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January 10, 2022

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MEMORANDUM

SUBJECT: **2,4-D Choline Salt and Glyphosate Dimethylammonium Salt:** 2022 Ecological Risk and Endangered Species Assessment for Use on Genetically-Modified Herbicide-Tolerant Corn, Soybean, and Cotton in Support of Registration Renewal Decision for Enlist One and Enlist Duo Products

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The Environmental Fate and Effects Division (EFED) has completed ecological risk assessments, including effects determinations for Federally listed threatened and endangered species, for the registration renewal of Enlist One and Enlist Duo products for use on 2,4-D (dichlorophenoxyacetic acid) choline- and glyphosate dimethylammonium-tolerant corn, soybeans, and cotton. This assessment of the potential risks from 2,4-D (dichlorophenoxyacetic acid) choline and glyphosate dimethylammonium to non-target non-listed taxa and potential effects to listed taxa and designated critical habitat from the use of Enlist One and Enlist Duo was based on proposed labels dated May 2021. Corteva submitted revised Enlist One and Enlist Duo labels in January 2022 to include mitigations that reduce the exposure of listed species to 2,4-D and glyphosate (see USEPA 2022 for more details). These mitigations were put in place as a response to the effects determination discussed in this document. An addendum to this effects determination (USEPA 2022) was developed, and new listed species and designated critical habitat effects determinations were made based on the revised labels.

2022 Ecological Risk and Endangered Species Assessment for Use on Genetically-Modified Herbicide-Tolerant Corn, Soybean, and Cotton in Support of Registration Renewal Decision for Enlist One and Enlist Duo Products

January 10, 2022

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Table of Contents

EXECUTIVE SUMMARY

SUMMARY OF THE ACTION AND PROPOSED USES

The current registration for 2,4-D (dichlorophenoxyacetic acid) choline salt (referred to as “2,4-D” throughout this assessment) use on genetically modified corn, cotton, and soybean crops is expiring on January 12, 2022. This document describes the EPA’s assessment of the potential risks to non-listed taxa and federally listed threatened or endangered species (listed species) from the use of the product Enlist Duo (2,4-D choline and glyphosate dimethylammonium salt; referred to as “glyphosate” throughout this assessment) and Enlist One (2,4-D choline) on tolerant corn, soybean, and cotton crops only. The labels also include conventional/non-GMO (genetically modified organism) corn, cotton and soybean uses as well as the maintenance of fallow acres to be planted with these crops; the assessments provided here also cover those uses. No other registered uses for 2,4-D or glyphosate are included in this assessment.

The 2,4-D and glyphosate products considered in this ecological risk assessment are:

- Enlist One® (contains 2,4-D choline salt; Reg. No. 62719- 695)
- Enlist Duo® (contains 2,4-D choline salt and glyphosate dimethylammonium salt; Reg. No. 62719-649)

Based on the submitted labels (May 2021; see **Appendix P**), EPA has generated two separate ecological risk assessments to evaluate the genetically modified organism (GMO) uses (*i.e*., use on corn, cotton, and soybean that have been genetically engineered to be tolerant to the herbicides) of 2,4-D and glyphosate. While application of the Enlist Duo product will result in the simultaneous environmental exposure to both 2,4-D and glyphosate at the time of application, the fate and environmental transport of the two active ingredients is driven by their physiochemical properties. Each of these components of the formulation will result in differential risk profiles following application of the product based on the different toxicity and exposure profiles of the two herbicide active ingredients. Beyond toxicity tests of these products on terrestrial plants, no combined exposure toxicity data are available for terrestrial or aquatic animals. Therefore, separate risk assessments are conducted for 2,4-D (**Section 2**) and glyphosate (**Section 3**). These risk assessments are then used to inform the potential risks to federally listed species in a combined effects determination for the Enlist One and Enlist Duo products (**Section 4**).

Each assessment relied upon the May 2021 product labels, which allow for three applications per year of 0.95 lbs acid equivalent (a.e.) 2,4-D/acre and 1.009 lbs a.e. glyphosate/acre (application rates were rounded to nearest 1.0 lb a.e./A for all exposure and risk modeling for both chemicals). The assessment considered these three applications per year to be made at different crop development stages; one application at 12 days prior to emergence (labeled as at plant, pre-plant or pre-emergence), and two over-the-top applications (at emergence, and 12 days after emergence). The maximum annual application rate, from the labeled products and inclusive of other applied 2,4-D products, is not to exceed an annual maximum of 3.0 lb a.e./A. Both products are labeled such that over-the-top applications may be made during the blooming stage of cotton and soybean. The over-the-top applications to corn are expected to cease prior to corn tasseling and pollen shedding.

The two Enlist products are for use on 2,4-D and glyphosate-tolerant corn, soybean, and cotton; non-GMO corn, cotton and soybean; as well as for the maintenance of fallow acres to be planted with these crops 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, 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 **Section 1.3** for complete details.

Each of the submitted labels includes the following requirements to address spray drift and runoff from the application of the products:

- Spray drift
  - Application equipment must use spray nozzles and pressure settings from an approved equipment list maintained at https://www.corteva.us/content/dam/dpagco/enlist/na/us/en/files/Enlist_Product_Use_Guide.pdf (accessed June 17, 2021).
  - Enlist products may only be tank-mixed with products that have been tested and found not to adversely affect the spray drift properties of Enlist Duo and Enlist One. A list of those products may be found at https://www.enlist.com/en/herbicides/approved-tank-mix.html (accessed June 17, 2021).
  - Application is only allowed by ground spray equipment.
  - Use may not exceed 24 inches boom height above the canopy.
  - Application can only occur when boom-height wind speed is less than 15 miles per hour.
  - Use is not allowed during an inversion.
  - Each product label requires a downwind 30-foot in-field spray drift setback (buffer) for all application sites adjacent to sensitive vegetation.
- Runoff
  - Enlist products may not be applied when rain is expected within 24 hours.
  - Treated fields may not be irrigated within 24 hours of application of Enlist products.

EPA structured this assessment to address the potential risks to non-target organisms, both listed and non-listed, that are located in three areas: on the treated field, in near-field areas (terrestrial landscapes within 100 ft of the field edge), and in the surrounding broader landscape (riparian, wetland, and other areas that may receive runoff from the field). The assessment considered spray drift and runoff control measures in assessing potential risks in each of these areas.

The U.S. Court of Appeals for the Ninth Circuit, in *National Family Farm Coalition, et al., v. U.S. EPA, et al.*, 966 F.3d 893 (9th Cir., 2020), held that EPA had failed to properly assess harm to monarch butterflies from increased 2,4-D use on milkweed (*Asclepias* spp.) in target fields from the Enlist Duo registration, and remanded EPA's decision to register Enlist Duo to the Agency without vacatur of the registration. The court stated that EPA was required to determine whether any effect on monarch butterflies due to control of on-field milkweed was "adverse" before determining whether any such effect was "reasonable." In response to the concerns raised by the 9th Circuit, each of the chemical assessments considers the potential for direct and indirect risks to monarch butterflies.

SUMMARY OF 2,4-D and GLYPHOSATE ECOLOGICAL RISK ASSESSMENTS

2,4-D

EPA's 2,4-D ecological assessment for non-listed taxa concludes that there are potential on-field (on the site of application) risks to terrestrial vertebrates (mammals, birds, amphibians, and reptiles), terrestrial invertebrates (including bees and monarch butterflies), and terrestrial plants. Risks are considered low for non-listed aquatic animals (fish, amphibians, and invertebrates) and aquatic plants. The spray drift reduction measures included on the labels reduce off-field risk from spray drift (for non-target animals and plants); however, there are still potential runoff risks for terrestrial and wetland plants. Monitoring data reinforce the risk conclusions for plants from runoff, as the data support this route of exposure for aquatic and emergent plants.

When considering the listed species level of concern, there are potential on-field risks for terrestrial animals that utilize corn, soybean and/or cotton fields, as well as several listed plant species that are considered on the field. There are also potential risks to terrestrial, wetland and aquatic plants that are exposed to runoff and listed animal species that depend upon plants in areas receiving runoff.

Table E-1 summarizes EPA's assessment regarding potential risks to non-target non-listed and listed taxa on and off the field from the use of 2,4-D (see **Section 2**) in the products subject to the terms and label restrictions described above.

For non-target plants located off the treated field, EPA's evaluation of the potential for off-field exposure to 2,4-D through spray drift and/or runoff, taking into account the restrictions on the labeling (May 2021), concludes the following about the potential risks:

- The mandatory spray drift control measures on the product labels, including the 30-foot downwind in-field spray drift set back, eliminate LOC exceedances due to spray drift for listed and non-listed off-field mammals, birds, reptiles, terrestrial phase amphibians, and terrestrial invertebrates. See **Section 2.5**.
- The same mandatory spray drift control measures eliminate LOC exceedances due to spray drift for listed and non-listed plant effects resulting from spray drift of these products. See **Section 2.6**.
- The mandatory runoff control measures on the May 2021 label provided some measure of reducing exposure; however, that label did not include enough measures to remove the determination of "may affect" for listed plants in terrestrial or wetland habitats that may receive runoff after application. Additionally, the measures on the May 2021 label were not sufficient to result in RQs below the FIFRA LOC for non-listed plants. See **Section 2.6**.
- Monitoring data reinforce the risk conclusions indicating that runoff is a relevant off-site transport pathway for 2,4-D. See **Section 2.2.1.2**.

Furthermore, because plants play an important role in terms of shelter, food, and habitat for animals, there are potential indirect effects to animals, primarily from the runoff exposure to plants. For monarch butterflies, in addition to direct risks on-field, there are potential indirect adverse effects from 2,4-D effects to on-field and off-field milkweeds.

Using listed-species levels of concern as a screen (USEPA 2004), EPA concluded that there are potential on-field effects to terrestrial animals that utilize corn, soybean and/or cotton fields as well as several listed plant species that are assumed to be on these types of fields based on FWS documentation of the species habitat or distribution. The Agency also found potential effects to terrestrial and wetland plants that are exposed to runoff and listed animal species that depend upon plants in terrestrial and wetland areas receiving runoff from Enlist treated corn, cotton or soybean fields. EPA further considered the potential effects to listed species by accounting for their taxon-specific life history and diets in the Effects Determination (**Section 4**). For information on EPA's listed species-specific analysis for the potential direct[1] and indirect effects from 2,4-D, see **Section 4**.

Table E-1 summarizes EPA's assessment regarding potential risks from 2,4-D to non-target non-listed and potential effects to listed taxa on and off the field from the use of Enlist One and Enlist Duo (based on the May 2021 label). Corteva submitted revised Enlist One and Enlist Duo labels in January 2022 to include mitigations that reduce the exposure of listed species to 2,4-D and glyphosate (see USEPA 2022 for more details). These mitigations were put in place as a response to EPA's effects determination based on the labels dated May 2021. An addendum to the effects determination (USEPA 2022) was generated, and new listed species and critical habitat effects determinations were made based on the revised labels.

[1] The term "direct effects" refers to decreases in the survival, growth, or reproduction of individuals of a listed species due to exposure to 2,4-D or glyphosate. The term "indirect effects" refers to impacts on individuals of a listed species that may be the result of the effects of 2,4-D or glyphosate on organisms on which the listed species depends upon for prey, pollination, habitat, and/or dispersal.

Table E-1. Summary of 2,4-D-Based Risk Quotients and Lines of Evidence Relevant to Potential Effects to Non-listed and Listed Species for Uses of Enlist One and Enlist Duo (May 2021 labeling).

Taxa	Exposure Duration	Risk Quotient (RQ) Range[1]	Potential Risk to Non-listed Species?[3]	Additional Information/Lines of Evidence	Do Direct Risks to Non-Listed Species Extend Beyond the Treated Field?	Potential Effects to Listed Taxa?[4,5]
Aquatic Animals (including fish, invertebrates, and aquatic phase amphibians)	Acute and Chronic	Not Calculated (non-definitive endpoints)	No	EECs are orders of magnitude below endpoint concentrations where no mortalities were observed.	Not Applicable	Direct: No Indirect: Yes
Mammals	Acute	<0.01-0.36	No	Exceedances of acute listed species LOC for small, medium, and large mammals based on shortgrass diet assumption. Listed small and medium size mammal RQs also exceed for all other dietary items except fruits/pods and seeds.	No (risk is limited to the field)	Direct: Yes Indirect: Yes
	Chronic	0.02-2.91 Dose based 0.02-0.34 Dietary based	Yes	Exceedances of chronic LOC for listed and non-listed small, medium, and large mammals based on shortgrass diet assumption. Small and medium size mammal RQs also exceed for all other dietary items except fruits/pods and seeds.		
Birds (including terrestrial phase amphibians and reptiles)	Acute	0.01-2.67 Dose based Dietary based Not Calculated	Yes	Exceedances of acute non-listed and listed species LOC for small and medium sized birds feeding on all exposed dietary items except fruits/pods and seeds.	No (risk is limited to the field)	Direct: Yes Indirect: Yes
	Chronic	0.02-0.38	No	--		

Taxa	Exposure Duration	Risk Quotient (RQ) Range[1]	Potential Risk to Non-listed Species?[3]	Additional Information/Lines of Evidence	Do Direct Risks to Non-Listed Species Extend Beyond the Treated Field?	Potential Effects to Listed Taxa?[4,5]
Bees and other terrestrial invertebrates	Acute Adult	<0.01	No	--	Not Applicable	Direct: Yes Indirect: Yes
	Chronic Adult	0.2-6.3	Yes	Chronic adult endpoint based on mortality. Systemic transport is of low risk concern. Enlist formulation-specific residue data supports risk conclusion for cotton and soybean.	No (risk is limited to the field)	
	Acute Larval	0.02-2.3	Yes	Acute endpoint based on mortality. Systemic transport is of low risk concern. Enlist formulation-specific residue data supports risk conclusion for cotton and soybean.	No (risk is limited to the field)	Direct: Yes Indirect: Yes
	Chronic Larval	>0.9-349	Yes	In the available studies, a NOAEL was not established. The assessment considered 2 different approaches to characterizing the potential for risk. Each of these approaches resulted in a conclusion of risk. Enlist formulation-specific residue data supports risk conclusion for cotton and soybean.	No (risk is limited to the field)	

Taxa	Exposure Duration	Risk Quotient (RQ) Range[1]	Potential Risk to Non-listed Species?[3]	Additional Information/Lines of Evidence	Do Direct Risks to Non-Listed Species Extend Beyond the Treated Field?	Potential Effects to Listed Taxa?[4,5]
Other (non-bee) Terrestrial Invertebrates	Acute	EEC to Endpoint Ratio Adult Not Calculated (non-definitive endpoints) Larval 0.015-2.37	Adult Not calculated Larval[2] Yes	RQs were not calculated; EECs were directly compared to available endpoints. Potential for acute risks based on using honey bee larval endpoint when exposures are based on either insect residue or vegetation residue estimates.	No (risk is limited to the field)	Direct: Yes Indirect: Yes
	Chronic	EEC to Endpoint Ratio Adult 0.08-1.34 Larval 1.94-127	Adult[2] Yes Larval[2] Yes	RQs were not calculated; EECs were directly compared to available endpoints. Potential for chronic risks based on using honey bee adult and larval endpoints when exposures are based on either insect residue or vegetation residue estimates.	No (risk is limited to the field)	

Taxa	Exposure Duration	Risk Quotient (RQ) Range[1]	Potential Risk to Non-listed Species?[3]	Additional Information/Lines of Evidence	Do Direct Risks to Non-Listed Species Extend Beyond the Treated Field?	Potential Effects to Listed Taxa?[4,5]
Vascular and Non-vascular Aquatic Plants	N/A	*Wetlands* Non-listed: 0.4-1.0 Listed: 2.4-6.3 *Non-Wetlands* Non-listed: <0.1-0.1 Listed: 0.2-0.8	Wetlands Yes Aquatic habitats No	Several exposure scenarios result in exceedances of the IC_{50} values and NOAECs in the available studies based on EECs in the wetland.	Yes Runoff from treated fields is predicted to cause threshold level effects to plant growth, reproduction, or survival in wetlands off the field.	Direct: Yes Indirect: Yes
Terrestrial Plants	Upland or Dry-Land Taxa	Non-Listed Taxa 0.1-9.3 Listed Taxa 0.1-12.3	Yes	A refined assessment was conducted considering all label requirements, various refinements of the model parameters, different application scenarios, and variability in species response in the toxicity data, and Species Sensitivity Distributions. Multiple lines of evidence suggest risk for terrestrial and wetland plants as a result of runoff-based exposure.	Yes Runoff from treated fields is predicted to cause threshold level effects to plant growth, reproduction, or survival off the field. Spray drift control measures on the label prevent risk from the spray drift route of exposure.	Direct: Yes Indirect: Yes
Terrestrial Plants	Wetlands, riparian areas and species within the flow-path of agricultural runoff	Non-Listed Taxa 0.4-26.9 Listed Taxa 0.4-35.5	Yes			Direct: Yes Indirect: Yes

[1] RQs reflect exposure estimates for 2,4-D and maximum application rates allowed on labels.
[2] RQs and LOC comparisons were not made for this taxon, potential risk was identified if the EECs exceeded the available endpoint.
[3] Non-Listed Level of Concern (LOC) Definitions: Terrestrial Vertebrates: Acute=0.5; Chronic=1.0; Terrestrial Invertebrates: Acute=0.4; Chronic=1.0; Aquatic Animals: Acute=0.5; Chronic=1.0; Plants: 1.0
[4] Listed Level of Concern (LOC) Definitions: Terrestrial Vertebrates: Acute=0.1; Chronic=1.0; Terrestrial Invertebrates: Acute=0.05; Chronic=1.0; Aquatic Animals: Acute=0.05; Chronic=1.0; Plants: 1.0
[5] All listed taxa with "Yes" indicated potential effects are considered further in the Effects Determination (Section IX of this document).

Glyphosate

EPA's glyphosate ecological assessment for non-listed taxa concludes that there are potential on-field (on the site of application) risks to birds, reptiles, and terrestrial phase amphibians, and terrestrial plants. Risks are considered low for mammals, terrestrial invertebrates, aquatic plants (vascular and non-vascular) and aquatic animals (fish, amphibians, and invertebrates). The spray drift reduction measures included on the labels reduce spray drift to the point that off-field risks for non-target animals are not expected; however, there is still potential risk to terrestrial and wetland plants from runoff. Monitoring data reinforce the risk conclusions for plants from runoff, as the data support this route of exposure for aquatic and emergent plants.

For species located off the treated field, EPA's evaluation of the potential for off-field exposure to glyphosate through spray drift and/or runoff, taking into account the restrictions on the labels, concludes the following about the potential risks:

- The mandatory spray drift control measures on the product labels, including the 30-foot downwind in-field spray drift set back, eliminate LOC exceedances due to spray drift for listed and non-listed off the treated field birds, reptiles, and terrestrial phase amphibians.
- The same mandatory spray drift control measures eliminate LOC exceedances due to spray drift for listed and non-listed plant effects resulting from spray drift of these products.
- The mandatory runoff control measures on the May 2021 label provided some measure of reducing exposure; however, that label did not include enough measures to remove the determination of "may affect" for listed plants in terrestrial or wetland habitats that may receive runoff after application. Additionally, the measures on the May 2021 label were not sufficient to result in RQs below the FIFRA LOC for non-listed plants.
- Monitoring data reinforce the risk conclusions indicating that runoff is a relevant off-site transport pathway for glyphosate.

Furthermore, because plants play an important role in terms of shelter, food, and habitat for animals, there are potential indirect effects to animals, primarily from the runoff exposure to plants. For monarch butterflies, there are potential indirect adverse effects from glyphosate effects to on-field and off-field milkweed.

Using listed-species levels of concern as a screen (USEPA 2004), EPA concluded that there are potential on-field effects to terrestrial animals that utilize corn, soybean and/or cotton fields as well as several listed plant species that are assumed to be on these types of fields based on FWS documentation of the species habitat or distribution. The Agency also found potential effects to terrestrial and wetland plants that are exposed to runoff and listed animal species that depend upon plants in terrestrial and wetland areas receiving runoff from Enlist treated corn, cotton or soybean fields. EPA further considered the potential effects to listed species by accounting for their taxon-specific life history and diets in the

Effects Determination (**Section 4**). For information on EPA's listed species-specific analysis for the potential direct[2] and indirect effects from 2,4-D, see **Section 4**.

Table E-2 summarizes EPA's assessment regarding potential risks from glyphosate to non-target non-listed and potential effects to listed taxa on and off the field from the use of Enlist One and Enlist Duo (based on the May 2021 label). Corteva submitted revised Enlist One and Enlist Duo labels in January 2022 to include mitigations that reduce the exposure of listed species to 2,4-D and glyphosate (see USEPA 2022 for more details). These mitigations were put in place as a response to EPA's effects determination based on the labels dated May 2021. An addendum to the effects determination (USEPA 2022) was generated, and new listed species and critical habitat effects determinations were made based on the revised labels.

[2] The term "direct effects" refers to decreases in the survival, growth, or reproduction of individuals of a listed species due to exposure to 2,4-D or glyphosate. The term "indirect effects" refers to impacts on individuals of a listed species that may be the result of the effects of 2,4-D or glyphosate on organisms on which the listed species depends upon for prey, pollination, habitat, and/or dispersal.

Table E-2. Summary of Glyphosate-Based Risk Quotients and Lines of Evidence Relevant to Potential Effects to Non-listed and Listed Species for Uses of Enlist One and Enlist Duo (May 2021 labeling).

Taxa	Exposure Duration	Risk Quotient (RQ) Range[1]	Potential Risk to Non-listed Species?[2]	Additional Information/Lines of Evidence	Do Direct Risks to Non-Listed Species Extend Beyond the Treated Field?	Potential Effects to Listed Taxa?[3,4]
Aquatic Animals (fish, invertebrates, aquatic phase amphibians)	Acute	< 0.01	No	--	Not Applicable	Direct: No Indirect: Yes
	Chronic	≤ 0.15				
Mammals	Acute	Not calculated (non-definitive endpoints)	No	Acute RQs not calculated; LOC exceedances not expected due to non-definitive (>) endpoints that are higher than exposures by at least 10x.	Not Applicable	Direct: No Indirect: Yes
	Chronic	0.01 – 0.08	No	--		
Birds (including terrestrial phase amphibians and reptiles)	Acute	<0.01 -0.16 (dose) Dietary based not calculated	No	Acute dietary RQ not calculated; LOC exceedances not expected due to non-definitive (>) toxicity endpoints that are higher than EECs by at least 7x.	Not Applicable	Direct: Yes Indirect: Yes
	Chronic	Not Calculated (non-definitive endpoints)	Yes	Potential for chronic risks to birds; effects on male weight gain in mallards reported at the lowest reliable test level.	Risk is uncertain, due to non-definitive (<) endpoint, but is expected to be low due to low drift as a result of the in-field buffer.	

Taxa	Exposure Duration	Risk Quotient (RQ) Range[1]	Potential Risk to Non-listed Species?[2]	Additional Information/Lines of Evidence	Do Direct Risks to Non-Listed Species Extend Beyond the Treated Field?	Potential Effects to Listed Taxa?[3,4]
Honey Bees and Other Non-Apis Terrestrial Invertebrates	Acute Adult	Not Calculated (non-definitive endpoints)	No	Risk is not expected. Endpoints are non-definitive (>); no mortality reported up to the highest level tested for both contact and oral toxicity. EECs are lower than the highest tested level for both contact and oral exposure.	Not Applicable	Direct: No Indirect: Yes
	Chronic Adult	Not Calculated (non-definitive endpoints)	No	Endpoints are non-definitive (>); no significant mortality reported up to the highest level tested. EECs do not exceed the test levels, so risk is not expected. There is some uncertainty as the study tested a different formulation.	Not Applicable	
	Acute Larval	Not Calculated (non-definitive endpoints)	No	Risk not expected. Acute and chronic laboratory-based toxicity data for larvae are not available. A colony feeding study using higher concentrations than the EEC reported no effects on larvae. There is uncertainty in extrapolating to other terrestrial invertebrates.	Not Applicable	Direct: No Indirect: Yes
	Chronic Larval	Not Calculated (non-definitive endpoints)	No			
Vascular and Non-vascular Aquatic Plants	N/A	<0.01	Wetlands No Aquatic habitats No	--	Not Applicable	Direct: No Indirect: Yes
Terrestrial Plants	Upland or Dry-land Taxa	Non-Listed Taxa 18 – 82 Listed Taxa 23.7 - 108	Yes	A refined assessment was conducted considering all label requirements, various refinements of the model parameters, different application scenarios, and	Yes Runoff from treated fields is predicted to cause threshold level effects to plant growth,	Direct: Yes Indirect: Yes

Taxa	Exposure Duration	Risk Quotient (RQ) Range[1]	Potential Risk to Non-listed Species?[2]	Additional Information/Lines of Evidence	Do Direct Risks to Non-Listed Species Extend Beyond the Treated Field?	Potential Effects to Listed Taxa?[3,4]
Terrestrial Plants	Wetlands, riparian areas and species within the flow-path of agricultural runoff	Non-Listed Taxa 38.6 - 199 Listed Taxa 50 - 262	Yes	variability in species response in the toxicity data. All evidence suggests potential risk to terrestrial plants and species within the flow-path from agricultural fields through runoff-based exposure.	reproduction, or survival off the field. Spray drift control measures on the label prevent risk from the spray drift route of exposure	Direct: Yes Indirect: Yes

[1] RQs reflect exposure estimates for glyphosate and maximum application rates allowed on labels.

[2] Non-Listed Level of Concern (LOC) Definitions: Terrestrial Vertebrates: Acute=0.5; Chronic=1.0; Terrestrial Invertebrates: Acute=0.4; Chronic=1.0; Aquatic Animals: Acute=0.5; Chronic=1.0; Plants: 1.0

[3] Listed Level of Concern (LOC) Definitions: Terrestrial Vertebrates: Acute=0.1; Chronic=1.0; Terrestrial Invertebrates: Acute=0.05; Chronic=1.0; Aquatic Animals: Acute=0.05; Chronic=1.0; Plants: 1.0

[4] All listed taxa with "Yes" indicated potential effects are considered further in the Effects Determination (Section IX of this document).

SUMMARY OF LISTED SPECIES EFFECTS DETERMINATION CONCLUSIONS

This assessment of the potential effects of 2,4-D (dichlorophenoxyacetic acid) choline and glyphosate dimethylammonium salt to listed taxa and designated critical habitat from the use of Enlist One and Enlist Duo was based on proposed labels dated May 2021. Corteva submitted revised Enlist One and Enlist Duo labels in January 2021 to include mitigations that reduce the exposure of listed species and designated critical habitat to 2,4-D and glyphosate (see USEPA 2022 for more details). These mitigations were put in place as a response to the effects determination discussed in this document. An addendum to this effects determination (USEPA 2022) was developed, and new listed species and critical habitat effects determinations were made based on the revised labels.

EPA evaluated whether the registration of Enlist One and Enlist Duo poses any reasonable expectation of discernible effects to listed (threatened and endangered) species and designated critical habitats (CH) within the action area in the listed species effects determination (**Section 4**[3]). Candidate species, proposed species, and experimental populations were not included in this assessment. The ESA effects determination makes use of the best available scientific information and considered both direct and indirect effects. The term "direct effects" refers to decreases in the survival, growth, or reproduction of individuals of a listed species due to exposure to 2,4-D or glyphosate. The term "indirect effects" refers to impacts on individuals of a listed species that may be the result of the effects of 2,4-D or glyphosate on organisms on which the listed species depends upon for prey, pollination, habitat and/or dispersal.

EPA determined whether the use of the Enlist products will have "No Effect" (NE) on a given listed species or designated CH or "May Affect" (MA) the species or designated CH. The U.S. Fish and Wildlife Service (USFWS) and National Marine Fisheries Service (NMFS) (collectively referred to as "the Services") regulations provide that the consultation obligation is triggered when an agency action MA one or more listed species or designated CH. For those species and designated CH that EPA determined MA, EPA further determined whether the action: "may affect but is not likely to adversely affect" the listed species or designated CH (NLAA); or "may affect and is likely to adversely affect" the listed species or designated CH (LAA). An LAA determination means that there is a discernible adverse effect to one or more individuals of a listed species or their designated CH. It is EPA's obligation under the Endangered Species Act (ESA) to ensure that the registration of the Enlist products does not jeopardize the continued existence of endangered or threatened species or destroy or adversely modify CH. Therefore, for those species with LAA determinations, EPA evaluated the likelihood that uses of Enlist could lead to a finding of jeopardy for the species or destruction or adverse modification of designated CH.

As summarized in **Section 4.3**, EPA determined that 408 listed species and 133 designated CH are within the action area of this Federal Action. EPA identified 24 listed animals on-field, 108 listed plants, five listed species with an obligate relationship with terrestrial plants, and 291 generalist species (relying on plants) as being in the action area. Refined risk-based analysis incorporating best available information on the life history of the species led to a conclusion of NE (no direct or indirect effects are anticipated) for: 18 on-field animals for Enlist One; 10 on-field animals for Enlist Duo; and 59 plants and 136

[3] Corteva submitted revised Enlist One and Enlist Duo labels to include mitigations that reduce the exposure of listed species to 2,4-D and glyphosate. These mitigations were put in place as a response to EPA's evaluation in Section 4 of this document. An addendum to this effects determination was generated, and new listed species and critical habitat effects determinations were made based on the revised labels. See USEPA 2022

generalist species for Enlist One and Enlist Duo. Similar analysis led to a MA determination for: 6 listed animals on-field for Enlist One; 14 listed animals on-field for Enlist Duo; 49 listed plants, 5 listed species with an obligate relationship with terrestrial plants, and 155 generalist species for Enlist One and Enlist Duo. Additionally, EPA identified 9 listed animal designated CH on-field, 6 listed plant designated CH, and 127 generalist designated CH within the action area of this Federal Action. Refined risk-based analysis incorporating best available information on the primary constituent elements (PCEs) and/or primary biological factors (PBFs) of the habitat led to a conclusion of NE for seven on-field animal designated CH, three plant designated CH and 63 generalist designated CH for Enlist One and Enlist Duo as no direct or indirect effects are anticipated. Similar analysis led to a MA determination for two on-field animal designated CH, three plant designated CH and 64 generalist designated CH for Enlist One and Enlist Duo. All species and designated CH where MA determinations were made are under the authority of FWS. NE determinations are made for all species and designated CH under the authority of NMFS.

For those species and designated CH for which MA determinations were made, EPA then determined whether the action "may affect, but is not likely to adversely affect" the listed species or designated CH (NLAA); or "may affect and is likely to adversely affect" the listed species or designated CH (LAA). Of the MA species, refined risk-based analysis incorporating best available information on the biology of the species led to a conclusion of NLAA for one on-field species, and 95 generalists for Enlist One and Enlist Duo. Similar analysis led to a LAA determination for 5 on-field species for Enlist One, 13 on-field species for Enlist Duo, 49 plants, five obligate animals and 60 generalist species for Enlist One and Enlist Duo. Of the MA determinations for designated CH, refined risk-based analysis incorporating best available information on the PCEs of the habitat led to a conclusion of NLAA for one animal species' on-field designated CH and 27 generalist species with designated CH for Enlist One and Enlist Duo. Similar analysis led to a LAA determination for one on-field designated CH, three plant species' designated CH, and 35 generalist species with designated CH for Enlist One and Enlist Duo.

EPA further evaluated the LAA species and designated CH, adopting an approach based on existing methodology to evaluate which species are likely to be jeopardized by uses of Enlist products or designated CH are likely to be adversely modified. The primary goal of performing this analysis was to identify listed species and designated CH for which EPA would need additional mitigations in order to avoid the likelihood of jeopardy or adverse modification. While meaningful and effective mitigations to reduce take for all LAA species and designated CH are needed, EPA recognizes that some species and designated CH may require more restrictive or special mitigations to avoid jeopardy. Of the species with LAA determinations, EPA identified two animals on-field for Enlist One and seven animals on-field for Enlist Duo, 45 plants, four obligate animals and 39 generalist species for Enlist One and Enlist Duo that EPA believes are likely to rise to a jeopardy determination and one plant species' designated CH and 33 generalist species' designated CH for Enlist One and Enlist Duo that EPA believes are likely to rise to adverse modification determinations.

Table E-3. Summary of Effects Determinations and Evaluations of Jeopardy and Adverse Modification

Taxon (of Listed Species)	Number of Species or Designated Critical Habitat with Determination[1]			
	NE	MA - NLAA	MA – LAA Not Likely J/AM	Likely J/AM
On-field Animal Species (Enlist One; Enlist Duo)	18; 10	1; 1	3; 6	2; 7
Plant Species	59	0	4	45
Obligate Species	0	0	1	4
Generalist Species	136	95	21	39
On-field CH	7	1	1	0
Plant CH	3	0	2	1
Obligate CH	0	0	0	0
Generalist CH	65	27	2	33

[1] Some species and designated CH have determinations in on- and off-field environmental compartments.
NE = No Effect; MA = May Affect; NLAA = Not Likely to Adversely Affect; LAA = Likely to Adversely Affect; J/AM = Jeopardy/Adverse Modification

1. Description of the Proposed Products for Registration, Label Restrictions and Structure of this Risk Assessment

1.1. Nature of Regulatory Action

Pursuant to the provisions of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Corteva submitted an application to the U.S. Environmental Protection Agency (EPA) for registration renewal of Enlist One (GF-3335) and Enlist Duo herbicide products for application to 1) Enlist corn and field corn, 2) Enlist soybean, and 3) Enlist cotton. All of these crops contain genetic traits that make them tolerant to the herbicides 2,4-D and glyphosate (both herbicides are active ingredient components of Enlist Duo). The genetic traits on the tolerant crops allow for application of 2,4-D choline 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 non-GMO corn, cotton and soybean, as well as for the maintenance of fallow acres to be planted with these crops. Ground boom application of these products is the only allowed 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.

1.2. Summary of Previous Agency Risk Assessments and Effects Determinations

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 (see **Appendix C** for a complete list of these assessments). 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.

1.3. Characterization of the Proposed Use of Enlist One and Enlist Duo

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

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

Label (EPA Reg. #)	Active Ingredient(s) (%)	Application Method Crop Growth Stage and Maximum Application[1] Rate, Application Interval
Enlist Duo® (42719-649)	2,4-D choline salt (24.4)[2] Glyphosate dimethylammonium salt (22.1)[3]	Ground Broadcast Spray **<u>Pre-plant (Burndown)</u>**[6] Single application at 0.95 lb a.e./A for 2,4-D choline and 1.009 lb a.e./A for glyphosate (12days preemergence)[4] **<u>Post-emergence</u>**[6]**<u>:</u>** 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 Minimum of 12 days between applications An annual maximum of 3 lbs a.e./A[4,6]
Enlist One® (42719-695)	2,4-D choline salt (55.7)[5]	

[1] Maximum single application rate lb a.e/A 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 Solo.
[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.

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 affect spray drift, the product labels require that applicators use only approved tank-mix partners from a list maintained by the registrants: https://www.corteva.us/content/dam/dpagco/enlist/na/us/en/files/Enlist_Product_Use_Guide.pdf (accessed June 17, 2021).

The application of these products is restricted to these 34 states:

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

Applications are prohibited in the following counties:

- Arizona: Yuma, Pinal or 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 GF3335 on land administered by the US Fish and Wildlife Service or National Park Service;
- Arkansas: Crawford, Franklin, Johnson, Little River, Logan, Montgomery, Polk, Scott, Sebastian, Sevier and Yell;
- Florida: 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;
- Kansas: Chautauqua, Cherokee, Cowley, Elk, Greenwood, Labette, Montgomery, Neosho, Wilson, and Woodson;
- Massachusetts: Nantucket;
- Missouri: Barton, Bates, Cedar, St. Clair and Vernon;
- Nebraska: Antelope, Blaine, Boone, Boyd, Brown, Cherry, Custer, Dawson, Frontier, Furnas, Garfield, Gosper, Greeley, Hayes, Holt, Hooker, Howard, Keya Paha, Knox, Lincoln, Logan, Loup, McPherson, Merrick, Nance, Phelps, Red Willow, Rock, Sherman, Thomas, Valley and Wheeler;
- Ohio: Athens, Butler, Fairfield, Guernsey, Hamilton, Hocking, Morgan, Muskingum, Noble, Perry, Vinton and Washington;
- Oklahoma: Adair, Atoka, Bryan, Carter, Cherokee, Choctaw, Cleveland, Coal, Craig, Creek, Delaware, Garvin, Haskell, Hughes, Johnston, Kay, Latimer, Le Flore, Lincoln, Love, Marshall, Mayes, McClain, McCurtain, McIntosh, Murray, Muskogee, Noble, Nowata, Okfuskee, Okmulgee, Osage, Ottawa, Pawnee, Payne, Pittsburg, Pontotoc, Pottawatomie, Pushmataha, Rogers, Seminole, Sequoyah, Tulsa, Wagoner and Washington;
- Rhode Island: Washington
- South Dakota: Bennett, Charles Mix, Gregory, Lyman, Mellette, Todd and Tripp;
- Tennessee: Wilson;
- Texas: Bowie, Cooke, Fannin, Grayson, Lamar and Red River

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, 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.

1.4. Organization of the Ecological Risk Assessments and Effects Determination

EPA conducted an ecological risk assessment on each of the individual active ingredients included in the Enlist One and Enlist Duo products. While application of the Enlist Duo product will result in the simultaneous environmental exposure to both 2,4-D choline and glyphosate at the time of application, the fate and environmental transport of the two active ingredients is driven by their physiochemical properties. Consequently, each of these components of the formulation will result in differential exposure and risk potential following application of the product. Additionally, the available toxicity data reflect exposure to each of the individual active ingredients or their environmental degradates. Beyond toxicity tests of these products on terrestrial plants, no combined exposure toxicity data are available for terrestrial or aquatic animals. Therefore, EPA conducted separate risk assessments for 2,4-D choline (**Section 2**) and glyphosate (**Section 3**). EPA then used these risk assessments to inform the potential effects to federally listed species in a combined effects determination for the Enlist One and Enlist Duo products (**Section 4**).

2. 2,4-D Choline: Ecological Risk Assessment (Not Including Listed Species Effects Determinations)

2.1. Problem Formulation

2.1.1. Mode of Action

2,4-D is a plant growth regulator (synthetic auxin herbicide) in the phenoxy or phenoxyacetic acid family that is most commonly used as a post-emergence herbicide for the selective control of broadleaf weeds. It causes disruption of multiple growth processes in susceptible plants by affecting proteins in the plasma membrane, interfering with ribonucleic acid (RNA) production, and changing the properties and integrity of the plasma membrane. Excessive cell division and the resulting growth destroy the plant's vascular transport system. Damage symptoms include growth and reproduction abnormalities, especially on new growth. Broadleaf plants experience stem and petiole twisting (epinasty), leaf malformations (parallel venation, leaf strapping, and cupping), undifferentiated cell masses and adventitious root formation on stems, and stunted root growth. Grass plants exhibit rolled leaves (onion leafing), fused brace roots, leaning stems, and stalk brittleness. Disruption of reproductive processes may occur resulting in sterile or multiple florets and nonviable seed production.

Consistent with the previous assessments on 2,4-D products for use on Enlist crops, EPA bridged the environmental fate and effects data used in this assessment across the 2,4-D acid and all of the supported Enlist salts (USEPA, 2016a, DP 428301). Registrant-submitted data indicate the 2,4-D Choline salts are rapidly converted to the free acid of 2,4-D amine salts. Additionally, the submitted effects data indicate equal toxicity of the acid and salts (based on acid equivalents). As a result, EPA determined that fate studies conducted with 2,4-D acid provide "surrogate data" for the 2,4-D Choline salts and that toxicity data across the acid and salts could generally be combined. Chemical structures of 2,4-D choline and 2,4-D amine salts are presented in USEPA 2016b, DP 424054. Further details regarding fate and transport laboratory and field studies submitted for 2,4-D can be found in the reference section of the 2016 Enlist Duo assessment (USEPA, 2016a, DP 428301).

2.1.2. Residues of Concern

As noted in **Appendix D (Table D-1),** there are several forms of 2,4-D, all of which, including 2,4-D choline, are expected to rapidly degrade into 2,4-D acid under typical field conditions. Therefore, the principal residue of concern is 2,4-D acid. Studies looking for other degradation and transformation products identified three major degradates: 1,2,4-benzenetriol (37% formed as a percent of applied), 2,4-dichlorophenol (2,4-DCP; 32.6% formed as a percent of applied), and chlorohydroquinone (CHQ; 16% formed as a percent of applied). Minor degradates included 4-chlorophenol, 4-CPA and 2,4-DCA. Although 2,4-DCP may be of toxicological concern, EPA did not include it as a residue of concern because it is primarily formed under anaerobic aquatic environments and is only a minor degradate in aerobic aquatic and terrestrial environments. Therefore, 2,4-DCP exposures will be limited within aquatic habitats relevant to most aquatic organisms for which exposure concentrations will be sufficiently low to result in no risk. The potential exposure of CHQ in the environment is likely to be low since it formed in aerobic aquatic environments to a significant extent only on day 27, and, from that point, dissipation was rapid (half-life of 5 days). EPA reported in previous ecological risk assessments (e.g., USEPA, 2016a) that estimated RQs for acute and chronic exposure were one to two orders of magnitude lower than the LOCs for aquatic organisms. Chemical structures for degradates and other information are provided in **Appendix D** (**Table D-2**).

2.2. Environmental Fate Characterization

In general, laboratory data suggest that the degradation of all forms of 2,4-D to 2,4-D acid is rapid to moderately rapid under aerobic terrestrial (DT_{50} of 2 to 12 days) and aerobic aquatic environments (DT_{50} of 15 days), but moderately persistent to persistent in anaerobic aquatic environments (DT_{50s} of 29 to 333 days). Further details regarding fate and transport laboratory and field studies submitted for 2,4-D can be found in the problem formulation document (USEPA, 2014a; DP 402410); and the preliminary risk assessment document (USEPA 2016b; DP 424054).

Therefore, in terms of off-field exposure, EPA assumed that 2,4-D choline degrades to the acid form. The physicochemical properties suggest that 2,4-D is soluble in water (569 mg/L) and has low soil sorption coefficients (average K_D of 0.52 mL/g and average Koc of 72 mL/g), which suggest it has the potential to move into surface water via runoff and erosion, and to groundwater via leaching. Because of these properties, the major route of transportation off the treated area for 2,4-D is runoff due to its moderate solubility and low adsorption onto soils. Although spray drift of 2,4-D choline may occur at application, the extent of the environmental exposure is greatly reduced because of application requirements and as a result exposure from spray drift is significantly less than that of runoff (see discussion in **Section 2.2.2**). Bioaccumulation of 2,4-D in fish is unlikely given the low value of the log *n*-octanol/water partition coefficient (log K_{ow} 0.18 at neutral pH). 2,4-D can exist in neutral or ionized forms, depending on the pH conditions of the environment.

The vapor pressure of 1.4×10^{-7} mm Hg and the estimated Henry's Law Constant of 7.16×10^{-11} atm-m^3/mol suggest that that the volatilization of 2**,**4**-**D is low from dry/moist soil surfaces. A field volatility study (MRID 48912102) was performed with 2,4-D choline salt and other 2,4-D formulations. The maximum flux rate of 1.88×10^{-8} g/m^2-s for 2,4-D vapor phase from 2,4-D choline from Arkansas suggests that 2,4-D Choline is less volatile than tested amine salts and 2,4-D ester formulations (USEPA 2016a and USEPA 2016b). Additional discussion of the field volatility of 2,4-D and off-field vapor exposure from application of 2,4-D choline and other 2,4-D products is provided in **Section 2.5.7.**

2.2.1. Aquatic Exposure Estimates

2.2.1.1. Modeling

EPA modeled estimated surface water concentrations of 2,4-D using the Pesticide in Water Calculator (PWC) version 2.001[4] coupled with the standard farm pond and wetland conceptual model. EPA selected all standard corn, soybean and cotton scenarios to assess runoff potential from vulnerable use sites. EPA used PWC scenarios to specify soil, climatic, and agronomic inputs in the Pesticide Root Zone Model (PRZM; USEPA, 2020a) and Variable Volume Water Body Model (VVWM). The PWC modeling intended to result in high-end water concentrations associated with a particular crop and pesticide within a

[4] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment#PWC

geographic region. Each PWC scenario is specific to a vulnerable area where the crop is commonly grown. Soil and agronomic data specific to the location are built into the scenario, and a specific climatic weather station providing 30 years of daily weather values is associated with the location. **Table 2-1** lists the input parameters EPA used for the PWC modeling of 2,4-D. EPA selected input parameters in accordance with EPA's guidance documents (USEPA, 2009; USEPA, 2010; USEPA, 2013).

Table 2-1. PWC Chemical Input Parameters for 2,4-D

Parameter	Input Value	Comments	Source
Molecular Weight	221.04 (g/mol)	Product Chemistry	USEPA, 2005
Solubility @ 25°C	569 (mg/L)		
Vapor Pressure	1.4 x 10^{-7} (mm Hg)		
Henry's Law Constant	2.92E-09 (Unitless)	Estimated value	PWC model
Foliar Half-life (days)	0	Default	
Adsorption-desorption distribution coefficient (K_D)[1]	0.52 (mL/g)	Represents the average K_D from five soils (0.30, 0.17, 0.28, 0.52 and 1.27 mL/g). Since 2,4-D can exist in ionic forms, mobility is pH dependent.	MRIDs 44117901, 42045302
Hydrolysis (pH 7)	0	Stable at pH 7	MRID 41007301
Aquatic Photolysis 40°N latitude) @ 25°C	12.98 days	--	MRID 41125306
Aerobic Soil Metabolism ($t_{1/2}$) @ 25°C	6.92 days	Represents the 90th percentile upper confidence limit on the mean of six half-lives (1.7, 4.6, 1.4, 12.4, 2.0 and 2.9 days)	MRIDs 43167501, 00116625
Aerobic Aquatic Metabolism ($t_{1/2}$) @ 25°C	Total Water/Sediment System 45 days	3x a single half-life value of 15 days	MRID 42045301
Anaerobic Aquatic Metabolism ($t_{1/2}$) @ 25°C	Total Water/Sediment System 321 days	Represents the 90th percentile upper confidence limit on the mean of three half-lives (333, 28.5 and 41.0) days	MRIDs 43356001, 42979201 and 41557901
Application rate	1.12 (kg ai/hectare)	--	Enlist Duo and Enlist Solo Labels
Number of applications per year	3	--	
Initial Application Date	-12 days	Pre-emergence date for all standard scenarios for corn, cotton, and soybean	
Interval between applications	12 days	--	
Application Method	Ground	--	

Parameter	Input Value	Comments	Source
Application efficiency	0.99	--	Offsite transport guidance[5]
Spray drift fraction	6.6 E-04	Based on average spray drift fraction in EPA farm pond	MRID 48844001
Heat of Henry	0	Default	
Benthic Compartment Depth	Standard Pond (APEZ) 0.05 m Variable Volume Water Model (VVWM) (WPEZ) 0.15 m	This input is on the waterbody tab to capture different depths of benthic layers for pond vs wetland.	Plant Assessment Tool (PAT)
[1]Although the coefficient of variation for K_{oc} is less than the coefficient of variation for K_D, indicating that pesticide binding to the organic matter fraction of the soil may explain some of the variability among the adsorption coefficients, the physicochemical properties of 2,4-D acid (ionic) and the propensity for 2,4-D to form metal-ligand complexes on surfaces of iron and aluminum oxides would suggest the K_D model is the most appropriate partitioning model. This model would account for sorption on both mineral and organic constituents in soils and sediments.			

