition to Enlist One and Enlist Duo, may occur to address new or emerging pest pressure (e.g., mosquitoes and other pests) in agricultural and nonagricultural settings. We anticipate some use of pesticides, including those in addition to Enlist One and Enlist Duo, may be used to directly or indirectly benefit listed species or their critical habitat. For example, future pesticide use is anticipated to be used to eliminate or reduce competing or predatory species within a species' habitat. While we are not aware of any such proposed projects at this time that would use Enlist One and Enlist Duo to specifically benefit listed species, we do anticipate that these or other pesticides will be used in the action area for this purpose over the life of the Action. Where implemented with appropriate avoidance and minimization measures to reduce the potential for lethal, sub-lethal, and indirect effects to listed species and their critical habitats, such projects could improve habitat conditions, thereby benefitting the species. However, in the absence of specific information for such activities, or for sufficient avoidance and minimization measures for other activities described above, we anticipate listed species will continue to be impacted as described previously in the Environmental Baseline section of this Opinion.

We also anticipate that conservation actions, such as habitat enhancement and restoration activities, will be undertaken in accordance with regional plans, recovery plans, and other planned or ongoing efforts. Where implementation is undertaken and successful, these activities are likely to benefit certain listed species and their habitats, food base, hosts, pollinators and other related species to varying degrees.

Given the broad geographic extent of the action area, many of the activities mentioned in the paragraphs above are expected within the ranges of various Federally listed wildlife, fish, and plant species, and could contribute to cumulative adverse, and in some cases beneficial, consequences to the species within the action area. We anticipate that species with small population sizes, high degrees of endemism or limited distributions, or slow reproductive rates will generally be more susceptible to cumulative effects than species with greater resilience and redundancy to stochastic events (i.e., via multiple stable or increasing populations). For example, narrow endemics confined to specific habitat locations may experience habitat degradation that in turn results in reductions in individuals or even localized extirpations. Where such a species is unable to recolonize or repopulate the habitat, species-level declines would be expected. Species with single or small numbers of populations may struggle to maintain sufficient numbers of individuals to persist where cumulative effects result in loss of individuals or habitat degradation. Designated and proposed critical habitats with essential physical and biological features that are affected by these activities may also experience varying levels of degradation or improvement from these activities.

CONCLUSION

Species Conclusion

This Opinion considers 22 species (Table 26). The proposed registration of Enlist One and Enlist Duo is not likely to jeopardize the continued existence of any of the species analyzed in this Opinion. Below we provide a summary of the rationale for our jeopardy determinations across species. Individual integration and synthesis summaries for each species can be found in Appendix B of this Opinion.

All of the species in this Opinion have vulnerabilities ranging from low to high, represented by a single population or few to many populations, with populations that may be declining, stable or increasing. While most listed species have isolated and fragmented populations, some of these species are less vulnerable to overall threats. The likelihood of exposure for these species' ranges from low to high, as demonstrated by the expected extent of overlap between species ranges or critical habitat areas with Enlist herbicide use sites and their associated runoff zones. We expect varying degrees of sublethal effects to growth or resource availability will occur with exposure to Enlist herbicides, ranging from low to high magnitudes, depending on the type of exposure (i.e., on-field dietary exposure, off-field runoff exposure) and the type of adverse effect (i.e., direct effects to growth and mortality or indirect effects to plant-based resources). Spatially refined exposure models provided additional information about the proportion of runoff scenarios that may reach levels in which we expect adverse effects to occur, which, for most species and critical habitats, indicate only a low likelihood of adverse effects. While we expect that a number of individuals for some species will experience mortality or sublethal effects (i.e., reduction in growth), or indirect effects, which will result in reduced fitness, reproduction, and dispersal for some individuals and populations, we do not expect these effects will appreciably reduce the likelihood of survival and recovery of these species in wild. Thus, it is the Service's biological opinion that the registration of Enlist One and Enlist Duo, as proposed, is not likely to jeopardize the continued existence of endangered or threatened species.