Since the major route of transportation off the treated area for 2,4-D choline products is runoff, EPA conducted modeling to evaluate runoff from pre- emergence- and post-emergence applications of 2,4-D for corn, cotton and soybean based on the PWC scenarios. EPA generated Estimated Environmental Concentrations (EECs) to refine and characterize aquatic and semi-aquatic exposures. The submitted labels allow for 3 applications of 1 lb a.e./A, intended for application at pre-emergence, emergence, and post-emergence crop growth stages. Relying upon the most sensitive MScorn scenario, EPA conducted additional analyses to evaluate the potential exposures following a single application at each of the three proposed times. The results indicate that later applications lead to lower EECs (detailed information is provided in the plant risk assessment (**Section 2.5**)).

Since the submitted labels have a prohibition for application within a 24-hr period of expected rain events as they relate to the application date, EPA incorporated a rainfast period into the PWC modeling. EPA simulated the PWC model using an advanced function tab to avoid 2,4-D application that coincides with rainfall within 48-hr. EPA used this application window to avoid any rain event within 24-hrs of application. An example of “Advance” tab with inputs is provided in the **Appendix E**.

The hydrologic soil groups (e.g., A, B, C and D) and management of an agricultural field such as tillage practices can influence the permeability of the surface soils on the field. Therefore, EPA modified the curve number in the PRZM estimations of runoff for selected scenarios based on a recent runoff assessment of dicamba (USEPA, 2018, DP 443732). In that assessment, EFED consulted with the Biological and Economic Analysis Division (BEAD) on the modeling parameters, and on current agronomic practices. BEAD recommended that EFED use a “good” classification and the inclusion of good agronomic practices that allow crop residues and pesticide to remain on the field. Based on BEAD

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[5] USEPA, 2013. Guidance on modeling off-site deposition of pesticides via spray drift for ecological and drinking water assessments

recommendations, EFED assessed aquatic exposure using revised curve numbers instead of default for fallow and default cropping for the hydrologic C and D soils listed in the **Appendix E**. The default curve numbers, and the revised curve numbers that EFED used reflect BEAD recommendation derived from the National Engineering Handbook (USDA-NRCS, 2004). Detailed descriptions are provided in the **Appendix E**.

The PRZM5/VVWM is also used along with the Plant Assessment Tool (PAT) to estimate pesticide concentrations in wetlands. The Plant Assessment Tool (PAT) is a mechanistic model that incorporates fate (*e.g.,* degradation) and transport (*e.g.,* runoff) data that are typically available for conventional pesticides, to estimate pesticide concentrations in terrestrial (TPEZ), wetland (WPEZ), and aquatic plant habitats (APEZ). For terrestrial plants, EPA modeled runoff and erosion using PRZM and spray drift using spray drift deposition values. The PRZM model uses a mixing cell approach to represent water within the active root zone area of soil, and accounts for flow through the terrestrial plant exposure zone (TPEZ) caused by both treated field runoff and direct precipitation onto the TPEZ. Pesticide loss from the TPEZ occur from transport (*i.e.*, washout and infiltration below the active root zone) and degradation. EPA modeled wetlands using PRZM/VVWM, which it then processed in PAT to estimate aquatic (mass per volume of water) and terrestrial (mass per area) concentrations. EPA modeled aquatic plant exposure using the PRZM/VVWM models and the standard farm pond. Detailed descriptions and results of this approach can be found in **Section 2.5**.

2.2.1.1.1. PWC Modeling Output

Table 2-2 presents PWC model-estimated concentrations of 2,4-D in surface water, commonly referred to as estimated environmental concentrations (EECs; USEPA, 2004 V.B.4) for the applications for use on 2,4-D-tolerant corn, cotton, and soybean. EPA used these EECs to calculate risk to aquatic animals and plants. EPA estimated the maximum 1-in-10-year EECs of 35.6 µg/L for the 1-day mean, 33.8 µg/L for the 21-day mean, and 27.6 µg/L for the 60-day mean based on the maximum annual application rate of 3.0 lbs/A for use on corn. For cotton, the maximum 1-in-10-year daily average, 21-day and 60-day EECs for 2,4-D are 30.0, 27.8,23.2 µg/L. For soybean, the 1-in-10-year daily average, 21-day and 60-day EECs for 2,4-D are 26.6, 24.1, and 20.2µg/L. Example output from the model run is provided in **Appendix E**.

Table 2-2 also provides PWC EECs for selected scenarios for a combination of rainfast period with modified curve numbers. The aquatic exposure using revised curve numbers for fallow and cropping for the hydrologic C resulted in an average reduction of 22% (ranged from 8 to 30%) for 1-in-10-year daily average EECs. Similar results were also observed for 1-in-10-year 21-day mean and 30-day mean EECs. Results also suggest that inclusion of good agronomic practices such as allow crop residues and pesticide to remain on the field can potentially reduce runoff loss from 2,4-D application sites.

Table 2-2. PWC Standard Pond Estimated Environmental Concentrations (EECs) for 2,4-D Assuming 3 x 1lb Applications with a 12-Day Reapplication Interval

Crops	Scenario	Estimated Environmental Concentrations (µg/L)		
		1-in-10-year Daily Average EEC	1-in-10-year 21-day mean EEC	1-in-10-year 60-day mean EEC
Corn	PWC Standard Scenario (Rainfast)			
	IAcornstd	13.9	12.4	9.7
	ILCornSTD	17.4	16.3	13.8
	INcornSTD	18.3	16.6	13.3
	KSCornStd	**35.6**[1]	**33.8**[1]	**27.6**[1]
	MNCornStd	15.6	14.9	12.6
	MScornSTD	32.8	30.2	26.8
	NCcornESTD	8.1	7.6	6.5
	NECornStd	29.5	27.0	22.3
	OHCornSTD	20.4	19.2	15.9
	PAcornSTD	10.4	9.9	8.3
Cotton	MScottonSTD	30.4	27.8	23.2
	NCcottonSTD	21.8	19.5	15.5
Soybean	MSsoybeanSTD	26.5	24.1	20.2
PWC Curve Number Adjusted Scenario (Rainfast)				
Corn	IACornSTD	11.3	9.0	7.0
	ILcorn	12.3	11.4	9.7
	MScornSTD	**26.9**[1]	**22.6**[1]	**18.9**[1]
	PAcornSTD	8.1	8.1	7.0
Cotton	MScottonSTD	23.4	21.2	17.5
	NCcottonSTD	17.2	14.9	11.3
Soybean	MSsoybeanSTD	24.4	22.1	19.2
[1] Bolded concentration are maximum concentrations in surface water.				

EPA used the PWC (VVWM) model along with the Plant Assessment Tool (PAT) to estimate pesticide concentrations in the WPEZ and APEZ zones (**Table 2-3**). The maximum EECs were 296.6 µg/L in the WPEZ and 35.6 µg/L in the APEZ zones. **Section 2.5.4** and **Table 2-28** provide more detailed analyses of wetland plant species potentially impacted by runoff exposures. The maximum EECs were 296.6 µg/L in the WPEZ and 35.6 µg/L in the APEZ zones. **Section 2.5.4** and **Table 2-28** provide more detailed analyses of wetland plant species potentially impacted by runoff exposures.

Table 2-3. PWC (VVWM) and Plant Assessment Tool (PAT) Estimated Environmental Concentrations (EECs) for 2,4-D Assuming 3 x 1 lb a.e. Applications with a 12-Day Reapplication Interval for the Wetland Plant Exposure Zone (WPEZ) and Aquatic Plant Exposure Zone (APEZ)

Crops	Scenario	Estimated Environmental Concentrations (µg/L) 1-in-10-year Daily Average EEC	
		Wetland Plant Exposure Zone (WPEZ)	Aquatic Plant Exposure Zone (APEZ)
Corn	IAcornstd	269.9	13.9
	ILCornSTD	222.8	17.4
	INcornSTD	165.3	18.3
	KSCornStd	242.8	**35.6**[1]
	MNCornStd	205.6	15.6
	MScornSTD	247.1	32.8
	NCcornESTD	131.9	8.1
	NECornStd	**296.6**[1]	29.5
	OHCornSTD	137.2	20.4
	PAcornSTD	110.5	10.4
Cotton	MScottonSTD	142.2	30.4
	NCcottonSTD	260.9	21.8
Soybean	MSsoybeanSTD	125.4	26.5

[1] Bolded concentration are maximum concentrations in WPEZ and APEZ zones

Runoff Analysis

EPA used the PWC model to calculate runoff masses for 2,4-D Choline for use in the USEPA standard pond with the MScornSTD and PAcornSTD field scenarios. The model estimated mass of pesticide in runoff based on 3 applications at a single maximum rate of 1.0 lb a.e./A assuming a 24-hr rainfast. EFED also estimated the mass of pesticide in runoff based on 2 and 1 post-emergence applications of 1.0 lb a.e./A. The transport of pesticide mass from the applied field to a water body ranged from 0.4 to 2.0% of the annual applied mass via runoff and erosion. Average estimated runoff flux rates ranged from 0.004 to 0.012 lbs a.e./A for PACorn and PAcorn respectively for 3 annual applications (**Table E-5, Appendix E**). Average estimated runoff flux rates ranged from 0.001 to 0.003 lbs a.e./A for PACorn and MScorn respectively for 1 post-emergence annual application. Detailed calculations are provided in the **Table E-5, Appendix E**. Additional runoff analyses are also provided in the Plant Risk Assessment **Section 2.5**.

While a 2,4-D Choline-specific field runoff study is not available, EPA evaluated several peer-reviewed 2,4-D runoff studies for relevant crop lands. EPA identified two studies (White *et al.*, 1976; Kenimer *et al.*, 1987) that provide practical information in calculating runoff flux from crop lands for comparison with PWC model generated runoff mass. EPA used the maximum runoff concentrations from these studies to estimate runoff flux following a calculation presented in the **Appendix E**. The estimated runoff flux rates for the White *et al.* (1976) and Kenimer *et al.* (1987) studies were 0.015 lbs ae/A and 0.005 lbs ae/A, respectively. EFED-calculated runoff fluxes from one application to the PAcorn and MScorn PWC scenarios were 0.001 and 0.003 lbs ae/A, respectively. The model-estimated fluxes are similar to that of the Kenimer *et al.* (1987) study; however, they are as much as an order of magnitude lower than that of the White *et al.* (1976) study. This difference between the White *et al*. (1976) study and PWC could be related to a larger runoff event simulated by White (1-in-50 year) than would be captured by the PWC estimates (average over a 30-year period).

Two studies from the literature evaluated the effectiveness of grassed buffers in removing 2,4-D from runoff (Cole *et al*. 1997; Asmussen *et al*. 1977). A well-maintained vegetative buffer could potentially intercept some amount of 2,4-D laden runoff (both soluble and sediment-bound) prior to reaching non-target areas. Cole *et al*. (1997) observed variable results in two different study periods when evaluating effectiveness of turf (bermudagrass) buffers (as long as 5 m). When antecedent soil moisture and runoff volume was lower, 2,4-D concentration reductions ranged 76-96%. At the same site, reductions in 2,4-D concentrations ranged 8-55% when antecedent soil moisture and runoff volume were higher. This suggests that turf buffers of 5 m or less are not effective at reliably reducing 2,4-D concentrations in runoff. Asmussen *et al*. (1977) observed approximately 70% retention of 2,4-D mass within a much longer 24-m grassed waterway. The studies suggest that the effectiveness of vegetative buffers is highly variable for 2,4-D. Detailed description of runoff loss and efficacy of vegetative buffer are provided in a recent document (USEPA, 2021a, DP 463523) and in **Appendix E**.

2.2.1.2. Monitoring

As part of the standard tiered approach for conducting pesticide risk assessments, EPA utilizes aquatic model estimates (**Section 2.2.1.1.1**) and when available measured pesticide concentrations from surface water monitoring programs. EPA evaluates the extent to which existing monitoring data may or may not be sufficient to describe the range of possible pesticide concentrations for exposure durations of interest.

The temporal and spatial variability of pesticide concentrations in surface water is typically not well characterized by periodic discrete samples, which represent snapshots of pesticide occurrence in specific locations. Short-term peaks in pesticide concentrations in surface water can occur because of seasonal or event-driven pesticide applications (e.g., infestation or public-health hazard) and streamflow conditions (Liess *et al*., 1999; Rabiet *et al*., 2010). Most monitoring data are collected on a non-daily basis and the number of sites is often limited. As a result, EPA is often unable to use measured pesticide concentration estimates as a quantitative exposure assessment for comparison to toxicity endpoints, particularly short-term (acute) endpoints. However, when measured concentrations exceed toxicity endpoints, additional analysis may be considered to allow EPA to better understand and interpret the available monitoring data. Additionally, EPA uses a qualitative weight-of-evidence approach to evaluate the spatial relevance of monitoring sites to aquatic habitats of interest.

2.2.1.2.1. 2,4-D Monitoring Data Results Summary

EPA evaluated available surface water monitoring data for 2,4-D from the Water Quality Portal (WQP). Data from the WQP often represent samples collected from a range of surface water types including flowing and non-flowing waterbodies, and may include samples from streams, rivers, ponds, reservoirs, wetlands, estuaries, and canals. Although the data are not targeted to specific sites where 2,4-D was used within the watershed, the data included 2,4-D detections. Since 1966, over 110,000 samples were collected and analyzed for 2,4-D, and 2,4-D has been detected in approximately 39% of those samples. A summary of data accessed on 05/28/2021 through the WQP is provided in **Table 2-4**.

Table 2-4. Summary of 2,4-D Data Accessed via the Water Quality Portal

Source	Number of Samples	Number of Non-Detections	Minimum Reported Concentration µg/L	Maximum Reported Concentration µg/L
NWIS	58,905	36,257	<0.0001	200
STORET	51,749	31,625	0.0032	170
Data accessed 5/26/2021 Years of collection include 1966-2021 NWIS (National Water Information System) STORET (STOrage and RETrieval Data Warehouse)				

Available data in the WQP are ambient, not targeted. Therefore, it is unknown whether recent monitoring data represent usage of Enlist products or a broader use of 2,4-D. Over the time period representing the monitoring data, there have been hundreds of registered formulated products containing 2,4-D, as well as hundreds of registered use sites (beyond just corn, cotton, and soybean which are relevant to Enlist). In addition, some uses registered on other labels allow for greater application rates than are allowed on the Enlist labels. Therefore, there is uncertainty as to the representativeness of the available monitoring data and the relevance to use of Enlist on corn, cotton, and soybeans.

When considering national level usage data of 2,4-D on all registered crops (USEPA, 2016b), the uses with the most 2,4-D applied include: pasture (9,400,000 lb a.e.), wheat (5,600,000 lb a.e.), soybean (4,400,000 lb a.e.), corn (4,300,000 lb a.e.), fallow (2,600,000 lb a.e.) and cotton (1,000,000 lb a.e.). Maximum registered single application rates for these uses are similar to the maximum rate for Enlist (pasture and fallow: 2 lb a.e./A; wheat: 1.25 lb a.e./A; corn and soybean: 1 lb a.e./A; USEPA 2016). Since corn, cotton, and soybean represent a large proportion of the annual lbs a.e. applied, and since the uses that represent the majority of the lbs a.e. applied are applied at similar application rates as Enlist, EPA assumed that the available monitoring data may be generally representative of Enlist applications to corn, cotton, and soybean. Enlist products have a 30 ft-foot in-field buffer and nozzle requirements that other 2,4-D formulations do not have. These requirements are intended to reduce drift transport to non-target areas. Monitoring data are expected to represent contributions from spray drift and runoff. When considering the possibility of application rates higher than Enlist products (i.e., >1 lb a.e./A) and drift reduction from the 30-ft in-field buffer for Enlist products, it is possible that monitoring data reflect concentrations that could be higher than what would be expected from use of Enlist products. However, these differences are not expected to result in concentrations that are substantially different than expected from Enlist labels. This is because the noted differences result in no greater uncertainty when compared to other uncertainties or variables that impact 2,4-D concentrations across the landscape.

Figure 2-1 shows the range of maximum measured concentrations collected per site per year (gray circles). It should be noted that these measured concentrations are unadjusted and do not account for the potential bias from non-daily sampling that are expected to bias concentrations low. The gray circles are formatted with transparency so that the darker the circle appears, the larger the number of sites with the same maximum measured concentration. Reference concentrations are provided in **Table 2-5** to place context around the measured concentrations of 2,4-D in surface water. These reference concentrations represent toxicity endpoints relevant to listed species in different aquatic or semi-aquatic environments. **Figure 2-1** shows that the measured concentrations (unadjusted) are above the wetland plant reference concentrations.

Figure 2-1. Maximum Measured Concentrations by Sample Site by Year for 2,4-D Data from the Water Quality Portal

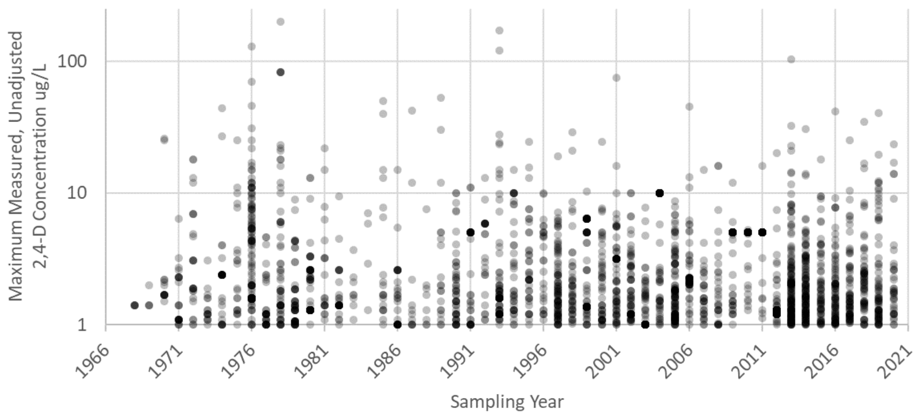


Table 2-5. Reference Concentrations for 2,4-D

Aquatic System	Reference Concentration[1] (µg a.e./L)	Taxa	Modeling Approach
Flowing (e.g., stream, rivers), Reservoirs	470	Aquatic plants	APEZ
Non-flowing (i.e., ponds)			
Wetland	5.6 (NOEC)	Listed Terrestrial Plant[2]	WPEZ
	7.4 (IC_{25})	Non-Listed Terrestrial Plant[2]	

1. This reference concentrations refers to the listed species endpoint
2. IC_{25} converted to a concentration equivalent (calculations provided in **Appendix H**)

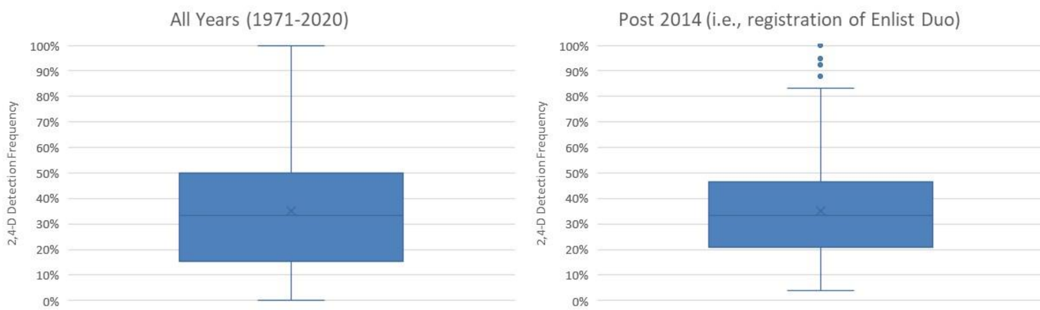
Figure 2-1 shows that the concentration profile for 2,4-D did not change significantly after the registration of Enlist products. Focusing on years post 2014 (*i.e.*, 2015-2020), 2016 had the lowest concentrations, but while the last two years were higher, the change is not outside the variability that existed in previous years.

The maximum detected value of 2,4-D is 200 µg/L (total) reported as a water grab sample measured by USGS on October 30, 1978 (USGS-261940080101100). Given this sample is from the 1970s, which may not represent currently registered uses of 2,4-D or analytical methods, available monitoring data were further examined. The data show several concentrations measured from samples collected prior to 2010 that exceed 35 µg a.e./L.

Focusing on monitoring data post 2014, the maximum detected value of 2,4-D was measured in a sample collected on February 21, 2018 (USGS-06800000; Maple Creek near Nickerson, Nebraska) which measured 34.5 µg/L. While this site is in an area (Dodge County) where 2,4-D may be applied, it is unknown if the measured concentration was the result of the use of an Enlist product. Based on sample timing, the detection could have been the result of an early or late season application of 2,4-D or the result of a runoff event associated with a 2,4-D application the prior year that remained on the field and was then transported off the field during a runoff event.

For site-years[6] with more than 13 samples collected per year, the median detection frequency is 33% (**Figure 2-2**). When only sampling years post 2014 (i.e., registration of Enlist Duo), the median detection frequency remains the same (**Figure 2-2**). This suggests that when 2,4-D containing products are applied, 2,4-D can reach aquatic systems and use of the Enlist product has not changed the detection frequency of 2,4-D in aquatic systems.

Figure 2-2. Box and Whiskers Plot of Sample Frequency for WQP Sites with more than 13 Samples Collected per Year



Surface water monitoring programs typically collect samples at most on a weekly or biweekly basis, even in programs with a relatively high sampling frequency. Generally, sample frequency is less frequent. **Figure 2-3** shows the range of the number of samples collected per site per year (gray circles) along with the number of sites sampled per year (red dash) for 2,4-D. The gray circles are formatted with transparency so that the darker the circle appears, the larger the number of sites with the same number of samples collected per year. Sample frequency has a significant impact on the ability to reliably estimate daily concentrations across a wide range of aquatic environments.

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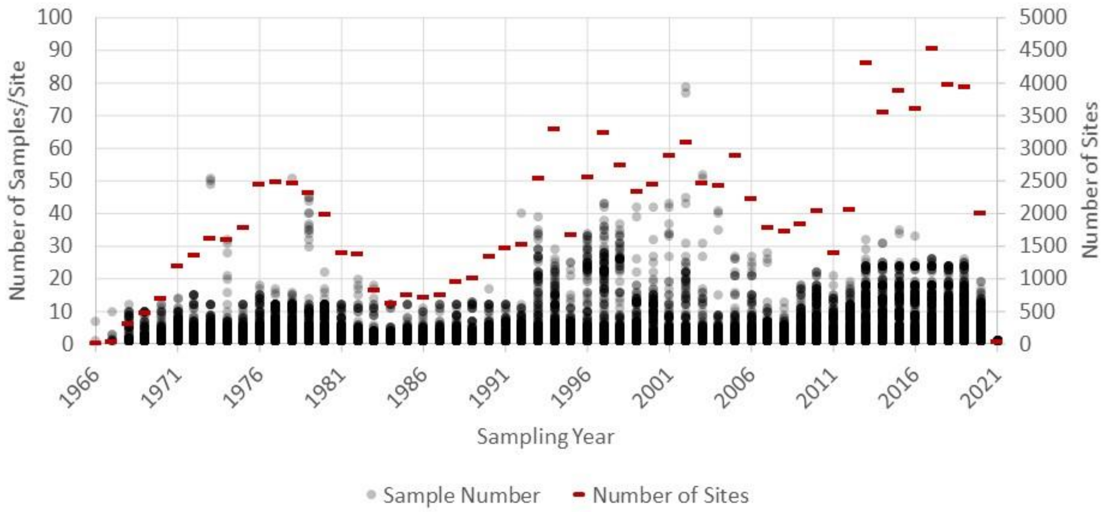
[6] A site-year is the combination of a monitoring site (location) with the year of sampling, such that any given monitoring site may have multiple years of sampling data. Nationwide there are 993 site-years available for 2,4-D with 13 or more samples per year.

Figure 2-3. Sampling Quantity Characteristics for 2,4-D Data from the Water Quality Portal



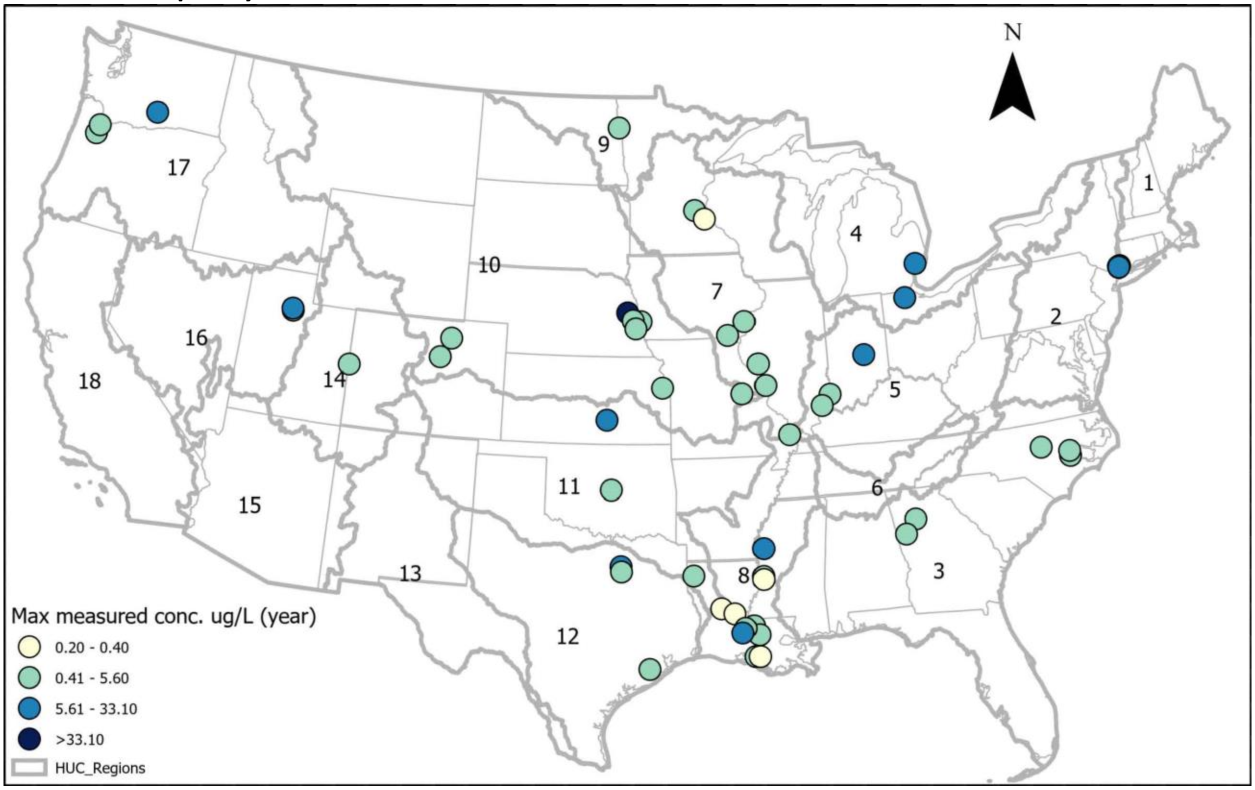
The sample number varies substantially across sites and the number of sites sampled varies by year. **Figure 2-3** illustrates a consistently high number of site samples in recent years (except 2020 and 2021 where all the data are not yet available) as well as the number of samples collected at each site in recent years (except 2021). The most samples collected at a site within a calendar year occurred in 2002 when 79 samples were collected. Sampling frequency at other sites and in other years is generally much lower, with the lowest being one sample per year. Most sites have low sample numbers.

To understand the available monitoring data and place context for the present action for Enlist, data need to be evaluated on a site-specific basis, as the sampling frequency, sample location, etc., may impact the interpretation of the data. Looking at monitoring data on a site-specific basis has been the recommendation of several FIFRA SAPs, as sampling frequency impacts monitoring data interpretation because sites with infrequent monitoring data may miss peak aquatic concentrations, which can impact both the estimation of short and longer-term exposure concentrations. In many cases, there are not enough data either on an annual, multi-year, or multi-site basis to reliably estimate pesticide concentrations for short-term exposure estimates.

Furthermore, given that 2,4-D has been registered for a long time, while the monitoring data indicate that it runs off, having a better understanding of the recent monitoring data and how it may relate to Enlist uses is important. Evaluation of the monitoring data on a site-specific basis permits evaluation of waterbody and watershed characteristics to place context around the available monitoring data.

Of the many sites with 2,4-D samples in the WQP datasets, EPA determined that 53 sites have ≥ 12 samples/year with 25% detection frequency for at least 3 years. Monitoring sites that have more frequent monitoring capture a fuller set of conditions with respect to pesticide usage and weather than sites with less frequent monitoring. Some models that are intended to estimate high-end values based on limited sampling frequency, such as SEAWAVE-QEX, use a criterion of 12 samples per year with 25% detection frequency for 3 or more years as a minimum sampling and detection frequency to allow for higher tier analysis. The range of measured concentrations for these sites is provided in **Figure 2-4**. Of the 53 sites, 11 had measured concentrations above the reference concentration for wetland plants. The highest measured concentration for these sites is 34.5 µg/L. This sample is reported as a dissolved water sample collected in Nebraska (USGS-06800000 Maple Creek near Nickerson) on February 21, 2018 as mentioned above. Again, while usage data are not available to determine if the measured concentration is associated with use of an Enlist product, Enlist Duo was registered for use in 2014. As such, it is possible that Enlist along with other products were being used at the time this sample was collected.

Figure 2-4. Maximum Measured 2,4-D Concentrations for WQP Sites with ≥ 12 Samples/Year with 25% Detection Frequency for At Least 3 Years



The maximum-measured concentrations shown in **Figure 2-4** are based on non-daily sampling data (*i.e.*, unadjusted). To have confidence that the monitoring data captures upper end estimated concentrations in a particular location and time, it would be necessary to perform higher tier analyses. Further analysis of available surface water monitoring data was completed using SEAWAVE-QEX, along with the development of 2,4-D-specific sampling bias factors. The analyses were not included in this assessment because (1) it is difficult to interpret the monitoring data in the context of the small number of 2,4-D products and use sites being considered for this registration action relative to the large number of 2,4-D products on the market and the associated diversity of use sites and label directions, (2) the diverse types of water bodies and environments included in the monitoring programs relative to the types of environments represented in this assessment; and (3) the concentrations (unadjusted) exceed the level of concern for wetland plants, which reduces the need for higher tier analyses of monitoring data for this assessment. Nonetheless, the higher-tier analyses are available upon request.

2.2.1.2.2. 2,4-D Surface Water Analysis Summary

In summary, there is a large amount of monitoring data available for 2,4-D with a high detection frequency (33%). These data permit confident estimation of upper end concentrations in surface water across all 2,4-D labels and use sites. These data suggest that 2,4-D is likely to runoff application sites and end up in a wide range of aquatic habitats. These concentrations cannot be tied to specific uses of 2,4-D including the use of Enlist product; however, these data confirm that concentrations of 2,4-D are expected to occur and exceed the wetland plant reference concentration frequently and across the landscape.

It is important to note that the magnitude of 2,4-D concentrations or the detection frequency has not notably changed since Enlist was registered.

Additional site characterization revealed that 2,4-D concentrations are likely to exceed the wetland plant reference concentration, and that these concentrations occur in areas where Enlist products could be used (i.e., corn, soybean, and cotton growing regions). The additional site characterization was not specific to a single ecologically relevant aquatic environment such as wetlands; however, the analysis indicates that there is high confidence that 2,4-D concentrations are above the wetland reference concentration for a wide-range aquatic system, and it is possible that these systems could be impacted by or could impact wetland areas. It is also important to note that wetlands are not expected to be static, as often there is channelized flow through wetland areas or overflow from adjacent flowing waterbodies due to overflow or tidal impacts.

Lastly, the 2,4-D concentrations derived from available monitoring data are in the range of those estimated using EPAs standard model output values.

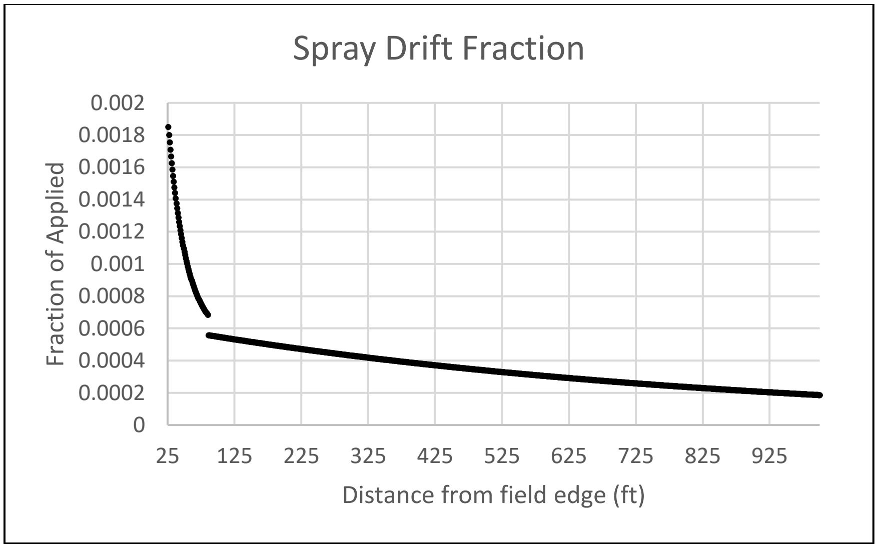
2.2.2. Spray Drift Analysis to Evaluate Potential Off-Site Risks to Terrestrial Plants, Birds, and Mammals

Label requirements for Enlist products include spray drift buffer setbacks (30 feet) and application nozzle restrictions designed to mitigate drift, and these must be considered before any conclusions can be reached as to the risks posed to terrestrial organisms beyond the treatment site. The Enlist product labels require the use of the product with an American Society of Agricultural and Biological Engineers (ASABE) S-572 droplet size classification of coarse/coarser spray quality. Therefore, EPA evaluated the distances to off-field LOC exceedances using the label-required droplet spectrum, combined with the results of a registrant submitted spray-drift field study (MRID 48844001). This study empirically measured spray drift deposition with various drift reduction nozzles and formulations. EPA considered this specific combination of nozzle and formulation as measured in the study to evaluate more realistic spray drift buffer requirements than were achievable using AgDRIFT modeling. The empirical deposition data show a biphasic distribution (**Figure A-1, Appendix A**; USEPA, 2016a, DP 428301) with a bias toward the edge of the application area. EPA truncated the deposition data from 0 to 10 feet and used 90th percentile data from 25 to 400 feet to determine buffer distances (**Figure A-1, Appendix A**). EPA developed the deposition curve using Exponential Decay, Double, 4 Parameter equation using SigmaPlot version 10.0. The Exponential Decay, Double, 4 Parameter (EDDP) and various coefficients are listed below (where x is the distance from the edge of field considering a 25 foot[7] in-field spray drift buffer; **Figure 2-5**.

$$\text{Fraction of applied} = (0.0013*\text{EXP}(-0.0388*x)) + (0.0006*\text{EXP}(-0.0012*x))$$

EPA considered the estimated spray drift fraction using the above equation appropriate, in that it accounts for spray drift limitation technologies employed for the product and label. EPA relied upon the edge of field drift fraction (0.00167) within each of the terrestrial animal and plant sections of **Section 2.4** and **Section 2.5** to determine if potential risk to these taxa would require greater than the labeled 30 ft in-field buffer to mitigate risks to the field.

Figure 2-5. 90th Percentile Deposition Curve of AIXR Nozzle for GF-2726 2,4-D Choline Formulation Distance



2.3. Ecological Effects Characterization

EPA used ecological effects data to estimate the toxicity of 2,4-D to surrogate species. EPA previously reviewed the ecotoxicity data for 2,4-D and the Enlist products in multiple ecological risk assessments (**Appendix C**). These data are summarized in **Section 2.3.1** and **Section 2.3.2**. This information is supplemented by various studies with terrestrial and aquatic plants, birds, and aquatic animals exposed to either the TGAI (Technical Grade Active Ingredient) or formulated 2,4-D received since the 2016 Enlist assessment (USEPA, 2016b). The results of these studies are described in this section.

EPA bridged data for acid, amine, ester and salt forms of 2,4-D for 2,4-D choline because all form 2,4-D acid (the residue of concern for this assessment; **Appendix B**). Available toxicity studies specific to 2,4-D choline indicate that toxicity is similar among the different forms of 2,4-D. For this assessment, 2,4-D choline salt specific data, when available, are discussed as well as the most sensitive 2,4-D acid equivalent data (see the 2016 2,4-D Draft Ecological Risk assessment USEPA, 2016b). EPA used only the most sensitive 2,4-D toxicity value from the broader 2,4-D dataset in risk quotient calculations to ensure

[7] Note: the label requires a 30 ft in-field downwind setback from sensitive vegetation.

that the selected endpoint was protective. There is a difference between the aquatic toxicity of 2,4-D esters and the amine, salts, and acid forms. This difference is driven by the hydrophobic nature of the ester compounds relative to the amines/salts/acid. The choline salt shares solubility properties like the amines/salts/acid forms, however since transformation of the choline salt to the acid is considered to happen quickly, the exposure in water is expected to be to the acid form. Consequently, only toxicity data from the amines/salts/acid are considered as 2,4-D choline analogs and used for comparison to aquatic exposure levels of 2,4-D choline. For terrestrial animals, EPA used the most sensitive 2,4-D toxicity value, regardless of the chemical form. In the case of terrestrial plants, EPA used Enlist Duo specific formulation data to address any dual active ingredient effects associated with the combinations of 2,4-D choline and glyphosate; no such data are available for Enlist One, but comparison across other formulation data (**Appendix B**) indicates similar toxicity to that seen with Enlist Duo.

A search of the ECOTOX database did not yield any 2,4-D choline toxicity studies. Therefore, EPA evaluated only studies submitted by the registrant to determine the effects of 2,4-D choline salt on non-target organisms. EPA conducted a search for 2,4-D acid and did not find reliable more sensitive endpoints than those listed in this assessment. EPA also reviewed the Ecological Incident Data System (IDS) to provide a refined characterization of the ecological effects for 2,4-D.

The aquatic and terrestrial effects endpoints that EPA utilized in the risk assessment are summarized briefly in **Table 2-6** below and **Sections 2.3.1, 2.3.2** and **2.3.2.4.** A full list of available studies is provided in **Appendix B**.

Table 2-6. Toxicity Values Used to Assess Risks from Use of 2,4-D on Enlist Corn, Cotton and Soybean

Species	Acute Endpoint	Species	Chronic Endpoint	MRID
Freshwater Fish				
Rainbow trout (*Oncorhynchus mykiss*)	LC_{50} > 48,000 µg a.e./L	Fathead minnow (*Pimphales promelas*)	NOAEC = 14,200 LOAEC = 37,600 µg a.e./L	48892401, 41767701
Estuarine Marine Fish				
Tidewater silverside (*Menidid beryllina*)	LC_{50} > 80,200 µg a.e./L	FW Fish Surrogate	NOAEC = 14,200 LOAEC = 37,600 µg a.e./L	42018301, 41767701
Aquatic Phase Amphibians				
Leopard frog tadpoles (*Rana pipiens*)	LC_{50} = 278,000 µg a.e./L	FW Fish Surrogate	NOAEC = 14,200 µg a.e./L[a]	44517306, 41767701
Freshwater Invertebrates				
Water flea (*Daphnia magna*)	EC_{50} = 25,000 µg a.e./L	Water flea (*Daphnia magna*)	NOAEC = 16,050 µg a.e./L	41158301, 42018303
Eastern oyster (Crassostrea virginica)	EC_{50} 49,600 µg a.e./L	ACR[b]	NOAEC = 31,800 µg a.e./L	41429003
Birds				
Northern bobwhite quail (*Colinus virginianus*)	LD_{50} = 218.7 mg a.e./kg-bw LC_{50} > 3,035 mg a.e./kg-diet	Northern bobwhite quail (*Colinus virginianus*)	NOAEC = 962 LOAEC >962 mg a.e./kg-diet	41644401, 41644403, 45336401
Mammals				
Rat (*Rattus norvegicus*)	LD_{50} = 441 mg a.e./kg-bw	Rat (*Rattus norvegicus*)	NOAEL = 55 LOAEL > 55 mg a.e./kg-bw	41413501, 00150557, 00130407,

Species	Acute Endpoint	Species	Chronic Endpoint	MRID
				47417902, 47417901
Terrestrial Invertebrates				
Honey bee (*Apis mellifera L.)*[c]	LD_{50} > 89.4 µg a.e./bee (adult contact) LD_{50} = 94 µg a.e./bee (adult oral) LD_{50} = 6 µg a.e./bee (larval oral)	Honey bee (*Apis mellifera L.)*	NOAEL = 5.1 µg a.e./bee/day (adult chronic) NOAEL < 0.459 µg a.e./bee/day (larval chronic)	48892404, 44517304, 50751201, 50282701, 50751301
Aquatic Plant Taxa [d]	**Non-listed Species Endpoint**	**Aquatic Plant Taxa [d]**	**Listed Species Endpoint**	**MRID**
Duckweed (*Lemna gibba*)	IC_{50} = 299 µg a.e./L	Duckweed (*Lemna gibba*)	NOAEC = 47 µg a.e./L	42712204
Freshwater diatom (*Navicula pelliculosa*)	IC_{50} = 3,880 µg a.e./L	Freshwater diatom (*Navicula pelliculosa*)	NOAEC = 1,412 µg a.e./L	41505903
Terrestrial Plant Taxa [d]	**Non-listed Species Endpoint**	**Terrestrial Plant Taxa [d]**	**Listed Species Endpoint**	**MRID**
Seedling Emergence [d]				
Dicot (Lambsquarters, *Chenopodium album*)	EC_{25} = 0.069 lbs ae/A	Dicot (Lambsquarters, *Chenopodium album*)	NOAEC = 0.060 lbs ae/A	49903203
Monocot (Onion, *Allium sp.*)	EC_{25} = 0.254 lbs ae/A	Monocot (Onion, *Allium sp.*)	NOAEC = 0.245 lbs ae/A	49903201
Vegetative Vigor [d]				
Dicot (Tomato, *Solanum lycopersicum*) – Vegetative Vigor	EC_{25} = 0.0099 lbs ae/A	Dicot (Tomato, *Solanum lycopersicum*) – Vegetative Vigor	NOAEC = 0.0075 lbs ae/A	49903202
Monocot (Wheat, *Triticum aestivum*) – Vegetative Vigor	EC_{25} = 0.048 lbs ae/A	Monocot (Wheat, *Triticum aestivum*) – Vegetative Vigor	NOAEC = 0.030 lbs ae/A	49903202
Species Sensitivity Distribution	Height SSD: HC_{10} = 0.0136 lbs a.e./A Weight SSD: HC_{10} = 0.0167 lbs a.e./A			

[a] Chronic toxicity data were not available for estuarine marine fish or freshwater amphibians, the freshwater fish chronic endpoint is used as a surrogate for these taxa.
[b] Chronic toxicity data were not available for estuarine marine invertebrates, an Acute to Chronic Ratio (ACR) was used as an alternative endpoint for these taxa (see **Section 2.3.1.2** for details).
[c] A NOAEC was not available for the larval honey bee chronic endpoint, further discussion and alternative endpoints are provided in **Section 2.3.2.3**.
[d] Terrestrial plant data, including discussion of the listed species endpoints, are discussed in depth in **Section 2.3.2.4**.

2.3.1. Aquatic Toxicity

EPA determined 2,4-D choline salt's effects on aquatic animals by evaluating freshwater and estuarine/marine fish and invertebrates, and freshwater amphibians. Acute and chronic studies were submitted for freshwater fish, freshwater invertebrates, and estuarine/marine fish, whereas only acute studies were submitted for estuarine/marine invertebrates and freshwater amphibians. For this risk assessment, EPA used a test organism surrogate approach, bridging tested taxa and effects endpoints to address data gaps in a manner consistent with the 2,4-D Registration Review Problem Formulation document for 2,4-D (USEPA, 2012). In general, 2,4-D choline salt is slightly toxic to fish and invertebrates, and practically non-toxic to aquatic-phase amphibians, on an acute basis.

2.3.1.1. Effects on Fish and Amphibians

An acute freshwater fish limit test for 2,4-D choline salt was available. Neither mortalities nor sub-lethal effects were observed. This study yielded the lowest registrant-submitted endpoint to date for 2,4-D moieties and classifies 2,4-D choline salt as "slightly toxic" to freshwater fish. Acute and chronic studies for other fish and amphibians were not available for 2,4-D choline salt; however, data were available for other 2,4-D forms. The lowest chronic endpoint for freshwater fish was in an early life stage study with the fathead minnow exposed to 2,4-D dimethylamine salt (MRID 41767701). The study found a decrease in length (most sensitive endpoint) as well as mortality at the highest two concentrations (98,100 and 62,900 µg ae/L) and weight loss at 37,600 µg ae/L. An acute estuarine/marine fish study with the tidewater silverside resulted in no mortality or sub-lethal effects. Chronic estuarine/marine fish endpoints were only available for ester forms of 2,4-D and so not applied to this risk assessment of a water-soluble salt. Owing to the similar sensitivity of freshwater and estuarine/marine fish on an acute basis, a reasonable assumption was made to use the freshwater fish chronic endpoint as a surrogate for estuarine/marine fish. An acute study with the leopard frog suggests that 2,4-D is "practically non-toxic" to freshwater amphibians (**Table 2-7**).

Table 2-7. Most Sensitive 2,4-D Toxicity Data for Fish and Amphibians

Exposure Duration and Taxon	Species	2,4-D Form Tested	Endpoint (µg a.e./L)	Study Classification	MRID
Acute Freshwater Fisht	Rainbow trout (*Oncorhynchus mykiss*)	2,4-D Choline Salt	96-h LC_{50} > 48,000	Acceptable	48892401
Chronic Freshwater Fish (Early Life Cycle) [1]	Fathead minnow (*Pimphales promelas*)	Dimethylamine salt of 2,4-D	**NOAEC = 14,200** LOAEC = 37,600 Based on Length	Acceptable	41767701
Acute Estuarine/Marine Fish	Tidewater silverside (*Menidid beryllina*)	Dimethylamine salt of 2,4-D	96-h LC_{50} > 80,240	Acceptable	42018301
Acute Freshwater Amphibians	Leopard frog tadpoles (*Rana pipiens*)	Dimethylamine salt of 2,4-D	**96-H LC_{50} = 278,000**	Acceptable	44517306

Bold = Value used for calculating risk quotients
[1]No Chronic data available for an estuarine fish species for the acid or salts. Owing to the low observed acute toxicity comparable to freshwater fish and non-definitive endpoints, the risk assessment uses the freshwater fish chronic effect level as a surrogate endpoint.

2.3.1.2. Effects on Aquatic Invertebrates

An acute freshwater invertebrate limit test for 2,4-D choline salt was available using the water flea. The most sensitive endpoint for acute exposures to freshwater invertebrates occurred in a water flea study conducted with 2,4-D acid (MRID 41158301). Sub-lethal effects were not reported for this study; the study classifies 2,4-D as "slightly toxic" to freshwater invertebrates on an acute basis. A freshwater invertebrate chronic study with the water flea yielded endpoints based on survival and reproduction (number of neonates produced, number of broods produced per daphnid, and brood size). An acute study with the eastern oyster classified 2,4-D as "slightly" toxic to estuarine/marine invertebrates. One mortality occurred at the highest treatment level (155 mg ae/L) and reduced feeding was also observed at this concentration. Shell growth reduction was noted in the other concentrations and the EC_{50} was based on this parameter (**Table 2-8**).

Estuarine/marine invertebrate chronic toxicity data were not available. A toxicity value can be estimated based on the assumption that the acute-to-chronic ratio (ACR) for freshwater invertebrates applies to estuarine/marine invertebrates also. Thus, EPA used the following equation to estimate a NOAEC for eastern oyster, the most sensitive estuarine/marine species on an acute basis (**Table 2-8**).

$$\frac{EC50\ (oyster)}{NOAEC\ (oyster)} = \frac{EC50\ (waterflea)}{NOAEC\ (waterflea)} = \frac{49{,}600}{X} = \frac{25{,}000}{16{,}050} = 31{,}800 = NOAEC\ (oyster)$$

Table 2-8. Effects of 2,4-D on Aquatic Invertebrates

Test	Species	2,4-D Form Tested	Endpoint (µg a.e./L)	Study Classification	MRID
Acute Freshwater Invertebrates	Water flea (*Daphnia magna*)	2,4-D Choline Salt	48-h EC_{50} >40,700	Acceptable	48892402
Acute Freshwater Invertebrates	Water flea (*Daphnia magna*)	2,4-D Acid	**48-h LC_{50} = 25,000**	Acceptable	41158301
Chronic Freshwater Invertebrates	Water flea (*Daphnia magna*)	Dimethylamine salt of 2,4-D	**NOAEC = 16,050** LOAEC = 25,473 Based on survival (18% reduction) and reproduction	Acceptable	42018303
Acute Estuarine/Marine Invertebrates	Eastern oyster (*Crassostrea virginica*)	Isopropylamine salt of 2,4-D	**96-h EC_{50} = 49,600**	Acceptable	41429003
Chronic Estuarine/Marine Invertebrates	Eastern oyster (*Crassostrea virginica*)	ACR	**NOAEC = 31,800**	ACR	ACR
Bold = Value used for calculating risk quotients ACR = Acute:Chronic Ratio approach for endpoint estimation					

2.3.1.3. Effects on Aquatic Plants

A non-vascular aquatic plant study for 2,4-D choline salt was available using green algae. Yield and growth rates were affected at the 23,300 µg ae/L treatment. Among all the forms of 2,4-D considered, the most sensitive endpoint for non-vascular plants was derived from a freshwater diatom study testing 2,4-D DMA and was based on growth inhibition. An aquatic vascular plant study tested with 2,4-D DEA on duckweed was available. The most sensitive endpoints were a reduction in frond number (40%

reduction) and plant number (35% reduction) at the LOAEC (88 µg a.e./L). At the two highest treatment levels (2,110 and 1,030 µg ae/L), colony break up and root destruction were observed; there was a statistically significant increase in frond chlorosis as well (**Table 2-9**).

Table 2-9. Effects of 2,4-D on Aquatic Plants

Test	Species	2,4-D Form Tested	Endpoint (µg a.e./L)	Study Classification	MRID
Non-vascular aquatic plant	Green algae (*Pseudokirchneriella subcapitata*)	2,4-D Choline Salt	IC_{50} > 45,850 NOAEC = 23,300 LOAEC = 45,850 Based on 24% reduction in yield	Acceptable	48892405
Non-vascular aquatic plant	Freshwater diatom (*Navicula pelliculosa*)	Dimethylamine salt of 2,4-D	**EC_{50} = 3,880** **NOAEC = 1,410** LOAEC = 1,925 Based on 43% reduction in growth	Acceptable	41505903
Vascular aquatic plant	Duckweed (*Lemna gibba*)	Diethanolamine salt of 2,4-D	**EC_{50} = 297** **NOAEC = 47** **LOAEC = 88** Based on 40% reduced frond number	Acceptable	42712204
Bold = Value used for calculating risk quotients					

2.3.2. Terrestrial Toxicity

EPA determined 2,4-D choline salt's effects on terrestrial organisms by evaluating toxicity data for birds, mammals, insects, and plants. Acute and chronic studies were submitted for birds, mammals and honey bees. Vegetative vigor and seedling emergence data were available for plants. In general, on an acute exposure basis, 2,4-D is practically non-toxic to terrestrial insects, slightly toxic to mammals, and moderately toxic to birds. There were no observed effects in chronic studies on birds. Chronic effects to mammals are observed following short term dietary exposures that exceed the elimination rate of the animal.

2.3.2.1. Effects on Birds

EPA evaluated 2,4-D toxicity to birds through acute oral dose exposure, acute dietary exposure, and chronic dietary exposure (**Table 2-10**). In the absence of toxicity data on terrestrial-phase amphibians and reptiles, EFED used bird toxicity data as a surrogate for these taxa (USEPA, 2016b).

The acute oral toxicity study with the northern bobwhite quail resulted in a classification of "moderately toxic" to birds on an acute oral basis. Three acute dietary studies were available, classifying 2,4-D choline salt as "practically non-toxic" on an acute dietary basis to birds. No mortalities occurred in the studies. The third study involved testing of 2,4-D acid with the passeriformes canary. This dietary study was performed in lieu of an acute oral study because reliable oral dosing with passeriformes was problematic due to regurgitation of the test substance and so testing shifted to a subacute dietary test. No mortalities were observed at doses as high as 4,790 mg/kg-diet.

The chronic reproduction study was performed with the northern bobwhite quail. No sub-lethal effects were observed for growth or reproduction at all test levels, resulting in a NOAEC of 962 mg a.e./kg-diet.

Table 2-10. Toxic Effects on Birds

Test	Species	2,4-D Form Tested	Endpoint (mg a.e./L)	Study Classification	MRID
Acute oral toxicity	Northern bobwhite quail (*Colinus virginianus*)	Triisopropanol amine salt of 2,4-D	**LD_{50} = 218.7 mg a.e./kg-bw**	Acceptable	41644401
Acute dietary toxicity	Canary (*Serinus canaria*)	2,4-D acid	LC_{50} > 4,790 mg a.e./kg-diet	Acceptable	49472501
Acute dietary toxicity	Northern bobwhite quail (*Colinus virginianus*)	Triisopropanol amine salt of 2,4-D	LC_{50} >3,035 mg a.e./kg-diet	Acceptable	41644402
Acute dietary toxicity	Mallard Duck (*Anas platyrhynchos*)	Triisopropanol amine salt of 2,4-D	LC_{50} >3,035 mg a.e./kg-diet	Acceptable	41644403
Chronic reproduction	Northern bobwhite quail (*Colinus virginianus*)	2,4-D acid	**NOAEC = 962 mg a.e./kg-diet** LOAEC > 962 mg a.e./kg-diet No observed effects	Acceptable	45336401

2.3.2.2. Effects on Mammals

EPA assessed the acute toxicity of 2,4-D choline salt to mammals using the oral gavage study conducted on laboratory rat. Based on the LD_{50}, 2,4-D choline salt is moderately toxic to mammals on an acute basis. **Table 2-11** presents acute and chronic endpoints incorporated as effects endpoints into the mammalian risk assessment.

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 ae/kg/day. See USEPA, 2014b for a detailed description of the analysis. The 55 mg/kg/day was considered to be a refined estimate of the threshold for chronic effects in the rat, following short-term exposures to 2,4-D, taking into account the pharmacokinetics information and serves as the endpoint for assessment of mammalian reproduction risks.

Table 2-11. Toxic Effects on Mammals from Acute and Chronic Oral Exposure to 2,4-D Choline Salt

Test	Species	2,4-D Form Tested	Endpoint (mg a.e./L)	Study Classification	MRID
Acute oral	Rat (*Rattus norvegicus*)	Triisopropanolamine salt of 2,4-D	**LD_{50} = 441 mg a.e./kg-bw**	Acceptable	41413501
Chronic reproduction/ developmental	Rat (*Rattus norvegicus*)	2,4-D generalized	**NOAEL = 55 LOAEL > 55 mg a.e./kg-bw/day**	HED Evaluation of all lines of evidence	00150557, 00130407, 47417902, 47417901

2.3.2.3. Effects on Terrestrial invertebrates

There are adult honey bee contact toxicity data for 2,4-D choline salt and another salt, 2,4-D dimethyl amine, and oral data for 2,4-D dimethylamine salt (**Table 2-12**). The available acute contact study with the dimethyl amine salt of 2,4-D is consistent with the 2,4-D choline results and produced a non-definitive endpoint that classifies 2,4-D as "practically non-toxic" to honey bees on an acute contact basis. Owing to the similar potency in the contact studies between the two salts, EPA used the 2,4-D choline endpoint for the acute contact effects endpoint and the data from the similar potency 2,4-D dimethyl amine salt oral study (which also identified 2,4-D as practically non-toxic on an acute oral basis), as the effects endpoints for terrestrial invertebrates.

The submitted adult honey bee chronic toxicity study (MRID 50751201) was acceptable (DP Barcode: 450376). This study reported a chronic no observed adverse effect level (NOAEL) of 5.3 µg a.e./bee/day (276 mg a.e./kg-diet) and a lowest observed adverse effect level (LOAEL) of 8.2 µg a.e./bee/day (668 mg a.e./kg-diet). Accounting for the weight of adult bees of 0.0881 g/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.

The submitted larval honey bee chronic study (MRID 50751301) was supplemental (DP Barcode: 450376). This study reported mortality to be the most sensitive endpoint in the study, with a LOAEL of 0.459 µg a.e./bee/day (LOAEC 11.9 mg a.e./kg-diet)**.** Because the study did not return a NOAEC and the most sensitive endpoint was lethality, EFED derived two approaches for characterizing effects of 2,4-D on insect larvae, including:

1. The LOAEL for mortality was used (i.e., 0.459 µg a.e./bee).

2. A threshold for the discernable effects level was predicted by considering the control and treatment variance of the binomial survival data from the study. Because a binomial minimum statistically detectible difference (MSDD) test for the Cochran Armitage trend test was not available, the number of observed mortalities in each treatment group were reduced by the same number until the Cochran Armitage test indicated that there was no statistical difference between the negative control and the lowest tested concentration (dose). Results indicate 15% mortality was the approximate MSDD. This MSDD was then applied to a Probit dose response

curve applied to the entire mortality data set to yield a corresponding dose at which 15% mortality would be predicted or an LD_{15} of 0.039 µg a.e./bee (LC_{15} of 2.91 mg a.e./kg-diet).

In **Section 2.4.3**, these endpoints are compared directly to the estimated exposure concentrations and indicate risk if the EECs are higher than the endpoint.

Table 2-12. Acute and Chronic Toxicity Endpoints for Bees Exposed to 2,4-D.

Study Type	Test Substance (% a.i.)	Test Species	Toxicity Value	MRID or ECOTOX No./ Classification	Comments
Terrestrial invertebrates					
Acute contact (adult)	2,4-D EHE TGAI 99% a.i.	Honey bee (*Apis mellifera L.)*	LD_{50} > 66 µg a.e./bee	44517301 Acceptable	Practically non-toxic
Acute oral (adult)	2,4-D choline TGAI 99% a.i.	Honey bee (*Apis mellifera L.*)	LD_{50} > 62.2 µg a.e./bee	48892404 Acceptable	Practically non-toxic 24% mortality at highest dose.
Chronic oral (adult)	2,4-D acid TGAI 97.5% a.i.	Honey bee (*Apis mellifera L.*)	NOAEL = 5.3 LOAEL = 8.2 µg a.e./bee NOAEC = 276 LOAEC = 668 mg a.e./kg-diet	50751201 Acceptable	71% mortality at LOAEC
Acute oral (larval)	2,4-D acid TGAI 98.8% a.i.	Honey bee (*Apis mellifera L.*)	LD_{50} = 6 µg a.e./larva LC_{50} = 156 mg a.e./kg-diet	50282701 Supplemental	Acute Single Dose Study not available. Day 8 larval mortality endpoints from this 22-day chronic study were determined suitable for quantitative use in risk assessment
Chronic oral (larval)[1]	2,4-D acid TGAI 97.5% a.i.	Honey bee (*Apis mellifera L.*)	NOAEL <0.459 LOAEL = 0.459 µg a.e./larvae NOAEC <11.9 LOAEC = 11.9 mg a.e./kg-diet Day 22 LD_{15} = 0.039 ug a.e./larvae (2.91 mg a.e./kg-diet)	50751301 Supplemental	Day 15 Mortality and Adult Emergence were both significantly different from control (respectively, 34% and 33% difference from controls) Day 8 Larval mortality 24% at highest dose. Estimated larval acute LD_{50} > 2.93 ug a.e./larvae

[1] Alternative endpoints for estimating chronic toxicity to larvae are described in the text of **Section 2.3.2.3**.

Open literature Data

The registrant, Corteva, submitted three white papers (MRIDs 51667101, 51667103, 51667104) and provided summaries of nine open literature studies in support of Enlist One and Enlist Duo registration renewal. EFED provided written reviews of the submitted studies to Corteva (USEPA, 2021c; DP 463524). For the reasons provided in the referenced documents, EPA found that nearly all of the studies were invalid for use in risk assessment. However, one supplemental study (Gupta and Bhattacharya, 2008) provides support for using the honey bee toxicity endpoint to assess chronic risks to non-apis terrestrial invertebrates, including butterflies.

Gupta and Bhattacharya (2008) conducted a chronic larval dietary feeding toxicity study with Jute Hairy caterpillar (*Spilarctia obliqua*) using an artificial diet dosed with several levels of a formulation of 2,4-D ethyl hexyl ester (PC CODE 030063). The tested formulated product is not a US registration and no further information could be located regarding its composition. However, EFED determined the study is useful for risk assessment for qualitative characterization of the risks to lepidopterans. The authors 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 (diet was provided for ~23 days of larval development). In comparison, in the honey bee larvae chronic toxicity study with 2,4-D acid (MRID 50751301), the 22-day mortality was 34% at 11.9 mg a.e./kg-diet, and the honey bee LC_{15} was estimated at 2.91 mg a.e./kg-diet. EFED considers the results of these studies as consistent. The Gupta and Bhattacharya (2008) study adds to the weight of evidence supporting the selected honey bee-based endpoint for evaluating the toxicity of 2,4-D to lepidopterans.

2.3.2.4. Effects in Plants

2.3.2.4.1. Greenhouse based toxicity studies

Enlist Duo is a mixture of 2,4-D choline salt and glyphosate dimethylammonium (glyphosate DMA) salt (formulation GF-2726). EPA required standard vegetative vigor (MRID 49903202) and seedling emergence (MRID 49903201) studies (Guidelines 850.4100 and 4150) with the Enlist Duo formulation for a suite of commonly tested plant species for which existing single-herbicide testing indicated plant sensitivity to 2,4-D or glyphosate. Surpassing the normal requirement of ten plant species, the Enlist Duo testing spanned 15 commonly tested monocot and dicot crop species: buckwheat, cabbage, corn, cucumber, mustard, oat, oilseed rape, onion, radish, sorghum, soybean, sugarbeet, sunflower, tomato, and wheat. In addition, the EPA required and received vegetative vigor (MRID 49903203) and seedling emergence (MRID 49903204) testing with three weed species identified in the patent data set as having the potential for exhibiting enhanced sensitivity to the 2,4-D choline/glyphosate combination in excess of simple addition of individual active ingredient effects. These species included lambsquarters (*Chenopodium album*), horseweed (*Conyza canadensis*), and quackgrass (*Agropyron repens*). EPA established plant endpoints for risk assessment using all of the available Enlist formulation data. Because this includes the formulation including both active ingredients, EPA addresses all concerns regarding potential synergistic effects on terrestrial plant height, weight and survival from the combined exposure of the two active ingredients.

Table 2-13 reports the growth effects endpoints for the tested monocot and dicot plants in vegetative vigor tests with Enlist Duo. These studies reported similar IC_{25} endpoints across most dicot species suggesting a broad toxicity across species. The monocot tested species generally showed less sensitivity to 2,4-D-alone in toxicity tests, and the estimates here may reflect some influence of the glyphosate DMA impacts. However, studies with 2,4-D acid and 2,4-D DMA have also illustrated the sensitivity of monocots to 2,4-D (USEPA, 2016b). For the Enlist Duo formulation, the most sensitive monocot IC_{25} was based upon dry weight reduction of wheat.

Table 2-14 reports the sublethal effects endpoints for the tested monocot and dicot plants in seedling emergence tests with Enlist Duo. These studies reported generally less sensitive endpoints than those for the vegetative vigor exposure route. Onion was the most sensitive tested monocot species which had a similar endpoint to several dicots. Other studies with 2,4-D acid and 2,4-D DMA have also illustrated the sensitivity of monocots to 2,4-D at the seedling emergence stage of plant development (USEPA, 2016b). For the dicots, several species reported no effects. The most sensitive monocot IC_{25} was based upon dry weight reduction of wheat.

The most sensitive monocot and dicot species, based on comparison of the EC_{25}'s, from the seedling emergence and vegetative vigor studies are selected to serve as the effects thresholds for the terrestrial plant risk assessment.

Table 2-13. Species Toxicity Endpoints for Vegetative Vigor Growth Stage Plants Exposed to Enlist Duo (2,4-D Choline and Glyphosate DMA) in Units of lbs of 2,4-D a.e./A

Test Species	Endpoint	NOEC	EC_{25}/IC_{25}	MRID
Dicots				
Buckwheat	Dry Weight	0.0075	0.020	49903202
Cabbage	Dry Weight	0.0018	0.030	49903202
Cucumber	Dry Weight	0.0038	0.035	49903202
Horseweed	Dry Weight	0.0075	0.019	49903204
Lambsquarters	Height	0.030	0.024	49903204
Mustard	Height	0.0038	0.011	49903202
Oilseed Rape	Dry Weight	0.015	0.038	49903202
Radish	Dry Weight	0.0075	0.016	49903202
Soybean	Dry Weight	0.015	0.020	49903202
Sugarbeet	Dry Weight	0.0075	0.022	49903202
Sunflower	Dry Weight	0.0075	0.022	49903202
Tomato	Dry Weight	**0.0075**	**0.0099**	49903202
Monocots				
Corn	Dry Weight	0.12	0.11	49903202
Oat	Dry Weight	0.060	0.21	49903202
Onion	Dry Weight	0.25	0.21	49903202
Quackgrass	Dry Weight	0.057	0.075	49903204
Sorghum	Height	0.12	0.21	49903202
Wheat	Dry Weight	**0.030**	**0.048**	49903202

Bold indicates the most sensitive endpoint

Table 2-14. Species Toxicity Endpoints for Seedling Emergence Growth Stage Plants Exposed to Enlist Duo (2,4-D Choline and Glyphosate DMA) in Units of lbs of 2,4-D a.e./A

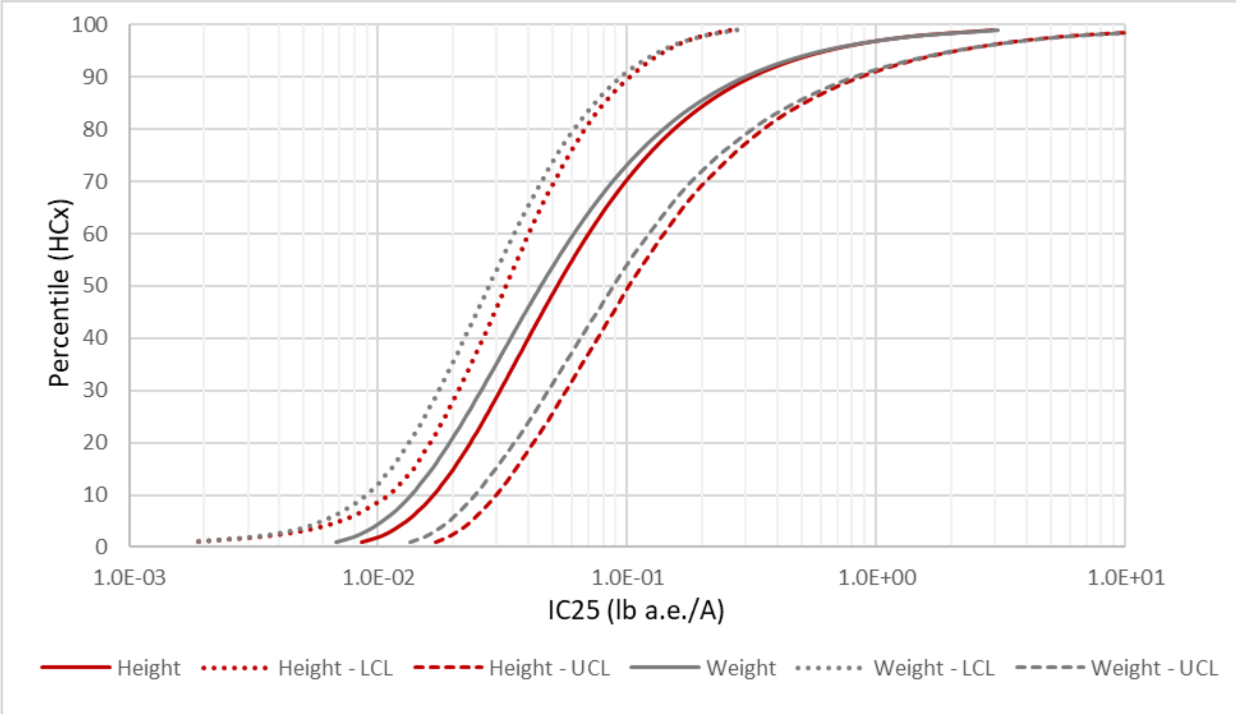
Test Species	Endpoint	NOEC	EC_{25}/IC_{25}	MRID
Dicots				
Buckwheat	Height	0.48	>0.96	49903201
Cabbage	Dry Weight	0.12	0.082	49903201
Cucumber	No Effects	0.96	>0.96	49903201
Lambsquarters	Dry Weight	**0.060**	**0.069**	49903203
Mustard	Dry Weight	0.12	0.16	49903201
Oilseed Rape	Dry Weight	0.12	0.42	49903201
Radish	Survival	0.25	0.25	49903201
Soybean	Dry Weight	0.96	>0.96	49903201
Sugarbeet	No Effects	0.49	>0.49	49955101
Sunflower	Dry Weight	0.96	>0.96	49903201
Tomato	Dry Weight	0.49	>0.96	49903201
Monocots				
Corn	No Effects	0.96	>0.96	49903201
Oat	Height	0.48	>0.96	49903201
Onion	Dry Weight	**0.245**	**0.254**	49903201
Sorghum	No Effects	0.96	>0.96	49903201
Wheat	No Effects	0.96	>0.96	49903201

Bold indicates the most sensitive endpoint
">" indicates the IC_{25} was predicted as above the highest tested concentration.

2.3.2.4.2. Species Sensitivity Distributions

Species Sensitivity Distributions (SSDs) were fit to inhibition concentrations (IC_{25} values) for vegetative vigor (VV) endpoints for plants exposed to 2,4-D choline. Separate SSDs for height and weight were developed to estimate Hazard Criterion (HC) values. For weight and height endpoints, the Gumbel distribution and normal distribution provided the best fit for the datasets, respectively (**Figure 2-6**). Akaike Information Criterion $(AIC)_c$ and weight and confidence limits for the different distributions (especially around the HC_{05} and HC_{50}) were used to determine the best fit. **Appendix I** describes the methods and supporting statistics for finding the best fit distributions.

Figure 2-6. SSDs for Plant Height and Weight Based on Monocot and Dicot Exposures to 2,4-D at Vegetative Vigor Growth Stages



The HCx (hazard criterion) estimates from SSDs are used to characterize the potential effects to listed species or their plant habitats. The HC_{05} is considered as an alternative threshold for characterizing individual listed species of plants. The HC_{10} and HC_{25} are thresholds for characterizing potential broader impacts across species, habitats, and communities. **Table 2-15** provides the HCx values for the height and weight SSDs shown in **Figure 2-6.**

Table 2-15. HCx Estimates (lbs a.e./A) from SSDs for IC_{25} Plant Height and Weight Endpoints from Vegetative Vigor Growth Stage Exposures to Enlist Duo.

Study Type	Endpoint	Percentile (HCx)						
		5	10	25	50	75	90	95
Vegetative vigor	Height	0.010	0.014	0.023	0.045	0.11	0.30	0.61
Vegetative vigor	Weight	0.013	0.017	0.027	0.052	0.12	0.31	0.62

2.3.2.4.3. Plant Reproduction and Visual Signs of Injury (VSI) Studies

In addition to the greenhouse studies discussed above, the registrant submitted several field studies that related plant damage in grape, cotton, and soybean to growth or yield endpoints (**Table 2-16**). The studies were all considered scientifically sound and appropriate for qualitative incorporation into a risk assessment. Being field studies, there was variation in the methodology and timing of measurements, as well as geography, soils, climate and products applied. Crop yields were measured at the end of the growing season or harvest. The growth endpoints, when available, were measured at multiple durations (e.g., 14 and 28 days after treatment). In addition to variation in field sites (location, year, month), some fields also received applications of pesticides (not herbicides) to control insects and other pests (see **Appendix B** for additional details). These variables introduce some uncertainty into the studies; however, they do simulate real-life field conditions. Consequently, the results of the studies are considered appropriate for risk characterization (**Table 2-16**).

As compared to the vegetative vigor studies described above, these studies often did not present enough data to estimate the 25% effect thresholds. Therefore, comparisons against the most sensitive height endpoint for vegetative vigor (tomato IC_{25} = 0.0099 lbs a.e./A) are done qualitatively. Following single applications, Ogg et al. 1991 reported no significant reductions in yield for grape, whereas all of the other studies reported reproduction effects from 15-63% at concentrations ranging from 0.022 to 0.16 lbs a.e./A. Everitt and Keeling had the greatest effect on yield (63% on average at 0.025 lbs a.e./A); however, there were less effects for plants exposed at later growth stages. Ogg et al. (1991) and Marple et al. (2008) each had studied the impact following multiple applications. In general, as more applications were made, lower application rates resulted in more significant effects. Ogg et al. (1991) reported 30% or greater reductions in yield after 2 or more applications at 0.00075 lbs a.e./A. Marple et al (2008) reported 35% reductions in yield following a single application of 0.0025 lbs a.e./A in the youngest plants, and 40 to 90% impacts to yield following 2 exposures at 0.000625 and 0.00125 lbs a.e./A respectively, and effects were observed for older growth stages.

The vegetative vigor study endpoints are based upon a single exposure event at a young growth stage. As compared to these reproduction studies, the observations for a single exposure result in similar or less effect than was observed for the vegetative vigor study. Therefore, the plant height endpoint from the vegetative vigor study represents a suitable surrogate for single exposure effects to plant yield. Because these studies were direct spray applications, the multiple exposure events are uniform in magnitude and plant coverage. Under environmental exposure conditions in the field following use, the exposure is likely more variable in terms of timing of plant growth stages, magnitude of exposure, and percent interception by the plant after each exposure; therefore, the multiple application data provided is an uncertainty in the establishment of endpoints.

Table 2-16. Percent of Plant Damage Producing Significant Effects in Growth/Yield for Grape, Cotton, and Soybean

Species	Percent Damage at Approximate Endpoint Dose Concentration	Endpoint (Observation)	Citation
Grape	20-50%	Yield (~30% reduction, after 2 or more applications at or above 0.00075 lbs a.e./A)	Ogg et al. 1991 Supplemental
Cotton	57% (single exposure) 40-97% (1-3 exposures)	Yield (35% effect, single exposure; approximately 40-90% effect following 2 exposures at 0.000625 and 0.00125 lbs a.e./A respectively)	Marple et al. 2008 Supplemental
Cotton	66% (average) Approximately the same for as % reduction in Yield	Yield (63% average at concentrations 0.025 lbs a.i./A; exposure at later development stages had less effect for similar rates of exposure at younger growth stages)	Everitt and Keeling 2009 Supplemental
Soybean	40-60%	Yield (VSI comparison made at approximately 25% reduction; most	Robinson et al. 2013 Supplemental

Species	Percent Damage at Approximate Endpoint Dose Concentration	Endpoint (Observation)	Citation
		sensitive growth stage was V5 with an ED_{20} = 0.022 lbs a.e./A)	
Soybean	30-35%	Yield (25-32% reduction at 0.1 lb a.e./A)	Andersen et al. 2004 Supplemental
Soybean	37-52%	Height (18-25% reduction at 0.16 lb a.e./A) Yield (15-25% reduction at 0.16 lb a.e./A)	Kelley et al. 2005 Supplemental

2.4. Risk Estimation and Characterization

EPA used the screening ecological risk assessment to generate a series of risk quotients (RQs) for broad taxonomic groups (e.g., mammals, birds, fish, etc.) that are the ratio of estimated exposures to acute and chronic effects endpoints (USEPA, 2004). EPA then compares these RQs to EPA established levels of concern (LOCs) to determine if risks to any taxonomic group are of concern. The LOCs address risks for both acute and chronic effects. Acute effects LOCs range from 0.05 for listed aquatic animals to 0.5 for aquatic non-listed animal species and 0.1 to 0.5 for terrestrial animals for listed and non-listed species. The LOC for chronic effects for all animal taxa (listed and non-listed) is 1.0. Plant risks are handled in a similar manner, but with different toxicity thresholds (NOAEC/EC_{05} and EC_{25}, respectively) used in RQ calculation for listed and non-listed species and a LOC of 1 is used to interpret the RQ. When a given taxonomic RQ is equal to or exceeds either the acute or chronic LOC for a taxonomic group, a concern for direct toxic effects is identified for that particular taxon. If RQs fall below the LOC for non-listed species, EPA makes a finding of no risk of concern. If the RQs fall below the LOC for listed species, EPA concludes that effects are not expected for that taxon and no further refinement is necessary to complete an Effects Determination for species within that taxon.

In this assessment, EPA presented the comparison of RQs with both the non-listed and the listed LOCs. With the exception of plants, the non-listed species comparisons are intended to communicate risks to inform the findings under FIFRA. Non-listed species RQs are also used to evaluate potential indirect effects to listed species due to impacts to their prey, pollinators, habitat and/or dispersal. In cases where RQs indicate a potential for direct or indirect effects to taxonomic groups, the results are carried through to the Effects Determinations in **Section 4**. In this section, EPA used the listed-species RQ:LOC comparisons to discriminate taxonomic groups requiring no further analyses from those where a species-specific risk assessment is needed to complete an Effects Determination. EPA's ecological risk assessment methods for endangered species that are used to make effects determinations are conducted using a tiered approach, beginning with highly conservative assumptions. When risks are not identified to listed species based on highly conservative assumptions, then EPA is confident that no discernable effects to species will occur. If risks to listed species are identified, then the assessment proceeds to a more refined tier that involves a spatially explicit analyses, incorporation of species life history, or other factors to describe potential effects on listed species and their critical habitat.

2.4.1. Aquatic Animal Risk Characterization

The maximum concentrations in the standard pond (**Table 2-17**) are three orders of magnitude below the non-definitive acute fish toxicity values with <50% mortality. All of the other available aquatic animal endpoints for acute and chronic exposure result in RQs below the acute and chronic LOCs (**Table 2-17**). Therefore, risk is considered low for aquatic animals.

Table 2-17 PWC Standard Pond Estimated Environmental Concentrations (EECs) for 2,4-D Assuming 3 x 1lb Applications with a 12-Day Reapplication Interval

Crops	Scenario	Estimated Environmental Concentrations (µg/L)		
		1-in-10-year Daily Average EEC	1-in-10-year 21-day mean EEC	1-in-10-year 60-day mean EEC
Corn	MScornSTD	71.9	67.7	57.5
Aquatic Animal	**Endpoints Acute; Chronic (µg/L)**	**Acute Fish/Amphibian RQ[2]**	**Chronic Invertebrate RQ[2]**	**Chronic Fish/Amphibian RQ[2]**
Fish	>40,000; 14,200	NA[1]	--	0.004
Amphibians	278,000; 14,200	0.0003	--	0.004
FW Invertebrates	25,000; 16,050	0.003	0.004	--
EM Invertebrates	49,600; 31,800	0.001	0.002	--

[1]RQs are not calculated for non-definitive endpoints.
[2]Bold values exceed the non-listed or listed acute LOCs (0.5, 0.05 respectively) or chronic LOCs (1.0).

2.4.2. Terrestrial Vertebrate Risk Characterization

2.4.2.1. On-Field Dietary

Terrestrial wildlife exposure estimates for birds and mammals typically focus on the dietary exposure pathway (USEPA, 2004). EPA considered the potential dietary exposure for terrestrial vertebrate wildlife based on consumption of 2,4-D residues on food items following spray (foliar) applications. EPA calculated EECs for birds[8] and mammals from consumption of dietary items on the treated field using T-REX v.1.5.2[9].

EPA derived dietary exposure estimates for terrestrial animals assumed to be consuming plants and arthropods in an area exposed to spray using the T-REX (Terrestrial Residue EXposure model) model (version 1.5.2). This model incorporates the Kenaga nomograph, as modified by Fletcher *et al.* (1994), which is based on a large set of actual field residue data. The upper limit values from the nomograph represent an approximation of the highest residue value observed in the data set (Hoerger and Kenaga 1972). Consideration is given to different types of feeding strategies for mammals and birds, including herbivores, insectivores and granivores. For dose-based exposures, three weight classes of birds (20, 100, and 1,000 g) and mammals (15, 35, and 1,000 g) are considered. EPA used the default foliar dissipation half-life of 35 days as well as an adjusted value of 8.8[10] days for risk estimation. EPA assumes a maximum single application rate of 1.0 lb a.e./A, 3 applications and a 12-day application interval to

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[8] Birds are also used as a proxy for reptiles and terrestrial-phase amphibians.
[9] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment#t-rex
[10] The foliar dissipation half-life has been modified from a default assumption of 35-days to 8.8 days on the basis of data for 2,4-D in Willis and McDowell (1987)

estimate terrestrial exposures of 2,4-D. The dose- and dietary-based EECs (upper bound Kenaga) on a variety of food items from the use of 2,4-D applied at the maximum labeled rates is provided below in **Table 2-18**, along with the full T-REX inputs and output. As the use rates are the same for Enlist One and Enlist Duo products applied to either soybean, cotton, or corn, T-REX EECs and RQs apply to all three crops.

Table 2-18. Summary of Dietary (mg a.e./kg-diet) and Dose-Based EECs (mg a.e./kg-bw) for 2,4-D as Food Residues for Terrestrial Vertebrates from Labeled Uses of Enlist or Enlist Duo on 2,4-D-Tolerant Crops (T-REX v.1.5.2, Upper-Bound Kenaga)

Food Type	Dietary-Based EEC (mg/kg-diet)	Dose-Based EEC (mg/kg-body weight)					
		Birds			Mammals		
		Small (20 g)	Medium (100 g)	Large (1000 g)	Small (15 g)	Medium (35 g)	Large (1000 g)
Soy, Cotton or Corn max annual ground application (3x 1 lb a.e./A, 12-day Interval)							
Short grass	369.51	420.83	239.98	107.44	352.30	243.48	56.45
Tall grass	169.36	192.88	109.99	49.24	161.47	111.60	25.87
Broadleaf plants	207.85	236.72	134.99	60.44	198.17	136.96	31.75
Fruits/pods	23.09	26.30	15.00	6.72	22.02	15.22	3.53
Arthropods	144.72	164.83	93.99	42.08	137.98	95.36	22.11
Seeds (granivore)[1]	23.09	5.84	3.33	1.49	4.89	3.38	0.78

[1] Seeds presented separately for dose – based EECs due to difference in food intake of granivores compared with herbivores and insectivores. This difference reflects the difference in the assumed mass fraction of water in their diets.

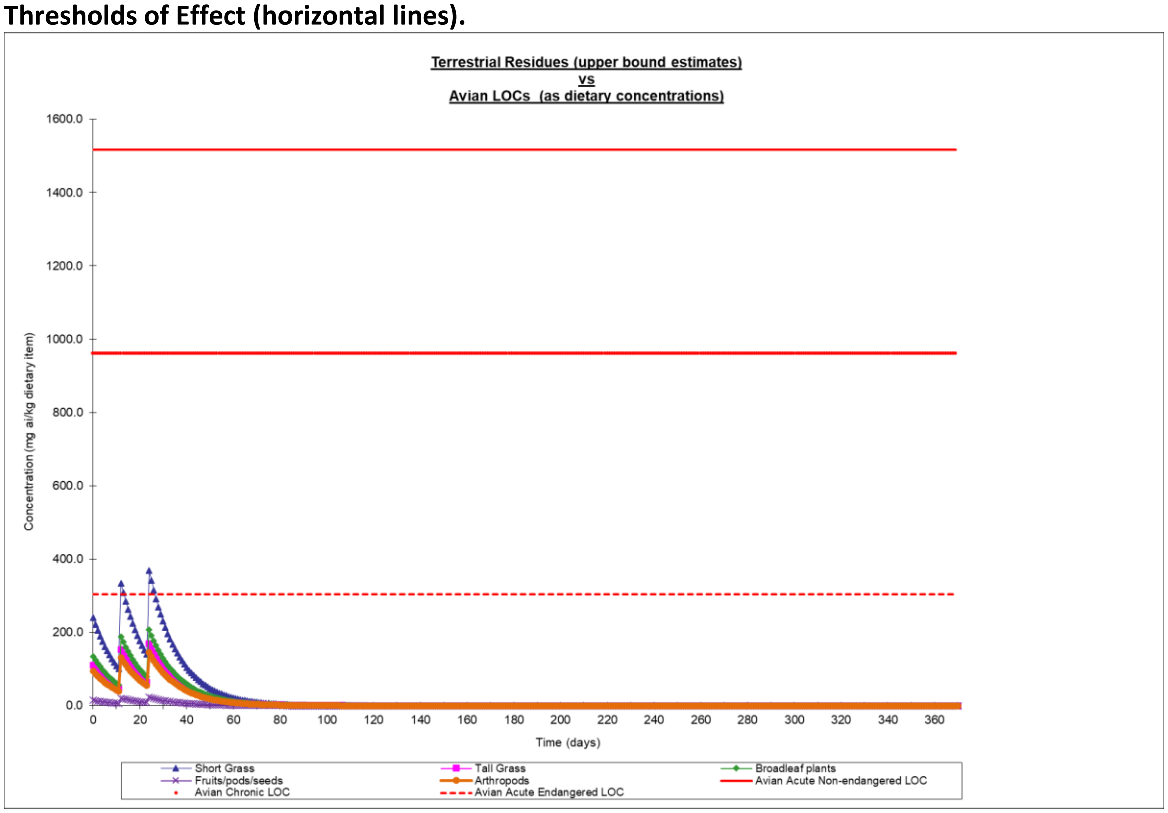
EPA generated RQ values based on the upper bound EECs shown in **Table 2-19** and toxicity values described in **Section 2.3.2**. For acute exposures to birds, dose-based RQs range from <0.01 to 2.7 based on upper-bound values and exceed the non-listed species LOC of 0.5 for small birds feeding on all dietary items except for fruits/pods/seeds and granivores, and for medium birds feeding on exposed short grass, tall grass, broadleaf plants and arthropods (**Table 2-19**). **Figure 2-7** shows that following 2 applications, the acute avian listed species LOC is exceeded by the shortgrass EECs and these continue to increase with the third application. Acute dietary-based RQs for birds were not calculated because there was not an available definitive endpoint. The EECs were orders of magnitude below the maximum tested dietary concentration, and no mortality was observed in the studies. Therefore, there are no acute dietary-based risks to birds; however, risk could not be precluded for acute dose-based exposure. For chronic exposures for birds, dietary-based RQs ranged from 0.02-0.38 and did not exceed the chronic LOC (1.0) for any dietary item. Therefore, EPA determined there were no chronic dietary-based risks of concern for birds.

Table 2-19. Acute and Chronic RQ Values for Birds, Reptiles, and Terrestrial-Phase Amphibians Exposed to 2,4-D Residues from the Use of Enlist Products on Enlist-Tolerant Crops (T-REX v. 1.5.2, Upper Bound Kenaga)

Food Type	Acute Dose-Based RQ LD_{50} = 218.7 mg a.e./kg-bw			Acute Dietary-Based RQ LC_{50} >10,000 mg a.e./kg-diet	Chronic Dietary RQ NOAEC = 962 mg a.e./kg-diet
	Small (20 g)	Medium (100 g)	Large (1000 g)		
Soybean/Corn/Cotton (3 apps x 1 lb a.e./A, 12-Day Interval)					
Herbivores/Insectivores					
Short grass	**2.67**	**1.20**	***0.38***	NC	0.38
Tall grass	**1.22**	**0.55**	***0.17***	NC	0.18
Broadleaf plants	**1.50**	**0.67**	***0.21***	NC	0.22
Fruits/pods	***0.17***	0.07	0.02	NC	0.02
Arthropods	**1.05**	***0.47***	***0.15***	NC	0.15
Granivores					
Seeds[1]	0.04	0.02	0.01	NC	0.02

Bolded values exceed the LOC for acute risk to non-listed species of 0.5 or the chronic risk LOC of 1.0.
Bold italicized numbers exceed the acute risk LOC for listed species (RQ > 0.1). The endpoints listed in the table are the endpoint used to calculate the RQ.
[1] Seeds presented separately for dose – based RQs due to difference in food intake of granivores compared with herbivores and insectivores. This difference reflects the difference in the assumed mass fraction of water in their diets.
N/A – dietary risk quotients for granivores is not estimated
NC – RQs are not calculated for non-definitive toxicity endpoints

Figure 2-7. Upper Bound TREX Avian EECs (plotted curves) and their Relationship to Acute and Chronic Thresholds of Effect (horizontal lines).



For mammals, the acute RQs from exposure to 2,4-D exceed the Agency's listed species LOCs (0.1) with acute dose-based RQs ranging from <0.01 to 0.36 (**Table 2-20**). Additionally, the dose-based chronic RQs exceed the Agency's LOCs for chronic risk (chronic dietary-based RQs range from 0.08 to 2.9) with all but the fruits/pods/seeds dietary items exceeding for small and medium size classes, and shortgrass exceeding for large mammals. Chronic dietary-based RQs do not exceed the Agency LOC for chronic risk from 2,4-D (RQs range from 0.02 to 0.34; **Table 2-21**). **Figure 2-8** shows that following 1 application, the chronic mammalian listed species LOC is exceeded by the shortgrass EECs and these continue to increase with the third application.

Table 2-20. Acute RQ Values for Mammals Exposed to 2,4-D Residues from the Use of Enlist Products (T-REX v. 1.5.2, Upper Bound Kenaga)

Food Type	Acute Dose-Based RQ LD_{50} = 441 mg a.e./kg-bw		
	Small (15 g)	Medium (35 g)	Large (1000 g)
Soybean/Corn/Cotton (3 apps x 1 lb a.e./A, 12-day interval)			
Herbivores/Insectivores			
Short grass	***0.36***	***0.31***	***0.17***
Tall grass	***0.17***	***0.14***	0.08
Broadleaf plants	***0.20***	***0.17***	0.09
Fruits/pods	0.02	0.02	0.01
Arthropods	***0.14***	***0.12***	0.07
Granivores			
Seeds[1]	0.01	<0.01	<0.01

Bolded values exceed the LOC for acute risk to non-listed species of 0.5 or the chronic risk LOC of 1.0.
Bold italicized numbers exceed the acute risk LOC for listed species (RQ > 0.1). The endpoints listed in the table are the endpoint used to calculate the RQ.
[1] Seeds presented separately for dose – based RQs due to difference in food intake of granivores compared with herbivores and insectivores. This difference reflects the difference in the assumed mass fraction of water in their diets.

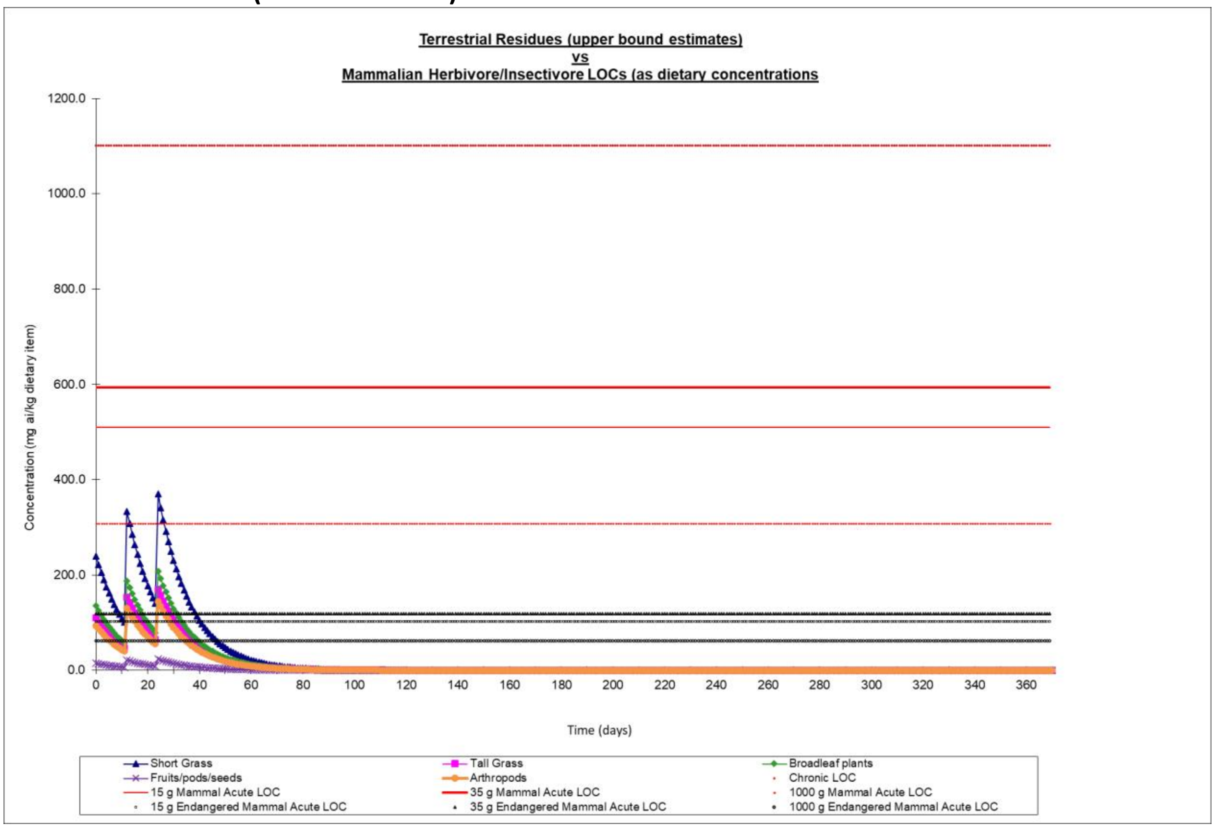
Table 2-21. Chronic RQ Values for Mammals Exposed to 2,4-D Residues from the Use of Enlist Products (T-REX v. 1.5.2, Upper Bound Kenaga)

Food Type	Chronic Dose-Based RQ NOAEL = 55 mg a.e./kg-bw			Chronic Dietary-Based RQ NOAEC = 1100 mg a.e./kg-diet
	Small (15 g)	Medium (35 g)	Large (1000 g)	
Soybean/Corn/Cotton (3 apps x 1 lb a.e./A, 12-day interval)				
Herbivores/Insectivores				
Short grass	**2.91**	**2.49**	**1.33**	0.34
Tall grass	**1.34**	**1.14**	0.61	0.15
Broadleaf plants	**1.64**	**1.40**	0.75	0.19
Fruits/pods	0.18	0.16	0.08	0.02
Arthropods	**1.14**	0.98	0.52	0.13
Granivores				
Seeds[1]	0.04	0.03	0.02	0.02

Chronic LOC is 1.0. The endpoints listed in the table are the endpoint used to calculate the RQ.
Chronic diet concentration NOAEC is based upon NOAEL and the daily diet consumption of laboratory rat, estimated in T-REX.
Bolded values exceed the LOC for acute risk to non-listed species of 0.5 or the chronic risk LOC of 1.0.