Table 27. Final jeopardy determinations for species that are likely adversely affected by the proposed registration of Enlist One and Enlist Duo

Entity ID	Species	Scientific Name	Mitigations	Final Jeopardy Determination
83	Attwater's greater prairie-chicken	*Tympanuchus cupido attwateri*	General mitigations, species-specific	No jeopardy
182	Bog turtle	*Glyptemys muhlenbergii*	General mitigations	No jeopardy
208	Dusky gopher frog	*Rana sevosa*	General mitigations	No jeopardy
558	Pecos sunflower	*Helianthus paradoxus*	General mitigations	No jeopardy

Entity ID	Species	Scientific Name	Mitigations	Final Jeopardy Determination
568	Spring Creek bladderpod	*Lesquerella perforate*	General mitigations, species-specific	No jeopardy
653	Brooksville bellflower	*Campanula robinsiae*	General mitigations	No jeopardy
764	Mohr's Barbara's buttons	*Marshallia mohrii*	General mitigations	No jeopardy
819	Green pitcher-plant	*Saracenia oreophila*	General mitigations	No jeopardy
852	Cooley's meadowrue	*Thalictrum cooleyi*	General mitigations	No jeopardy
875	Sensitive joint-vetch	*Aeschynomene virginica*	General mitigations	No jeopardy
891	Decurrent false aster	*Boltonia decurrens*	General mitigations	No jeopardy
960	Pondberry	*Lindera melissifolia*	General mitigations	No jeopardy
967	Rough-leaved loosestrife	*Lysimachia asperulaefolia*	General mitigations	No jeopardy
976	Canby's dropwort	*Tiedemannia canbyi*	General mitigations	No jeopardy
982	Godfrey's butterwort	*Pinguicula ionantha*	General mitigations	No jeopardy
994	Alabama canebrake pitcher-plant	*Sarracenia alabamensis*	General mitigations	No jeopardy
1028	Virginia sneezeweed	*Helenium virginicum*	General mitigations	No jeopardy
1881	Whorled Sunflower	*Helianthus verticillatus*	General mitigations	No jeopardy
3412	Dakota Skipper	*Hesperia dacotae*	General mitigations	No jeopardy
6617	Neches River rose-mallow	*Hibiscus dasycalyx*	General mitigations	No jeopardy
9386	Panama City crayfish	*Procambarus econfinae*	General mitigations	No jeopardy
10147	Poweshiek skipperling	*Oarisma poweshiek*	General mitigations	No jeopardy

With the exception of the Attwater's prairie-chicken, we expect animal species are only at risk of exposure to Enlist herbicides through runoff, as these species are unlikely to occur on-field and consume contaminated food items. These species then are only at risk of indirect effects resulting from adverse effects to plant species that provide food or habitat given that we do not expect dermal contact is a significant source of exposure. As such, we expect the required runoff mitigations included in the label will be sufficiently protective of these species. All animal species analyzed in this Opinion have low to high overlap with runoff areas, indicating a wide range of exposure likelihoods. With the exception of the Panama City crayfish, none of the animal species analyzed in this Opinion are especially reliant on plant species that are sensitive to Enlist herbicides (i.e., herbaceous forbs), indicating that these species would experience only a low magnitude of adverse indirect effects. Spatially refined runoff exposure models indicate that all of these animal species are not likely to experience more than low levels of adverse effects from runoff exposure as the majority of runoff scenarios are not likely to cause more than low levels of adverse effects to plant food or habitat resources. Thus, animals that are dependent on herbaceous forbs, such as the Panama City crayfish, are still likely to experience no more than low levels of adverse effects.

Unlike the other animal species, the Attwater's prairie-chicken is also at risk of adverse effects from consuming contaminated food items on Enlist herbicide use sites. This risk cannot be reduced through runoff mitigation measures. Thus, to reduce the remaining risk to individual Attwater's prairie-chickens resulting from on-field exposure, the EPA adopted a species-specific mitigation measure that reduces the allowable number of applications of Enlist herbicides in areas where Attwater's prairie-chickens are likely to be exposed. With this measure, we now expect that concentrations of 2,4-D on food and forage items will not cause more than low levels of adverse effects to prairie-chickens, and thus, are not likely to jeopardize the species.