N/A – dietary risk quotients for granivores is not estimated
[1] Seeds presented separately for dose – based RQs due to difference in food intake of granivores compared with herbivores and insectivores. This difference reflects the difference in the assumed mass fraction of water in their diets.

Figure 2-8. Upper Bound TREX Avian EECs (plotted curves) and their Relationship to Acute and Chronic Thresholds of Effect (horizontal lines).



2.4.2.2. Spatial Extent of Off-Field Risks from Spray Drift of Residues

For any terrestrial vertebrate results where LOCs were exceeded through route of off-site spray deposition, off-field risks are calculated using the empirical spray drift data provided in MRID 48844001. EPA found that spray drift was a biphasic decay function with distance, where the predominant reduction in deposition occurred 0-25 ft from the downwind edge of the application zone. As noted above, the Enlist labels include a 30 ft infield buffer off set for areas down wind of an application. At 30 ft from the edge of the field, the deposition is 0.167% of the application rate. When an application rate of 0.00167 lb a.e./A is entered into T-REX, all RQs are below the non-listed and listed species LOCs for acute and chronic exposures. Therefore, there are no off field risks (direct effects) to birds, terrestrial-phase amphibians, reptiles and mammals from spray drift of 2,4-D onto non-target habitats.

2.4.2.3. Spatial Extent of Off-Field Risks to Birds and Mammals from Volatility Based Exposure

EPA used the Screening Tool for Inhalation Risk (STIR) model to consider if animals exposed to vapor phase 2,4-D may be at risk. EPA concluded that exposure through inhalation was not likely significant (**Appendix J**) and therefore not of concern.

2.4.3. Honey Bee Risk Characterization

The uses being assessed for 2,4-D include Enlist corn, soybean, and cotton crops, which produce pollen and/or nectar attractive to bee species (USDA, 2017). Bees may also consume both pollen and nectar from 2,4-D-exposed flowering weed species. The pollen and nectar may contain 2,4-D residues either from direct spray or resulting from systemic uptake of 2,4-D residues. Bees (both *Apis* and non-*Apis*) may potentially be exposed on or off the field to direct sprays of 2,4-D to Enlist corn, cotton or soybean plants from subsequent deposition on attractive floral resources.

2.4.3.1. Honey Bee Tier I Exposure Estimates

EPA estimated contact and dietary exposure separately using different approaches specific to different plant exposure assumptions (e.g., direct spray or systemic transport from soil). The Bee-REX model (Version 1.0) calculates default (*i.e*., high end, yet reasonably conservative) EECs for contact and dietary routes of exposure for foliar, soil, and seed treatment applications. Refer to **Appendix J** for a sample output from Bee-REX for 2,4-D. Additional information on bee-related exposure estimates, and the calculation of risk estimates in Bee-REX, can be found in the *Guidance for Assessing Pesticide Risks to Bees* (USEPA *et al.*, 2014).

2.4.3.2. Tier I Risk Estimation (Contact Exposure)

2.4.3.2.1. On-Field Risk

Since bees are potentially exposed to 2,4-D through use on Enlist corn, soybean, and cotton plants, the next step in the risk assessment process was to conduct a Tier 1 risk assessment. By design, the Tier 1 assessment begins with (high-end) model-generated (foliar and soil treatments) or default (seed treatments) estimates of exposure via contact and oral routes. For contact exposure, only the adult (forager and drones) life stage is considered since this is the relevant caste of honey bees (*i.e*., since other bees are in-hive, the presumption is that they would not be subject to contact exposure). Furthermore, toxicity testing protocols have only been developed for acute exposures. Effects are defined by laboratory exposures to groups of individual bees (which serve as surrogates for solitary non-*Apis* bees and individual social non-*Apis* bees).

On the basis of acute contact exposure to adult honey bees, RQs could not be calculated because a definitive honey bee endpoint was not available. The highest EECs are more than an order of magnitude lower than the highest tested concentration (66 μg a.e./bee). Therefore, EPA determined there is no acute risk to bees from 2,4-D exposure following the use of the Enlist One and Enlist Duo products (**Table 2-22**).

Table 2-22. Default Tier I Adult, Acute Contact Risk Quotients for Honey bees Foraging on Enlist Crops from Bee-REX (v1.0)

Use Pattern	Bee Attractiveness[3]	Max. Single Application Rate	Dose (μg a.e./bee per 1 lb a.e./A)	Contact Dose (μg a.e./bee)	Acute RQ[1,2]
Soybean	Y (nectar & pollen)	1.0 lb a.e./A	2.7	2.7	NC
Cotton	Y (nectar only)	1.0 lb a.e./A	2.7	2.7	NC
Corn	Y (pollen only)	1.0 lb a.i./A	2.7	2.7	NC

[1] Based on a 48-h acute contact LD_{50} of > 66 µg a.i./bee (MRID 00036935). Acute LOC = 0.4.

[2] NC = not calculated because of non-definitive endpoint

[3] Y indicates: Yes

2.4.3.3. Tier I Risk Estimation (Oral Exposure)

2.4.3.3.1. On-Field Risk

For oral exposure, the Tier I assessment considered the caste of bees with the greatest oral exposure (foraging adults). If risks are identified, then other factors can be considered for refining the Tier I risk estimates. These factors include other castes of bees and any available information on empirical residues in pollen and nectar applicable to the crops of interest. As noted in **Section 2.4.2.3**, submitted acute oral data for adult honey bees reported no mortality (Contact LD_{50} > 66 µg a.e./bee; Oral LD_{50} > 62.2 µg a.e./bee) and EECs (Acute Contact EEC = 2.7 µg a.e./bee; Acute Oral EEC = 32 µg a.e./bee) are well below those highest tested concentrations. Therefore, EPA found that there are no acute risks of concern for adult bees.

The attractiveness of these floral resources to honey bees differs across the three crops. As such, different assumptions were made regarding the potential exposure through pollen and nectar. These assumptions included limiting EECs to just those dietary items that the adult bee would forage upon and bring back to the hive.

Soybean provides both a pollen and nectar route of exposure for adult and larval bees and cotton provides a primarily nectar route of exposure for adult and larval bees. Compared to the EECs, there are likely no acute risks to adult bees. The larval acute LOCs for non-listed and listed bees are exceeded for larval bees for both crops (RQs = 2). Chronic risks were identified for adult bees (max chronic RQs of 6.3 for both crops, which are above the chronic LOC of 1.0). EPA determined there is chronic oral risk for larval bees for the use on soybeans and cotton as the RQs (>29.6 and >28.8) are above the chronic LOC of 1.0. The RQs are represented as ">" because the endpoint used to derive the RQ was a LOAEL, and no NOAEL is available. The alternative approaches to estimating the endpoint each result in exceedances of the LOC as well (**Table 2-23**).

With corn, pollen is the primary route of exposure for bees. The applications under the current label are unlikely to be applied during tassel and pollen shedding; however, the estimates in **Table 2-23** provide the EECs and RQs assuming spray deposition on the pollen. Compared to the EECs, there are likely no acute risks to adult or larval bees. The analysis also resulted in chronic RQs for adult bees, and larval acute RQs below the LOCs. For chronic risks to larvae, all three approaches to address the uncertainty in the toxicity endpoint result in exceedances of the LOC (RQs range from >0.9 to 10.2).

In summary, acute risks are considered low for adult bees for all three crops. For soybean and cotton, there are acute risks for larvae and chronic risks for adult and larval bees. For corn, there are chronic risks for larval bees.

Table 2-23. Tier 1 Oral Risk Quotients for Adult Nectar Forager and Larval Worker Honey Bees from Bee-REX (v1.0) following foliar application of 2,4-D.

Use Pattern	Max. Single Appl. Rate	Bee Caste/Task	Oral Dose (μg a.i./bee)	Acute Oral RQ[1,3]	Chronic Oral RQ[2,3]
Soybean	1.0	Adult nectar forager	32	NC	**6.3**
		Larval worker	13.6	**2.3**	**LOAEL based >29.6 LD_{15} based = 349**
Cotton[4]	1.0	Adult nectar forager	32	NC	**6.3**
		Larval worker	13.2	**2.2**	**LOAEL based >28.8 LD_{15} based = 338**
Corn[5]	1.0	Adult nectar forager	0.0045	NC	0.21
		Larval worker	0.396	0.07	**LOAEL based >0.9 LD_{15} based = 10.2**

[1] Adult acute oral RQs were not calculated (NC) due to lack of a definitive LD_{50}.
[2] **Bolded** RQ value exceeds (or potentially exceeds) the non-listed acute risk LOC of 0.4, Listed acute LOC of 0.05, or chronic LOC of 1.0.
[3] Based on a 10-d chronic NOAEL of 5.3 µg a.e./bee/d for adults (MRID 50751201); an 8-day LD_{50} of 6 µg a.e./bee for larvae (acute oral; MRID 50282701); and a 22-d chronic NOAEL of <0.459 µg a.e./bee/d; 28-day LD_{15} = 0.039 µg a.e./larvae (MRID 50751301).
[4] Although the application rate is the same, larval exposures in cotton are very slightly smaller due to the lack of attractive pollen to adult bees (pollen is an insignificant portion of adult forager diet).
[5] Although the application rate is the same, adult and larval exposures in corn are significantly smaller due to the lack of attractive nectar to adult foragers).

Refinement of the Exposure Assumptions for Honey Bees -Systemic Transport

Because there are pre-bloom applications in all three crops, EPA conducted another evaluation to consider the potential systemic transport and resulting contamination of pollen and nectar. This refinement relied upon the BeeREX model, the Log Kow (2.14) and the Koc (72). **Table 2-24** provides the EECs ("Oral dose") assuming the different dietary items for each crop. For all three uses, risks are low for acute and chronic exposures to adult bees. Risks are also low for adult and larval bees from corn. For soybean and cotton, there is some evidence that there may be risk to larval bees; however, since only one of three lines of evidence suggest a concern, there is uncertainty.

Table 2-24. Tier 1 Refined – Systemic Transport Assumption: Oral Risk Quotients for Adult Nectar Forager and Larval Worker Honey Bees from Bee-REX (v1.0)

Use Pattern	Max. Single Appl. Rate	Bee Caste/Task	Oral Dose (µg a.i./bee)	Acute Oral RQ[1,3]	Chronic Oral RQ[2,3]
Soybean	1.0	Adult nectar forager	0.24	NC	<0.1
		Larval worker	0.10	0.02	LOAEL based >0.2 **LD_{15} based = 2.6**
Cotton[4]	1.0	Adult nectar forager	0.24	NC	<0.1
		Larval worker	0.10	0.02	LOAEL based >0.2 **LD_{15} based = 2.6**
Corn[5]	1.0	Adult nectar forager	0.04	NC	<0.1
		Larval worker	0.003	<0.01	LOAEL based >0.1 LD_{15} based <0.1

[1]Adult acute oral RQs were not calculated (NC) due to lack of a definitive LD_{50}.
[2] **Bolded** RQ value exceeds (or potentially exceeds) the non-listed acute risk LOC of 0.4, Listed acute LOC of 0.05, or chronic LOC of 1.0.
[3] Based on a 10-d chronic NOAEL of 5.3 µg a.e./bee/d for adults (MRID 50751201); an 8-day LD_{50} of 6 µg a.e./bee for larvae (acute oral; MRID 50282701); and a 22-d chronic NOAEL of <0.459 µg a.e./bee/d; 28-day LD_{15} = 0.039 µg a.e./larvae (MRID 50751301).
[4] Although the application rate is the same, larval exposures in cotton are very slightly smaller due to the lack of attractive pollen to adult bees (pollen is an insignificant portion of adult forager diet).
[5] Although the application rate is the same, adult and larval exposures in corn are significantly smaller due to the lack of attractive nectar to adult foragers).

As indicated in the guidance for BeeREX, the model used to estimate pesticide concentrations in pollen and nectar from soil treated plants is based on a dataset of non-ionic organic chemicals (referred to as "the Briggs model"; Briggs *et al.* 1982 and 1983; with modifications by Ryan *et al.* 1988). The Briggs model is designed to estimate pesticide concentrations in plant shoots (based on studies with barley); the concentrations in plant shoots are assumed to be representative of concentrations in pollen and nectar. When assessing exposure and associated risks to bees from soil treatments of ionic chemicals, there is uncertainty associated with the magnitude of exposure estimated by the BeeREX model. In a separate set of experiments, Briggs *et al.* (1987) investigated uptake of several ionic organic chemicals (including 2,4-D and 3,5-D) in barley. Results indicated that as pH of water (soil pore water) decreased, ionic chemical concentrations in shoots increased. The optimal soil pH for most plants ranges 5.5-7 (https://www.cropnutrition.com/efu-soil-ph). When considering the data presented by Briggs *et al.* (1987) for ionic chemicals at pH 5 and 7, concentrations of 2,4-D in shoots were 1.3 to 22x higher in solutions with pH 5 compared to pH 7. The ratios of concentrations in stems to water of the ionic chemicals at pH 5 are more similar to non-ionic chemicals with similar Log Kow values (from 1982 and 1983[11]). Since pH 5 is lower than the optimal soil pH for plant growth, EECs generated using BeeREX for soil treatments are expected to be conservative. In cases where soil pH is higher than 5, EECs may overpredict exposure; for example, at pH 7, available data for 2,4-D indicate that concentrations are an order of magnitude lower than predicted by BeeREX.

[11] Note: the pH of the tests with non-ionic organic chemicals was not provided by Briggs *et al.* (1982 or 1983).

Refinement of the exposure assumptions for honey bees – Empirical Residue Data

As part of the submission for registration, Corteva submitted three studies (MRIDs 51557401, 55157402 and 51241602) containing 2,4-D residue data from Enlist One applications to corn, cotton and soybeans. For each crop, residues were collected from three separate locations. Corn field trials were in Iowa, Missouri, and South Dakota. Cotton field trials were in Missouri, Texas, and California. Soybean trials were in North Carolina, Indiana, and Missouri. Three foliar applications were made at the pre-emergence stage, approximately two weeks prior to full bloom, and at full bloom to each crop at a nominal application of Enlist One rate ranged from 0.95 lb to 1.0 lb a.e./A. Various plant matrices (e.g., pollen, nectar, leaves) for corn, cotton and soybean were collected at five different sampling intervals from each trial to analyze for residue concentrations. **Table 2-25** provides the maximum measured and maximum average residues in nectar and pollen for cotton and soybean from each of the three locations.

Based upon the comparison of the chronic larval LOAEL-based endpoint (0.459 ug a.e./larvae), the residues in pollen and nectar (maximum and mean concentrations) result in exceedances of the LOCs (**Table 2-25**). These results support the conclusion that applications to these crops at the proposed rates likely result in risk of mortality for individual bees and other terrestrial invertebrates.

Table 2-25. Maximum Measured and Maximum Mean Average Residues in Nectar and Pollen for Soybean and Cotton and Pollen for Corn from Tested Locations.

Study Site	Matrix	Maximum (mg/kg)	Maximum Mean (mg/kg)	Adult Chronic BeeREX estimated RQ[1,4]		Larval Acute BeeREX estimated RQ [1,4]		Larval Chronic BeeREX estimated RQ (NOAEL Based) [1,4]	
				Maximum Residue Based RQ	Maximum Mean Residue RQ	Maximum Residue Based RQ	Maximum Mean Residue RQ	Maximum Residue Based RQ	Maximum Mean Residue RQ
MRID 51557401 (Soybean)									
Mebane, NC	Nectar	1.1	0.84	0.06	0.05	0.02	0.02	>0.3	>0.2
West Point, IN	Nectar	15	5.1	0.8	0.3	**0.33**	**0.13**	**>4.4**	**>1.8**
Renick, MO	Nectar	11	11	0.6	0.6	**0.26**	**0.26**	**>3.4**	**>3.4**
Mebane, NC	Pollen	1.9	1.7	NA[2]		NA[2]		NA[2]	
West Point, IN	Pollen	54	54						
Renick, MO	Pollen	63	63						
MRID 51241601 (Cotton)									
Fisk, MO	Nectar	44.6	28.2	**2.5**	**1.6**	**0.89**	**0.56**	**>11.7**	**>7.4**
Levelland, TX	Nectar	15.8	9.4	0.9	0.5	**0.32**	**0.19**	**>4.1**	**>2.5**
Sanger, CA	Nectar	6.9	4	0.4	0.2	**0.14**	**0.08**	**>1.8**	**>1.1**
Fisk, MO	Pollen	5.4	4.6	NA[3]		NA[3]		NA[3]	
Levelland, TX	Pollen	38.3	21						
Sanger, CA	Pollen	11.4	5.1						
MRID 51557402 (Corn)									
Richland, IA	Pollen	0.033	0.025	<0.01		<0.01		<0.01	
Mobery, MO	Pollen	0.063	0.031						
Aurora, SD	Pollen	0.410	0.029						

[1] **Bolded** RQ value exceeds (or potentially exceeds) the non-listed acute risk LOC of 0.4, Listed acute LOC of 0.05, or chronic LOC of 1.0.

[2] RQs for Soybean assume measured residues in both pollen and nectar for each region and residue estimate.

[3] cotton pollen is an insignificant portion of adult forager diet so residues in cotton pollen were not combined with nectar residues to calculate RQs.

[4] Based on a 10-d chronic NOAEL of 5.3 µg a.e./bee/d for adults (MRID 50751201); an 8-day LD_{50} of 6 µg a.e./bee for larvae (acute oral; MRID 50282701); and a 22-d chronic NOAEL of <0.459 µg a.e./bee/d; 28-day LD_{15} = 0.039 µg a.e./larvae (MRID 50751301).

Bold RQs exceed the LOCs

EPA relies on honey bees as a surrogate for other bee pollinators. Data on individual honey bees provides information appropriate for solitary bee species. Previous analysis (USEPA *et al*., 2014) of food consumption rates (of pollen and nectar) for individuals of several species of bees suggests that honey bees are similar or protective of other species. Therefore, honey bees represent an appropriate surrogate for assessing individual level risks to other nectar and pollen-foraging species of bees.

2.4.3.3.2. Off-Field Risk

In addition to bees foraging on the treated field, bees may also be foraging in fields adjacent to the treated fields. As noted above, the Enlist labels include a 30 ft infield buffer off set for areas down wind of an application. At 30 ft from the edge of the field, the deposition is 0.167% of the application rate. When an application rate of 0.00167 lb a.e./A is entered into Bee-REX, all RQs are below the LOCs for acute and chronic exposures. Therefore, there are no off field risks to bees from spray drift of Enlist products onto non-target habitats.

2.4.4. Other Terrestrial Invertebrates

2.4.4.1. Use of Honey Bee Toxicity Data as a Surrogate for Non-Apis Terrestrial Invertebrate Species

A search of ECOTOX[12] was conducted on 8/21/2021, and no new reliable terrestrial invertebrate toxicity data were identified in the search. The registrant for Enlist One and Enlist Duo submitted several open literature studies reporting results of studies on lepidopterans. EPA reviewed these articles and determined that none of them changed the exposure or toxicity assumptions based on the honey bee data (see **Section 2.3.2.3** for details). Therefore, EPA relied on honey bee effects data for this evaluation of impact to other terrestrial invertebrate species (*e.g.,* beetles, butterflies, etc.) and is based on the surrogate species approach; whereby, the effects endpoints established for honey bees are used to represent the sensitivity of other invertebrate species. To use this data to evaluate risks to other terrestrial invertebrates, EPA converted the dose-based endpoints to concentration-based endpoints that it then compared to potential exposures based on T-REX exposures for arthropods and potential dietary items. As the acute data for honey bees were non-definitive (*i.e.,* no mortalities were observed at the highest tested doses), EPA considered the much more sensitive chronic honey bee endpoints for this comparison (**Section 2.3.2.3**). Therefore, the chronic analysis presented is protective of any potential acute effects.

2.4.4.2. Monarch Butterfly Risk Characterization

Adult monarchs are generalist nectar feeders that visit a variety of flowering plants. For reproduction, however, monarch adults lay their eggs on a suite of species in the genus *Asclepius*, collectively known as milkweeds, and a related species, *Cynanchum laeve* (Pocius et al., 2017). The most abundant of the

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[12] The ECOTOXicology Knowledgebase (ECOTOX) https://cfpub.epa.gov/ecotox/help.cfm

monarch host plants in the U.S. is *Asclepius syriaca* (common milkweed), which grows in disturbed areas including agricultural fields, field edges, roadsides, and pastures. Milkweeds are needed for the successful development of monarch larvae (caterpillars). Potential effects to monarchs are considered in on-field and off-field areas through dietary exposures to larvae and adults consuming milkweed leaves and nectar, respectively. Since no toxicity data for adult or larval monarchs are available, the honey bee toxicity data are relied upon as a surrogate for the risk estimation. The potential for indirect effects to monarchs through the impact of 2,4-D on milkweed plants is discussed in **Section 2.4.4.2**.

2.4.4.2.1. On-Field Risks to Monarch Butterflies

Milkweed is listed as a target weed on the Enlist Duo product label. According to the label, direct applications of the product to "suppress" milkweed are recommended when milkweed plants are in the early bud stage of growth. It is also possible that milkweed may be present on the field when Enlist One or Enlist Duo are applied to control non-milkweed pests. In terms of potential monarch exposure, monarch larvae may be present on milkweed plants at the time of Enlist and Enlist Duo application, which will result in on-field monarch exposure (see **Section 2.5.3.1**). Therefore, EPA conducted a risk assessment for monarch larvae that are actively feeding on "on-field" milkweed. There could also be an exposure to adults that are laying eggs or foraging on nectar from plants located on field.

EFED conducted an exposure and risk assessment for direct spray of 2,4-D onto monarchs located on the treated field. Since larvae (caterpillars) consume milkweed, the broadleaf plant EEC from T-REX (i.e., 135 mg a.e./kg-food from a single application) was used to represent exposure to larvae. For adults, which consume nectar, the BeeREX EEC for nectar (i.e., 110 mg a.e./kg-food) was used. The best available toxicity data for terrestrial invertebrates is represented by the honey bee data described in **Section 2.3.2.3**. Since 2,4-D is practically non-toxic to adult honey bees on an acute exposure basis, acute exposures to adult monarchs is assumed to not be of concern. For acute exposure to larvae, the EEC of 110 mg a.e./kg-diet is less than the LC_{50} (156 mg a.e./kg-diet). For chronic exposures to larvae, the chronic endpoints (<11.9 and 2.91 mg a.e./kg-diet) are exceeded by the EEC of 110 mg a.e./kg-diet. For adults, the EEC for nectar is below the NOAEC of 276 mg ai/kg-diet. Therefore, EPA concluded that 2,4-D poses a chronic risk to monarch larvae located on milkweed of corn, cotton and soybean fields. Risk is considered low for adult monarchs.

2.4.4.2.2. Off-Field Risks to Monarch Butterflies

Monarch larvae may also be located on milkweed adjacent to the treated fields. As noted above, the Enlist labels include a 30 ft infield buffer off set for areas down wind of an application. At 30 ft from the edge of the field, the deposition is 0.167% of the application rate. When an application rate of 0.00167 lb a.e./A is entered into T-REX, the broadleaf plant EEC is 0.23 mg a.e./kg-food. This is well below the chronic toxicity endpoints for larvae (<11.9 and 2.91 mg a.e./kg-diet). Therefore, there are no off-field risks to monarchs from spray drift of 2,4-D onto non-target habitats.

2.4.4.3. Other Non-Apis Terrestrial Invertebrates Risk Characterization

2.4.4.3.1. On-Field Risk Evaluation for Other Non-Apis Invertebrates

EFED conducted an exposure and risk assessment for terrestrial invertebrates following direct spray of Enlist One and Enlist Duo located on the treated field. Since terrestrial invertebrates may consume a wide variety of diet items, this assessment considered the consumption of plant parts (e.g., seeds, fruits, leaves), arthropods, and bird or mammal carrion. EPA estimated exposure with T-REX (**Table 2-26**). Carrion dietary EECs were based upon shortgrass estimated exposures for mammals and birds (352.3 mg a.e./kg and 420.8 mg a.e./kg respectively) and considered the feeding rate, assimilation rate and hourly depuration rate for each (see **Appendix G** for details). For adults, the best available toxicity data for terrestrial invertebrates is represented by the honey bee data described in **Section 2.3.2.3**. Since 2,4-D is practically non-toxic to adult honey bees on an acute exposure basis, acute exposures to adult invertebrates is assumed to not be of concern. For acute risks to larvae, the EECs for most dietary items exceed the LC_{50} of 156 mg a.e./larva following 3 applications, but only for shortgrass following a single application. For chronic exposure to adults, the EECs for short grass after 3 applications are above the NOAEC of 276 mg ai/kg-diet, all other dietary items have EECs lower than the NOAEC and are also below the NOAEC following a single application. For chronic exposures to larvae, the chronic endpoints (11.9 and 2.91 mg a.e./kg-diet) are exceeded by all dietary EECs assuming either 3 applications or a single application. Therefore, EPA concluded that 2,4-D poses a risk to larval terrestrial invertebrates located on Enlist corn, cotton and soybean fields. The results of this analysis suggest that there may be exceedance of the adult chronic endpoint. However, these are considered to be limited and of only marginal exceedance. Therefore, chronic risk to non-apis adult terrestrial invertebrates is considered low.

Table 2-26. Summary of Dietary (mg a.e./kg-diet) EECs for 2,4-D as Food Residues for Terrestrial Invertebrates and Risk Estimations from Labeled Uses of Enlist or Enlist Duo on 2,4-D-Tolerant Crops

Food Type	Dietary-Based EEC (mg/kg-diet)	Acute Larval Invertebrate Ratios 156 mg a.i./kg-diet	Chronic Adult Invertebrate Ratios[3] 276 mg a.i./kg-diet	Chronic Larval Invertebrate Ratios[3]	
				11.9 mg a.e./kg-diet	2.91 mg a.e./kg-diet
Soy, Cotton or Corn max annual ground application (3x 1 lb a.e./A, 12-d interval)					
Short grass[1]	369.51	**2.37**	**1.34**	**31.05**	**126.98**
Tall grass[1]	169.36	**1.09**	0.61	**14.23**	**58.20**
Broadleaf plants[1]	207.85	**1.33**	0.75	**17.47**	**71.43**
Fruits/pods[1]	23.09	0.15	0.08	**1.94**	**7.93**
Arthropods[1]	144.72	0.93	0.52	**12.16**	**49.73**
Seeds (granivore) [1]	23.09	0.15	0.08	**1.94**	**7.93**
Avian Carrion[2]	234.53	**1.50**	0.85	**19.71**	**80.59**
Mammalian Carrion[2]	162.36	**1.04**	0.59	**13.64**	**55.79**
Soy, Cotton or Corn max annual ground application (1 application of 1 lb a.e./A)					
Short grass[1]	240.00	**1.54**	0.87	**20.17**	**82.47**
Tall grass[1]	110.00	0.71	0.40	**9.24**	**37.80**
Broadleaf plants[1]	135.00	0.87	0.49	**11.34**	**46.39**
Fruits/pods[1]	15.00	0.10	0.05	**1.26**	**5.15**
Arthropods[1]	94.00	0.60	0.34	**7.90**	**32.30**
Seeds (granivore) [1]	15.00	0.10	0.05	**1.26**	**5.15**
Avian Carrion[2]	152.33	0.98	0.55	**12.80**	**52.35**
Mammalian Carrion[2]	105.45	0.68	0.38	**8.86**	**36.24**

[1] EEC estimated with T-REX v.1.5.2, Upper-Bound Kenaga, See section 2.4.1 for model details

[2] Carcass dietary concentrations estimated with a depuration model that accounts for assimilation rate and body burden within the carrion. (See **Appendix G** for details).

[3] Ratios > 1 indicate that the dietary EEC is greater than the endpoint and there is a potential for risk.

2.4.4.3.2. Off-Field Risks

Terrestrial invertebrates may also be located in habitats adjacent to the treated fields. As noted above, the Enlist labels include a 30 ft in-field buffer off set for areas down wind of an application. At 30 ft from the edge of the field, the deposition is 0.167% of the application rate. When an application rate of 0.00167 lb a.e./A is entered into T-REX, the broadleaf plant EEC is 0.23 mg a.e./kg-food. This is well below the chronic toxicity endpoints for adult (276 mg a.e./kg-diet) and larvae (11.9 and 2.91 mg a.e./kg-diet). Therefore, as a result of the spray drift controls on the label, there are no off-field risks to terrestrial invertebrates from 2,4-D following spray drift of Enlist Duo or Enlist One onto non-target habitats.

2.5. Plant Risk Assessment

2.5.1. Summary of the Risk Assessment Approach for 2,4-D

EPA relied upon the Plant Assessment Tool (PAT)[13] for estimating environmental exposure. PAT is a mechanistic model that incorporates fate (e.g., degradation) and transport (e.g., runoff) data that are typically available for conventional pesticides, to estimate pesticide concentrations in terrestrial, wetland, and aquatic plant habitats. PAT was developed to enable more efficient evaluations of exposure than have traditionally been carried out through post processing of PRZM/PWC/and VVWM output files (as was conducted for previous Enlist evaluations, e.g., USEPA 2016b), as well as to bring runoff exposures into consistency with the runoff approaches and assumptions considered for predicting aquatic EECs (e.g., standard pond EECs). For terrestrial plants, runoff and erosion are initially modeled using PRZM and spray drift is modeled using the Enlist Duo (GF-2726, a premixed of 2,4-D choline salt and glyphosate dimethylamine salt) specific deposition curves (**Section 2.2.2**). These are imported into PAT and the model uses a mixing cell approach to represent water within the active root zone area of soil, and accounts for flow through the terrestrial plant exposure zone (TPEZ) caused by both treated field runoff and direct precipitation onto the TPEZ. Pesticide loss from the TPEZ occur from transport (*i.e.*, washout and infiltration below the active root zone) and degradation. EPA modeled wetlands using PRZM/VVWM, which are then processed in PAT to estimate aquatic (mass per volume of water) and terrestrial (mass per area) concentrations. EPA modeled exposure for aquatic plants using the PRZM/VVWM models and the standard farm pond conceptual model, which are imported into PAT to provide further characterizations of the exposure and potential risk.

2.5.2. Spray Drift Control Measures Impacts to Off-Field Plant Risk

EPA considered the mandatory spray drift control measures of wind speed and inversion limits, requirements for spray nozzles, and boom height limits by the distance to plant effect predictions presented in the off-field analysis with PAT. PAT allows for the input of specific drift deposition curves, and in the case of these products, the specific drift curves used for establishing the maximum drift thresholds for the product and any tank mix with the product. The requirements of the label describe the limitations of the tank mix combinations and tested spray nozzles to prevent the potential for off field risks to plants as a result of spray drift when combined with the 30-foot setback requirement such

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[13] Visit this website for more information on PAT: https://www.epa.gov/endangered-species/models-and-tools-national-level-listed-species-biological-evaluations-triazine#Aquatic

that the edge of the downwind field when plants are present adjacent to the field should result in a fraction of 0.00167 of the applied rate (0.00167 lbs a.e./A). This rate is below all of the estimated NOAEC concentrations in the tested species (lowest NOAEC = 0.0018 lbs a.e./A for cabbage); therefore, risks from spray drift alone are limited to the field.

2.5.3. Terrestrial Plant Exposure Zone (TPEZ): Runoff and Spray Drift from a Treated Field Deposited onto a Non-Target Terrestrial (Upland) Plant Area Next to the Field

The TPEZ is intended to represent a non-target terrestrial (non-inundated) plant community immediately adjacent to a treated field, which is exposed to pesticide via sheet flow[14] and spray drift from the treated field (based on the label restrictions spray drift is a minor contributor to total EECs). The TPEZ is defined as an area adjacent to the treated field with a length of 316 m (equal to the length of the edge of the treated field), and a width of 30 m. The width of the TPEZ represents the distance that overland surface flow can travel before sheet flow transitions to concentrated flow. The TPEZ assumes that runoff to an area immediately adjacent to the treated field is in the form of sheet flow that carries pesticides dissolved in water and sorbed to eroded sediment. Beyond this distance over which runoff moves as sheet flow, runoff is assumed to become concentrated into rivulets, gullies, *etc*., which is represented by evaluations with the Wetland Plant Exposure Zone (WPEZ) and Aquatic modules of PAT. For the TPEZ, EFED used a mixing cell approach in the model to represent water within the active root zone area of soil, which accounts for flow through the TPEZ caused by both treated field runoff and direct precipitation onto the TPEZ. Pesticide loss from the TPEZ occur from transport (*i.e*., washout and infiltration below the active root zone) and degradation. **Table 2-27** provides the resulting EECs and RQs for the most sensitive seedling emergence and vegetative vigor-based endpoints.

The submitted labels allow for 3 applications of 1 lb a.e./A, intended for application at pre-emergence, emergence and post-emergence crop growth stages. Based upon the EECs, all PWC scenarios result in exceedances of the vegetative vigor dicot non-listed and listed LOCs (RQ range 3.7-9.3 and 4.8-12.3, respectively). Although less sensitive than dicots, LOCs were exceeded for both non-listed and listed monocots (RQ range 0.8-1.9 and 1.2-3.1, respectively). As discussed in **Section 2.3.2.4**, the seedling emergence endpoints are less sensitive than vegetative vigor. There were non-listed LOC exceedances for seedling emergence for 4 scenarios (RQ range 0.5-1.3); and the listed species LOC was exceeded for several scenarios (RQ range 0.6-1.5).

EPA conducted additional analyses to evaluate the potential risks following a single application at each of the three proposed times. The results indicate that later applications had lower EECs but still exceeded the non-listed and listed LOCs for vegetative vigor (dicot RQ range 3.0-7.6 and 4-10.0, respectively). The lower EECs at the later growth stages is related to the curve number for post-emergent applications, which is lower than pre-emergent applications; therefore, there is more infiltration and less potential for runoff. Field management practices (e.g., no-till or conservation tillage) can improve infiltration on field and can be modeled by adjusting the curve number in PWC modeling. The results with regard to exceedances are mixed, such that some scenarios drop by about 50% while others are practically unchanged. Considering the results of these modeling adjustments, the risks are generally reduced for a no-till or conventional tillage practice versus traditional tilling, and reduced number of applications. Exceedances of the LOCs for dicots and monocots persist despite these

[14] A continuous film of water flowing over the soil surface which is not concentrated into channels.

adjustments. All evaluated application and characterization scenarios resulted in exceedances of the non-listed and/or listed LOCs.

Several incidents involving Enlist One or Enlist Duo were submitted to EPA. These incidents all reported significant damage (dozens to hundreds of acres) to cotton grown in Mississippi, Oklahoma and Texas. Limited information is available on these incidents, so the determination of route of exposure cannot be made. However, several incidents report hundreds of acres or >60% crop damage, suggesting that volatility-based exposure may have played a role. This route of potential exposure is discussed in **Section 2.5.7**.

In conclusion, the proposed 30-foot spray drift setback from sensitive vegetation is sufficient to prevent exposures that would result in exceedances of the most sensitive terrestrial plant endpoints, therefore, potential risks from spray drift are considered to be low. The results indicate that there are potential risks to non-listed and potential effects to listed terrestrial plants within 100 ft of Enlist corn, Enlist soybean or Enlist cotton fields as a result of surface runoff (i.e., sheet-flow). Beyond this distance from the edge of the field, the surface runoff is expected to transition into concentrated flow resulting in transport to wetland, riparian and aquatic habitats downgradient (USEPA, 2020c; PAT User Manual for ESA[15]).

[15] Available in the zip file "Plant Assessment Tool (PAT), v. 2.0 (ZIP)" at https://www.epa.gov/endangered-species/models-and-tools-national-level-listed-species-biological-evaluations-triazine#Terrestrial

Table 2-27. Terrestrial Plant Exposure Zone: RQs for Most Sensitive Terrestrial Plant Taxa and Associated EECs

Scenario Summary	EEC (lbs/A)	Seedling Emergence[1]				Vegetative Vigor[2]			
		Non-Listed Monocot RQ	Listed Monocot RQ	Non-Listed Dicot RQ	Listed Dicot RQ	Non-Listed Monocot RQ	Listed Monocot RQ	Non-Listed Dicot RQ	Listed Dicot RQ
Proposed label: 3 applications of 1 lb a.e./A at pre-emergence, emergence and post emergence, application interval 12 days.									
IAcornSTD	0.061	0.2	0.2	0.9	**1.0**	**1.3**	**2.0**	**6.1**	**8.1**
ILCornSTD	0.060	0.2	0.2	0.9	**1.0**	**1.2**	**2.0**	**6.0**	**8.0**
INCornSTD	0.054	0.2	0.2	0.8	0.9	**1.1**	**1.8**	**5.5**	**7.2**
KSCornSTD	0.060	0.2	0.2	0.9	**1.0**	**1.3**	**2.0**	**6.1**	**8.0**
MNCornSTD	0.047	0.2	0.2	0.7	0.8	**1.0**	**1.6**	**4.8**	**6.3**
MscornSTD	0.075	0.3	0.3	**1.1**	**1.3**	**1.6**	**2.5**	**7.6**	**10.0**
NCcornESTD	0.036	0.1	0.1	0.5	0.6	0.8	**1.2**	**3.7**	**4.8**
NECornStd	0.075	0.3	0.3	**1.1**	**1.2**	**1.6**	**2.5**	**7.6**	**10.0**
OHCornSTD	0.071	0.3	0.3	**1.0**	**1.2**	**1.5**	**2.4**	**7.2**	**9.5**
PAcornSTD	0.045	0.2	0.2	0.7	0.7	**0.9**	**1.5**	**4.5**	**6.0**
MSsoybeanSTD	0.051	0.2	0.2	0.7	0.9	**1.1**	**1.7**	**5.2**	**6.8**
MScottonSTD	0.054	0.2	0.2	0.8	0.9	**1.1**	**1.8**	**5.5**	**7.2**
NCcottonSTD	0.092	0.4	0.4	**1.3**	**1.5**	**1.9**	**3.1**	**9.3**	**12.3**
2 applications of 1 lb a.e./A at 12 and 24 days post emergence									
IAcornSTD	0.025	0.1	0.1	0.4	0.4	0.5	0.8	**2.5**	**3.3**
ILCornSTD	0.050	0.2	0.2	0.7	0.8	**1.1**	**1.7**	**5.1**	**6.7**
INCornSTD	0.033	0.1	0.1	0.5	0.5	0.7	**1.1**	**3.3**	**4.4**
KSCornSTD	0.055	0.2	0.2	0.8	0.9	**1.2**	**1.8**	**5.6**	**7.4**
MNCornSTD	0.037	0.1	0.1	0.5	0.6	0.8	**1.2**	**3.7**	**4.9**
MscornSTD	0.054	0.2	0.2	0.8	0.9	**1.1**	**1.8**	**5.4**	**7.2**
NCcornESTD	0.040	0.2	0.2	0.6	0.7	0.8	**1.3**	**4.0**	**5.3**
NECornStd	0.069	0.3	0.3	**1.0**	**1.1**	**1.4**	**2.3**	**6.9**	**9.2**
OHCornSTD	0.066	0.3	0.3	**1.0**	**1.1**	**1.4**	**2.2**	**6.7**	**8.9**
PAcornSTD	0.033	0.1	0.1	0.5	0.6	0.7	**1.1**	**3.4**	**4.4**
MSsoybeanSTD	0.049	0.2	0.2	0.7	0.8	**1.0**	**1.6**	**5.0**	**6.6**
MScottonSTD	0.067	0.3	0.3	**1.0**	**1.1**	**1.4**	**2.2**	**6.7**	**8.9**
NCcottonSTD	0.073	0.3	0.3	**1.1**	**1.2**	**1.5**	**2.4**	**7.3**	**9.7**
Single 1 lb a.e./A application, different timing									
MScornSTD Pre-emergence	0.075	0.3	0.3	**1.1**	**1.3**	**1.6**	**2.5**	**7.6**	**10.0**
MScornSTD Emergence	0.038	0.2	0.2	0.6	0.6	0.8	**1.3**	**3.9**	**5.1**

MScornSTD Post-emergence	0.044	0.2	0.2	0.6	0.7	0.9	**1.5**	**4.4**	**5.8**
MSsoybeanSTD Pre-emergence	0.038	0.1	0.2	0.6	0.6	0.8	**1.3**	**3.8**	**5.1**
MSsoybeanSTD Emergence	0.033	0.1	0.1	0.5	0.6	0.7	**1.1**	**3.4**	**4.5**
MSsoybeanSTD Post-emergence	0.031	0.1	0.1	0.5	0.5	0.7	**1**	**3.2**	**4.2**
MScottonSTD Pre-emergence	0.045	0.2	0.2	0.7	0.7	0.9	**1.5**	**4.5**	**6**
MScottonSTD Emergence	0.030	0.1	0.1	0.4	0.5	0.6	**1**	**3**	**4**
MScottonSTD Post-emergence	0.032	0.1	0.1	0.5	0.5	0.7	**1.1**	**3.2**	**4.2**
Single 1 lb a.e./A Application, Post Emergence									
IAcornSTD	0.013	0.1	0.1	0.2	0.2	0.3	0.4	**1.3**	**1.7**
ILCornSTD	0.023	0.1	0.1	0.3	0.4	0.5	0.8	**2.4**	**3.1**
INCornSTD	0.019	0.1	0.1	0.3	0.3	0.4	0.6	**1.9**	**2.5**
KSCornSTD	0.033	0.1	0.1	0.5	0.6	0.7	**1.1**	**3.4**	**4.4**
MNCornSTD	0.014	0.1	0.1	0.2	0.2	0.3	0.5	**1.4**	**1.9**
NCcornESTD	0.025	0.1	0.1	0.4	0.4	0.5	0.8	**2.5**	**3.3**
NECornStd	0.056	0.2	0.2	0.8	0.9	**1.2**	**1.9**	**5.6**	**7.4**
OHCornSTD	0.030	0.1	0.1	0.4	0.5	0.6	**1.0**	**3.0**	**4.0**
PAcornSTD	0.021	0.1	0.1	0.3	0.4	0.4	0.7	**2.1**	**2.8**
NCcottonSTD	0.036	0.1	0.1	0.5	0.6	0.8	**1.2**	**3.7**	**4.8**
Curve Number Adjustment									
MScorn, 3 apps	0.075	0.3	0.3	**1.1**	**1.3**	**1.6**	**2.5**	**7.6**	**10.0**
Mscorn 2 apps post 12 and 24 days	0.043	0.2	0.2	0.6	0.7	0.9	**1.4**	**4.4**	**5.8**
MScorn, 1 post-emergence app	0.033	0.1	0.1	0.5	0.5	0.7	**1.1**	**3.3**	**4.4**
IAcorn 3apps	0.036	0.1	0.1	0.5	0.6	0.7	**1.2**	**3.6**	**4.8**
IAcorn 2 apps post 12 and 24 days	0.022	0.1	0.1	0.3	0.4	0.5	0.7	**2.3**	**3**
IAcorn 1 post emergence app	0.009	<0.1	<0.1	0.1	0.2	0.2	0.3	0.9	**1.2**
PAcorn 3 apps	0.042	0.2	0.2	0.6	0.7	0.9	**1.4**	**4.2**	**5.5**
Mscotton 3 apps	0.065	0.3	0.3	0.9	**1.1**	**1.3**	**2.2**	**6.5**	**8.6**
MScotton 2 apps post 12 and 24 days	0.043	0.2	0.2	0.6	0.7	0.9	**1.4**	**4.3**	**5.7**
Mscotton 1 post emergence app	0.022	0.1	0.1	0.3	0.4	0.5	0.7	**2.2**	**2.9**
NCcotton 3 apps	0.064	0.3	0.3	0.9	**1.1**	**1.3**	**2.1**	**6.4**	**8.5**
Mssoybean 3 apps	0.044	0.2	0.2	0.6	0.7	0.9	**1.5**	**4.5**	**5.9**

Highlighted cells indicate the RQ exceeds either the Non-Listed or Listed LOC

[1]Seedling emergence endpoints: Dicot - IC25 = 0.069, NOAEC = 0.06 lbs a.e./A; Monocot – IC25 = 0.254, NOAEC 0.245 lbs a.e./A

[2]Vegetative vigor endpoints: Dicot - IC25 = 0.0099, NOAEC = 0.0075 lbs a.e./A; Monocot – IC25 = 0.048, NOAEC 0.030 lbs a.e./A

2.5.3.1. Potential Effects to Milkweed Plants and Indirect Effects to Monarch Butterflies

Migratory monarch populations are in decline, both in numbers and overall health (USFWS, 2020). There is extensive discussion in the literature about which stressors are influencing declines of monarch populations. In the 2020 Status of the Species Assessment released by the U.S. Fish and Wildlife Service (USFWS), the primary drivers affecting monarch health are listed as: "loss and degradation of habitat (from conversion of grasslands to agriculture, widespread use of herbicides, logging/thinning at overwintering sites in Mexico, senescence and incompatible management of overwintering sites in California, urban development, and drought), continued exposure to insecticides, and effects of climate change" (USFWS, 2020). Of these different factors, "widespread use of herbicides" is of particular interest for potential impacts on milkweed.

The U.S. Court of Appeals for the Ninth Circuit, in *National Family Farm Coalition, et al., v. U.S. EPA, et al.*, 966 F.3d 893 (9th Cir., 2020), directed EPA to "address the evidence that monarch butterflies may be harmed by the destruction of milkweed on target fields in determining whether the registration of Enlist Duo will lead to any 'unreasonable adverse effect' on the environment." *Id*. at 930. This section discusses the potential for indirect effects on monarchs through loss of milkweed.

On-field milkweed is expected to be present in a subset of corn, cotton, and soybean fields. Once a common agricultural weed, populations of milkweed in agricultural fields declined substantially with the widespread adoption of over-the-top herbicide applications on herbicide-resistant crops (Pleasants and Oberhauser, 2013; Stenoien, 2018; Pleasants 2017). On-field milkweed now appears to be extirpated in some areas (Stenoien, 2018); however, milkweed still occurs on some fields. Milkweed remains a minor weed in field crops (USEPA, 2021b). Between 2015 through 2019, an average of 850,000 acres of corn, cotton, or soybean were treated to control milkweed (USEPA, 2021b). This estimate does not account for fields where milkweed was present but was not the target weed of herbicide applications. Therefore, milkweed may be present on corn, cotton, and soybean fields.

Monarchs are expected to make use of milkweed located on fields as host plants (Oberhauser *et al.* 2001). The extent to which monarchs make use of remaining on-field milkweed has not been directly surveyed. When milkweed is rare or sparsely distributed, female monarchs are expected to be able to find and oviposit on host plants (Kral-O'Brien *et a*l., 2020). Monarchs can fly long distances and have a conservatively estimated perceptual range of 125 m (Fisher and Bradbury, 2021), so large expanses of agricultural fields would not be a barrier to locating milkweed. Insects use multiple sensory modalities, including long distance olfaction, to locate and identify host plants and nectar sources, and host plants would stand out within a monoculture of non-hosts more than they would stand out in a diverse plant community (Bruce *et al.* 2005; Kerr *et al*. 2017; Knudsen *et al.* 2017). Therefore, monarchs are expected to be on-field when milkweed is present.

Toxicity data for milkweed are not available for 2,4-D. Using a surrogacy approach, effects to on-field milkweed are expected. However, 2,4-D is not generally recommended as a target herbicide for milkweed, which suggests that milkweed toxicity endpoints may be less sensitive than the endpoints identified in the vegetative vigor studies. The level of effects expected for on-field milkweed are therefore uncertain and application of 2,4-D may suppress, but may not eliminate, on-field milkweed. If Enlist Duo is applied prior to when adult monarch butterflies pass through an area, impacts could include a decline in reproduction of an individual through reduction of available oviposition sites. If Enlist Duo applications occur after oviposition occurs, potential effects to monarchs include caterpillar mortality from loss of the host milkweed.

Overall, EFED anticipates that application of Enlist Duo may decrease the growth of milkweed, which could have an impact on monarchs at the field level. It is unknown if use of Enlist Duo would have a population level impact. As indicated in the FWS status of species assessment, an increase of millions of stems of milkweed are needed to reverse the monarch populations' downward trajectories (USFWS, 2020). Therefore, a decrease in milkweed stems (including those on field) is contrary to what is needed to recover this species.

2.5.4. Wetland Plant Exposure Zone (WPEZ): Runoff and Spray Drift from a Treated Field Deposited into a Non-Target Wetland Area

The WPEZ is intended to represent a non-target wetland plant community that is exposed to pesticide via overland flow[16] and spray drift. The wetland can be immediately adjacent to the treated field or some undetermined distance away and be exposed via spray drift and runoff or from runoff only. The WPEZ is intended to represent a plant community that can exist in a saturated to flooded environment, such as a depression or shallow wetland that would collect and hold runoff from upland areas. This wetland system is considered to be protective of other surface-fed wetland systems (*e.g.*, permanently flooded; riparian) such that it is allowed to dry-down (concentrating contaminants), has a finite volume (considers standing water exposure), and would receive all of the runoff from an adjacent treated field. Similar to the TPEZ discussed above, the contribution to concentration from spray drift deposition (although limited because of the label restrictions) is considered along with runoff only for the dimensions of the WPEZ. The WPEZ is defined as a one hectare (ha) wetland receiving inputs from the adjacent 10-ha field. Within the WPEZ, two depth zones are defined: a standing water zone and a saturated soil pore-water (15 cm benthic) zone. The maximum depth of standing water is set to 15 cm, but this water is allowed to dry down to a minimum depth of 0.5 cm using algorithms from the Variable Volume Water Model (VVWM). The model excludes comparison of standing water concentrations to aquatic taxa (e.g., *Lemna sp*.; green algae; diatoms) when water depth is less than 0.5 (see **Section 2.5.6** for aquatic plant results). For comparison to terrestrial plant endpoints, the total mass per area is calculated by tallying the total mass in the water column plus that in the benthic layer and expressing it on an area-normalized basis (lbs a.e./A).

As discussed for the TPEZ, several different considerations of the proposed use were evaluated. **Table 2-28** provides the WPEZ EECs and RQs for all evaluated characterizations. The risks from the proposed use extend across both seedling emergence and vegetative vigor-based endpoints with LOC exceedances for both non-listed and listed species (RQ range 0.8-35.5). The temporal aspect of application (pre-, post-, and emergence) did not refine the risks substantially, but the non-listed aquatic plants no longer have an LOC exceedance. Similarly, the consideration of a single application made post-emergence or differing infiltration assumptions (curve number) did result in reduced EECs, but risk was determined across all scenarios for non-listed and listed vegetative vigor growth stage monocots and dicots and for seedling emergence growth stage dicots.

[16] Water flow that moves in swales, small rills, and gullies

As described previously, monitoring data for 2,4-D permits confident estimation of upper end concentrations in surface water (**Section 2.2.1.2**). These data not only suggest that 2,4-D is likely to runoff application sites and end up in a wide range of aquatic habitats. They also support the conclusions of risk to wetland species because they are detections downstream from wetland environments. While these concentrations cannot be tied to specific uses of 2,4-D including the use of Enlist products, they confirm exposure and exceed the EECs that trigger wetland plant risk.

Table 2-28. Wetland Plant Exposure Zone: Most Sensitive Terrestrial Wetland Plant Taxa and Associated EECs and RQs

Scenario Summary	Terrestrial Plant EEC (lbs a.e./A)	Seedling Emergence[1]				Vegetative Vigor[2]			
		Non-Listed Monocot RQ	Listed Monocot RQ	Non-Listed Dicot RQ	Listed Dicot RQ	Non-Listed Monocot RQ	Listed Monocot RQ	Non-Listed Dicot RQ	Listed Dicot RQ
Proposed label: 3 applications of 1 lb a.e./A at pre-emergence, emergence and post emergence, application interval 12 days.									
IAcornSTD	0.20	0.8	0.8	**2.9**	**3.4**	**4.2**	**6.7**	**20.4**	**26.9**
ILCornSTD	0.15	0.6	0.6	**2.1**	**2.4**	**3.0**	**4.9**	**14.7**	**19.5**
INCornSTD	0.13	0.5	0.5	**1.9**	**2.2**	**2.8**	**4.4**	**13.4**	**17.7**
KSCornSTD	0.24	0.9	**1.0**	**3.4**	**4.0**	**4.9**	**7.9**	**24.0**	**31.7**
MNCornSTD	0.16	0.6	0.6	**2.3**	**2.6**	**3.3**	**5.3**	**16.0**	**21.1**
MScornSTD	0.27	**1.0**	**1.1**	**3.9**	**4.4**	**5.5**	**8.9**	**26.9**	**35.5**
NCcornESTD	0.10	0.4	0.4	**1.4**	**1.6**	**2.0**	**3.3**	**9.9**	**13.1**
NECornStd	0.22	0.9	0.9	**3.2**	**3.7**	**4.6**	**7.4**	**22.3**	**29.4**
OHCornSTD	0.15	0.6	0.6	**2.2**	**2.5**	**3.1**	**5.0**	**15.1**	**20.0**
PAcornSTD	0.12	0.5	0.5	**1.7**	**2**	**2.5**	**3.9**	**11.9**	**15.7**
MSsoybeanSTD	0.16	0.6	0.7	**2.3**	**2.7**	**3.3**	**5.3**	**16.1**	**21.3**
MScottonSTD	0.20	0.8	0.8	**2.8**	**3.3**	**4.1**	**6.5**	**19.7**	**26.0**
NCcottonSTD	0.21	0.8	0.8	**3.0**	**3.5**	**4.3**	**6.9**	**21**	**27.7**
2 applications of 1 lb a.e./A at 12 and 24 days post emergence									
IAcornSTD	0.11	0.4	0.5	**1.7**	**1.9**	**2.4**	**3.8**	**11.5**	**15.2**
ILCornSTD	0.13	0.5	0.5	**1.9**	**2.2**	**2.7**	**4.4**	**13.3**	**17.5**
INCornSTD	0.09	0.4	0.4	**1.4**	**1.6**	**2.0**	**3.2**	**9.6**	**12.7**
KSCornSTD	0.21	0.8	0.9	**3.1**	**3.5**	**4.4**	**7.1**	**21.4**	**28.2**
MNCornSTD	0.11	0.4	0.4	**1.5**	**1.8**	**2.2**	**3.5**	**10.7**	**14.2**
MScornSTD	0.18	0.7	0.7	**2.6**	**3.0**	**3.8**	**6.0**	**18.3**	**24.2**
NCcornESTD	0.11	0.4	0.4	**1.5**	**1.8**	**2.2**	**3.5**	**10.6**	**14.0**
NECornStd	0.20	0.8	0.8	**2.9**	**3.3**	**4.1**	**6.6**	**20.0**	**26.4**
OHCornSTD	0.20	0.8	0.8	**2.9**	**3.3**	**4.1**	**6.6**	**19.9**	**26.3**
PAcornSTD	0.09	0.4	0.4	**1.3**	**1.5**	**1.9**	**3.1**	**9.4**	**12.4**
MSsoybeanSTD	0.15	0.6	0.6	**2.2**	**2.6**	**3.2**	**5.1**	**15.5**	**20.4**
MScottonSTD	0.15	0.6	0.6	**2.2**	**2.6**	**3.2**	**5.1**	**15.6**	**20.6**
NCcottonSTD	0.22	0.9	0.9	**3.1**	**3.6**	**4.5**	**7.2**	**21.9**	**28.9**
Single 1 lb a.e./A application, different timing									
MScornSTD Pre-emergence	0.16	0.6	0.7	**2.4**	**2.7**	**3.4**	**5.4**	**16.5**	**21.8**

MScornSTD Emergence	0.25	**1.0**	**1.0**	**3.7**	**4.2**	**5.3**	**8.4**	**25.6**	**33.7**
MScornSTD Post-emergence	0.15	0.6	0.6	**2.2**	**2.5**	**3.1**	**5.0**	**15.2**	**20.1**
MSsoybeanSTD Pre-emergence	0.1	0.6	0.6	**2.1**	**2.4**	**3.0**	**4.8**	**14.6**	**19.3**
MSsoybeanSTD Emergence	0.1	0.3	0.3	**1.0**	**1.2**	**1.5**	**2.3**	**7**	**9.3**
MSsoybeanSTD Post-emergence	0.1	0.5	0.5	**1.7**	**1.9**	**2.4**	**3.9**	**11.8**	**15.6**
MScottonSTD Pre-emergence	0.2	0.9	0.9	**3.3**	**3.8**	**4.7**	**7.6**	**22.9**	**30.3**
MScottonSTD Emergence	0.2	0.6	0.7	**2.3**	**2.7**	**3.4**	**5.4**	**16.4**	**21.6**
MScottonSTD Post-emergence	0.1	0.3	0.3	**1.2**	**1.3**	**1.7**	**2.6**	**8**	**10.6**
Single 1 lb a.e./A application, post emergence									
IAcornSTD	0.07	0.3	0.3	**1.0**	**1.1**	**1.4**	**2.2**	**6.8**	**9.0**
ILCornSTD	0.07	0.3	0.3	**1.0**	**1.1**	**1.4**	**2.3**	**6.9**	**9.1**
INCornSTD	0.06	0.3	0.3	0.9	**1.1**	**1.3**	**2.1**	**6.4**	**8.5**
KSCornSTD	0.14	0.5	0.6	**2.0**	**2.3**	**2.9**	**4.6**	**14.1**	**18.6**
MNCornSTD	0.05	0.2	0.2	0.7	0.8	**1.0**	**1.6**	**4.9**	**6.4**
MScornSTD	0.15	0.6	0.6	**2.2**	**2.5**	**3.1**	**5.0**	**15.2**	**20.1**
NCcornESTD	0.06	0.2	0.2	0.9	**1.0**	**1.2**	**2.0**	**6.0**	**8.0**
NECornStd	0.14	0.5	0.6	**2.0**	**2.3**	**2.8**	**4.5**	**13.7**	**18.1**
OHCornSTD	0.15	0.6	0.6	**2.2**	**2.5**	**3.1**	**5.0**	**15.1**	**20.0**
PAcornSTD	0.04	0.2	0.2	0.6	0.7	0.9	**1.4**	**4.3**	**5.6**
Curve Number Adjustment									
MScorn, 3 apps	0.21	0.8	0.9	**3.0**	**3.5**	**4.4**	**7.0**	**21.2**	**28.0**
Mscorn 2 apps post 12 and 24 days	0.13	0.5	0.5	**1.9**	**2.2**	**2.7**	**4.3**	**13.1**	**17.3**
MScorn, 1 post-emergence app	0.10	0.4	0.4	**1.5**	**1.7**	**2.1**	**3.4**	**10.2**	**13.4**
IAcorn 3apps	0.15	0.6	0.6	**2.2**	**2.5**	**3.1**	**5.0**	**15.1**	**19.9**
IAcorn 2 apps post 12 and 24 days	0.05	0.2	0.2	0.8	0.9	**1.1**	**1.7**	**5.3**	**7.0**
IAcorn 1 post emergence app	0.09	0.3	0.4	**1.3**	**1.4**	**1.8**	**2.9**	**8.7**	**11.5**
PAcorn 3 apps	0.10	0.4	0.4	**1.4**	**1.6**	**2.1**	**3.3**	**10.0**	**13.1**
Mscotton 3 apps	0.23	0.9	0.9	**3.4**	**3.9**	**4.8**	**7.7**	**23.4**	**30.8**
MScotton 2 apps post 12 and 24 days	0.13	0.5	0.5	**1.9**	**2.2**	**2.7**	**4.3**	**13.1**	**17.2**
Mscotton 1 post emergence app	0.07	0.3	0.3	**1.0**	**1.1**	**1.4**	**2.2**	**6.7**	**8.9**
NCcotton 3 apps	0.17	0.7	0.7	**2.5**	**2.8**	**3.5**	**5.7**	**17.2**	**22.7**
Mssoybean 3 apps	0.16	0.6	0.6	**2.3**	**2.6**	**3.3**	**5.2**	**15.9**	**21.0**

Highlighted cells indicate the RQ exceeds either the Non-Listed or Listed LOC

[1]Seedling emergence endpoints: Dicot - IC25 = 0.069, NOAEC = 0.06 lbs a.e./A; Monocot – IC25 = 0.254, NOAEC 0.245 lbs a.e./A

[2]Vegetative vigor endpoints: Dicot - IC25 = 0.0099, NOAEC = 0.0075 lbs a.e./A; Monocot – IC25 = 0.048, NOAEC 0.030 lbs a.e./A

2.5.5. Consideration of the Diversity of Terrestrial and Wetland Species Potentially Impacted by Runoff Exposures.

EPA also considered the diversity of plants that may be impacted by exposures through runoff for the proposed uses. This comparison relies upon the Species Sensitivity Distributions (SSDs) generated for the IC_{25}s from the vegetative vigor studies (**Section 2.3.2.4.2**). **Figure 2-9.** MSCorn (3 apps) TPEZ and WPEZ EECs as Related to the SSDs for Plant Height and Plant Weight
illustrates the TPEZ and WPEZ highest EECs (MSCorn 3 apps) as they relate to the SSDs. These results suggest that approximately 60% and 90% of plant IC_{25}s would be exceeded in the TPEZ and WPEZ, respectively. Comparisons against other scenarios discussed in **Sections 2.5.3** and **2.5.4** result in similar exceedances. These results illustrate the broad-spectrum 2,4-D toxicity and potential risk to species and habitats in terrestrial and wetland environments from runoff.

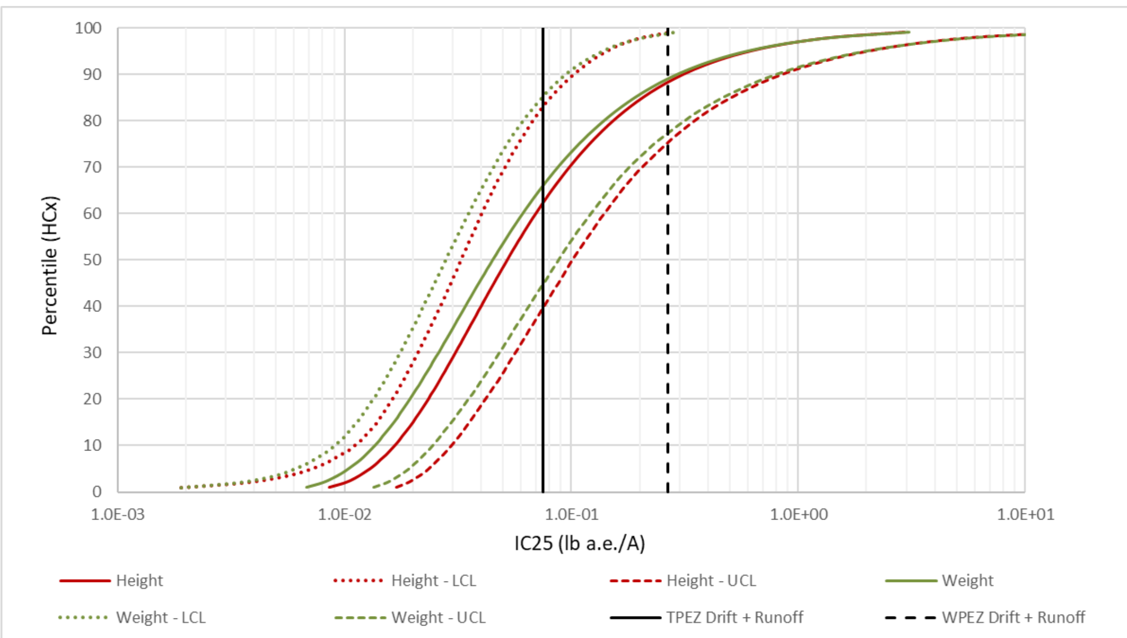


Figure 2-9. MSCorn (3 apps) TPEZ and WPEZ EECs as Related to the SSDs for Plant Height and Plant Weight

2.5.6. Aquatic Plants in the WPEZ and Aquatic Plant Exposure Zone (APEZ): Runoff and Spray Drift from a Treated Field Deposited into a Wetland or Pond.

Aquatic plants are considered within the WPEZ and the Aquatic Plant Exposure Zone (APEZ) which are intended to represent environments where aquatic vascular and non-vascular plants are exposed to pesticide via runoff and/or spray drift. The APEZ aquatic community is the same as the current standard pond model used in aquatic animal assessments[17]. The evaluation considers that the pond community

[17] USEPA. 2016. The Variable Volume Water Model, Revision A. USEPA/OPP 734S16002. https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment#PWC

can be immediately adjacent to the treated field or some undetermined distance away and be exposed via spray drift and runoff or from runoff only.

Based on the current label, the EECs for the WPEZ do not result in a significant risk potential for non-listed wetland vascular plants (RQs $\leq$ 1) but exceed the LOC for listed aquatic plants in wetlands (RQs 1-6.3). The APEZ EECs do not exceed listed or non- listed LOCs (**Table 2-29**). Overall, the relatively low EECs suggest generally low risk to aquatic species of plants; however, potential effects were identified for listed species of vascular aquatic plants in wetlands.

Table 2-29. RQs for Vascular and Non-Vascular Aquatic Plants as a Result of Comparison to the EECs in the WPEZ and APEZ

Scenario Summary	WPEZ					APEZ				
	EEC (µg/L)	Non-Vascular Aquatic Plants[1]		Vascular Aquatic Plants[2]		EEC (µg/L)	Non-Vascular Aquatic Plants[1]		Vascular Aquatic Plants[2]	
		Non-Listed RQ	Listed RQ	Non-Listed RQ	Listed RQ		Non-Listed RQ	Listed RQ	Non-Listed RQ	Listed RQ
Proposed label: 3 applications of 1 lb a.e./A at pre-emergence, emergence and post emergence, application interval 12 days.										
IAcornSTD	281.5	0.1	0.2	0.9	**6.0**	28.0	<0.01	<0.01	0.1	0.6
ILCornSTD	222.8	0.1	0.2	0.7	**4.7**	17.4	<0.1	<0.1	0.1	0.4
INCornSTD	165.3	<0.1	0.1	0.6	**3.5**	18.3	<0.1	<0.1	0.1	0.4
KSCornSTD	242.8	0.1	0.2	0.8	**5.2**	35.6	<0.1	<0.1	0.1	0.8
MNCornSTD	205.6	0.1	0.1	0.7	**4.4**	15.6	<0.1	<0.1	0.1	0.3
MScornSTD	247.1	0.1	0.2	0.8	**5.3**	32.8	<0.01	<0.01	0.1	0.7
NCcornESTD	131.9	<0.1	0.1	0.4	**2.8**	8.1	<0.1	<0.1	<0.1	0.2
NECornStd	296.6	0.1	0.2	1	**6.3**	29.5	<0.1	<0.1	0.1	0.6
OHCornSTD	190.3	<0.01	0.1	0.6	**4**	19.9	<0.01	<0.01	0.1	0.4
PAcornSTD	110.5	<0.1	0.1	0.4	**2.4**	10.4	<0.1	<0.1	<0.1	0.2
MSsoybeanSTD	125.4	<0.1	0.1	0.4	**2.7**	26.5	<0.1	<0.1	0.1	0.6
MScottonSTD	142.2	<0.1	0.1	0.5	**3**	30.4	<0.1	<0.1	0.1	0.6
NCcottonSTD	260.9	0.1	0.2	0.9	**5.6**	21.8	<0.1	<0.1	0.1	0.5
Single 1 lb a.e./A application, different timing										
MScornSTD Pre-emergence	111.65	<0.1	0.1	0.4	**2.4**	21.5	<0.1	<0.1	0.1	0.5
MScornSTD Emergence	130.8	<0.1	0.1	0.4	**2.8**	31.2	<0.1	<0.1	0.1	0.7
MScornSTD Post-emergence	127.5	<0.1	0.1	0.4	**2.7**	14.8	<0.1	<0.1	<0.1	0.3
MSsoybeanSTD Pre-emergence	103.9	<0.1	0.1	0.3	**2.2**	21.1	<0.1	<0.1	0.1	0.4
MSsoybeanSTD Emergence	60.0	<0.1	<0.1	0.2	**1.3**	5.3	<0.1	<0.1	<0.1	0.1
MSsoybeanSTD Post-emergence	90.4	<0.1	0.1	0.3	**1.9**	14.8	<0.1	<0.1	<0.1	0.3
MScottonSTD Pre-emergence	179.6	<0.1	0.1	0.6	**3.8**	13.8	<0.1	<0.1	<0.1	0.3
MScottonSTD Emergence	100.5	<0.1	0.1	0.3	**2.1**	19.7	<0.1	<0.1	0.1	0.4
MScottonSTD Post-emergence	72.4	<0.1	0.1	0.2	**1.5**	7.5	<0.1	<0.1	<0.1	0.2
Single Post Emergence Application										
IAcornSTD	50.2	<0.1	<0.1	0.2	**1.1**	13.9	<0.1	<0.1	<0.1	0.3
ILCornSTD	84.4	<0.1	0.1	0.3	**1.8**	5.9	<0.1	<0.1	<0.1	0.1
INCornSTD	48.1	<0.1	<0.1	0.2	**1**	4.9	<0.1	<0.1	<0.1	0.1
KSCornSTD	137.9	<0.1	0.1	0.5	**2.9**	13.2	<0.1	<0.1	<0.1	0.3
MNCornSTD	92.3	<0.1	0.1	0.3	**2**	3.4	<0.1	<0.1	<0.1	0.1
NCcornESTD	74.7	<0.1	0.1	0.2	**1.6**	3.4	<0.1	<0.1	<0.1	0.1

NECornStd	115.4	<0.1	0.1	0.4	**2.5**	11.8	<0.1	<0.1	<0.1	0.3
OHCornSTD	137.2	<0.1	0.1	0.5	**2.9**	8.3	<0.1	<0.1	<0.1	0.2
PAcornSTD	33.8	<0.1	<0.1	0.1	0.7	3.2	<0.1	<0.1	<0.1	0.1
NCcottonSTD	90.9	<0.1	0.1	0.3	**1.9**	9.7	<0.1	<0.1	<0.1	0.2
Curve Number Adjustment										
MScornModified, 3 apps	229.5	0.1	0.2	0.8	**4.9**	26.9	<0.1	<0.1	0.1	0.6
MScornModified, 1 post app	77.9	<0.1	0.1	0.3	**1.7**	8.3	<0.1	<0.1	<0.1	0.2
IAcornSTD 3apps	279.1	0.1	0.2	0.9	**5.9**	11.3	<0.1	<0.1	<0.1	0.2
IAcorn 1 post app	39.1	<0.01	<0.01	0.1	0.8	4.2	<0.01	<0.01	<0.01	0.1
PAcornSTD 3 apps	77.8	<0.1	0.1	0.3	**1.7**	8.1	<0.1	<0.1	<0.1	0.2
Mscotton 3 apps	119.5	<0.1	0.1	0.4	**2.5**	23.4	<0.1	<0.1	0.1	0.5
MScotton 1 post app	58.7	<0.01	<0.01	0.2	**1.2**	4.4	<0.01	<0.01	<0.01	0.1
NCcotton 3 apps	185.3	<0.1	0.1	0.6	**3.9**	17.2	<0.1	<0.1	0.1	0.4
Mssoybean 3 apps	123.0	<0.1	0.1	0.4	**2.6**	24.4	<0.1	<0.1	0.1	0.5

Highlighted cells indicate the RQ exceeds either the Non-Listed or Listed LOC

[1] Non-Vascular aquatic plant: LC_{50} = 3880, NOAEC = 1410 µg a.e./L

[2] Vascular aquatic plant: LC_{50} = 299, NOAEC = 47 µg a.e./L

2.5.7. Volatile Emission Impacts to Off-Field Plant Risk

Plant toxicity data for volatility exposure

There are two plant toxicity studies that evaluated plant responses to 2,4-D vapor exposures. The first of these is a greenhouse study (MRID 48911801); the second is plant data that were collected within a field volatility study (MRID 48912102) and are discussed below.

In the greenhouse study (MRID 48911801), grape, cotton, tomato, and soybean plants were placed in 10-gallon fish aquariums and a hot plate was used to vaporize 2,4-D acid. Plants were exposed to the vapor for 0.25, 0.5, 1, 2, 4 and 8-hour intervals to arrive at time varied cumulative concentrations with air circulated at a rate of 20 L/min to minimize condensation in the chamber. Periodic air sampling occurred to measure the concentration in the chambers and this concentration was then converted to an exposure per hour basis. After exposure, the plants were removed from the chambers and placed in a greenhouse facility and visual injury (%VSI) ratings were taken at 7 and 14 days after treatment (0% = no injury; 100% = dead plant). The study did not use controls.

Concentrations in air were similar across different durations of exposure (within 2x), so EFED estimated the concentration of exposure as the average of the different concentrations (1.527 µg ae/m^3/h). The authors (MRID 48911801) presented the percent effect (visual injury) as compared to the cumulative concentration collected over the duration (e.g., 12.03 µg ae/m^3/h for 8 hrs). However, these cumulative concentrations did not necessarily represent a cumulative dose, and concentrations in plant tissues were not provided to calculate the cumulative dose.

Injury symptoms included cupping of the leaves at the margins, epinasty, and twisting of the foliage. On seedling grapes, swelling or increased growth on the main stem at the point of attachment of a petiole was also observed. After 8 hours of exposure, the 14 day %VSI was highest for grape (80%) and cotton (50%), but soybean and tomato also showed damage (34% and 30% respectively; **Table 2-30**).

Table 2-30. Day 14 Plant Effects Following Different Durations of Exposure

Avg Concentration µg a.e./m^3 (standard deviation)	Duration (hours)	Cumulative Concentration (µg a.e./m^3)	Day 14 Visual Signs of Injury (%)			
			Grape	Cotton	Tomato	Soybean
1.504 (0.38)	8	12.03	80.4	49.7	33.6	29.7
1.358 (0.46)	4	5.43	63.6	42.4	19.7	19.1
1.217 (0.27)	2	2.43	34.4	28.8	16.9	10.5
1.15 (0.30)	1	1.15	6.9	26.3	10.8	1.5
1.733 (0.50)	0.5	0.87	1.9	9.2	5.7	1.1
2.2 (0.20)	0.25	0.55	0.3	4.6	0.6	0.2

To fully utilize the plant response data in terms of the potential effect compared to environmental exposure, EFED generated regressions of %VSI and duration of exposure. **Table 2-31** provides the different regressed exposure times that corresponded to different %VSI levels. Notably, based on the regressions, less than one hour of exposure may impact grapes, cotton or tomato at levels exceeding 10% VSI. 20% VSI can be reached with exposure durations that range from <1 hour for cotton to ~4 hours for soybean.

Table 2-31. Calculated Time of Exposure Required to Achieve Different Levels of Percent Visual Signs of Injury Assuming an Average Air Concentration of 1.527 µg ae/m³/h

% Effect	Time of Exposure (hours)			
	Grape	Cotton	Tomato	Soybean
5	0.6	0.3	0.5	1.2
10	0.9	0.4	0.8	2.1
20	1.3	0.9	2.6	4.2
50	3.1	7.8	*>8hrs*[a]	*>8hrs*[a]

[a] Effects in exceedance of 50% were not observed for this species.

Estimating Air Concentrations and Risk to Plants

EPA also considered potential effects from the volatilization of 2,4-D choline salt after application to fields. Information regarding the effects of vapor phase 2,4-D-choline on grapes and cotton was contained within a vapor-flux study conducted in Fowler, IN (MRID 48912102). In this study, fields were treated with a 2,4-D choline salt formulation at an application rate of 5 lb a.e./A, which is 5x higher than the proposed application rates. Plants were placed near a series of air monitoring stations approximately one hour after the herbicide was applied; this ensured that all of the effects were the result of vapor-phase exposures rather than spray drift. Air monitoring stations were set at 5 and 15 m beyond the field's edge and several sets were located along each side of the field to capture air concentrations that differed as a result of wind direction. **Table 2-32** provides maximum, minimum, and average concentrations observed for various sampling periods. Plants were also placed directly on the field; no air monitoring station was associated with these plants. These plants were left in the field for 3 days after treatment and then removed to a greenhouse where they were observed for 27 days. Phytotoxic effects (qualitative) were scored on a scale of 0 (no damage) to 100% (dead plant) and compared with controls. The only plants to show outward signs of damage were the grape (0.6% damage) and cotton (40% damage) plants located directly on the field. This study suggests that under the conditions of the test, at 5 m from the edge of the field, plants do not sustain damage from the volatilization of 2,4-D choline salt from a treated field.

Table 2-32. Observed Air Concentrations in a Vapor-Flux Study in Fowler, IN

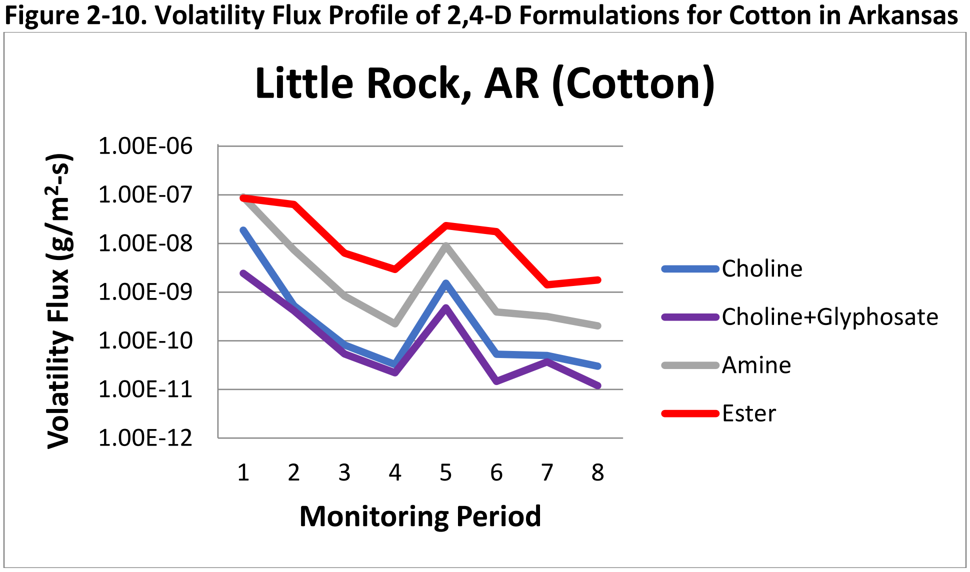
Treatment	Period	Hours after treatment	Concentration (µg/m³)		
			Minimum	Maximum	Mean
2,4-D Choline	1	5	0.001	0.9214	0.0838
	2	11	<LOQ	0.0107	0.0035
	3	17	<LOQ	0.0215	0.0022
	4	23	<LOQ	0.0885	0.0152
	5	34	<LOQ	0.0036	0.0006
	6	47	<LOQ	0.0062	0.0005
	7	59	<LOQ	0.0031	0.0005
	8	70	<LOQ	0.0010	0.0002

Considering the results from the field vapor-flux study, and the laboratory vapor-phase study, EPA took a conservative approach in selecting endpoints to characterize risk from vapor-phase transport. The laboratory study (MRID 48911801) had a relatively constant exposure concentration over the various durations of exposure (1.527 µg ae/m³/hr) and effects on the plants increased with time. The observed effects in the field study indicated that after three days of exposure cotton plants on the field were observed to have 40% VSI. In the laboratory study, the response of cotton to the exposure over time resulted in a plateauing of effect at or near this same effect level (4-hr = 42% VSI; 8-hr = 50% VSI).

Estimating air flux rates

The vapor pressure of 1.4 × 10^{-7} mm Hg and the estimated Henry's Law constant of 7.16 x10^{-11} atm-m^3/mol (**Table 2-1**) suggest that volatilization of 2,4-D is low from dry/moist soil surfaces. In 2010, field volatilization of 2,4-D ethylhexyl ester (EHE), 2,4-D dimethylamine salt (DMA) and 2,4-D choline salt was examined from three bare plots in Fowler, IN and three cropped plots at Farmland, IN (MRID 48912102). In 2011, additional field volatilization experiments of 2,4-D formulations were conducted in cropped plots from Little Rock, AR and Ty Ty, GA. In 2011, these experiments included an additional fourth treatment, a 2,4-D choline plus glyphosate experimental formulation, with all treatments being made to plant canopies (MRID 48912102). The majority of 2,4-D mass loss occurred within 12 hours following application from all the treated plots). From the submitted field volatility studies, the Agency estimated the volatility flux of 2,4-D choline, 2,4-D dimethylamine salt (DMA), and 2,4-D ethylhexyl ester (EHE) for various sites. In general, the field volatility results suggest that the estimated volatility flux rate of 2,4-D from the choline salt is lower than that from the DMA and EHE formulations. More detailed flux rate information can be obtained from previous ecological risk assessment documents (USEPA, 2016a; USEPA, 2016b). The highest volatility fluxes from 2,4-D Choline from AR are depicted in **Figure 2-10.**

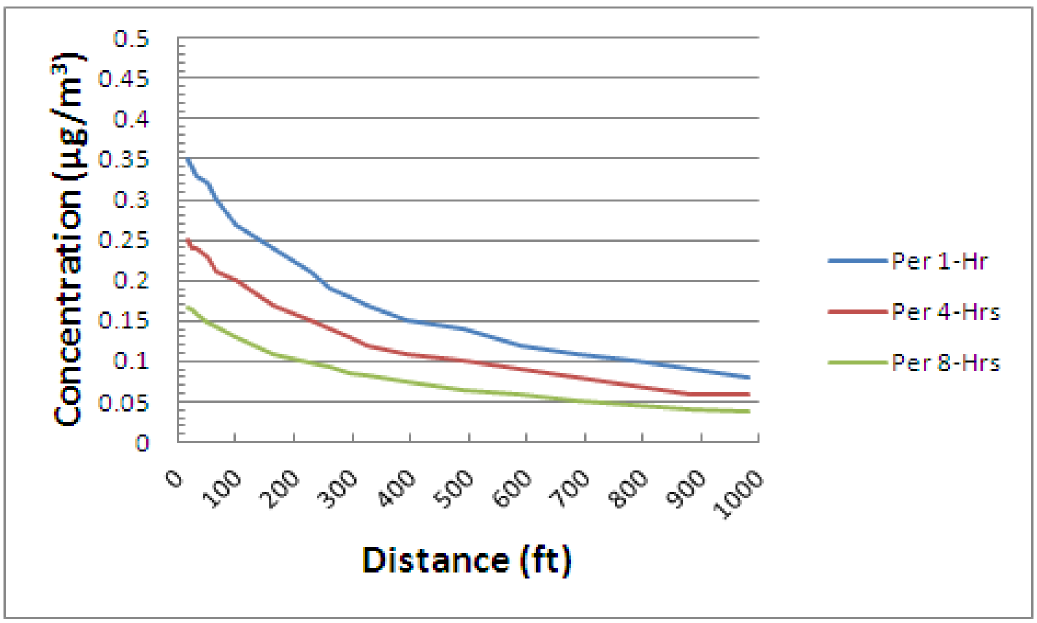
Figure 2-10. Volatility Flux Profile of 2,4-D Formulations for Cotton in Arkansas



2.5.7.1. Near-Field Volatility Modeling

EPA used the Probabilistic Exposure and Risk Model for Fumigants (PERFUM, v. 2.5.1, 7/2/2008)[18] to estimate the vapor phase concentrations of 2,4-D in the atmosphere. PERFUM incorporates actual weather data and flux distribution estimates, and then accounts for changes and altering conditions. The Agency-estimated highest volatility flux of 1.88 x 10^{-8} g/m^2-s from 2,4-D Choline (Little Rock, AR) was used in the PERFUM modeling. EPA calculated upper-bound EECs in air near the edge of a field or at a downwind distance from a treated field using PERFUM. Atmospheric 90th percentile concentrations for three exposure durations (1 hour, 4 hours and 8 hours) are depicted at various distances. EPA selected these durations based on the data in the plant vapor-phase laboratory toxicity study (MRID 48911801). EPA compared the PERFUM EECs to the IC_{10} (0.66 ug a.e./m3) and IC_{20} (1.32 ug a.e./m3) endpoints for visual signs of injury as well as to the average air concentration (1.527 ug a.e./m3) in the study. **Figure 2-11** shows the vapor phase curves for the three different exposure durations. What is illustrated in this figure is that the edge of field concentrations at 1-, 4-, and 8-hour durations are approximately 2, 3, and 4 times lower than the IC_{10}. Therefore, the model predicts no adverse damage to plants off-field for applications to 80 acres or less.

Figure 2-11. Estimated 90th Percentile Vapor Phase Curves for Various Exposure Durations of 2,4-D on an 80-Acre Treated Area



Plant exposure in the field may occur over longer durations than those tested in the greenhouse study and may show different magnitudes of response than that observed in the greenhouse. The field studies conducted with 2,4-D choline included plant toxicity components but did not establish endpoint data that was coupled with air concentrations. As discussed above, cotton when placed on the field one hour after application had 40% VSI after 36 hours of exposure, however these were not paired with air samplers. The field study flux data suggests that significant volatility occurred during the first 4-6 hours after treatment and dropped to low volatility for the remainder of the test. Considering the greenhouse

[18] http://www.epa.gov/opp00001/science/models_pg.htm.

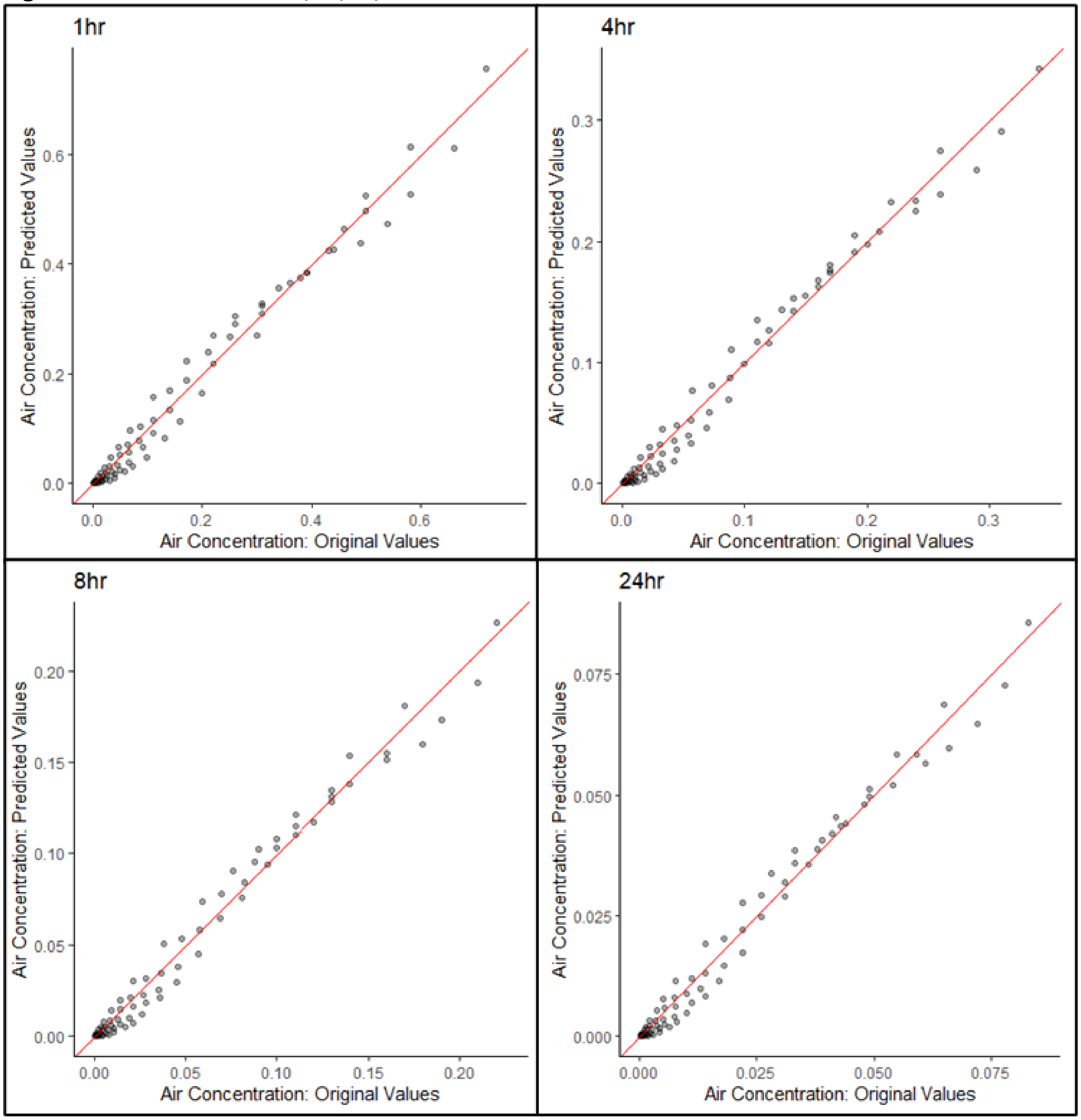
studies, the time to achieve maximum observed effect for cotton (50%) was 8 hours; however, it only took 30 minutes of exposure to reach ~10% VSI and one hour to achieve 25% VSI. These effect durations are similar between the greenhouse and field and add to the confidence that the greenhouse study reflects exposures and effect responses similar to those experienced by cotton plants in the field.

2.5.7.2. Potential for Wide Area (Landscape Level) Volatility Risks

Incident data from Mississippi and Oklahoma, presented in **Section 2.6**, include several Enlist One and Enlist Duo related incidents on cotton that had high percent crop damage (>60% of field had visual signs of injury). Details on the exposure and conditions of the damage relative to Enlist Duo use areas are not available. The magnitude of impact (% acres and number of acres) suggests that volatility and/or spray drift exposure may have played a role in these incidents, but more details would be needed to confirm the route of exposure. The analysis provided in the previous section reflects the model estimation of air concentrations from a single treated (80-acre) field. While it informs the potential exposure near a treated field, and low risk was concluded, it does not address the potential contribution from the use of the products across larger landscapes (e.g., 500 or 1,000 acres) and the resulting potential for more widespread exposures and plant damage.

One of the constraints on estimating concentrations over larger landscapes is that the limit on the field size that PERFUM can handle is 160 acres. This is considerably smaller than the potential landscape area that could be treated within a few days. To address this, EPA developed a screening-level approach for predicting off-field air concentrations for larger treated acreages (up to 2,000 acres). EPA used PERFUM to estimate concentrations at the smaller field sizes (10, 20, 40, 80, and 160 acres) for a range of distances from the application site (1 to 1,500m). EPA conducted PERFUM model runs for a range of durations (1hr, 4hr, 8hr, and 24hr), and the 90th and 95th percentile for each model run was recorded. EPA used these PERFUM output data to fit a series of generalized linear regression models in which field size and off-field distance were used to predict concentration for each duration (1hr, 4hr, 8hr, and 24hr) and percentile (90th and 95th), resulting in a set of eight fitted equations (**Appendix F**). EPA fit these models in R statistical software version 4.1.1. To account for non-normal error distributions, models included a log-link function and square-root transformed independent variables (size and distance). EPA evaluated best-fit models and selected the model using the Akaike information criterion (AIC) method. EPA also visually evaluated the model fit by comparing PERFUM output values and predictive model outputs (**Figure 2-12**; 90th percentiles in **Appendix F**). EPA then used these predictive multiple regression models to provide a set of extrapolated concentrations at larger field sizes (up to 2,000 acres).

Figure 2-12. Model Fit of 1-, 4-, 8-, and 24-Hour 95th Percentile Air Concentrations.