With the exception of the Spring Creek bladderpod, we do not expect any listed plants will occur on-field. Thus, these plant species are only at risk of runoff exposure and will be protected through the required runoff mitigations included in the label. All of the listed plant species in Table 26 have low to moderate overlaps with Enlist herbicide runoff areas. When exposed, species like the Godfrey's butterwort, Brooksville bellflower, Pecos sunflower, and Virginia sneezeweed may experience moderate to high magnitudes of adverse effects; however, these species have ranges with only a very small extent of overlap with runoff areas. As only a few individual plants are likely to be exposed, we do not expect that the registration of Enlist or Enlist Duo is likely to jeopardize these species. In contrast, species like the American chaffseed, Neches River rose mallow, pondberry, and sensitive joint-vetch, have substantial levels of overlap between their ranges and runoff areas. However, we expect exposed individuals of these species to experience only low magnitudes of adverse effects due to either habitat preferences that preclude the accumulation of high concentrations of Enlist herbicides (e.g., tidal marshes will have increased transport of pesticides out of the sensitive joint-vetch's habitat) or their inherent physiology that makes them less sensitive to Enlist herbicide AIs (i.e., the pondberry, which is a woody shrub). For the remaining species, spatially refined runoff exposure models indicate that, while there may be substantial overlap between species ranges and runoff areas that may result in potentially high magnitudes of effects, the majority of runoff scenarios are unlikely to cause more than low levels of adverse effects to individuals. Thus, exposure to Enlist herbicides is not anticipated to appreciably reduce survival or recovery of these species and, therefore, the registration of Enlist One and Enlist Duo is not likely to jeopardize these species.

Unlike other plant species, the Spring Creek bladderpod is likely to occur on Enlist herbicide use sites, indicating that runoff conservation measures are not sufficient to protect this species. To reduce the remaining risk to the Spring Creek bladderpod from on-field exposure, the EPA and technical registrants proposed a species-specific mitigation measure that restricts the timing of herbicide application to periods after seed set has occurred, which will substantially reduce the risk of adverse effects to individuals. Considering the general runoff measures included on the labels for the Enlist herbicides and the species-specific mitigations adopted for the Spring Creek bladderpod, it is the Service's Opinion that the registration of Enlist and Enlist Duo is not likely to jeopardize the continued existence of the Spring Creek bladderpod.

Critical Habitat Conclusion

Destruction or adverse modification means a direct or indirect alteration that appreciably diminishes the value of critical habitat as a whole for the conservation of a listed species. Through this consultation, we determined pertinent elements of the PBFs of proposed and designated critical habitats that are susceptible to effects from Enlist herbicides. These elements fall within the following categories: (1) plant species, groups, or communities that provide habitat and/or physical structures to support listed species, (2) plant-based food resources, (3) plant-based features required for reproduction or recruitment, and (4) habitat that is free of chemical contaminants that might prevent the use of critical habitat by listed species.

This Opinion considers five critical habitats (Table 27). Based on the critical habitat analysis described in the *Effects of the Action* section, adverse effects are anticipated for some critical habitats. With the exception of the whorled sunflower, we expect adverse effects will generally be low to all the critical habitats considered in this Opinion. The Pecos sunflower and Panama City crayfish critical habitats have a low extent of overlap between designated critical habitat and Enlist herbicide use sites or runoff areas, indicating that only a small portion of critical habitat is likely to experience exposure and adverse effects from the Action. While some critical habitats have a high extent of overlap with runoff areas, such as critical habitat for the Dakota skipper or Poweshiek skipperling, we anticipate low levels of adverse effects will occur, as the necessary plants required for PBF function (i.e., native grasses) are not likely to experience more than low levels of adverse effects.

For whorled sunflower critical habitat, the EPA adopted a mitigation measure that restricts Enlist herbicide application within 60-meters of designated critical habitat due to a high extent of overlap between designated critical habitat and runoff areas. We anticipate that this restriction will sufficiently reduce the likelihood of runoff exposure such that no more than low levels of adverse effects are expected in the designated habitat of the whorled sunflower.

Based upon the above discussion, and as described in detail in Appendix B-3, it is the Service 's biological opinion that the registration of Enlist and Enlist Duo is not likely to result in the destruction or adverse modification of the critical habitats listed in Table 27.

Table 28. Final destruction and adverse modification determinations for critical habitats that are likely adversely affected by the registration of Enlist One and Enlist Duo.

Entity ID	Species	Scientific Name	Mitigations	Final Determinations
558	Pecos sunflower	*Helianthus paradoxus*	General mitigations	No destruction or adverse modification

Entity ID	Species	Scientific Name	Mitigations	Final Determinations
1881	Whorled Sunflower	*Helianthus verticillatus*	General mitigations, critical habitat-specific	No destruction or adverse modification
3412	Dakota Skipper	*Hesperia dacotae*	General mitigations	No destruction or adverse modification
9386	Panama City crayfish	*Procambarus econfinae*	General mitigations	No destruction or adverse modification
10147	Poweshiek skipperling	*Oarisma poweshiek*	General mitigations	No destruction or adverse modification