EPA then compared the estimated air concentrations from the screening approach to the toxicity endpoints from the greenhouse study (MRID 48911801) in terms of the cumulative exposure based IC_{10} (0.66 µg a.e./m^3) and IC_{20} (1.32 µg a.e./m^3) endpoints for visual signs of injury as well as to the average air concentration (1.527 µg a.e./m^3) in the study. Comparisons using the 95th percentile one hour concentration estimates indicated that the IC_{10} and IC_{20} are exceeded at ~100 and 500 acres respectively (**Figure 2-13**). The comparison at 1,000 acres for the 1-hour duration resulted in exceedances of the two IC_{10} and IC_{20} at <100 m and <20 m respectively. As discussed above, significant visual signs of injury for cotton were observed after approximately one hour of exposure to air concentrations of 1.527 µg a.e./m^3. EPA predicted this concentration off-field for the 95th percentile at the 1-hour duration when applications were made to greater than 500 acres. Modeled results for the 4-, 8- and 24-hour durations result in lower estimated concentrations, such that the air concentrations of 1.527 µg a.e./m^3 were not estimated for the off-field areas. Considering application to 2,000 acres (maximum modeled), the 1-, 4- and 8-hour durations had exceedances of the IC_{10} out to 200, 50 and 10 m respectively. The maximum acres treated also resulted in the exceedance of the IC_{20} for the 1- and 4-hour duration out to 100 and 5 m (Figure 2-14) respectively.

There are general patterns, such that the frequency and magnitude of the exceedance increases with increasing acres treated (**Figure 2-13**). However, 4- and 8-hour durations have fewer exceedances and the 24-hour estimated concentrations do not exceed the thresholds. **Figure 2-14** illustrates these exceedances from the perspective of distance, and shows the exceedances are proximal to the field at 4 and 8 hours, and shows the maximum distance (<200 m) for the 2,000 acres at 1-hour. When considering these against the 90th percentile concentrations (**Appendix F**), there are exceedances of the IC_{10} or IC_{20} thresholds were similarly observed at the 1-hour time period but only reached out to 50 m for the largest acreages and were near field for the 4-hour duration as well. The low frequency and low magnitude of the exceedances at both the 95th and 90th percentiles suggest that 2,4-D choline has a low potential for wide area damage as a result of larger acreage applications.

Figure 2-13. Estimated 95th Percentile 1-, 4-, 8-, and 24-Hour Air Concentrations for Different Field Sizes. Black solid line = IC_{10}. Black dashed line = IC_{20}; Red solid line = mean air concentration (1.527 ng m^3) that showed effects in the greenhouse study.

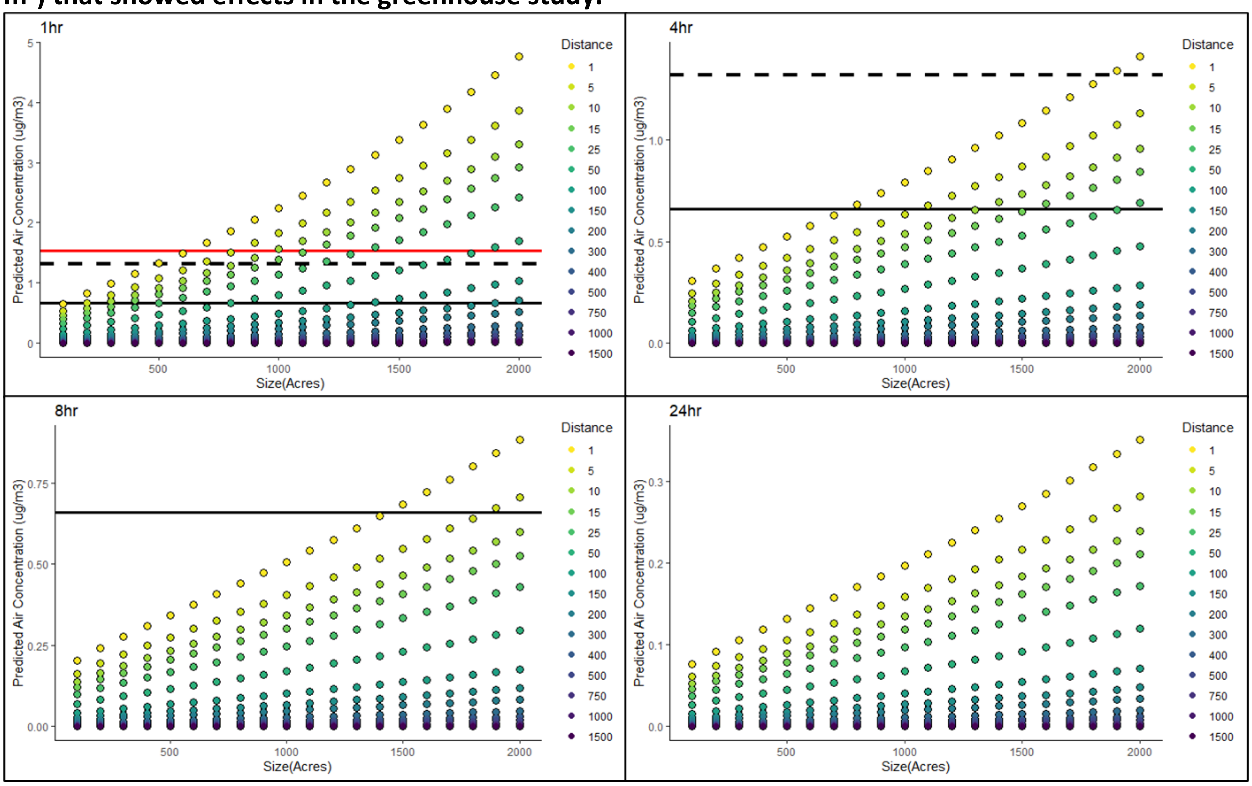
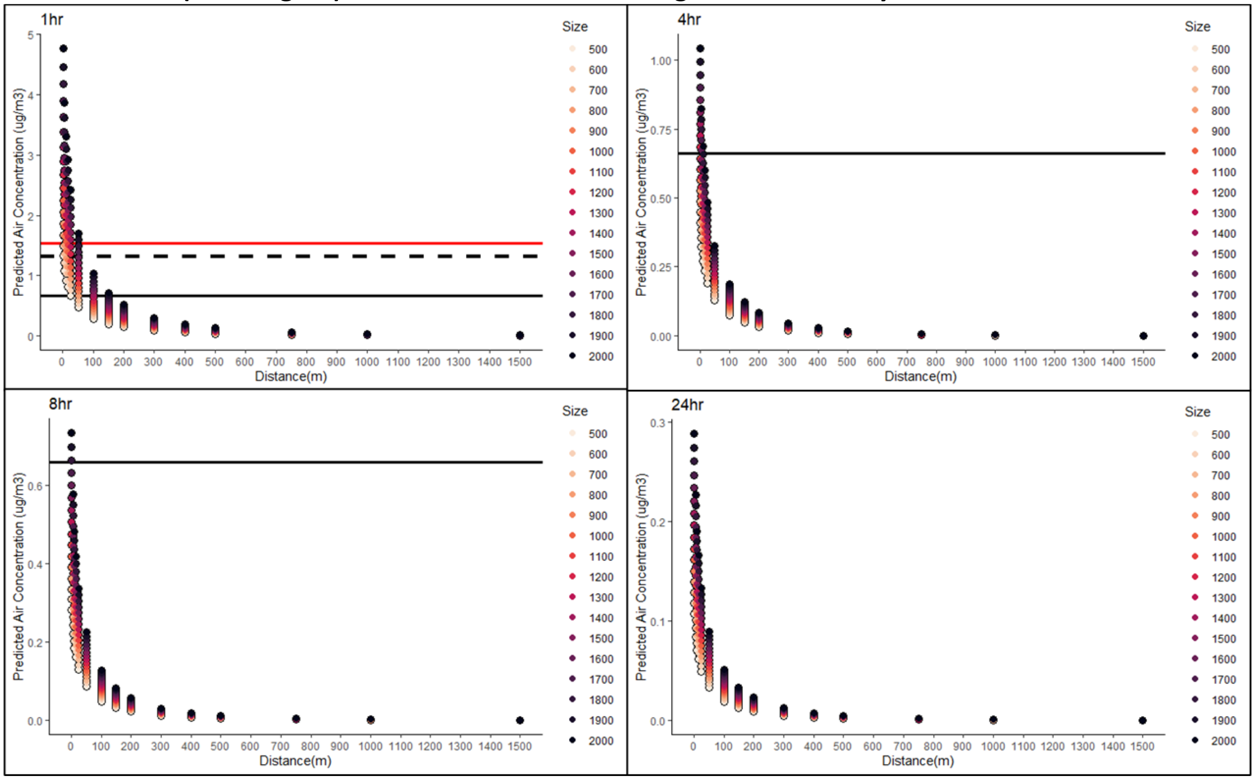


Figure 2-14. Estimated 95th Percentile 1-, 4-, 8-, and 24-Hour Air Concentrations at Off-Field Distances for Varying Field Sizes. Black solid line = IC_{10}. Black dashed line = IC_{20}; Red solid line = mean air concentration (1.527 ng m^3) that showed effects in the greenhouse study.



There are several uncertainties with regard to the concentrations in air over large landscapes that may have high adoption rates of Enlist One or Enlist Duo. The analysis above is based on the highest volatility flux rate from a field study in Arkansas (MRID 48912102). However, the PERFUM derived concentrations using the meteorological conditions may differ across sites. Three additional volatility flux studies were conducted in IN (two sites) and GA (one site), which provided lower flux rates than the AR study. The conclusions presented above are intended to be protective of other areas of the country where soybean, cotton, and corn are grown. Based on the available studies, the analysis suggests that it is likely protective. For instance, the 4-hour, 95th percentile estimated air concentrations nearest the field (0.23 µg a.e./m^3) for a 10-acre treated field were compared to those air concentrations reported in the field volatility study (~4.3 acres treated; maximum and mean measured 0.92 and 0.084 µg a.e./m^3, respectively; MRID 48912102), suggesting that the PERFUM estimates are approximating similar concentrations measured in the study.

EPA believes that when qualitatively considering other factors such as the dynamic nature of air movement, deposition, differential exposure concentrations, differential time of application, and exposure durations across these landscapes, the likelihood of wide area risks from volatility are low. Furthermore, where data is available comparing to compare VSI to plant height, weight, or yield endpoints, the %VSI that was observed was generally significantly higher than the effect observed for these other endpoints (**Table 2-16; Section 2.3.2.4.3**). This suggests that the IC_{10} and IC_{20} thresholds established in the volatility exposure study discussed in this section are conservative. Based on all of the information available, EPA concluded that there is a low potential for air concentrations of volatilized

2,4-D from the use of Enlist One or Enlist Duo to exceed the volatility IC_{10} and IC_{20} thresholds, and low likelihood that volatility-based exposures would result in significant impacts to off-field plant growth or reproduction.

2.6. Analysis of Incident Data

The Incident Data System (IDS) was reviewed for incidents related to 2,4-D choline salt (PC 051505) from January 2016 to August 2021. This search excluded incidents classified as "unlikely" or "unrelated" and only includes incidents with the certainty categories of "possible" or "probable." **Table 2-33** provides a listing of the reported incidents that are associated with 2,4-D. For one of the incident reports, the certainty that the incident was due to 2,4-D exposure is probable given that the symptoms noted were consistent with 2,4-D damage. For the remaining reports, the certainty that the incidents were due to 2,4-D exposure is possible due to the lack of definitive evidence. Additionally, all incidents reported were related to drift. Four of the incidents were noted as being wind-related drift, while the remaining incidents did not specify the type of drift or off-site movement.

Additional incidents are reported to the Agency in aggregated form. Pesticide registrants report certain types of incidents to the Agency as aggregate counts of incidents occurring per product per quarter. Ecological incidents reported in aggregate reports include those categorized as "minor fish and wildlife" (W-B), "minor plant" (P-B), and "other non-target" (ONT) incidents[19]. "Other non-target" incidents include reports of adverse effects to insects and other terrestrial invertebrates. Incidents related to 2,4-D choline salt (PC 051505) were reviewed from its registration in 2016 to August 2021. In terms of aggregate incidents, the IDS database includes 170 minor "plant damage" incidents (PB), and no reports of minor "wildlife" incidents (WB) or "other nontarget" incidents (ONT). Aggregate incident reports do not contain information on the specific use site or plants affected. Unless additional information on these aggregated incidents become available, they are assumed to be representative of registered uses of 2,4-D choline salt.

The number of actual incidents associated with 2,4-D may be higher than what is reported to the Agency. Incidents may go unreported since side effects may not be immediately apparent or readily attributed to the use of a chemical. Although incident reporting is required under FIFRA Section 6(a)(2), the absence of reports in IDS does not indicate that the chemical has no effects on wildlife; rather, it is possible that incidents are unnoticed and unreported.

[19] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/guidance-using-incident-data-evaluating-listed-and#aggregate

Table 2-33. Incident Reports Associated with 2,4-D Choline Salt from the Incident Data System (IDS)

Incident Number	Year	State	Product and Additional Active Ingredients	Legality	Certainty Index	Affected Species	Observed effects
I032671-00011	2019	OK	Enlist One	Undetermined	Probable	Cotton	Unknown
I032671-00012	2019	MS	Enlist One	Undetermined	Possible	Cotton	800 acres of 1250 acres, or 64%
I032671-00013	2019	MS	Enlist One	Undetermined	Possible	Cotton	160 acres of 260 acres, or 62%
I032671-00014	2019	MS	Enlist One	Undetermined	Possible	Cotton	100 acres of a 200 acre field, or 50%
I033400-00006	2018	TX	Enlist Duo	Undetermined	Possible	Cotton	Unknown
I033400-00007	2018	TX	Enlist Duo	Undetermined	Possible	Cotton	Unknown
I033400-00008	2018	TX	Enlist Duo	Undetermined	Possible	Cotton	Unknown
I033441-00003	2018	OK	Enlist Duo	Undetermined	Possible	Cotton	140 acres or 100%
I033441-00007	2018	OK	Enlist Duo	Undetermined	Possible	Cotton	30 acres of an 88 acre field, or 34%
I033442-00030	2018	OK	Enlist Duo	Undetermined	Possible	Cotton	100 acres of a 218 acre field, or 46%
I033442-00031	2018	OK	Enlist Duo	Undetermined	Possible	Cotton	88 acres or 100%
I033442-00032	2018	OK	Enlist Duo	Undetermined	Possible	Cotton	154 acres or 100%
I033442-00033	2018	OK	Enlist Duo	Undetermined	Possible	Cotton	156 acres or 100%
I033442-00034	2018	OK	Enlist Duo	Undetermined	Possible	Cotton	115 acres or 100%
I033442-00035	2018	OK	Enlist Duo	Undetermined	Possible	Cotton	57 acres or 100%
I033455-00019	2019	OK	Enlist Duo	Undetermined	Possible	Cotton	13 acres

Prairie Rivers Network Summary

In 2018, Prairie Rivers Network (PRN) launched a volunteer monitoring program to investigate the increase in landowner reports of suspected herbicide injury to trees and other broadleaf plants (Prairie Rivers Network 2020). PRN's volunteer monitoring program is not intended to identify the cause of injuries, but merely to serve as a rapid ecological assessment in order to document the presence and prevalence of symptoms of possible off-target herbicide exposure through the documentation of visual symptoms consistent with plant growth regulator (PGR) herbicide exposure. In order to verify exposure of herbicides in species or locations that were of particular interest, a small number of tree leaf samples were analyzed for PGR herbicides. Results confirmed that 15 of 24 tree leaf samples had detectable levels of 2,4-D residues present at the time of sampling. PRN's monitoring program concluded that symptoms of possible off-target herbicide injury were frequent and widespread, and present in a wide variety of plant types in the regions that were monitored. A total of 70 species were monitored and all showed symptoms. It wasn't possible to link the symptomology presented in the report to those of precent visual signs of injury. Ratings of symptom severity ranged from light to severe and varied by location and species. In 2018, 45 out of 49 locations had at least one species with symptoms that were moderate or greater in severity. Of those, 29 had species where symptoms were severe. In 2019, 59 of the 83 locations had species with symptoms that were moderate or higher; 28 species had symptoms that were severe.

3. Glyphosate: Ecological Risk Assessment (Not Including Listed Species Effects Determinations)

3.1. Problem Formulation

3.1.1. Mode of Action

Glyphosate acid (CAS number 1071-83-6) [*N*-(phosphonomethyl)glycine] is an herbicide belonging to the phosphono amino acid class of pesticides. Glyphosate is a foliar, non-selective, systemic herbicide widely used to control weeds in agricultural crops and non-agricultural sites. Glyphosate is a potent and specific inhibitor of the enzyme 5-enolpyruvylshikimate 3-phosphate (ESPS) synthase. This enzyme is the sixth enzyme on the shikimate pathway, and it is essential for the biosynthesis of aromatic amino acids (e.g., tyrosine, tryptophan, and phenylalanine) and other aromatic compounds in algae, higher plants, bacteria, and fungi. Inhibition of this enzyme leads to plant cell death. The shikimate pathway is absent in animals.

Glyphosate [*N*-(phosphonomethyl)glycine] is an acid, and it can also be associated with different counter cations to form salts. Glyphosate acid will be a zwitterion (presence of both negative (anionic) and positive (cationic) electrostatic charges) in the environment because of the presence of a carboxylate, phosphonate, and amine functional groups. These functional groups allow glyphosate to form metal-ligand complexes with Fe, Cu, Mn, Zn, Al, Ca in soil, sediment, and aquatic environments (Popvoc, *et al*., 2001).

Several salts of glyphosate are currently marketed, as well as the acid, and are considered as the active ingredient in toxicity tests and end-use products. Enlist Duo is formulated with the dimethylammonium salt of glyphosate. The parent acid is the chemical species that exhibits herbicidal activity and so is the chemical stressor considered in this risk assessment regardless of the salt, unless otherwise specified.

Available toxicity tests use a variety of glyphosate salts. In order to have comparable results across toxicity tests, each salt is considered in terms of its glyphosate equivalent, (acid equivalent; a.e.), determined by multiplying the application rate by the acid equivalence ratio, defined as the ratio of the molecular weight of *N*-(phosphonomethyl) glycine to the molecular weight of the salt. **Table 3-1** shows the salts of glyphosate that may be used as the source of the actual herbicide-active chemical species. For this assessment, the acid and all salt species are referred to collectively as “glyphosate” throughout this document.

Table 3-1. Identification of Glyphosate and the Corresponding Salts

Counter Cation	PC Code	CAS No.	Acid Equivalence Ratio
Glyphosate acid (no counter cation)	417300	1071-83-6	1
Isopropyl amine	103601	38641-94-0	0.74
Monoammonium	103604	114370-14-8	0.94
Diammonium	103607	40465-66-5	0.83
N-methylmethanamine	103608	34494-07-7	0.79
Potassium	103613	39600-42-5; 70901-20-1	0.81

3.1.2. Residues of Concern

EPA addresses potential risks to non-listed taxa and potential effects to federally listed threatened or endangered species (listed species) from glyphosate exposure expected from three applications of Enlist Duo at 1 lb a.e./A on corn, cotton, and soybean fields. Risks from the 2,4-D component of Enlist Duo are addressed in **Section 2**.

Along with significant mineralization to carbon dioxide, the major metabolite of glyphosate is aminomethylphosphonic acid (AMPA). It was detected in all laboratory studies except for the abiotic hydrolysis studies. The laboratory and field dissipation data indicate that AMPA is substantially more persistent than glyphosate.

AMPA is generally less toxic than the parent glyphosate to terrestrial taxa, therefore the assessment for glyphosate is considered protective. Where there are exceptions to this, as in earthworms, assessment of AMPA is provided.

3.2. Environmental Fate Characterization

The major transport routes off the treated area for glyphosate include runoff and spray drift. Glyphosate has a high solubility (12,000 mg/L), low octanol-water partitioning coefficient (<0.001), low vapor pressure ($9.8\text{x}10^{-10}$ torr), and low Henry's Constant ($2.1\text{x}10^{-14}$ atm-m^3 $mole^{-1}$). These data suggest that glyphosate has a low potential for volatilization and bioaccumulation. EPA assumed that the glyphosate salts dissociate rapidly to form glyphosate acid and the counter ion. Because glyphosate acid will be a zwitterion (presence of both negative (anionic) and positive (cationic) electrostatic charges) in the environment, it is expected to speciate into dissociated species of glyphosate acid as well as glyphosate-metal complexes in soil, sediment, and aquatic environments. Glyphosate can form various metal complexes (Popov et al., 2001). The formation of glyphosate-metal complexes with iron and aluminum promotes a high sorption affinity of glyphosate on the surfaces of Fe and Al oxides in soils and sediments (McBride, 1994).

The main routes of dissipation are microbial transformation under aerobic conditions [aerobic soil metabolism $T_{1/2}$ = 1.8 (25^{o}C) - 109 days (20^{o}C), aerobic aquatic metabolism $T_{1/2}$ = 14.1 (25^{o}C) - 518 days (20^{o}C], and runoff. Glyphosate is expected to reach surface water primarily through runoff; however, transport also occurs through spray drift. Glyphosate is expected to travel in overland flow as both dissociated species and sorbed to eroded sediment as glyphosate-metal complexes. Environmental factors (e.g., soil pH) can affect the extent to which glyphosate remains sorbed to eroded sediment during transport. Therefore, depending on the environmental conditions, glyphosate may become available for plant uptake and foliar/stem contact during overland flow as well as in water bodies. Examination of available ambient surface water monitoring data suggests that glyphosate can be present in the dissolved and suspended phases in surface water. While the data cannot be linked to specific glyphosate applications, the data confirms offsite transport of glyphosate to adjacent and downstream aquatic environments.

Additional details on the fate of glyphosate are provided in Chapter 3 of the Biological Evaluation (USEPA, 2020b) and Registration Review Preliminary Ecological Risk Assessment for Glyphosate (USEPA, 2015).

3.2.1. Aquatic Exposure Estimates

3.2.1.1. Modeling

EPA modeled likely surface water concentrations of glyphosate using PWC (v 2.001)[4] coupled with the standard farm pond and wetland conceptual model. EPA selected all standard corn, soybean, and cotton scenarios to assess runoff potential from vulnerable use sites. EPA used PWC scenarios to specify soil, climatic, and agronomic inputs in PRZM[20] and VVWM. The PWC modeling intended to result in high-end water concentrations associated with a particular crop and pesticide within a geographic region. Each PWC scenario is specific to a vulnerable area where the crop is commonly grown. Soil and agronomic data specific to the location are built into the scenario, and a specific climatic weather station providing 30 years of daily weather values is associated with the location. **Table 3-2** list the input parameters EPA used for the PWC modeling of glyphosate. EPA selected input parameters in accordance with EPA's guidance documents (USEPA, 2009; USEPA, 2010; USEPA, 2013).

Since the major route of transportation off the treated area for 2,4-D choline product containing glyphosate is runoff, EPA conducted modeling to evaluate runoff from pre- emergence- and post-emergence applications of glyphosate for corn, cotton and soybean based on the PWC scenarios. EPA generated estimated Environmental Concentrations (EECs) to refine and characterize aquatic and semi-aquatic exposures. The submitted labels allow for 3 applications of 1 lb a.e./A, intended for application at pre-emergence, emergence, and post-emergence crop growth stages. Relying upon the most sensitive MScorn scenario, EPA conducted additional analyses to evaluate the potential exposures following a single application at each of the three proposed times. The results indicate that later applications lead to lower EECs and detailed information is provided in the plant risk assessment (**Section 3.5**).

Since the submitted labels have a prohibition of application within a 24-hr period of expected rain events as they relate to the application dates, EPA incorporated a rainfast period into PWC modeling. EPA simulated the PWC model using an advanced function tab to avoid 2,4-D application date that

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[20] USEPA. 2020a. PRZM5 A Model for Predicting Pesticides in Runoff, Erosion, and Leachate Revision B. USEPA/OPP.

coincides with rainfall within 48-hr. EPA used this window to avoid any rain event within 24-hrs of application. An example of "Advance" tab with inputs was provided in the **Appendix E**.

The hydrologic soil groups (e.g., A, B, C and D) and management of an agricultural field such as tillage practices can influence the permeability of the surface soils on the field; therefore, EPA modified the curve number in the PRZM estimations of runoff for selected scenarios based on a recent runoff assessment of dicamba (USEPA, 2018, DP 443732). In that assessment, EFED consulted with the Biological and Economic Analysis Division (BEAD) on the modeling parameters, and on current agronomic practices. BEAD recommended that EFED use a "good" classification and the inclusion of good agronomic practices that allow crop residues and pesticide to remain on the field. Based on BEAD recommendations, EFED assessed aquatic exposure using revised curve numbers instead of default for fallow and default cropping for the hydrologic C and D soils listed in the **Appendix E**. The default curve numbers, and the revised curve numbers that EFED used reflect BEAD recommendation derived from the National Engineering Handbook (USDA-NRCS, 2004). Detailed descriptions are provided in the **Appendix E**.

Table 3-2. Glyphosate PWC Inputs

Parameter (units)	Value (s)	Source	Comments
Organic-carbon Normalized Soil-water Distribution Coefficient (K_f (L/kg))	175	MRID 44320646, 00108192	Mean K_f (64, 9.4, 90, 470, 700 (MRID 44320646), 62,90, 70, 22, and 175 (MRID 00108192)[a]
Water Column Metabolism Half-life or Aerobic Aquatic Metabolism Half-life (days) 25 °C	381	MRID 41723601 PMRA 1161822	Upper 90th percentile confidence bound of the mean half-life= 189.7+(1.886*175.8)/SQR(3) Average=189.7 SD=175.8 $T_{n-1,90}$ =1.886 n=3 (14, 188[a] and 366[a])
Benthic Metabolism Half-life or Anaerobic Aquatic Metabolism Half-life (days) 25 °C	208	MRID 41723701, 42372502	Upper 90th percentile confidence bound of the mean half-life= 203.33+(1.886*4.509)/SQR(3) Average=203.33 SD=4.509 $T_{n-1,90}$ =1.886 n=3
Aqueous Photolysis Half-life @ pH 7 (days) and Reference Latitude 40° N latitude, 25°C	Stable (0)	MRID 41689101, 44320643	
Hydrolysis Half-life (days)	Stable (0)	MRID 00108192, 44320642	

Parameter (units)	Value (s)	Source	Comments
Soil Half-life or Aerobic Soil Metabolism Half-life (days) 25 °C	29	MRID 44320645, 44125718, 42372501 PMRA 1161813 Al-Rajab, 2010	Upper 90th percentile confidence bound of the mean half-life= 16.19+(1.415*25.37)/SQR(8) Average=16.19 SD=25.37 $T_{n-1,90}$ =1.415 n=8 (1.8, 2.0, 2.6, 5.5[b], 7.5, 13.6[b], 19.4[b], 77.1[b])
Molecular Weight (g/mol)	169.08	Calculated	
Vapor Pressure (Torr) at 25°C	9.75E-10		
Solubility in Water @ 25°C, pH not reported (mg/L)	12,000	Product Chemistry	
Heat of Henry	0	Default	
Application rate	1.12 (kg ai/hectare)	--	
Number of applications per year	3	--	
Initial Application Date	-12 days		Pre-emergence date for all standard scenarios for corn, cotton, and soybean
Interval between applications	12 days	Product labels	==
Application method	Ground		
Application Efficiency	0.99 (ground)	Default	==
Spray Drift Fraction	6.6 E-04	MRID 48844001	Based on average spray drift fraction in EPA farm pond
Heat of Henry	0	Default	
Benthic Compartment Depth	Standard Pond (APEZ) 0.05 m Variable Volume Water Model (VVWM) (WPEZ) 0.15 m	This input is on the waterbody tab to capture different depths of benthic layers for pond vs wetland.	Plant Assessment Tool (PAT)

a. Although the coefficient of variation for K_{foc} is less than the coefficient of variation for K_f, indicating that pesticide binding to the organic matter fraction of the soil may explain some of the variability among the adsorption coefficients, the physicochemical properties of glyphosate (ionic) and the propensity for glyphosate and AMPA to form metal-ligand complexes on surfaces of iron and aluminum oxides would suggest the Freundlich model is the most appropriate partitioning model. This model would account for sorption on both mineral and organic constituents in soils and sediments.
b. Half-lives corrected from 20°C to 25°C using Q10 temperature correction equation.

3.2.1.1.1. PWC Modeling Output

Table 3-3 presents PWC model-estimated concentrations of glyphosate in surface water, commonly referred to as estimated environmental concentrations (EECs; USEPA, 2004 V.B.4) for the intended uses. EPA used these EECs to calculate risk to aquatic animals and plants. EPA estimated the maximum 1-in-10-year daily average, 21-day and 60-day EECs for glyphosate are 14.6, 11.9, and 10.5 µg/L. For cotton, the maximum 1-in-10-year daily average, 21-day and 60-day EECs for glyphosate are 14.1, 10.8, and 10.1 µg/L. For soybean, the 1-in-10-year daily average, 21-day and 60-day EECs for glyphosate are 11.8, 9.3, and 8.0 µg/L. Example output from the model run is provided in **Appendix E**.

Table 3-3 also provides PWC EECs for selected scenarios for a combination of rainfast period with modified curve numbers. The aquatic exposure using revised curve numbers for fallow and cropping for the hydrologic C resulted in an average reduction of 7% (ranged from 4 to 19%) for 1-in 10-year daily average EECs except for MScotton scenario, where 4% higher EECs for revised CN scenario. However, 9-11% reductions were observed for 1-in 10-year 21-day mean and 30-day mean EECs. In general, results also suggest that inclusion of good agronomic practices such as allow crop residues and pesticide to remain on the field can potentially reduce runoff loss of glyphosate from 2,4-D Choline containing glyphosate application sites.

Table 3-3. PWC Standard Pond Estimated Environmental Concentrations (EECs) for Glyphosate Assuming 3 x 1lb Applications with a 12-Day Reapplication Interval

Crops	Scenario	Estimated Environmental Concentrations (µg/L)		
		1-in-10-year Daily Average EEC	1-in-10-year 21-day mean EEC	1-in-10-year 60-day mean EEC
Corn	**PWC Standard Scenario (Rainfast)**			
	IAcornstd	13.3	10.0	9.5
	ILCornSTD	**14.6**	**11.9**	**10.5**
	INcornSTD	7.8	5.2	3.9
	KSCornStd	9.9	7.1	6.2
	MNCornStd	6.7	4.9	3.8
	MScornSTD	13.2	11.7	11.3
	NCcornESTD	9.5	6.5	5.7
	NECornStd	12.5	8.5	6.8
	OHCornSTD	13.8	10.9	10.0
	PAcornSTD	10.6	8.0	7.0
Cotton	MScottonSTD	13.5	11.5	11.1
	NCcottonSTD	12.5	10.6	9.9
Soybean	MSsoybeanSTD	12.6	9.8	8.5
PWC Curve Number Adjusted Scenario (Rainfast)				
Corn	IAcorn	12.7	8.6	8.3
	ILcorn	11.8	9.2	10.3
	MScornSTD	12.0	**11.2**	**10.3**
	Pacorn	9.7	7.1	6.2
Cotton	MScottonSTD	**14.1**	10.8	10.1
	NCcottonSTD	11.5	9.2	8.7
Soybean	MSsoybeanSTD	11.8	9.3	8.0

Bolded concentration are maximum concentrations in surface water.

3.2.1.2. Monitoring

In summary, EPA utilized a tiered approach for evaluating available monitoring data to conserve resources by only using time-intensive, refined risk assessment methods requiring more complex data analysis for pesticides failing [i.e., concentration of pesticide in surface water estimated with models or observed or estimated from measured pesticide concentration data exceeds the level of concern) at lower tiers. For more information on the standard approach used to evaluate monitoring data see **Section 2.2.1.2**.

3.2.1.2.1. Glyphosate Monitoring Data Results

EPA evaluated available surface water monitoring data for glyphosate from the WQP. As previously mentioned, data from the WQP represent samples collected from a wide range of surface water types. Although the data are not targeted to specific sites where glyphosate was used within the watershed, glyphosate usage occurred based on the detections of glyphosate. Since 1987, roughly 20,000 samples have been collected and analyzed for glyphosate. Glyphosate was detected in approximately 62% of those samples. A high-level summary of data downloaded on May 25, 2021, is provided in **Table 3-4.**

Table 3-4. Summary of Glyphosate Data Accessed via the Water Quality Portal

Source	Number of Samples	Number of Non-Detections	Minimum Reported Concentration µg/L	Maximum Reported Concentration µg/L
NWIS	10,478	3,609	0.02	200
STORET	8,531	3,584	0.01	590
Data accessed 5/25/2021 Years of collection include 1987-2021 NWIS (National Water Information System) STORET (STOrage and RETrieval Data Warehouse)				

Given the nature of ambient surface water monitoring data, it is unknown if the recent monitoring data represent usage of Enlist Duo or glyphosate usage on corn, cotton, and soybean. Over the time represented in the monitoring data, there have been many registered formulated products containing glyphosate as well as a wide range of registered use sites. In addition, some uses registered on other labels allow greater application rates than are allowed on the Enlist Duo. Therefore, there is uncertainty as to the representativeness of the available monitoring data and the relevance to uses of Enlist Duo on corn, cotton, and soybean.

When considering national level usage data of glyphosate on all registered crops (USEPA, 2020), the uses with the most glyphosate use are soybean (114,100,000 lb a.e.), corn (90,400,000 lb a.e.), cotton (19,500,000 lb a.e.), and fallow land (13,900,000 lb a.e.). Maximum registered single application rates for these uses generally range between 1.55-3.75 lb a.e./A (4.65-6.0 lb a.e./A/year); however, much higher application rates are permitted for forestry and residential sites. Since corn, cotton and soybean represent a large proportion of the annual lbs a.e. applied annually and average use rates for soybean (1.0 a.e./A), corn (0.97 a.e./A), and cotton (0.82 a.e./A) are roughly equal to application rates for Enlist Duo, EPA assumed that the available monitoring data in agricultural areas may be generally representative of Enlist Duo applications to corn, cotton and soybean. Enlist products have a 30 ft-foot in-field buffer and nozzle requirements that other glyphosate containing formulations do not have. These requirements are intended to reduce drift transport to non-target areas. Monitoring data are expected to represent contributions from spray drift and runoff. When considering the possibility of application rates higher than Enlist products (i.e., >1 lb a.e./A) and drift reduction from the 30-ft infield buffer for Enlist products, it is possible that monitoring data reflect concentrations that could be higher than what would be expected from use of Enlist Duo. However, these differences are not expected to result in concentrations that are different in agricultural areas than expected from Enlist Duo. This is because the noted differences result in no greater uncertainty when compared to other uncertainties or variables that impact glyphosate concentrations across the landscape.

Figure 3-1 shows the range of maximum measured (unadjusted) glyphosate concentrations collected per site per year (gray circles). The gray circles are formatted with transparency so that the darker the circle appears, the larger the number of sites with the same maximum measured concentration. There are some noted horizontal stratification of points across years. This is based on the analytical detection methods available at the time. Several reference concentrations are provided in **Table 3-5** to place context around the measured concentrations of 2,4-D in surface water. These reference concentrations represent toxicity endpoints relevant to listed species in different aquatic or semi-aquatic environments. **Figure 3-1** also shows that the concentrations are above the wetland plant reference concentrations but are lower than the aquatic plant reference concentration. As such, this triggers a more in-depth monitoring data evaluation.

Figure 3-1. Maximum Measured Concentrations by Sample Site by Year for Glyphosate Data from the Water Quality Portal

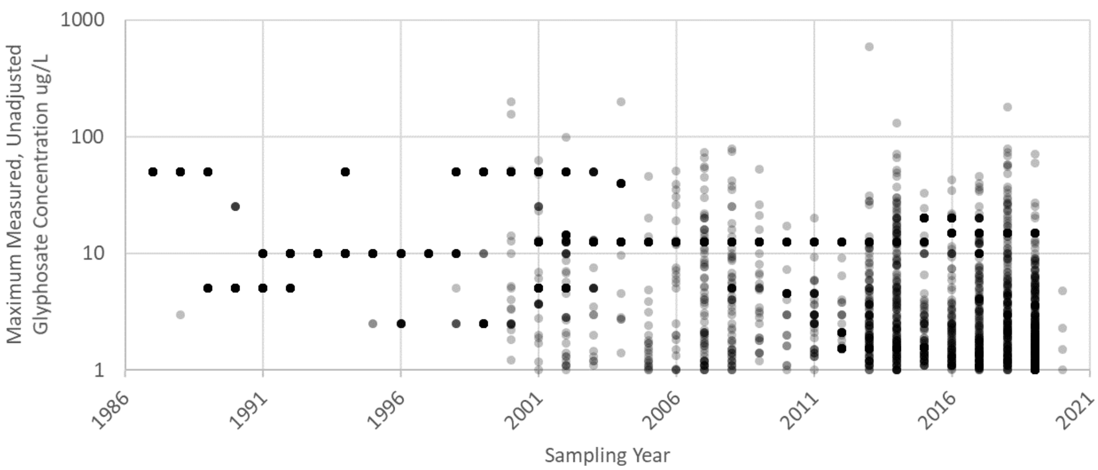


Table 3-5. Reference Concentrations for Glyphosate

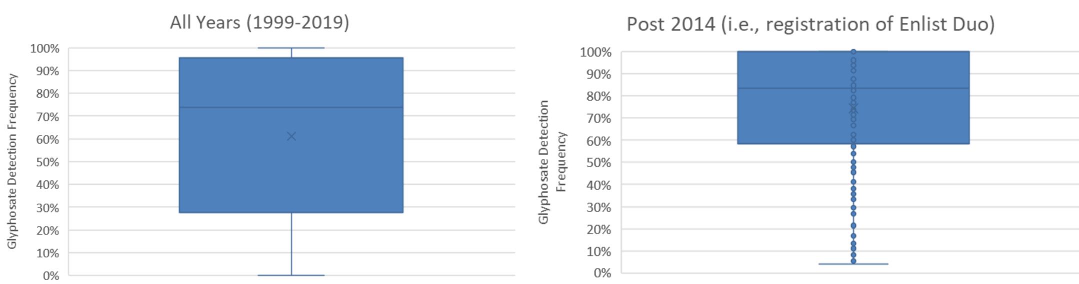
Aquatic System	Reference Concentration[1] (µg a.e./L)	Taxa	Modeling Approach
Flowing (e.g., stream, rivers), Reservoirs	11,400	Aquatic Plants	APEZ
Non-flowing (i.e., ponds)			
Wetland	5.8 (NOEC)	Listed Terrestrial Plant[2]	WPEZ
	7.5 (IC_{25})	Non-listed Terrestrial Plant[2]	

1. This reference concentrations refers to the listed species endpoint
2. IC_{25} converted to a concentration equivalent (calculations provided in **Appendix H**)

The maximum detected value of 590 µg/L (total) was reported as a water grab sample measured by CEDEN in California (CEDEN-312BCJ Bradley Channel at Jones Street) on December 16, 2013. This is outside the Enlist Duo use areas. However, based on visual inspection, this site appears to be a channel adjacent to an agricultural field. Focusing on monitoring data post-2014, several of the highest measured glyphosate concentrations occurred in areas not expected to reflect use of Enlist Duo (e.g., urban areas, California). The highest measured (42.8 µg/L) glyphosate concentration that is relevant to an Enlist Duo use area was measured in Minnesota (MNDA_PESTICIDE-S002-015) on June 13, 2016. This site is in a highly agricultural influenced watershed.

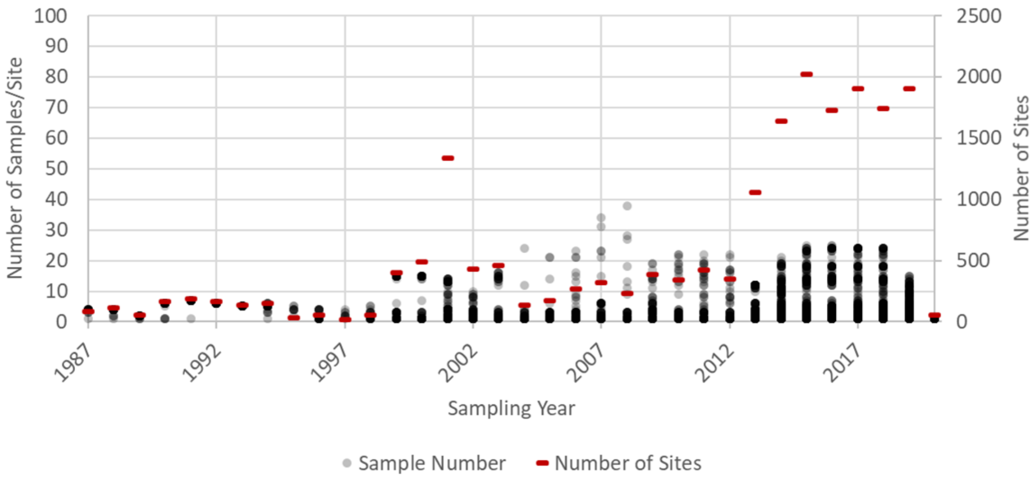
For site-years (443) with more than 13 samples collected per year, the median detection frequency is 74% (**Figure 3-2)**. When only sampling years post-2014 (i.e., registration of Enlist Duo) are considered, the median detection frequency is 83% (**Figure 3-2**). This suggests that when glyphosate containing products are applied, glyphosate often reaches aquatic systems. The detection frequency is slightly (9%) higher for years when Enlist Duo was registered.

Figure 3-2. Box and Whiskers Plot of Sample Frequency for Water Quality Portal sites with more than 13 Samples Collected per Year



As previously mentioned, surface water monitoring programs typically do not collect data daily or even on a weekly basis. Most sampling occurs far less frequently for glyphosate. **Figure 3-3** shows the range of the number of samples collected per site per year (gray circles) along with the number of sites sampled per year (red dash) for glyphosate. The gray circles were formatted with transparency so that the darker the circle appears, the larger the number of sites with the same number of samples collected per year. Sample frequency has a significant impact on the ability to estimate daily concentrations.

Figure 3-3. Sampling Quantity Characteristics for Glyphosate Data from the Water Quality Portal

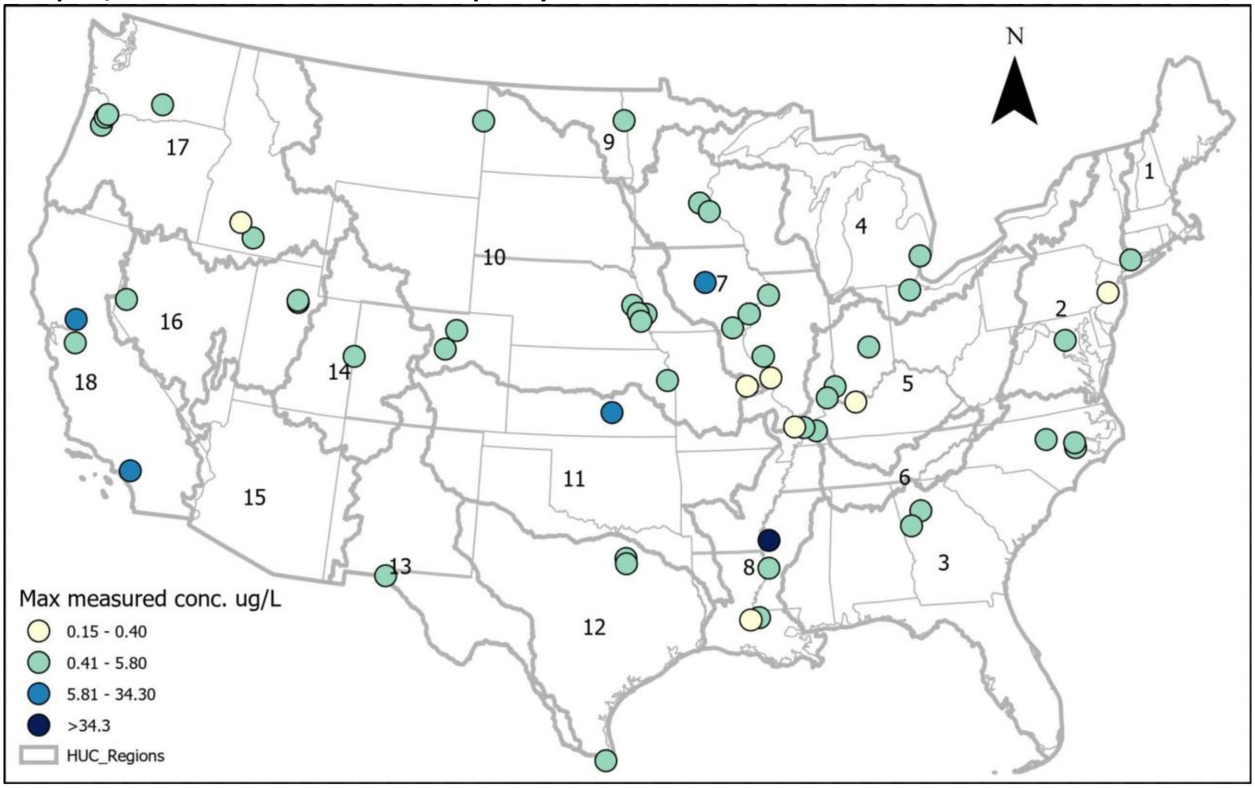


The sample number varies substantially across sites, and the number of sites sampled varies by year. **Figure 3-4** illustrates a consistently higher number of sites sampled in recent years compared to historical data as well as the number of samples collected at each site in recent years. The most samples collected at a site within a calendar year occurred in 2008 when 38 samples were collected. Sampling frequency at other sites and in other years is generally much lower, with the lowest being one sample per year. Most sites have low sample numbers.

As previously mentioned, to understand the available monitoring data and place context for the present action for Enlist products, data need to be evaluated on a site-specific basis, as the sampling frequency, sample location, etc. may impact the interpretation of the data. Given that glyphosate has been registered for a long time, while the monitoring data indicate that it moves from the site of application to aquatic environments, having a better understanding of the recent monitoring data and how it may relate to Enlist Duo is important. Furthermore, evaluation of the monitoring data on a site-specific basis permits evaluation of waterbody and watershed characteristics to place context around the available monitoring data.

Of the many sites with glyphosate samples in the WQP dataset, 57 sites were determined to have ≥ 12 samples/year with 25% detection frequency for at least 3 years. Of the 57 sites, five had concentrations above the wetland plant reference concentration. The highest measured concentration for these sites is 73 µg/L. This sample is reported as a dissolved water sample collected in Mississippi (USGS-07288650 Bogue Phalia near Leland) on October 19, 2007. Based on satellite images, this site appears to be a small stream adjacent to agricultural fields. This sample was collected prior to the registration of Enlist Duo.

Figure 3-4. Maximum Measured Glyphosate Concentrations for Water Quality Portal Sites with ≥ 12 Samples/Year with 25% Detection Frequency for At Least 3 Years



The maximum-measured concentrations shown in **Figure 3-4** are based on non-daily sampling data (i.e., unadjusted). To have confidence in the upper end estimated concentrations it would be necessary to run SEAWAVE-QEX or apply sampling bias factors. Further analysis of available surface water monitoring data was completed using SEAWAVE-QEX, along with the development of glyphosate-specific sampling bias factors. This information can be made available upon request. Because several glyphosate concentrations (unadjusted) exceed the level of concern for wetland plants, it was not deemed necessary to proceed to that level of refinement in this assessment.

3.2.1.2.2. Glyphosate Surface Water Analysis Summary

In summary, in recent years there is a reasonable amount of data that can be used to estimate upper bound concentrations of glyphosate in surface water across a range of aquatic environments. These data not only suggest that glyphosate is likely to occur in surface water, but concentrations are likely to be above the reference concentration for wetland plants. It is important to note that these concentrations cannot be tied to specific uses of glyphosate because of the nature of ambient monitoring data, but concentrations are expected to occur and exceed the wetland plant reference concentrations in areas of the country where Enlist Duo can be used. A majority of the referenced data refers to monitoring sites located in flowing systems and have not been linked to wetlands. However, it is possible for flowing surface water to be linked to wetland environments as previously discussed.

Moreover, the monitoring data concentrations are generally within the range of model estimated concentrations and, in some cases, the measured concentrations are higher than the model estimates. Spray drift analysis to evaluate potential off-site risks to terrestrial plants, birds, and mammals

Label requirements for Enlist products include spray drift buffer setbacks (30 feet) and application nozzle restrictions designed to mitigate drift, and these must be considered before any conclusions can be reached as to the risks posed to terrestrial organisms beyond the treatment site. The Enlist product labels require the use of the product with an American Society of Agricultural and Biological Engineers (ASABE) S-572 droplet size classification of coarse/coarser spray quality. Therefore, EPA evaluated the distances to off-field LOC exceedances using the label-required droplet spectrum, combined with the results of a registrant submitted spray-drift field study (MRID 48844001). This study empirically measured spray drift deposition with various drift reduction nozzles and formulations. EPA considered this specific combination of nozzle and formulation as measured in the study to evaluate more realistic spray drift buffer requirements than were achievable using AgDRIFT modeling. The empirical deposition data show a biphasic distribution (**Figure A-1, Appendix A**; USEPA 2016a, DP 428301) with a bias toward the edge of the application area. EPA truncated the deposition data from 0 to 10 feet and used 90th percentile data from 25 to 400 feet to determine buffer distances (**Figure A-1, Appendix A**). The deposition curve was developed using Exponential Decay, Double, 4 Parameter equation using SigmaPlot version 10.0. The Exponential Decay, Double, 4 Parameter (EDDP) and various coefficients are listed below (where x is the distance from the edge of field considering a 25 foot[21] in-field spray drift buffer; **Figure 3-5**).

$$\text{Fraction of applied} = (0.0013*\text{EXP}(-0.0388*x)) + (0.0006*\text{EXP}(-0.0012*x))$$

The edge of field drift fraction (0.00167) is relied upon within each of the terrestrial animal and plant sections of **Sections 3.4** and **3.5** to determine if potential risk to these taxa would require greater than the labeled 30 ft in-field buffer to mitigate risks to the field.

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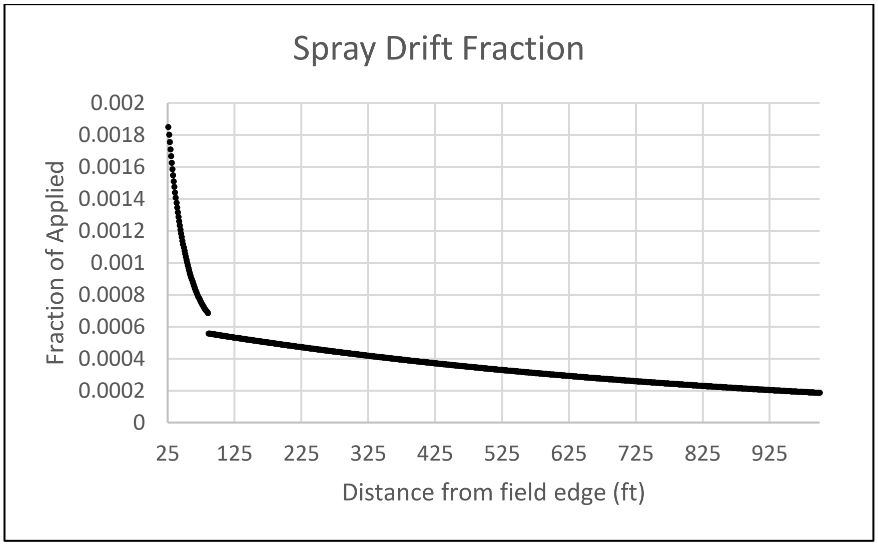
[21] Note: the label requires a 30 ft in-field downwind setback from sensitive vegetation.

Figure 3-5. 90th Percentile Deposition Curve of AIXR Nozzle for GF-2726 2,4-D Choline Formulation Distance



3.3. Ecological Effects Characterization

EPA described the toxicity of a pesticide to different organisms, and the uncertainties and assumptions associated with the toxicity studies in an effects characterization.

Toxicity data are available for glyphosate in both the open literature and from registrant-submitted toxicity tests. The aquatic and terrestrial effects endpoints selected for use in this risk assessment are summarized in **Table 3-6** below**.** A summary of registrant-submitted toxicity studies is available in **Appendix B**. For a comprehensive discussion of the available toxicity data, see Chapter 2 of the Draft Glyphosate Biological Evaluation (BE; USEPA, 2020b).

Table 3-6. Ecological Toxicity Values Used to Assess Risks to Various Taxa from Use of Glyphosate

Species	Acute Endpoint	Chronic Endpoint	MRID[1]
Freshwater Fish	LC_{50} = 43,000 µg a.e./L	NOAEC = 25,700 µg a.e/L	44320630, 00108171
Estuarine/Marine Fish	LC_{50} > 180,000 µg a.e./L	NOAEC = 11,000 µg a.e./L	00025390, 48718011
Aquatic-phase Amphibians	LC_{50} = 103,200 µg a.e./L	NOAEC = 1,800 µg a.e./L	43839601, 46650501
Freshwater Invertebrates	EC_{50} =53,200 µg a.e./L	NOAEC = 9,220 µg a.e./L	00162296, NA[2]
Estuarine/Marine Invertebrates	EC_{50} = 40,000 µg a.e./L	NOAEC = 6,110 µg a.e./L	00034702, NA[2]
Aquatic Vascular Plants	IC_{50} = 11,900 µg a.e./L	NA	44320638
Aquatic Non-Vascular Plants	IC_{50} = 11,400 µg a.e./L	NA	40236904
Birds	LD_{50} >2000 mg a.e./kg-bw (passerines) ED_{50} = 2,819 mg a.e./kg-bw (regurgitation in passerines)	NOAEC < 501 mg a.e./kg-diet (effects on male mallard weight gain)	4893406, 108107/37765, 48876602

	LC_{50} > 4,570 mg a.e./kg-diet (non-passerines)		
Mammals	LD_{50} > 4,860mg a.e./kg-bw	NOAEL = 500 mg a.e./kg/day LOAEL = 1,500 mg a.e./kg/day	46760505, 41621501
Terrestrial Invertebrates	LD_{50} > 100 µg a.e./bee (adult contact) LD_{50} > 180 µg a.e./bee (adult oral)	NOAEC > 187 µg a.e./bee/d (adult chronic, non-Enlist Duo formulation) Larval NOAEC not available	50603803
Terrestrial Plant Taxa[1]	**Non-listed Species Endpoint**	**Listed Species Endpoint**[1]	**MRID**
Dicot (Tomato, *Solanum lycopersicum*) – Vegetative Vigor	EC_{25} = 0.010 lbs ae/A	NOAEC = 0.0078 lbs ae/A	49903202
Monocot (Wheat, *Triticum aestivum*) – Vegetative Vigor	EC_{25} = 0.051 lbs ae/A	NOAEC = 0.031 lbs ae/A	49903202
Monocots and Dicots	EC_{25} > 5 lb a.e./A		40159301

[1] Terrestrial plant data, including discussion of the listed species endpoints, are discussed in depth in the terrestrial plant risk assessment (**Section 3.5; toxicity studies are described in Section 3.3.2.6**).
[2] Calculated using an Acute-to-Chronic Ratio
>Greater than values designate non-definitive endpoints where no effects were observed at the highest level tested, or effects did not reach 50% at the highest concentration tested (USEPA, 2011).
< Less than values designate non-definitive endpoints where growth, reproductive, and/or mortality effects are observed at the lowest tested concentration.

3.3.1. Aquatic Toxicity

Aquatic toxicity data for glyphosate as the technical grade active ingredient (TGAI) are presented in **Table 3-7**. Glyphosate salts in aqueous environments quickly dissociate to the acid form. Technical glyphosate is considered representative of toxicity of a variety of glyphosate salts when compared as acid equivalents (a.e.).

The endpoint 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 (CI = 30.6 – 53.5 mg a.e/L; MRID 44320630). In a chronic fish life cycle study conducted with technical glyphosate and fathead minnows, there were no reported effects up to the highest concentration tested, 25.7 mg a.e./L (MRID 00108171).

Less data are available on the toxicity of technical and formulated glyphosate to estuarine/marine fish, but the available data suggest similar sensitivity as freshwater fish. In an open literature study by Le Mer *et al.*, 2013, the effects of technical glyphosate exposure on estuarine/marine threespine stickleback larvae (*Gasterosteus aculeatus*) were examined in two consecutive years. In this study, there was <11% mortality in the fish exposed to glyphosate over the 42-day exposure period, and there were no significant differences in wet weight, body length, sex ratio, or condition of juvenile fish between glyphosate-exposed fish and control in either test year at concentrations up to 0.100 mg/L. A chronic NOAEC value for estuarine/marine fish was calculated using an acute to chronic ratio (ACR) as follows:

> Acute fathead minnow LC_{50} = 69.4 mg a.e./L (MRID 00162296)
> Chronic fathead minnow NOAEC = 25.7 mg a.e./L (MRID 00108171)
> ACR = 69.4/25.7 = 2.7
> Acute sheepshead minnow LC50 = 240 mg a.e./L (MRID 44320632)
> Chronic sheepshead minnow calculated NOAEC = 240/2.7 = 88.9 mg a.e./L.

As there were no reported effects in the chronic fathead minnow study, the NOAEC reported was the highest concentration tested (25.7 mg a.e./L). There is uncertainty in using the non-definitive NOAEC value in the ACR calculation, as it is a conservative approach.

The available data for aquatic phase amphibians are limited but suggest that the freshwater fish endpoint is protective of acute toxicity to aquatic phase amphibians. An acute study on the Australian tree frog (*Litoria moorei*) reported an LC_{50} of 150 mg a.e./L (MRID 43839601). A chronic study showed no effects on the Leopard frog (*Rana pipiens*) up to the highest level tested (1.3 mg a.e./L; MRID 46650501).

The acute toxicity endpoint for freshwater aquatic invertebrates in the risk characterization section is from the study on early fourth instar midge larvae with and EC_{50} value of 53.2 mg a.e./L (MRID 00162296). In a chronic toxicity test with *Daphnia magna* and technical glyphosate IPA salt, the reported NOAEC and LOAEC are 49.9 and 95.7 mg a.e./L, respectively (MRID 44320631). However, as the acute toxicity test with the midge was the most sensitive, the acute to chronic ratio approach was used using acute and chronic *Daphnia magna* data and acute midge toxicity data. As there were two reliable acute toxicity values for *Daphnia*, the geometric mean of the two values was used to derive a single acute toxicity value (based on EFED guidance on acute to chronic ratios). The calculated chronic NOAEC for freshwater invertebrates was 9.22 mg a.e./L.

For estuarine/marine invertebrates, acute toxicity testing with technical glyphosate and the Pacific oyster embryos (*Crassostrea gigas*) reported an acute toxicity EC_{50} value of 40 mg a.e./L. This endpoint incorporated normal larvae development as opposed to strictly mortality. For the estuarine/marine copepod, *Acartia tonsa*, a 48 hr acute LC_{50} toxicity values of 35.5 mg a.e./L was reported for glyphosate acid in the open literature (Tsui and Chu, 2003); it is noted that analytical recovery was reported as approximately 53% in the saltwater media and test concentrations corrected for percent recovery.

Chronic toxicity data for estuarine/marine invertebrates for use in the risk estimation are not available. Therefore, a chronic toxicity value was calculated using the acute to chronic ratio approach, using acute and chronic *Daphnia magna* data and acute copepod data from Tsui and Chu 2003. A chronic NOAEC value was calculated for estuarine/marine invertebrates using an acute to chronic ratio (ACR) as follows:

Chronic *Daphnia magna* NOAEC = 49.9 mg a.e./L (MRID 0124763)
Geometric mean of two acute *Daphnia magna* EC_{50}s[1] = 288 mg a.e./L
ACR = 288/49.9 = 5.77
Acute copepod LC_{50} = 35.3 mg a.e./L (Tsui and Chu, 2003)
Calculated chronic NOAEC = 35.3/5.77 = 6.11 mg a.e./L.

[1]128.1 mg a.e./L (MRID 44320631; static test system; 95.6% a.e.; EC_{50} based on measured concentrations) and 647.4 mg a.e./L (MRID 00108172; static test system; 83% a.e.; based on nominal test concentrations.)

The *Daphnia* ACR was also used to calculate a chronic freshwater NOAEC for the midge (*Chironomus plumosus*) because the available acute studies showed that midges are more sensitive than *Daphnia* to glyphosate. The chronic freshwater endpoint for midges was calculated as follows:

Daphnia ACR = 5.7 (as above)
Acute midge LC50 = 53.3 mg a.e./L
Calculated chronic midge NOAEC = 53.2/5.77 = 9.22 mg a.e./L;

The calculated midge NOAEC was used at the chronic freshwater invertebrate endpoint for risk analysis.

Numerous acute toxicity studies for aquatic-phase amphibians are available in the open literature for technical grade glyphosate, as discussed in the 2020 glyphosate draft BE (USEPA, 2020b). The most sensitive endpoint is from an acute study that showed technical glyphosate is practically non-toxic to the Australian tree frog (*Litoria moorei*), with a 96h LC_{50} of 103.2 mg a.e./L. This supports the use of the acute fish endpoints as protective surrogates for aquatic phase amphibians. A chronic study on the Leopard frog (*Rana pipiens*) showed no effects up to 1.8 mg a.e./L, which was the highest level tested.

Toxicity tests for aquatic vascular and nonvascular plants result in EC_{50}s of 11.4 and 11.9 mg a.e./L, respectively (MRID 44320638 and 40236904). The glyphosate draft BE presents a Species Sensitivity Distribution (SSD) for aquatic plants, with an HC_{05} of 5 mg a.e./L, incorporating both registrant-submitted and open literature studies (USEPA, 2020b).

Toxicity tests using the other salts of glyphosate are assumed to be representative of gly-DMA, as each salt will dissociate rapidly in the aqueous environment to release the parent acid. The results of toxicity tests are therefore presented in glyphosate acid equivalents (a.e.).

Table 3-7. Glyphosate Aquatic Toxicity Endpoints used in Risk Estimation

Assessment Endpoint	Species	% a.e.*	Toxicity Values (mg a.e./L)*	Toxicity Category[1]	MRID (Date)	Comment
Acute Toxicity to Freshwater Fish	Bluegill sunfish (*Lepomis macrochirus*)	87.3	96-hr. LC_{50}: 43 Slope: NA	Slightly toxic	44320630 (1995) Acceptable	NOAEC = 30.6 mg a.e./L (based on mortalities at 56 mg a.e./L)
Chronic Toxicity to Freshwater Fish	Fathead minnow (*Pimephales promelas*)	96.7	NOAEC: 25.7	NA	00108171 (1975) Acceptable	Highest concentration tested
Acute Toxicity to Aquatic-phase Amphibians	Australian tree frog (*Litoria moorei*)	NA	96-hr LC_{50}: 103.2 Slope: NR	Practically non-toxic	43839601 (1995) Supplemental	NOAEC: NR
Chronic Toxicity to Aquatic-phase Amphibians	Leopard frog (*Rana pipiens*)	Assumed ~100	NOAEC: 1.8	NA	46650501 (2004) Supplemental	Highest concentration tested; no effects
Acute Toxicity to Freshwater Invertebrates	Midge (*Chironomus plumosus*)	96.7	48-hr EC_{50}: 53.2 Slope: NR	Slightly toxic	00162296 (1979) Acceptable	NOAEC: NR
Chronic Toxicity to Freshwater Invertebrates	Midge (*Chironomus plumosus*)	NA	NOAEC (calculated): 9.22	NA	NA	Based on acute-to-chronic ratio
Acute Toxicity to Estuarine/Marine Fish	Sheepshead minnow (*Cyprinodon variegatus*)	96	96-hr. LC_{50}: 240	Practically nontoxic	44320632 (1996) Acceptable	NOAEC = 100 mg a.e./L (based on quiescent fish in 180 mg a.e./L)
Chronic Toxicity to Estuarine/ Marine Fish	Sheepshead minnow (*Cyprinodon variegatus*)	NA	NOAEC (calculated): 88.9	NA	NA	Based on acute-to-chronic ratio
Acute Toxicity to Estuarine/Marine Invertebrates	Mollusk - Pacific oyster (*Crassostrea gigas*)	96	48-hr EC_{50}: 40 Slope: NA	Slightly toxic	44320634 (1996) Acceptable	NOAEC = 32 mg a.e./L (based on % normal larvae at 56 mg a.e./L)
Chronic Toxicity to Estuarine/Mari	Amphipod (*Acartia tonsa*)	NA	NOAEC (calculated): 6.11	NA	NA	Based on acute-to-chronic ratio

Assessment Endpoint	Species	% a.e.*	Toxicity Values (mg a.e./L)*	Toxicity Category[1]	MRID (Date)	Comment
ne Invertebrates						
Acute Toxicity to Non-vascular Aquatic Plants	Bluegreen algae (*Anabaena flos-aquae*)	96.6	4-day EC_{50}: 11.4 Slope: 3.53	NA	40236904 (1987) Acceptable	NOAEC: NR
Acute Toxicity to Vascular Aquatic Plants	Duckweed (*Lemna gibba*)	95.6	14-day EC_{50}: 11.9 Slope:NR	NA	44320638 (1996) Supplemental	NOAEC:1.3

*a.e. = expressed in terms of acid equivalents for glyphosate; NA = not available; NR = not reported
[1]Categories of acute toxicity for aquatic organisms (U.S. EPA, 2004) based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic. Toxicity categories for aquatic plants have not been defined.

3.3.2. Terrestrial Toxicity

3.3.2.1. Avian

Acute dose-based toxicity data on selected avian species are available for technical glyphosate, several formulations and the AMPA degradate (**Table 3-8**). No mortality was observed in the available dose-based acute studies conducted with technical glyphosate at concentrations up to 4,570 mg a.e./kg-bw. For the passerine species, canary, the acute LD_{50} was >2,000 mg a.e./kg bodyweight. In this 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. In the 2015 PRA, an effective dose (ED_{50}) based on regurgitation was calculated to be 2,819 mg ae/kg-bw. The endpoint for acute avian toxicity for technical glyphosate is based on the regurgitation endpoint calculated in the 2015 preliminary risk assessment (USEPA, 2015).

Table 3-8. Avian Acute Dose-based Toxicity Data for Technical Glyphosate

Test Substance	Species	% a.i.[1]	LD_{50} mg a.e./kg bw[1,2] (Confidence Intervals)	Reference
Glyphosate	Bobwhite quail (*Colinus virginianus*)	83	>3196.3 (N.A.)	MRID 00108204
	Bobwhite quail (*Colinus virginianus*)	98.5	>4570 (N.A.)	MRID 00076492
	Canary (*Serinus canaria*)	96	>2,000 (N.A.)	MRID 48934206

1 a.e. = acid equivalent
2 N.A. = not available

Acute dietary-based toxicity data on selected avian species are available for technical glyphosate (**Table 3-9**). No mortalities were observed in the available sub-acute dietary-based exposure studies conducted with technical grade or formulated glyphosate at concentrations up to 4,971 mg a.e./kg-diet.

Table 3-9. Avian Acute Dietary-based Toxicity Data for Technical Glyphosate

Test Substance	Species	% a.i.	LC_{50} mg a.e./kg diet[1]	Reference
Glyphosate	Mallard duck (*Anas platyrhynchos*)	98.5	>4,570.4	108107/37765
	Bobwhite quail (*Colinus virginianus*)	95.6	>1,912	MRID 44320626
	Mallard duck (*Anas platyrhynchos*)	95.6	>4,971.2	MRID 44320627
	Bobwhite quail (*Colinus virginianus*)	95.6	>4,971.2	MRID 44320628

1 a.e. = acid equivalent

Limited data are available on the effects of technical or formulated glyphosate on the growth and reproduction of birds (**Table 3-10**). In an avian reproduction study evaluating the toxicity of technical glyphosate on mallard duck, significant decreases of 99%, 59%, and 127% in male body weight gain were observed at the low-, mid-, and high- test concentration, respectively (LOAEC = 501 mg a.e./kg; MRID 48876602). Treatment-related decreases in hatchling and 14-day body weights were observed at the highest and lowest test concentrations. There were no treatment-related effects on eggs laid, embryo viability, or eggshell thickness in this study. An additional qualitative study from the open literature, Kubena *et al*. 1981, reports effects of 50% reduction in male and female body weights for domesticated chickens (*Gallus gallus domesticus*) at glyphosate concentrations of 4,505 mg a.e./kg-diet. There are two additional registrant submitted studies conducted with technical glyphosate, where effects on growth or reproduction were not observed following exposure to either mallards or bobwhite quail up to concentrations of 830 mg a.e./kg-diet (MRID 00111953 and 00108207). In the mallard study (MRID 00111953), body weights were not examined by sex, rather an overall treatment group mean body weight was reported (5 pens each containing 5 males and 2 females). At 830 mg a.e./kg-diet, body weight gain decreased 25% compared to the control and one mortality was reported. An additional study on mallards reported no reproductive or body weight effects at 30 mg-kg diet, also reported by overall treatment group and not examined by sex (MRID 00113457). 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.

Table 3-10. Effects of Glyphosate on Avian Growth and Reproduction

Test Substance	Species	% a.i.	NOAEC mg a.e./kg diet[1]	LOAEC mg a.e./kg diet[1]	Effect	Reference
Technical Glyphosate	Mallard duck (*Anas platyrhynchos*)	96	<501	501	Male weight gain and offspring weight	MRID 48876602
	Mallard duck (*Anas platyrhynchos*)	83	830	-	25% reduction in weight gain at 830 mg a.e./kg-diet, not statistically significant, not separated by sex	MRID 00111953
	Bobwhite quail (*Colinus virginianus*)		830	-	No effects reported	MRID 00108207
	Mallard duck (*Anas platyrhynchos*)	90.4	30	-	No effects reported	MRID 00113457
Glyphosate isopropylamine	Chicken (*Gallus gallus domesticus*)	NR	608	6,080	50% reduction in male and female body weight by day 7	E162010 Kubena *et al.* 1981

1 a.e. = acid equivalent

3.3.2.2. Mammals

The available acute toxicity studies on the technical material indicate that glyphosate is practically non-toxic to mammals on an acute exposure basis with resulting LD_{50} values greater than the highest concentration tested up to 4,860 mg a.e./kg-bw (MRID 46760505).

Two 2-generation mammalian reproduction studies with the rat are available for glyphosate. In the first study, the parental/systemic NOAEL is 500 mg/kg/day in both sexes and the LOAEL is 1,500 mg/kg/day based on decreased body weight gain (MRID 41621501, 1990). The offspring NOAEL is 500 mg/kg/day in both sexes with a LOAEL of 1,500 mg/kg/day based on decreased body weight gain during lactation. 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 (MRID 48865101-48865105, 2007/2012), the parental/systemic NOAEL is 15,000 mg/kg-diet in both sexes (equivalent to 1,234/1,273 mg/kg/day in males/females, respectively) as parental toxicity and reproductive toxicity were not observed at any test level. The offspring 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.

3.3.2.3. Other Terrestrial Vertebrates

A study in the open literature evaluated the effects of technical glyphosate on terrestrial-phase amphibians, considered for qualitative use only because of limitations in study reporting (E162011). In an acute oral study by McComb *et al*. 2008 (E162011), the 96-hr LD_{50} value for field-collected (prior exposure history unknown) rough-skinned newts (*Taricha granulosa*) exposed to technical glyphosate was greater than 2,600 mg/kg-bw (unsure if adjusted to acid equivalents). This value is consistent with the endpoints for birds from studies conducted with technical glyphosate. Control mortality was not reported in the study.

As no additional data are available on glyphosate toxicity to reptiles in relevant units, the available toxicity data for birds are used as a surrogate for reptiles.

3.3.2.4. Honey Bees

Definitive mortality endpoints are not available for honey bees for technical glyphosate. However, available data point to low direct toxicity to honey bees. Mortality did not reach 50% in any of the available adult acute contact or oral studies for studies with technical glyphosate, technical glyphosate with an adjuvant, and glyphosate formulations (**Table 3-11**). Acute larval studies are not available. Some chronic larval data are available for a glyphosate formulation from a supplemental field colony-feeding study (Thompson *et al.* 2014).

At test termination in an acute contact study with technical glyphosate (LD_{50} >100 µg/bee), there was 38% mortality (27% after correcting for control mortality) at the limit dose (MRID 00026489) (**Table 3-11**). Acute contact and oral toxicity studies using technical glyphosate with an adjuvant (Agral 90), reported 48-hour LD_{50} values of >103 and >182 µg a.e./bee, respectively. No mortality or clinical signs of toxicity were observed (MRID 48876603).

Chronic toxicity data for adults is not available for technical glyphosate. A chronic oral study using a formulation (MON 0139, GLY-IPA, 46.1% a.e) showed no significant mortality, up to 179.9 µg a.e./bee/day (MRID 50603803). A NOAEC for effects on food consumption was established at 234 mg a.e./kg-diet, with a 57% effect at the LOAEC of 595 mg a.e./kg-diet. The corresponding food consumption LOAEL in terms of dietary dose is 9.5 µg a.e./bee/day.

A combined semi-field residue and colony-feeding study is also available (Thompson *et al*. 2014; also referenced in EFSA 2014 report). This study provides relevant information on 1) glyphosate residues in *Phacelia* nectar and pollen collected by honey bees after overspray application of a formulation at 1.92 lb a.e./A measured, and 2) oral toxicity of technical glyphosate to honey bee larvae in a colony feeding study. The authors further attempt to link the dose rates used in the feeding study to the level of larval exposure expected in the field, using the measured quantity of pollen and nectar returned to the hive during the residue study and prior literature on pollen and nectar consumption rates.

The highest test level evaluated in the effects portion of the study was 255 mg a.e./L measured, meant to be twice as high as the expected exposure from applications at 1.92 lb a.e./A. Evaluation of mortality/morbidity, appearance, larval weight and residue were conducted (16-day test duration). There was no significant effect on survival of eggs, young or old larvae or on larvae weight. Conclusions on adult mortality and other colony level impacts could not be drawn.

Acute and chronic laboratory-based toxicity data (*i.e.*, mortality and emergence data) for honey bee larvae are not available. The Thompson *et al*. 2014 colony feeding study is used to evaluate larval mortality for relevant exposure pathways and application rates.

Table 3-11. Honey Bee Toxicity Data

Test Type	Endpoint	Test Substance	Reference	Study Classification	Comments
Adult					
Acute Oral	48 hr LD_{50}: >100 µg/bee NOAEL: N.R.	TGAI (98.5%)	MRID 00026489	Acceptable	
	48 hr LD_{50}: >182 µg/bee NOAEL: 182	TGAI (97.6%) + Adjuvant (Agral 90)	MRID 48876603	Acceptable	No mortality or clinical signs of toxicity reported
Acute Contact	48 hr LD_{50}: >103 µg/bee NOAEL: 103	TGAI (97.6%) + Adjuvant (Agral 90)	MRID 48876603	Acceptable	No mortality or clinical signs of toxicity reported
	48 hr LD_{50}: >100 µg/bee NOAEL: N.R.	TGAI (98.5%)	MRID 00026489	Acceptable	27% mortality after correcting for control mortality at the limit dose
Chronic oral	LD_{50}: > 170 µg a.e./bee/day NOAEL: 170 µg a.e./bee/day	Formulation (MON 0139, GLY-IPA, 46.1% a.e.)	MRID 50603803	Acceptable	No statistically significant levels of mortality reported.
Larvae					
Not available					
Colony-level					
Semi-field residue (greenhouse) and field colony feeding	No significant effects on survival of eggs, young or old larvae or on larvae weight, up to highest dose level.	Formulation (MON5227, 30.68% a.e. as glyphosate IPA) for residue study; technical grade IPA salt of glyphosate for colony feeding (46.14% w/w glyphosate ae)	MRID 50603805, Thompson et al 2014	Supplemental	Stage 1. Residue analysis in pollen and nectar of flowering *Phacelia tanacetifolia* treated with 1.92 lb a.e./A formulation; Stage 2. Colonies supplied sucrose solution spiked with TGAI.

3.3.2.5. Toxicity Data on Other Terrestrial Invertebrates

An oral and contact study conducted with bumblebees (*Bombus terrestris*) reported no treatment-related mortality or behavioral abnormalities up to the highest dose of a glyphosate formulation (MON0139), resulting in a non-definitive LD_{50} and NOAEC that were both > 412 µg a.e./org (MRID 50603801).

Additional toxicity studies with other types of terrestrial invertebrates (*i.e.*, predatory mites, earthworms, parasitic wasps) are also available, with generally no effects reported up to the highest dose tested. In a study with a predatory mite, the 7-day LD_{50} value was reported as 1,200 g a.e./ha (1.1 lb/A) for a formulated product (MRID 45767105). This study also reported an 85% reduction in eggs laid at 216 g a.e./ha (0.193 lb a.e./A).

Soil exposure tests have been conducted for annelids and several arthropod species. In these studies, 50% mortality was not reached, so an LC_{50} could not be established. An acute toxicity test with technical glyphosate showed no mortality effects up to the highest level tested, resulting in a non-definitive LC_{50} > 472.8 mg a.e/kg soil (MRID 50603804).

AMPA toxicity:
Sublethal effects of a major glyphosate degradate, AMPA, have been reported for earthworms. 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.

3.3.2.6. Effects in Plants

Enlist Duo is a mixture of 2,4-D choline salt and glyphosate dimethylammonium (glyphosate DMA) salt (formulation GF-2726). The EPA required standard vegetative vigor (MRID 49903202) and seedling emergence (MRID 49903201) studies (Guidelines 850.4100 and 4150) with the Enlist Duo formulation for a suite of commonly tested plant species for which existing single-herbicide testing indicated plant sensitivity to 2,4-D or glyphosate. Surpassing the normal requirement of ten plant species, the Enlist Duo testing spanned fifteen commonly tested monocot and dicot crop species: buckwheat, cabbage, corn, cucumber, mustard, oat, oilseed rape, onion, radish, sorghum, soybean, sugarbeet, sunflower, tomato, and wheat. In addition, the Agency required and received vegetative vigor (MRID 49903203) and seedling emergence (MRID 49903204) testing with three weed species identified in the patent data set as having the potential for exhibiting enhanced sensitivity to the 2,4-D choline/glyphosate combination in excess of simple addition of individual active ingredient effects. These species included lambsquarters (*Chenopodium album*), horseweed (*Conyza canadensis*), and quackgrass (*Agropyron repens*). EPA established plant endpoints for risk assessment using all of the available Enlist formulation data.

Because this includes the formulation including both active ingredients, EPA addressed all concerns regarding potential synergistic effects on terrestrial plant height, weight and survival from the combined exposure of the two active ingredients.

Table 3-12 reports the sublethal effects endpoints for the most sensitive monocot and dicot plants from commonly tested species for vegetative vigor with the Enlist Duo formulation. The most sensitive monocot and dicot species based on comparison of the EC_{25} are selected to serve as the effects thresholds for the terrestrial plant risk assessment and the effects of any risk mitigation buffers.

The most sensitive Enlist Duo endpoints are similar to endpoints established during registration review for formulations with glyphosate as the sole active ingredient (USEPA, 2015). For comparison, the most sensitive endpoint used in the 2015 PRA was 0.07 lb a.e./A, derived from cucumber (*Cucumis sativus*), which is roughly 2x higher than the cucumber endpoint in the Enlist Duo test (0.037 lb a.e./A). In the glyphosate Draft Biological Evaluation (BE), a species sensitivity distribution (SSD) was generated using available registrant-submitted and open literature studies on a variety of plant species (USEPA, 2020b). The fifth percentile (HC_{05}) from the SSD was 0.02 lb a.e./A, which is similar to the most sensitive endpoint from the Enlist Duo study. As expected for glyphosate, the most sensitive endpoint in each case is from a dicot.

Table 3-12. Vegetative Vigor Endpoints, Adjusted for Glyphosate Acid Equivalents (lb a.e./A)[1]

Species	Endpoint	NO EC	EC_{25}/IC_{25}
Buckwheat	Dry Weight	0.0078	0.021
Cabbage	Dry Weight	0.0019	0.032
Corn	Dry Weight	0.13	0.11
Cucumber	Dry Weight	0.0039	0.037
Mustard	Height	0.0039	0.011
Oat	Dry Weight	0.063	0.21
Oilseed Rape	Dry Weight	0.016	0.040
Onion	Dry Weight	0.26	0.22
Radish	Dry Weight	0.0078	0.017
Sorghum	Height	0.13	0.22
Soybean	Dry Weight	0.016	0.10
Sugarbeet	Dry Weight	0.0078	0.023
Sunflower	Dry Weight	0.0078	0.023
Tomato	**Dry Weight**	**0.0078**	**0.010**
Wheat	**Dry Weight**	**0.031**	**0.051**

[1]Bolded values used as endpoints

Milkweed is listed as a target species on the Enlist Duo label. Information on toxicity to milkweed (*Asclepias* sp.) from glyphosate exposure is available in the open literature. In a paper by Egan *et al*., 2014, the 28-day ED_{25} value (effective dose), based on biomass, for common milkweed from foliar exposure to Roundup Powermax (540 g a.e./L) in greenhouse experiments was reported as 141 g a.e./ha (0.126 lb a.e./A, 95% CI of 0.036-0.29). In another study by White and Boutin, 2007, a 28-d IC_{25} value of 46 g a.i./ha (0.04 lb a.i./A, 95% CI of 0.03-0.07), based on biomass) was reported for milkweed after exposure to Round-Up Original. The adsorption, translocation, and metabolism of glyphosate in common milkweed were also examined and results indicated that an insignificant amount of metabolism of glyphosate occurred after 20 days in both milkweed roots and leaves above the treated leaves (Wyrill *et al*. 1976). The reported 28-d $IC(ED)_{25}$ values for milkweed reported by White and Boutin (2007) are similar to the most sensitive vegetative vigor terrestrial plant IC_{25} value used in the 2015 PRA (0.074 lb a.e./A; MRID 44320636), and less sensitive than the IC_{25} for Enlist Duo (0.010 lb a.e./A based on tomato dry weight).

Studies on formulations containing glyphosate as the sole active ingredient show no effects on seedling emergence greater than 25% (IC_{25} > 5 lb a.e./A; MRID 40159301; MRID 44125712; MRID 44320635). Additionally, NOAECs were reported as the highest concentration tested or were not reported. This is expected, given the mode of action of glyphosate. The reported effects of Enlist Duo on seedling emergence are therefore likely to be the result of 2,4-D exposure rather than glyphosate.

3.4. Risk Estimation and Characterization

The screening ecological risk assessment generates a series of risk quotients (RQs) for broad taxonomic groups (e.g., mammals, birds, fish, etc.) that are the ratio of estimated exposures to acute and chronic effects endpoints (USEPA, 2004). EPA then compares these RQs to EPA established levels of concern (LOCs) to determine if risks to any taxonomic group are of concern. The LOCs address risks for both acute and chronic effects. Acute effects LOCs range from 0.05 for listed aquatic animals to 0.5 for aquatic non-listed animal species and 0.1 to 0.5 for terrestrial animals for listed and non-listed species. The LOC for chronic effects for all animal taxa (listed and non-listed) is 1.0. Plant risks are handled in a similar manner, but with different toxicity thresholds (NOAEC/EC_{05} and EC_{25}, respectively) used in RQ calculation for listed and non-listed species and a LOC of 1 is used to interpret the RQ. When a given taxonomic RQ exceeds either the acute or chronic LOC for a taxonomic group, a concern for direct toxic effects is identified for that particular taxon. If RQs fall below the LOC for non-listed species, EPA makes a finding of no risk. If the RQs fall below the LOC for listed species, EPA concludes that effects are not expected for that taxon and no further refinement is necessary to complete an Effects Determination for species within that taxon.

In this assessment, EPA presented the comparison of RQs with both the non-listed and the listed LOCs. With the exception of plants, the non-listed species comparisons are intended to communicate risks to inform the findings under FIFRA. Non-listed species RQs are also used to evaluate potential indirect effects to listed species due to impacts to their prey, pollinators, habitat and/or dispersal. In cases where RQs indicate a potential for direct or indirect effects to taxonomic groups, the results are carried through to the Effects Determinations in **Section 4**. In this section, EPA used the listed-species RQ:LOC comparisons to discriminate taxonomic groups requiring no further analyses from those where a species-specific risk assessment is needed to complete an Effects Determination. EPA's ecological risk assessment methods for endangered species that are used to make effects determinations are conducted using a tiered approach, beginning with highly conservative assumptions. When risks are not identified to listed species based on highly conservative assumptions, then EPA is confident that no discernable effects to species will occur. If risks to listed species are identified, then the assessment proceeds to a more refined tier that involves a spatially explicit analyses, incorporation of species life history, or other factors to describe potential effects on listed species and their critical habitat.

3.4.1. Aquatic Organism Risk Characterization

The highest EEC from **Table 3-3** was the 1-in-10-year daily average for corn applications, 14.6 µg/L in the standard pond.

The lowest acute aquatic toxicity endpoint among all aquatic animal taxa, both vertebrate and invertebrate, is 35.3 mg a.e./L for amphipods (USEPA, 2015), which results in an acute RQ < 0.01. Risks to listed or non-listed aquatic organisms are expected to be low.

The lowest chronic aquatic toxicity endpoint among all animal taxa, including both vertebrates and invertebrates, is NOAEC = 6.11 mg a.e./L for amphipods (ACR) (USEPA, 2015). The highest RQ = 0.15 for chronic risks, which is lower than the chronic LOC of 1. Both acute and chronic risks to listed or non-listed species are expected to be low.

For aquatic plants, no RQs exceed the Agency LOC (1.0 for both listed and non-listed aquatic plants). Tier I RQs are <0.01 for both vascular and non-vascular aquatic plants, using the technical glyphosate endpoints for *Anabaena flos-aquae* (11.4 mg a.e./L) and *Lemna gibba* (11.9 mg a.e./L).

3.4.1.1. Potential Off-Field Extent of Risk to Listed Non-Vascular Aquatic Plants from Spray Drift of Glyphosate Residues

Risk from runoff and/or spray drift to listed or non-listed aquatic plant species expected to be low, due to low toxicity.

3.4.2. Terrestrial Vertebrate Risk Characterization

3.4.2.1. On-Field Dietary

Terrestrial wildlife exposure estimates for birds and mammals typically focus on the dietary exposure pathway (USEPA, 2004). EPA considered this route of exposure as well as potential exposures to spray droplet inhalation or vapor-phase exposure. EPA based potential dietary exposure for terrestrial vertebrate wildlife on consumption of 2,4-D residues on food items following spray (foliar) applications. EPA calculated EECs for birds[22] and mammals from consumption of dietary items on the treated field using T-REX v.1.5.2[23].

EPA calculated exposures to bees using the Bee-REX v1.0 tool, while for other terrestrial invertebrates EPA estimated the on-field screening exposure through application of the T-REX model, estimating a pesticide concentration in insects following exposure to direct pesticide spray or any residues ingested from exposed diet. The risk assessment sections on bees and other terrestrial invertebrates (**Sections 3.3.2.4** and **3.3.2.5**) describe the exposure assessment and risk characterization for this taxon in more detail.

EPA derived exposure estimates for terrestrial animals assumed to be in an area exposed to spray drift using the T-REX (Terrestrial Residue EXposure model) model (version 1.5.2). This model incorporates the Kenaga nomograph, as modified by Fletcher *et al.* (1994), which is based on a large set of actual field residue data. The upper limit values from the nomograph represent an approximation of the highest residue value observed in the data set (Hoerger and Kenaga 1972). EPA used the glyphosate-specific foliar dissipation half-life of 12 days for risk estimation previously established in the 2015 PRA (USEPA 2015). EPA assumed a maximum single application rate of 1.0 lb a.e./A, 3 applications and a 12-day application interval to estimate terrestrial exposures of glyphosate. The dose- and dietary-based EECs (upper bound Kenaga) on a variety of food items from the use of glyphosate applied at the maximum labeled rates is provided below in **Table 3-13**, along with the full T-REX inputs and output. Consideration is given to different types of feeding strategies for mammal and birds, including herbivores, insectivores and granivores. EPA estimated dose-based exposures for three weight classes of birds (20 g, 100 g, and 1,000 g) and three weight classes of mammals (15 g, 35 g, and 1,000 g). As the use rates are the same

[22] Birds are also used as a proxy for reptiles and terrestrial-phase amphibians.
[23] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment#t-rex

for Enlist Duo products applied to soybean, corn, and cotton, EPA predicted on-field residues to be identical.

Table 3-13. Summary of Dietary (mg a.e./kg-diet) and Dose-Based EECs (mg a.e./kg-bw) for Terrestrial Vertebrates from Labeled Uses of Glyphosate in Enlist Duo® Application (T-REX v.1.5.2, Upper-Bound Kenaga)

Food Type	Dietary-Based EEC (mg/kg-diet)	Dose-Based EEC (mg/kg-body weight)					
		Birds			Mammals		
		Small (20 g)	Medium (100 g)	Large (1000 g)	Small (15 g)	Medium (35 g)	Large (1000 g)
Glyphosate-tolerant soybean, cotton, and corn max annual ground (3 x 1 lb a.e./A, 12-d interval)							
Short grass	420	478	272	122	400	277	64.2
Tall grass	193	219	125	56.0	184	127	29.4
Broadleaf plants/small insects	236	269	153	68.7	225	156	36.1
Fruits/pods/seeds (dietary only)	26.3	29.9	17.1	7.63	25.0	17.3	4.01
Arthropods	165	187	107	47.8	157	108	25.1
Seeds (granivore)[1]	N/A	6.64	3.79	1.70	5.56	3.84	0.89

[1] Seeds presented separately for dose – based EECs due to difference in food intake of granivores compared with herbivores and insectivores. This difference reflects the difference in the assumed mass fraction of water in their diets.

Acute risk to birds, reptiles, and terrestrial phase amphibians

The highest dose-based EEC (20g birds feeding on short grass, upper bound Kenaga, EEC = 478 mg ae/kg-bw) is less than the doses with no mortality in the bobwhite quail studies (3,196.3 mg a.e./kg-diet) and no mortality or regurgitation in the canary study (2,000 mg a.e./kg-bw). For dietary exposure, the highest EEC (420 mg/kg-diet) is an order of magnitude less than the highest concentration tested (4971 mg a.e./kg-diet) for the bobwhite quail and mallard duck, which both reported no treatment-related mortality. Based on this analysis, EPA anticipated no acute risks from exposure to glyphosate for listed or non-listed species of birds, reptiles, or terrestrial-phase amphibians.

Chronic risk to birds, reptiles, and terrestrial-phase amphibians

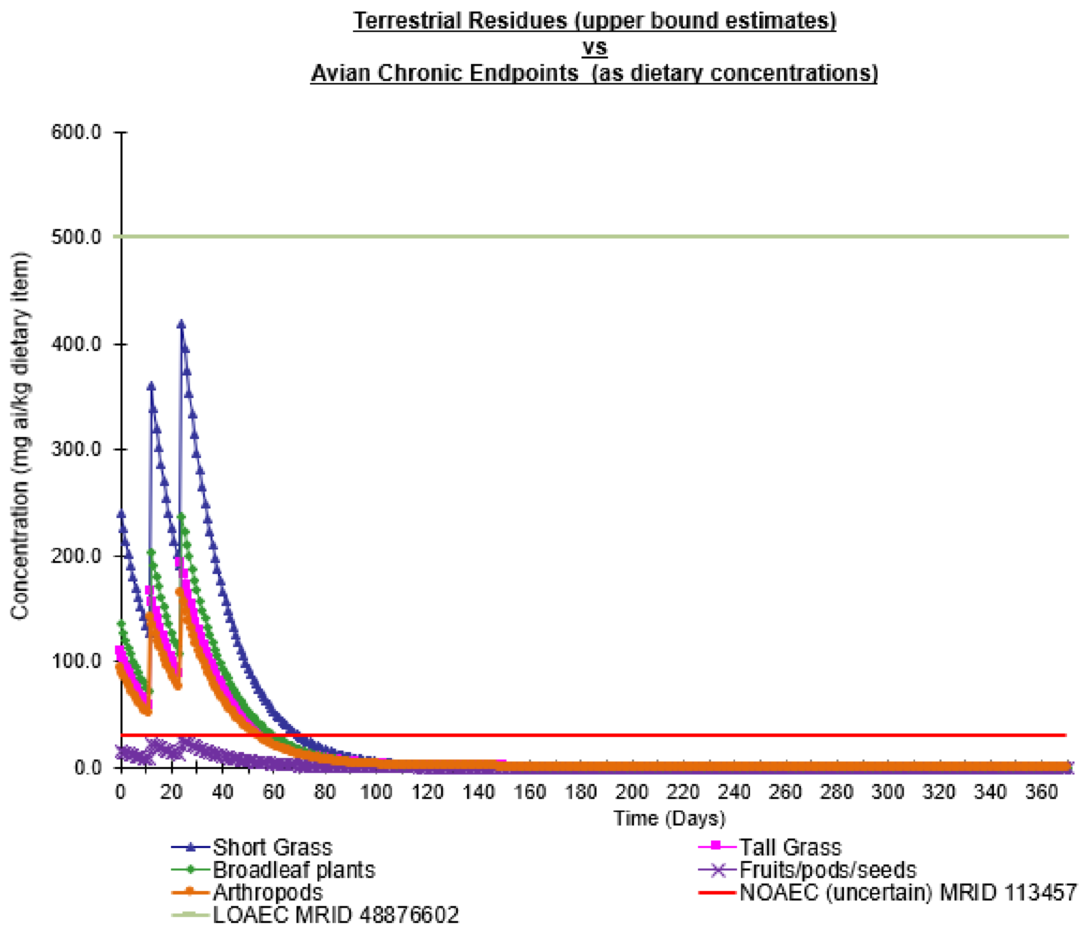
Peak EEC values are lower than the available chronic NOAECs for mortality and reproduction and risk is not expected either on- or off-field for those parameters.

However, chronic risk to birds, amphibians, and reptiles is expected on field due to potential effects on growth (weight gain), with some uncertainty due to the available toxicity data. The most sensitive endpoint in an avian reproduction study reported effects at all tested concentrations, so chronic avian RQs are not presented. Instead, EECs are compared to the non-definitive (<) LOAEC.