INCIDENTAL TAKE STATEMENT

Section 9 of the ESA and, in some cases, regulations issued for threatened species pursuant to section 4(d) of the ESA prohibit the take of endangered and threatened species, respectively, without special exemption. Take is defined as to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture or collect, or to attempt to engage in any such conduct. Harm is defined by the regulations as an act that actually kills or injures wildlife. Such act may include significant habitat modification or degradation where it actually kills or injures wildlife by significantly impairing essential behavior patterns, including breeding, feeding, or sheltering (50 CFR 17.3). Incidental take is defined as takings that result from, but are, not the purpose of, carrying out of an otherwise lawful activity conducted by the Federal agency or applicant (50 CFR 402.02). Under the terms of section 7(b)(4) and section 7(o)(2), taking that is incidental to and not intended as part of the agency action is not considered to be prohibited taking under the ESA provided that: 1) the action is not likely to jeopardize the continued existence of listed species or the action agency implements a reasonable and prudent alternative to avoid the likelihood of jeopardy, and; (2) such taking is in compliance with the terms and conditions of this Incidental Take Statement.

AMOUNT OR EXTENT OF TAKE

In this Opinion, we describe the types of adverse effects anticipated for listed animals in the species-specific analyses in Appendix B and the *Conclusion* section above. Overall, we anticipate that the general and species-specific conservation measures implemented for this Action will substantially decrease the level of adverse effects from decreased availability of plant-based food and habitat resources off-field. Thus, while these impacts are likely to adversely affect listed species and critical habitat, we do not anticipate these effects will rise to the level of take. We also expect these measures to substantially reduce adverse effects to animals exposed to Enlist pesticides from consumption of contaminated food items on-field. For the Attwater's greater prairie-chicken, we expect the Action to result in infrequent adverse effects from on-field exposure over the course of the action, such that we expect take of no more than one individual throughout the duration of the Action.

REASONABLE AND PRUDENT MEASURES

"Reasonable and prudent measures" (RPMs) are those actions the Service believes necessary or appropriate to minimize the impacts (i.e., amount or extent) of incidental take. (50 CFR 402.02). The Service believes the following RPM will minimize the impact of incidental take of listed species from the Action.

1. EPA shall use its authorities under FIFRA to minimize impacts of incidental take to the listed species addressed in this Incidental Take Statement.

Terms and Conditions

To be exempt from the prohibitions of section 9 and section 4(d) of the ESA, the EPA must comply with the following terms and conditions, which implement the reasonable and prudent measures described above.

As part of the RPM and Terms and Conditions described below, we anticipate monitoring and reporting will be needed to confirm our assumptions in our Opinion, as well as the assumptions outlined in EPA's BE. We anticipate that data collection will continue to occur over the duration of the action on variable time schedules and that we will gain information on an annual basis (e.g., incident data, status of label changes during the first two years), while other data set updates or collection will be available after longer intervals. For the initial annual reporting, the Service expects that the first report will be transmitted no later than one year following release of the final Opinion, as described below.

To implement RPM #1, EPA shall:

1. Provide annual reports to the FWS summarizing all information collected and analyzed as a result of monitoring and reporting required under the Terms and Conditions described below.
    a. The first annual report shall be submitted no later than one year following release of the final Opinion
    b. Each annual report will include, at a minimum ecological incident data.
    c. EPA shall set up annual meetings with the FWS to review annual report findings and species and critical habitat status updates relevant to this Opinion. Annual meetings can be organized to cover the needs of multiple FIFRA consultations over time, as appropriate and mutually agreeable.
2. Ensure that label changes (described in the *Description of the Action*) are implemented in a timely manner according to the timeline outlined below and provide confirmation on the status of that implementation to the Service. These label changes that are part of the Action include species-specific measures that will be incorporated as Endangered Species Protection Bulletins.
    a. EPA will ensure these activities occur within the following timeline:
        i. Within 60 days of this Opinion, EPA shall notify the registrants of label language changes incorporated as part of the Action and the requirements for registrants to submit amended labels per the registrant commitment letters.
        ii. Within 18 months of the issuance date of this Opinion, EPA shall review and act on the registrants' amended labels.
        iii. Within 18 months of the issuance date of this Opinion, EPA shall implement Endangered Species Protection Bulletins using the *Bulletins Live! Two* system.

b. EPA shall provide confirmation to the Service that all label changes have been completed and Endangered Species Protection Bulletins have been posted no later than 18 months after the date of this Opinion. EPA will provide status and confirmation as part of any annual reports and meetings.

3. Compile and evaluate available data to detect changes in estimations of Enlist One and Enlist Duo exposure to ESA listed species and critical habitat designations described in this Opinion related to Enlist herbicide concentrations in the environment and ecological incidents.

    a. Water quality monitoring data: EPA shall evaluate available water quality monitoring data for exceedances of values reported in the Biological Evaluation and for trends that indicate 2,4-D and glyphosate concentrations in waterways within the action area are either increasing or decreasing.

        i. No later than 12 months following the release of the Opinion, EPA shall perform a trend analysis in the initial annual report to include water quality monitoring data from all years since those provided in the BE. EPA will include a summary of any such information, including any relevant information that either supports or amends the validity of the assumptions in the Opinion. Results will be included in the first annual report (*date: one year following release of final Opinion*). Following this initial report, EPA will perform this trend analysis again in five years, and then every five years thereafter. EPA shall notify the Service of any known changes in environmental concentrations of Enlist pesticides from those predicted in the interim between reports.

        ii. EPA shall coordinate with the Service to identify sources that provide water quality monitoring data and will use sources that are mutually deemed relevant by EPA or the Service.

    b. Ecological incidents: EPA shall compile and evaluate available ecological incident data to determine if those data suggest that labeled uses of Enlist herbicides have caused unforeseen ecological impacts.

        i. EPA shall include this information in its annual reports to the Service, and specify any information related to Enlist-specific incidents for any species. This includes any information regarding:

            1. Any ecological incidents reported as a result of non-compliance with labels or other factors.

            2. All minor and major ecological incidents attributable to the application of products containing Enlist.

            3. Where no reports were submitted, EPA shall document this in the annual report referenced in Paragraph 1.

    c. Overlap data:

        i. No later than 12 months following the release of USDA NASS Census of Agriculture updates (which are conducted every 5 years), EPA shall evaluate whether there are meaningful changes in the geographic footprint of corn, cotton, or soybean use layers that would appreciably change the extent of overlap between listed species range or designated or proposed critical habitat with on- or off-field exposure areas. For example, an evaluation of the change in CDL layers, census information, or other spatial data over time may be used to confirm whether the assumptions in the BE and BO on potential use locations/geographic areas remain valid. Findings shall be included in annual reports to the Service in years when NASS updates of this data triggers this analysis. In the event the analysis reveals that no meaningful changes have occurred, this result shall also be acknowledged in the annual report.

ii. EPA will work with registrants and other stakeholders to better understand the geographic extent of use where recent or use-specific landcover data is changing. Additional information received shall be provided in annual reports to FWS.

4. Provide training and education to pesticide users and applicators.

    a. EPA will work with the Service to include Enlist herbicides as part of the development of a multi-lingual, voluntary, generic pesticides/listed species training modules for its website. Within this training, EPA will highlight new Enlist herbicide requirements for listed species. EPA will describe new runoff mitigation requirements, including use of the mitigation picklist, its point system, and definitions and descriptions of picklist mitigation practices. EPA will provide a link to this voluntary training/educational material within the specific Enlist Bulletins.

    b. EPA will review the training modules and work to update them to improve understanding of ESA issues and compliance with ESA requirements for Enlist herbicide labels 5 years after the release of the BO.

    c. EPA will seek and implement ways to increase use of ESA training modules by licensed applicators through existing stewardship programs, such as those developed and offered by the technical registrants. Examples of such activities include providing optional training modules to states for adoption into their training and licensing programs as they deem appropriate or developing partnerships with agricultural extension specialists, academic groups, and professional societies to help increase the reach of training materials.

    d. The Enlist registrant will expand its existing stewardship programs to ensure that applicators in counties newly added to labels have access to training, and that all applicators receive training regarding the use of EPA's Bulletins Live! Two system to access and implement species-specific Bulletins. Registrants will provide annual reports on the type and extent of training delivered, including number of participants, for five years following the release of the BO.

CONFERENCE REPORT

CONFERENCING ON PROPOSED AND CANDIDATE SPECIES AND PROPOSED CRITICAL HABITAT

The Service undertook formal consultation for most endangered and threatened species and designated critical habitat, and these listed resources are addressed in this Opinion. The Act requires a Federal agency to conference if their action is likely to jeopardize a species proposed for listing or that is likely to destroy or adversely modify critical habitats proposed for designation (ESA 7(a)(4)). Recommendations resulting from that conference are advisory (i.e., they are not required) because the species or critical habitat is the subject of a proposed rule and the prohibition against jeopardy and adverse modification under ESA section 7(a)(2) only applies to listed species and critical habitat designations. Conferencing can be conducted informally, or can follow the format of a formal consultation under 7(a)(2).

In this case, because the duration of the Action is 15 years, the Agencies agreed it would be prudent to use this opportunity for EPA to conference with the Service on the effects to species that are proposed for listing and critical habitats proposed for designation. In addition, although not required, the Agencies agreed to evaluate candidate species that may be proposed in the near future in this Conference. By conferencing now, any future consultation required under 7(a)(2) when a species listing or critical habitat designation is finalized may be streamlined, and in some cases, conferences can satisfy the consultation requirements under 7(a)(2). The EPA did not consult on any proposed species or critical habitats. The Service will work with the EPA to complete any required analyses for these species and habitats and the results will be incorporated into the final biological opinion.

Upon completion of this conference, EPA may elect to adopt any of the recommendations provided by the Service, including any of the reasonable and prudent measures to minimize incidental take for the proposed and candidate species and proposed critical habitat. In the future, upon listing of the species or designation of critical habitat, the EPA can request the Service adopt the conference opinion as a biological opinion to satisfy the EPA's 7(a)(2) requirement.

CONSERVATION RECOMMENDATIONS

Section 7(a)(1) of the ESA directs Federal agencies to use their authorities to further the purposes of the ESA by carrying out conservation programs for the benefit of the endangered and threatened species. Conservation recommendations are discretionary agency activities to minimize or avoid adverse effects of a proposed action on ESA-listed species or critical habitat, to help implement recovery plans or develop information (50 C.F.R. §402.02).

The following conservation recommendations would provide information and support for future consultations involving upcoming FIFRA registration authorizing use of pesticide active ingredients that may affect ESA-listed species and critical habitats:

1. Improve reporting by initiating an interagency committee to work with stakeholders and other interested parties to devise a methodology(s) or program to better understand and more comprehensively track usage of chemicals in the field. Implementation of methodologies or programs for tracking usage may include various tasks. For example, one option may include setting up or overseeing a volunteer data collection program regarding agricultural and non-agricultural pesticide usage.

2. Develop a conservation program for endangered and threatened species in collaboration with stakeholders and Agencies that specifically addresses threats to listed species and how implementation of FIFRA programs and collaboration with pesticide registrants and other stakeholders can help to ameliorate those threats.

3. Develop a conservation banking, in-lieu fee, and/or environmental market-based initiative, through a cooperative effort with pesticide registrants and stakeholders, designed to voluntarily offset impacts to listed species and designated critical habitats from multiple pesticides that may pose similar threats.

4. Work with other appropriate Federal, state, and local partners to study the efficacy of conservation practices in reducing pesticide loading to sensitive areas. Topics may include the width, structure and complexity of buffer strips, swales, riparian areas, other vegetation types, use of in field native vegetation buffers and cover crops, precision agriculture technologies and other strategies that have the potential to reduce adverse impacts to listed species.

5. Develop methods and models that better describe and quantify pesticide persistence and fate and transport to assist in analyses for future pesticide consultations. For example, models may be used to better quantify exposures resulting from runoff exposure and how they might differ from on-field exposures or greenhouse toxicity studies.

6. Sponsor additional research to support new technological devices or procedures to further reduce effects to ESA-listed resources.

7. Work with stakeholders and growers to develop conservation guidelines to be posted on EPA’s website.

8. Facilitate outreach to large growers so they are educated about the issues and work with the agencies to minimize impacts to listed species and critical habitat.

REINITIATION NOTICE

Issuance of a final biological opinion will conclude formal consultation on the Action outlined in the request. As provided in 50 CFR 402.16, reinitiation of formal consultation is required and shall be requested by the Federal agency or by the Service, where discretionary Federal involvement or control over the action has been retained or is authorized by law and : (1) If the amount or extent of taking specified in the incidental take statement is exceeded; (2) If new information reveals effects of the action that may affect listed species or critical habitat in a manner or to an extent not previously considered; (3) If the identified action is subsequently modified in a manner that causes an effect to the listed species or critical habitat that was not considered in the biological opinion or written concurrence; or (4) If a new species is listed or critical habitat designated that may be affected by the identified action.

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