As discussed in **Section 3.3.2.1**, effects on male mallard weight gain were seen at all tested concentrations, with significant decreases of 99%, 59%, and 127% observed at the low-, mid-, and high-test concentration, respectively (LOAEC = 501 mg a.e./kg; MRID 48876602). A supplemental study on mallards showed no reproductive or body weight effects up to 30 mg/kg-diet (MRID 00113457). This study deviated from current EPA protocols and did not separate data by sex, and so should be interpreted with caution. However, it provides some information for examining effects at low dietary concentrations.

Peak EECs are lower than the level at which effects were reported for male mallard duck weight gain. Without a definitive NOAEC, risk is assumed. To explore the uncertainty related to making this risk call with low EECs**, Figure 3-6** presents the TREX upper-bound Kenaga EECs over time, relative to the sublethal LOAEC (501 mg a.e./kg-diet; MRID 48876602) and the lowest available NOAEC at which no effects, significant or otherwise, were reported for bodyweight (MRID 113457).

Figure 3-6 Upper-Bound Kenaga Dietary EECs Over Time



The threshold for effects to weight gain is not known but is lower than 501 mg a.e./kg-diet. Of the dietary items modeled, chronic risk is most likely for animals feeding on short grass during the first 70-80 days after the first application. Peak EECs for granivorous birds are consistently lower than the uncertain NOAEC described in MRID 00113457, and chronic risk is not considered likely.

Acute and chronic risk to mammals

Definitive acute toxicity endpoints were not available for mammals, so RQs are not reported. Numerous studies have been conducted with no mortalities at rates up to 4,860 mg a.e./kg-bw for the lab rat (MRID 46760505). The highest mammalian EEC is 400 mg a.e./kg-bw (for a 15g mammal feeding on short grass), which is more than 10x less than the highest tested concentration with no mortality.

Two 2-generation mammalian reproduction studies with the rat are available. The most sensitive endpoint was the offspring NOAEL of 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. Chronic RQ values range from 0.01 to 0.08 (**Table 3-14**). Overall, based on these analyses, acute or chronic mammal risk is not expected from glyphosate use.

Table 3-14. Chronic RQ Values for Mammals Exposed to Glyphosate Residues from the Use of Enlist Duo (T-REX v. 1.5.2, Upper Bound Kenaga)

Food Type	Chronic Dietary-Based RQ
	NOAEC = 2720 mg a.e./kg-diet[1]
Short grass	0.08
Tall grass	0.04
Broadleaf plants	0.05
Fruits/pods/seeds	0.01
Arthropods	0.03

[1] The chronic LOC is 1.0. The endpoints listed in the table are the endpoint used to calculate the RQ. Chronic diet concentration NOAEC is based upon NOAEL and the daily diet consumption of laboratory rat, estimated in T-REX.

3.4.2.2. Off-Field Risks from Spray Drift of Glyphosate Residues

For any terrestrial vertebrate results where LOCs were exceeded, off-field risks screening, through route of off-site spray drift deposition, is based on empirical spray drift data provided in MRID 48844001. EPA found that spray drift was a biphasic decay function with distance, where the predominant reduction in deposition occurred 0-25 ft from the downwind edge of the application zone reaching a fraction of application at 30 ft of 0.00167. Therefore, based on the 30 ft in-field spray drift buffer requirement on the label, off-field spray drift deposition of glyphosate would be equal to or less than 0.00167 times the application rate.

The analysis above indicated no on-field acute or chronic risk to mammals or acute risk to birds from exposure to glyphosate residues in dietary items. Potential chronic risk to birds, reptiles, and terrestrial phase amphibians was identified based on reduced weight gain from on-field exposure to glyphosate residues in dietary items. Using the established drift fraction (0.00167), the peak EEC at the edge of the field for short grass is 420 mg a.e./kg-diet x 0.00167 = 0.701 mg a.e./kg-diet. This level of exposure is not expected to result in effects on weight gain and chronic risk is therefore not expected off-field.

3.4.3. Honey Bee Risk Characterization

The uses being assessed for glyphosate include Enlist corn, soybean, and cotton crops, which produce pollen and/or nectar attractive to bee species (USDA, 2017). Bees may also consume both pollen and nectar from glyphosate-exposed flowering weed species. The pollen and nectar may contain glyphosate residues either from direct spray or resulting from systemic uptake of glyphosate residues. Bees (both *Apis* and non-*Apis*) may potentially be exposed on or off-the field to direct sprays of glyphosate to Enlist corn, cotton or soybean plants from subsequent deposition on attractive floral resources.

3.4.3.1. Bee Tier I Exposure Estimates

EPA estimated contact and dietary exposure separately using different approaches specific to different application methods. The Bee-REX model (Version 1.0) calculates default (*i.e*., high end, yet reasonably conservative) EECs for contact and dietary routes of exposure for foliar, soil, and seed treatment applications. See **Appendix J** for a sample output from Bee-REX for glyphosate. Additional information on bee-related exposure estimates and the calculation of risk estimates in Bee-Rex can be found in the *Guidance for Assessing Pesticide Risks to Bees* (USEPA *et al.*, 2014).

3.4.3.2. Tier I Risk Estimation (Contact Exposure)

3.4.3.2.1. On-Field Risk

Since bees are potentially exposed to glyphosate through use on corn, soybean and cotton plants and both on and off the treated field, the next step in the risk assessment process is to conduct a Tier 1 risk assessment. By design, the Tier 1 assessment begins with (high-end) model-generated (foliar and soil treatments) or default (seed treatments) estimates of exposure via contact and oral routes. For contact exposure, only the adult (forager and drones) life stage is considered since this is the relevant caste of honey bees (*i.e*., since other bees are in-hive, the presumption is that they would not be subject to contact exposure). Effects are defined by laboratory exposures to groups of individual bees (which serve as surrogates for solitary non-*Apis* bees and individual social non-*Apis* bees).

Definitive mortality endpoints are not available for honey bee acute contact exposure, as mortality did not reach 50% in any available studies. At test termination in the acute contact study with technical glyphosate (LD_{50} >100 µg a.e./bee), there was 38% mortality (27% after correcting for control mortality) at the limit dose (MRID 00026489). The BeeRex estimated contact dose is 2.7 ug a.e./bee, so risk is not expected.

3.4.3.3. Tier I Risk Estimation (Oral Exposure)

3.4.3.3.1. On-Field Risk

For oral exposure, EPA considered the caste of bees with the greatest oral exposure (foraging adults) in the Tier I assessment. If risks are identified, then other factors can be considered for refining the Tier I risk estimates. These factors include other castes of bees and any available information on empirical residues in pollen and nectar applicable to the crops of interest.

On-field exposure through nectar and pollen are expected for soybean and cotton. On-field honey bee exposure from corn is expected only through pollen, via systemic uptake and transport of glyphosate from pre-bloom application. Systemic exposure is not expected to exceed direct application at bloom; therefore, the at-bloom analysis is considered protective of corn uses.

Definitive mortality endpoints are not available for honey bee adult acute oral exposure, as mortality did not reach 50% in any of the available studies, which tested up to 187 ug a.e./bee. The available chronic adult toxicity test was conducted with a formulated product; tests using technical glyphosate are not available. The adult chronic toxicity test shows no statistically significant mortality up to the highest level tested (170 µg a.e./bee/day). The maximum EEC for an adult worker foraging on both nectar and pollen is 32 ug a.e./bee. Acute and chronic risk is not expected for adult honey bees.

Acute and chronic laboratory-based toxicity data (*i.e.*, mortality and emergence data) for toxicity to honey bee larvae are not available. Instead, EECs are compared to the values tested in a supplemental semi-field residue and colony feeding study that tested a different glyphosate formulation (Thompson *et al*. 2014).

The semi-field study measured maximum residues in *Phacelia tanacetifolia* nectar and pollen for rates that exceed the proposed Enlist Duo rate (Thompson *et al*. 2014). In a complementary colony-feeding study, honey bee colonies were fed treated sucrose (technical GLY-IPA) with test concentrations based on the mean residue concentration up to 255 mg a.e./L, which is intended to represent double the estimated residue concentration from the residue study. There was no significant effect on survival of eggs, young or old larvae, or on larvae weight (Thompson *et al*. 2014). These test levels exceed the EEC calculated in Bee-REX (110 mg a.e./kg-diet), so risks to larval honey bees are considered low. This study does not track other measures of colony strength (e.g., adult mortality). This colony-level data provides relevant information on the potential effects of glyphosate on honey bee larvae, which are tended and provisioned by adult honey bees, but may underestimate risk to less social terrestrial invertebrates (*e.g.* solitary bees)

3.4.3.3.2. Off-Field Risk

On-field risk is expected to be low, therefore off-field risk is not expected.

3.4.4. Other Terrestrial Invertebrates

3.4.4.1. Use of Honey Bee Toxicity Data as a Surrogate for Non-Apis Terrestrial Invertebrate Species

A search of ECOTOX[24] for toxicity information on all forms of glyphosate was conducted in 2020, in support of the Draft Biological Evaluation for glyphosate (USEPA, 2020b). Potential endpoints from that search have not been evaluated for FIFRA use. Therefore, honey bee effects data were relied upon for this evaluation of impact to other terrestrial invertebrate species (*e.g.,* beetles, butterflies, etc.) and is

[24] The ECOTOXicology Knowledgebase (ECOTOX) https://cfpub.epa.gov/ecotox/help.cfm

based on the surrogate species approach, whereby the effects endpoints established for honey bees are used to represent the sensitivity of other invertebrate species. To use this data to evaluate risks to other terrestrial invertebrates, EPA converted the dose-based endpoints to concentration-based endpoints that it then compared to potential exposures based on T-REX exposures for arthropods and potential dietary items. As the acute data for honey bees were non-definitive (*i.e.,* no mortalities were observed at the highest tested doses), EPA considered the chronic honey bee endpoints for this comparison (**Section 3.3.2.4**). The chronic analysis presented is considered protective of any potential acute effects.

3.4.4.2. Monarch Butterfly Risk Characterization

EPA considered the potential risks to monarch butterflies (*Danaus plexippus* L.) from glyphosate application, including both direct effects to monarchs and indirect effects resulting from effects on host plants and nectar resources. The monarch butterfly is an iconic species well known for its multigenerational migration between summer breeding grounds, found in the U.S. and Canada, and overwintering habitat in central Mexico. Additional populations that are thought to be predominantly non-migratory are established in several states and territories (e.g., Hawai'i, Puerto Rico, Florida), as well as abroad (USFWS, 2020). The range of adult monarchs in the United States is therefore broad and includes landscapes dominated by agricultural land use.

Adult monarchs are generalist nectar feeders that visit a variety of flowering plants. For reproduction, however, monarchs specialize on a suite of species in the genus *Asclepius*, collectively known as milkweeds, and a related species, *Cynanchum laeve* (Pocius *et al*., 2017).

In this risk assessment, milkweed plants are functionally divided into two separate categories relative to the labeled use application area: plants that are "on-field" and "off-field." Potential effects to monarchs are considered in these on-field and off-field areas through direct exposures through diet. Since no glyphosate toxicity data for adult or larval monarchs are available, the honey bee toxicity data are relied on as a surrogate for the risk estimation. Additionally, potential indirect effects of reduced on-field milkweed availability are discussed in **Section 3.5.3.1**.

3.4.4.2.1. On-Field Risks to Monarch Butterflies

Milkweed is listed as a target weed on the Enlist Duo product label. According to the label, direct applications of the product to "suppress" milkweed are recommended when milkweed plants are in the early bud stage of growth. In terms of potential monarch exposure, monarch larvae may be present on target milkweed plants at the time of Enlist Duo application, which would result in on-field monarch exposure. Therefore, EPA conducted an ecological risk assessment for monarch larvae that are actively feeding on "on-field" milkweed. There could also be an exposure to adults that are laying eggs; however, it is more likely that the larval life stage will be present at the time of an application. Furthermore, there is a potential for adult foraging of nectar on-field from flowering plants, including milkweed.

Adult Monarch Exposure and Risk Characterization

EFED used the Bee-REX (v1.0) model to calculate exposures for foraging bees with pollen and nectar as food sources. Bee-REX relies on a single application of pesticide and the maximum single application rate for Enlist Duo as glyphosate is 1.0 lb a.i./ acre. Bee-REX returns an estimated concentration of glyphosate in pollen and nectar of 110 mg a.e./kg (for adult monarchs only nectar is applicable). Bee-REX uses the pollen and nectar residue estimates to derive a dietary dose, which is compared to a dose-based effects endpoint. However, the exposure dose is calibrated to the body weight and ingestion rate of honey bees, not monarch butterflies. Since a weight and an ingestion rate are unavailable for the adult monarch butterfly, comparison to honey bee consumption rates is not possible. In addition, definitive endpoints are not available for honey bees, so RQs could not be calculated. Consequently, the Bee-REX nectar residue estimates are compared directly to the highest dietary concentration at which there were no mortalities reported in the available honey bee studies (9,450 mg a.e./kg-diet; MRID 50603803). The expected dietary exposure is over an order of magnitude less than the test level at which no mortalities were reported.

Risk Conclusion for Dietary Exposure Risks to Adult Monarch Butterfly

There are several uncertainties in using the estimated toxicity to honey bees to be representative of toxicity to monarchs, including both uncertainty from using individual honey bee toxicity as a surrogate and from using the results of a study conducted on a different formulation. Based on the available information, considering the totality of the evidence pointing toward relatively low direct toxicity to terrestrial invertebrates through dietary exposure, risk to adult monarchs nectaring on treated plants is expected to be low.

Larval Monarch Exposure and Risk Characterization

Dietary glyphosate exposure is expected between the time of application and milkweed senescence. Exposure time may be extended in glyphosate resistant populations of milkweed. To account for exposure based solely on consumption of vegetative matter (treated milkweed) by monarch larvae, the T-REX broadleaf plant EECs (236 mg/kg) were used. Toxicity information for monarch caterpillars is not available and no laboratory data on acute or chronic toxicity to honey bee larvae are available to serve as surrogate endpoints. Thompson *et al.* 2014 tested up to 301 mg a.e./kg diet with no larval effects reported for honey bees. Use of this study introduces some uncertainty due to the nature of the available test, since honey bee larvae are tended by adults and monarch caterpillars are not.

Based on the available data showing no effects on honey bee larvae at exposure levels that were designed to mimic application rates similar to the application rates of glyphosate in Enlist Duo, and which exceed the glyphosate EECs from the Enlist Duo application rate, plus the expectation of reduced dietary exposure due to milkweed senescence, on-field risk to larval monarchs from direct effects is expected to be low.

3.4.4.2.2. Off-Field Risks to Monarch Butterflies

Because on-field direct risks to monarchs are expected to be low, off-field risks are likewise expected to be low.

3.4.4.3. Other Non-Apis Terrestrial Invertebrate Risk Characterization

EPA conducted screening estimations of terrestrial invertebrate exposure using two methods. The first method involves a direct comparison of insect residues in exposed animals (exposure originating from contact with the pesticide incidentally and by impingement of spray as well as any consumption of contaminated diet). EPA compared this estimate to the tested honey bee effects endpoints expressed in terms of mass of pesticide /mass of exposed insect. The second route of exposure is dietary exposure expressed as a concentration of pesticide in vegetation receiving pesticide application. EPA compared this second exposure estimation to the effects endpoint expressed on a mass of pesticide/mass of diet basis. The exposure estimates for any terrestrial invertebrate warranting more refined estimation methods (where screening estimates and available biological data for a species indicate a potential concern) are included in the individual species effects determinations.

3.4.4.3.1. On-Field Exposure to Terrestrial Invertebrates

EPA estimated on-field screening exposure for terrestrial invertebrates through the application of the T-REX model (v 1.5.2), as described in **Section 3.4.4.3**, which estimates a pesticide concentration in insects following exposure to direct pesticide spray and any residues ingested from diet also receiving direct spray. EPA estimated residues using three 1 lbs a.e./A applications with a 12-day retreatment interval between each. Dietary EECs were based on the T-REX predictions for the most conservative vegetation (short grass), yielding a diet concentration EEC of 420 mg/kg-diet, and whole terrestrial arthropod dose estimates were based on T-REX predicted EECs for terrestrial arthropods yielding an estimated dose 164.5 mg/kg-bw.

3.4.4.3.2. Off-Field Exposure to Terrestrial Invertebrates

For any terrestrial invertebrate results where LOCs were exceeded, off-field risks screening, through route of off-site spray drift deposition, draws from the methods applied in the 2016 effects determination which is based on empirical spray drift data provided in MRID 48844001. EPA found that spray drift was a biphasic decay function with distance, where the predominant reduction in deposition occurred 0-25 ft from the downwind edge of the application zone reaching a fraction of application at 25 ft of 0.001075. This phase of the function was then followed by a much shallower decay function beyond 25 feet from the edge. Therefore, any off-field spray drift deposition of glyphosate above 0.001075 times the application rate occurs at a distance closer than 25 ft from the field edge and any off-field spray drift deposition lower than 0.001075 times the application rate occurs at a distance further than 25 ft from the edge.

3.4.4.4. On-Field Risk Evaluation for Non-Apis Terrestrial Invertebrates

Definitive endpoints for TGAI toxicity are not available, so the following analyses compare the highest test level from the adult acute and chronic oral tests (182 ug a.e/bee and 3,666 mg a.e./kg-diet, respectively) at which no significant mortality was reported.

The maximum dietary EEC exposure estimate from T-Rex (420 mg a.e./kg-diet) is lower than the dietary levels tested. Both acute and chronic risks are expected to be low for adults. Use of the available honey bee data results in low risk under all scenarios modeled for non-bee terrestrial invertebrates.

Additional toxicity studies with other types of terrestrial invertebrates (*i.e.*, predatory mites, earthworms, parasitic wasps) are also available. A study on technical glyphosate toxicity to earthworms reported reduced biomass and fecundity noted in Earthworms (*Eisenia fetida*) at the LOAEL, 5,000 mg a.e./kg soil (E170666). No mortality was reported. In a study with a predatory mite, the contact toxicity 7-day LD_{50} value was reported as 1,200 g a.e./ha (1.1 lb a.e./A) for a formulation (MRID 45767105). The single application rate is lower than the contact LD_{50} for the predatory mite, so risk is not expected.

3.4.4.5. Off-Field Risk Evaluation for Non-Apis Terrestrial Invertebrates

No risks are identified on-field; therefore, no risks are expected off-field.

3.5. Plant Risk Assessment

3.5.1. Summary of the Risk Assessment Approach for Glyphosate

EPA considers reduction in growth, survival, and reproduction as regulatory endpoints. In this assessment, EPA evaluated available studies to determine the appropriate in-field setbacks to address the potential for off-field effects to non-target organisms.

For estimating environmental exposure, EPA relied upon the Plant Assessment Tool (PAT) v. 1.0. PAT is a mechanistic model that incorporates fate (e.g., degradation) and transport (e.g., runoff) data that are typically available for conventional pesticides to estimate pesticide concentrations in terrestrial, wetland, and aquatic plant habitats. For terrestrial plants, EPA modeled runoff and erosion using PRZM and spray drift using AgDRIFT or user-defined deposition values. The model uses a mixing cell approach to represent water within the active root zone area of soil, and accounts for flow through the terrestrial plant exposure zone (TPEZ) caused by both treated field runoff and direct precipitation onto the TPEZ. Pesticide loss from the TPEZ occur from transport (*i.e.*, washout and infiltration below the active root zone) and degradation. EPA modeled wetlands using PRZM/VVWM, which are then processed in PAT to estimate aquatic (mass per volume of water) and terrestrial (mass per area) concentrations. EPA modeled aquatic plants exposure using the PRZM/VVWM models and the standard farm pond.

3.5.2. Spray Drift Control Measures Impacts to Off-Field Plant Risk

EPA accounted for the mandatory spray drift control measures of wind speed and inversion limits, requirements for spray nozzles, and boom height limits by the distance to plant effect predictions presented in the off-field analysis with PAT. PAT allows for the input of specific drift deposition curves, and in the case of these products, the specific drift curves used for establishing the maximum drift thresholds for the product and any tank mix with the product. The requirements of the label describe the limitations of the tank mix combinations and tested spray nozzles to prevent the potential for off field risks to plants as a result of spray drift when combined with the 30 ft setback requirement such that the edge of the downwind field when plants are present adjacent to the field should result in a fraction of 0.00167 of the applied rate (0.00167 lbs a.e./A). This rate is below the estimated NOAEC concentration in the tested species (lowest NOAEC = 0.0019 lbs a.e./A for cabbage), therefore risks from spray drift are limited to the field.

3.5.3. Terrestrial Plant Exposure Zone (TPEZ): Runoff and Spray Drift from a Treated Field Deposited onto a Non-Target Terrestrial (Upland) Plant Area Next to the Field

The TPEZ is intended to represent a non-target terrestrial (non-inundated) plant community immediately adjacent to a treated field, which is exposed to pesticide via sheet flow[25] and spray drift from the treated field. The TPEZ is defined as an area adjacent to the treated field with a length of 316 m (equal to the length of the edge of the treated field), and a width of 30 m. The width of the TPEZ represents the distance that overland surface flow can travel before sheet flow transitions to concentrated flow. The TPEZ assumes that runoff to an area immediately adjacent to the treated field is in the form of sheet flow that carries pesticides dissolved in water and sorbed to eroded sediment. Glyphosate is expected to move in sheet flow primarily sorbed to eroded sediment. The equilibrium of sorbed and non-sorbed glyphosate will be influenced by environmental conditions and soil characteristics (e.g., pH), which can be influenced by plant root exudates. Beyond this distance over which runoff moves as sheet flow, the runoff is assumed to become concentrated into rivulets, gullies, *etc*., and is then evaluated in the WPEZ and Aquatic Modules of PAT. For the TPEZ, the model uses a mixing cell approach to represent water within the active root zone area of soil, and accounts for flow through the TPEZ caused by both treated field runoff and direct precipitation onto the TPEZ. Pesticide loss from the TPEZ occur from transport (*i.e*., washout and infiltration below the active root zone) and degradation.

Table 3-15 provides the resulting EECs and RQs for vegetative vigor endpoints. As discussed in Section 2.4.2, seedling emergence endpoints are less sensitive than vegetative vigor; multiple studies show that the glyphosate IC_{25} for seedling emergence exceeds 5 lb a.e./A. Effects on seedling emergence from Enlist Duo are therefore likely attributable to 2,4-D, rather than glyphosate. Seedling emergence RQs are therefore not presented in Table 3-15 and risk to seedling emergence from glyphosate is considered low.

The submitted labels allow for three applications of 1 lb a.e./A, intended for application at pre-emergent, emergence and post-emergence crop growth stages. Because the submitted labels have a prohibition of application within a 24- or 48-hr period of expected rainfall, the following analyses account for a 48-hr rainfast. Based upon the EECs, all PWC scenarios result in exceedances of the vegetative vigor non-listed and listed LOCs (RQ range 3.2-70.9 and 5.2-93.5, respectively).

The highest terrestrial EECs resulted from the MScotton, MScorn, and OHcorn scenarios (0.73, 0.71, and 0.71 lb a.e./A, respectively). The lowest terrestrial EECs resulted from the MNcorn scenario (0.16 lb a.e./A). Extending the application interval from 12d to 30d in the MScorn scenario reduced the EEC from 0.71 lb a.e./A (non-listed RQs = 14.2 – 69.4) to 0.61 lb a.e./A (non-listed RQs = 12.0 – 58.7). All further multiple application analyses were carried out using the 12d interval.

Relying upon the MScorn, MScotton, and MSsoybean scenarios, EPA conducted additional analyses to evaluate the potential risks following a single application at each of the three proposed times. The

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[25] A continuous film of water flowing over the soil surface which is not concentrated into channels.

results indicate that later applications had lower EECs than earlier applications but maintained the exceedance of the non-listed and listed LOCs for vegetative vigor (non-listed RQs = 6.1 – 9.8 and 5.4 – 26.7 for pre- and postemergence, respectively). The lower EECs at the later growth stages is related to the curve number for post-emergent applications, which is lower than pre-emergent applications, therefore there is more infiltration and less potential for runoff. Further consideration of the impact of the curve number is discussed below.

Because the later applications were resulting in lower EECs and risk, a third set of analyses across all scenarios considered the risk relative to a single application of 1 lb a.e./A applied 12 days after emergence. This resulted in continued vegetative vigor-based LOC exceedances for all scenarios (non-listed RQs range from 1.0 – 25.1).

Lastly, the management of an agricultural field can influence the permeability of the surface soils on the field, therefore the curve number relied upon in the PRZM estimations of runoff was modified for seven scenarios, including MScorn and MScotton. Based on BEAD recommendations, EFED assessed aquatic exposure using the curve numbers of 88 instead of default 87 for fallow and 82 instead of default 84 during cropping for the hydrologic C soil**.** Both multiple and single application assumptions resulted in exceedances of the listed and non-listed LOCs for all scenarios run.

The proposed 30 ft spray drift setback from sensitive vegetation is sufficient to prevent exposures that would result in exceedances of the most sensitive terrestrial plant endpoints, therefore potential risks from spray drift are considered low. However, the results indicate that there are potential risks to non-listed and potential effects to listed terrestrial plants within 30 m of Enlist corn, Enlist soybean or Enlist cotton fields as a result of surface runoff (i.e., sheet-flow). Beyond this distance from the edge of the field, the surface runoff is expected to transition into concentrated flow resulting in transport to wetland, riparian and aquatic habitats downgradient.

Table 3-15. Terrestrial Plant Exposure Zone: RQs for Most Sensitive Terrestrial Plant Taxa and Associated Runoff + Drift EECs, with Rainfast

Scenario Summary	EEC (lbs a.e./A)	Seedling Emergence	Vegetative Vigor			
			Monocot		Dicot	
			Non-Listed	Listed	Non-Listed	Listed
Proposed label: 3 applications of 1 lb a.e./A at pre-emergence, emergence and post emergence, application interval 12 days.						
IAcornSTD	0.48	NC	**9.5**	**15.3**	**46.4**	**61.2**
ILCornSTD	0.51		**10.1**	**16.3**	**49.4**	**65.2**
INCornSTD	0.18		**3.5**	**5.6**	**17.1**	**22.6**
KSCornSTD	0.28		**5.4**	**8.8**	**26.6**	**35.1**
MNCornSTD	0.16		**3.2**	**5.2**	**15.6**	**20.6**
MScornSTD	0.71		**14.2**	**22.9**	**69.4**	**91.5**
NCcornESTD	0.23		**4.5**	**7.3**	**22.2**	**29.2**
NECornStd	0.33		**6.5**	**10.5**	**31.8**	**41.9**
OHCornSTD	0.71		**14.1**	**22.7**	**69.0**	**91.0**
PAcornSTD	0.34		**6.7**	**10.9**	**33.0**	**43.5**
MSsoybeanSTD	0.46		**9.0**	**14.6**	**44.3**	**58.4**
MScottonSTD	0.73		**14.5**	**23.4**	**70.9**	**93.5**
NCcottonSTD	0.54		**10.6**	**17.2**	**52.1**	**68.7**
Single 1 lb a.e./A application, different timing						
MScornSTD Pre-emergence	0.37	NC	**7.3**	**11.8**	**35.7**	**47.0**
MScornSTD Emergence	0.37		**7.3**	**11.8**	**35.9**	**47.3**
MScornSTD Post-emergence	0.30		**5.9**	**9.5**	**29.0**	**38.2**
MSsoybeanSTD Pre-emergence	0.31		**6.0**	**9.8**	**29.6**	**39.0**
MSsoybeanSTD Emergence	0.14		**2.8**	**4.5**	**13.7**	**18.0**
MSsoybeanSTD Post-emergence	0.18		**3.5**	**5.6**	**17.0**	**22.4**
MScottonSTD Pre-emergence	0.31		**6.1**	**9.8**	**29.8**	**39.2**
MScottonSTD Emergence	0.29		**5.7**	**9.2**	**27.9**	**36.8**
MScottonSTD Post-emergence	0.28		**5.4**	**8.8**	**26.7**	**35.1**
Single Post Emergence Application						
IAcornSTD	0.20	NC	**4.0**	**6.5**	**19.7**	**25.9**
ILCornSTD	0.24		**4.6**	**7.5**	**22.7**	**30.0**

INCornSTD	0.050		**1.0**	**1.6**	**4.8**	**6.4**
KSCornSTD	0.11		**2.2**	**3.5**	**10.7**	**14.1**
MNCornSTD	0.062		**1.2**	**2.0**	**6.0**	**7.9**
NCcornESTD	0.10		**2.0**	**3.2**	**9.7**	**12.8**
NECornStd	0.81		**1.6**	**2.6**	**7.8**	**10.3**
OHCornSTD	0.26		**5.1**	**8.3**	**25.1**	**33.1**
PAcornSTD	0.11		**2.2**	**3.5**	**10.7**	**14.1**
NCcottonSTD	0.22		**4.3**	**7.0**	**21.2**	**27.9**
Curve Number Adjustment						
MScornModified, multiple applications, at 1 lb a.e./A	0.63	NC	**12.5**	**20.1**	**61.1**	**80.5**
MScornModified, single post-emergence application at 1 lbs a.e./A	0.26		**5.1**	**8.2**	**24.8**	**32.7**
MScottonModified, multiple apps at 1 lb/A	0.66		**13.0**	**21.0**	**63.8**	**84.1**
MSsoybeanModified, multiple applications at 1 lb a.e./A	0.43		**8.5**	**13.7**	**41.6**	**54.8**
NCcottonModified, multiple applications at 1 lb a.e./A	0.48		**9.5**	**15.4**	**46.6**	**61.5**
PAcornModified, multiple applications at 1 lb a.e./A	0.32		**6.2**	**10.1**	**30.5**	**40.3**
IAcornModified, multiple applications at 1 lb a.e./A	0.45		**8.8**	**14.3**	**43.3**	**57.0**
Proposed label: 3 applications of 1 lb a.e./A at pre-emergence, emergence and post emergence, application interval 30 days.						
MScornSTD, multiple applications, at 1.12 lbs a.i./A, Drift + Runoff EEC	0.61	NC	**12.0**	**19.4**	**58.7**	**77.4**

Bold and highlighted text indicates listed or non-listed LOC exceedance.
NC = Not Calculated

3.5.3.1. Potential Effects to Milkweed and Indirect Effects on Monarch Butterflies

Milkweed is a dicotyledonous perennial plant that is a target weed for suppression on the Enlist Duo label and as such, may be exposed to glyphosate from direct applications on-field as well as from spray drift and/or runoff off-field. Because glyphosate-specific endpoints are available, the least sensitive vegetative vigor endpoint for glyphosate (0.126 lb a.e./A) was compared to the MScorn EECs assuming a single post-emergence application. This resulted in maximum RQs of 2.4 and 5.0 for TPEZ and WPEZ, respectively. Effects to milkweed are therefore expected to exceed the LOC on-field and RQs exceed the LOC off-field as well, due to runoff.

Migratory monarch populations are in decline, both in numbers and overall health (FWS, 2020). There is extensive discussion in the literature about which stressors are influencing declines of monarch populations. In the 2020 Status of the Species Assessment released by the U.S. Fish and Wildlife Service (USFWS), the primary drivers affecting monarch health are listed as: “loss and degradation of habitat (from conversion of grasslands to agriculture, widespread use of herbicides, logging/thinning at overwintering sites in Mexico, senescence and incompatible management of overwintering sites in California, urban development, and drought), continued exposure to insecticides, and effects of climate change” (USFWS, 2020). Of these different factors, “widespread use of herbicides” is of particular interest for potential impacts on milkweed.

The U.S. Court of Appeals for the Ninth Circuit, in *National Family Farm Coalition, et al., v. U.S. EPA, et al.*, 966 F.3d 893 (9th Cir., 2020), directed EPA to “address the evidence that monarch butterflies may be harmed by the destruction of milkweed on target fields in determining whether the registration of Enlist Duo will lead to any ‘unreasonable adverse effect’ on the environment.” *Id*. at 930. This section discusses the potential for indirect effects on monarchs through loss of on-field milkweed.

On-field milkweed is expected to be present in a subset of corn, cotton, and soybean fields. Once a common agricultural weed, populations of milkweed in agricultural fields declined substantially with the widespread adoption of over-the-top herbicide applications on herbicide-resistant crops (Pleasants and Oberhauser, 2012; Stenoien, 2018; Pleasants, 2017). On-field milkweed now appears to be extirpated in some areas (Stenoien, 2018); however, milkweed still occurs on some fields. Milkweed remains a minor weed in field crops (USEPA, 2021b). Between 2015 and 2019, an average of 850,000 acres of corn, cotton, or soybean were treated to control milkweed (USEPA, 2021b). This estimate does not account for fields where milkweed was present but was not the target weed of herbicide applications. Therefore, milkweed may be present on corn, cotton, and soybean fields.

Monarchs are expected to make use of milkweed located on fields as host plants (Oberhauser *et al.* 2001). The extent to which monarchs make use of remaining on-field milkweed has not been directly surveyed. When milkweed is rare or sparsely distributed, female monarchs are expected to be able to find and oviposit on host plants (Kral-O’Brien *et a*l., 2020). Monarchs can fly long distances and have a conservatively estimated perceptual range of 125m (Fisher and Bradbury, 2021), so large expanses of agricultural fields would not be a barrier to locating milkweed. Insects use multiple sensory modalities, including long distance olfaction, to locate and identify host plants and nectar sources, and host plants would stand out within a monoculture of non-hosts more than they would stand out in a diverse plant community (Bruce et al. 2005; Kerr et al. 2017; Knudsen *et al.* 2017). Therefore, monarchs are expected to be on-field when milkweed is present.

Glyphosate application is expected to suppress or eliminate on-field milkweed, which is listed as a target weed on the Enlist Duo label. If Enlist Duo is applied prior to when adult monarch butterflies pass through an area, impacts could include a decline in reproduction of an individual through reduction of available oviposition sites. If Enlist Duo applications occur after oviposition occurs, potential effects to monarchs include caterpillar mortality from loss of the host milkweed.

Overall, EFED anticipates that application of Enlist Duo may decrease the growth of milkweed, which could have an impact on monarchs at the field level. It is unknown if use of Enlist Duo would have a population level impact. As indicated in the USFWS status of species assessment, an increase of millions of stems of milkweed are needed to reverse the monarch populations' downward trajectories (USFWS, 2020). Therefore, a decrease in milkweed stems (including those on field) is contrary to what is needed to recover this species.

3.5.4. Wetland Plant Exposure Zone (WPEZ): Runoff and Spray Drift from a Treated Field Deposited into a Non-Target Semi-Aquatic Plant Area

The WPEZ is intended to represent a non-target wetland plant community that is exposed to pesticide via overland flow[26] and spray drift. The wetland can be immediately adjacent to the treated field or some undetermined distance away and be exposed via spray drift and runoff or from runoff only. The WPEZ is intended to represent a plant community that can exist in a saturated to flooded environment, such as a depression or shallow wetland that would collect and hold runoff from upland areas. This wetland system is considered to be protective of other surface-fed wetland systems (*e.g.*, permanently flooded; riparian) in that it is allowed to dry-down (concentrating contaminants), has a finite volume (considers standing water exposure), and would receive all of the runoff from an adjacent treated field. Similar to the TPEZ discussed above, the contribution to concentration from spray drift deposition is considered along with runoff only for the dimensions of the WPEZ. The WPEZ is defined as a one hectare (ha) wetland receiving inputs from the adjacent 10-ha field. Within the WPEZ two depth zones are defined: a standing water zone and a saturated soil pore-water (15 cm benthic) zone. The maximum depth of standing water is set to 15 cm, but this water can dry down to a minimum depth of 0.5 cm using algorithms from the Variable Volume Water Model (VVWM). The model excludes comparison of standing water concentrations to aquatic taxa (e.g., *Lemna sp*.; green algae; diatoms) when water depth is less than 0.5 cm. To obtain an EEC in lbs a.e./A, the total mass per area is calculated by tallying the total mass in the water column plus that in the benthic layer and expressing it on an area-normalized basis.

The PWC and subsequently PAT model consider 30 years of meteorological data in the EEC estimation. Because the submitted labels have a prohibition of application within a 24- or 48hr period of expected rainfall, all analyses presented account for this rainfast.

As discussed for the TPEZ, several different considerations of the proposed use were evaluated. **Table 3-16** provides the WPEZ EECs and RQs for all these evaluated characterizations. The risks from the proposed use extend across all scenarios for vegetative vigor-based endpoints, with LOC exceedances for both non-listed and listed species (RQ range 5.0-234). The temporal aspect of application (pre-, post,

[26] Water flow that moves in swales, small rills, and gullies

and emergence) did not reduce the RQs to below the LOC. Similarly, the consideration of a single application made post-emergence or differing infiltration assumptions (curve number) did result in reduced EECs. These reduced EECs remain above the endpoints, therefore there is potential risk to non-listed and potential effects to listed wetland vascular plants. Because glyphosate alone is not expected to result in significant effects on seedling emergence at application rates up to 5 lb a.e/A, RQs were not calculated for seedling emergence and risk to seedling emergence is not expected.

Monitoring data described in Section 3.3.1 and detailed in Appendix N point to the presence of glyphosate in sampled water bodies due to glyphosate usage including, but not limited to, usage of Enlist Duo since it was registered in 2014. As discussed in **Section 3.3.1**, the measured glyphosate concentrations in the sampled water bodies are not exclusively due to use of Enlist Duo, and the contribution of Enlist Duo cannot be distinguished from other labeled uses of glyphosate. However, glyphosate detection in the monitored agricultural areas and the range of concentrations seen from different sampling sites and times, support the proposition that glyphosate run-off is reaching non-target water bodies under currently labeled uses of glyphosate, and Enlist Duo is not expected to be an exception.

Table 3-16. Wetland Plant Exposure Zone: Most Sensitive Terrestrial and Aquatic Plant Taxa and Associated Runoff + Drift EECs and RQs, with Rainfast

Scenario Summary	EEC (lbs a.e./A)	Seedling Emergence	Vegetative Vigor			
			Monocot		Dicot	
			Non-Listed	Listed	Non-Listed	Listed
Proposed label: 3 applications of 1 lb a.e./A at pre-emergence, emergence and post emergence, application interval 12 days.						
IAcornSTD	1.12	NC	**22.2**	**35.9**	**108.9**	**143.6**
ILCornSTD	1.21		**23.9**	**38.6**	**117.2**	**154.5**
INCornSTD	0.25		**5.0**	**8.1**	**24.6**	**32.5**
KSCornSTD	0.43		**8.6**	**13.8**	**41.9**	**55.3**
MNCornSTD	0.29		**5.8**	**9.3**	**28.2**	**37.2**
MScornSTD	1.83		**36.2**	**58.5**	**177.5**	**234.0**
NCcornESTD	0.58		**11.4**	**18.4**	**55.9**	**73.6**
NECornStd	0.53		**10.4**	**16.8**	**51.1**	**67.3**
OHCornSTD	1.37		**27.1**	**43.8**	**133.0**	**175.3**
PAcornSTD	0.81		**15.9**	**25.8**	**78.2**	**103.1**
MSsoybeanSTD	0.86		**16.9**	**27.3**	**82.8**	**109.2**
MScottonSTD	1.37		**27.2**	**43.9**	**133.3**	**175.7**
NCcottonSTD	1.6		**31.6**	**51.1**	**155.1**	**204.4**
Single 1 lb a.e./A application, different timing						
MScornSTD Pre-emergence	0.72	NC	**14.2**	**22.9**	**69.6**	**91.7**
MScornSTD Emergence	0.70		**13.7**	**22.1**	**67.0**	**88.3**
MScornSTD Post-emergence	0.63		**12.4**	**20.0**	**60.7**	**80.0**
MSsoybeanSTD Pre-emergence	0.40		**7.9**	**12.8**	**38.7**	**51.0**
MSsoybeanSTD Emergence	0.86		**16.9**	**27.4**	**83.0**	**109.4**
MSsoybeanSTD Post-emergence	0.26		**5.0**	**8.1**	**24.7**	**32.6**
MScottonSTD Pre-emergence	0.47		**9.4**	**15.1**	**45.9**	**60.5**
MScottonSTD Emergence	0.24		**4.8**	**7.7**	**23.3**	**30.7**
MScottonSTD Post-emergence	0.42		**8.4**	**13.5**	**41.1**	**54.2**
Single Post Emergence Application						
IAcornSTD	0.40	NC	**7.8**	**12.6**	**38.2**	**50.4**
ILCornSTD	0.40		**7.9**	**12.8**	**38.8**	**51.1**

INCornSTD	0.084		**1.7**	**2.7**	**8.2**	**10.8**
KSCornSTD	0.15		**2.9**	**4.7**	**14.3**	**18.8**
MNCornSTD	0.10		**2.0**	**3.2**	**9.8**	**12.9**
NCcornESTD	0.21		**4.2**	**6.8**	**20.7**	**27.3**
NECornStd	0.16		**3.1**	**5.0**	**15.2**	**20.1**
OHCornSTD	0.51		**10.0**	**16.2**	**49.2**	**64.8**
PAcornSTD	0.23		**4.6**	**7.5**	**22.6**	**29.9**
NCcottonSTD	0.52		**10.3**	**16.7**	**50.7**	**66.9**
Curve Number Adjustment						
MScornModified, multiple applications, at 1 lb a.e./A	1.52	NC	**30.0**	**48.5**	**147.3**	**194.1**
MScornModified, single post-emergence application at 1 lb a.e./A	0.51		**10.1**	**16.4**	**49.6**	**65.4**
MScottonModified, multiple apps at 1 lb/A	1.21		**23.8**	**38.5**	**116.9**	**154.1**
MSsoybeanModified, multiple applications at 1 lb a.e./A	0.78		**15.4**	**25.0**	**75.7**	**99.8**
NCcottonModified, multiple applications at 1 lb a.e./A	1.29		**25.4**	**41.1**	**124.7**	**164.3**
PAcornModified, multiple applications at 1 lb a.e./A	0.69		**13.5**	**21.9**	**66.4**	**87.5**
IAcornModified, multiple applications at 1 lb a.e./A	0.98		**19.5**	**31.4**	**95.4**	**125.7**
Proposed label: 3 applications of 1 lb a.e./A at pre-emergence, emergence and post emergence, application interval 30 days.						
MScornSTD, multiple applications, at 1.12 lbs a.i./A	1.75	NC	**34.6**	**55.9**	**169.7**	**223.7**

Bold and highlighted text indicates listed or non-listed LOC exceedance.

NC = Not Calculated

3.5.5. Consideration of the Diversity of Terrestrial and Wetland Species Potentially Impacted by Runoff Exposures

EPA also considered the diversity of plants that may be impacted by exposures through runoff for the proposed uses. This comparison focuses on the vegetative vigor endpoints from registrant submitted studies. While submitted data were also available for seedling emergence, those endpoints were determined to be less reliable because of uncertainties due to significant effects on survival. In addition, multiple studies on formulations containing glyphosate alone reported that effects did not reach 25% reduction in any measured seedling emergence endpoints at test levels up to 5 lb a.e./A, which resulted in non-definitive (> 5 lb a.e./A) endpoints. As mentioned earlier, the vegetative vigor endpoints are more sensitive than those from seedling emergence studies. **Table 3-17** provides the RQs for all tested monocot and dicot species in the submitted vegetative vigor studies as a result of comparison to the EECs in the TPEZ and WPEZ for the MScotton and MScorn scenarios, respectively. These results illustrate the broad-spectrum toxicity and risk of glyphosate to both monocots and dicots through runoff. The most sensitive monocot and dicot species were selected for the TPEZ and WPEZ analyses based on the most sensitive IC_{25}.

Table 3-17. Risk Quotients for all Tested Monocot and Dicot Species, Rainfast with Modified Curve Number[1]

Species	TPEZ - EEC 0.66 lbs a.e./A		WPEZ - EEC 1.52 lbs a.e/A	
	Non-listed RQ	Listed RQ	Non-listed RQ	Listed RQ
Dicots				
Tomato	**63.8**	**84.1**	**147.3**	**194.1**
Cabbage	**20.8**	**342.7**	**48.1**	**790.9**
Mustard	**58.9**	**168.2**	**136.0**	**388.3**
Radish	**39.5**	**84.1**	**91.3**	**194.1**
Buckwheat	**31.8**	**84.1**	**73.4**	**194.1**
Sugarbeet	**28.6**	**84.1**	**65.9**	**194.1**
Sunflower	**28.1**	**84.1**	**64.9**	**194.1**
Cucumber	**17.8**	**168.2**	**41.1**	**388.3**
Oilseed Rape	**16.5**	**41.9**	**38.1**	**96.6**
Soybean	**6.6**	**41.9**	**15.1**	**96.6**
Monocots				
Wheat	**13.0**	**21.0**	**30.0**	**48.5**
Corn	**5.7**	**5.3**	**13.3**	**12.1**
Oat	**3.1**	**10.5**	**7.1**	**24.3**
Onion	**3.0**	**2.5**	**7.0**	**5.8**
Sorghum	**2.9**	**5.3**	**6.8**	**12.1**

[1]Bold and highlighted text indicates LOC exceedance.

3.5.6. Aquatic Plant Risk Characterization

For aquatic plants, no RQs exceed the Agency LOC (1.0 for both listed and non-listed aquatic plants). Tier I RQs are <0.01 for both vascular and non-vascular aquatic plants, using the technical glyphosate endpoints for *Anabaena flos-aquae* (11.4 mg a.e./L) and *Lemna gibba* (11.9 mg a.e./L). Risks are expected to be low.

3.5.7. Volatile Emission Impacts to Off-Field Plant Risk

The potential for volatilization of glyphosate from soil and water is expected to be low due to the low vapor pressure and low Henry's Law constant (**Table 3-2**). Several studies have shown both glyphosate and AMPA detections in rainwater near use locations. In most cases, these detections were found during the spraying season in the vicinity of local use areas and can be attributed to spray drift rather than to volatilization or long-range transport (Baker *et al*., 2006; Quaghebeur *et al*., 2004). The highest concentrations were found in urban locations. At one site in Belgium that was 5 m from a spraying location in an urban parking lot, glyphosate was detected in rainwater for several months following a single application (Quaghebeur *et al*., 2004). Deposition was measured to be 205 µg a.i./m^2 at one week after spraying and 0.829 µg/m^2 two months after spraying. These data suggest that volatilization of glyphosate from hard surfaces is possible despite its low vapor pressure. Risk via this pathway is not expected from use of Enlist Duo.

3.6. Analysis of Incident Data

As part of the draft Biological Evaluation for Glyphosate (USEPA, 2020b), the Ecological Incident Information System (EIIS) and the (AIMS) were searched for incidents of adverse effects to wildlife, fish, invertebrates, and plants resulting from exposure to glyphosate since the registration of glyphosate through September 2020. Twelve incidents were found for Enlist Duo, all of which affected cotton via drift.

Since the registration of glyphosate and its salts (PC Codes 417300, 103601, 103604, 103607 and 103608 and 103613 (all active registrations)), there have been 1,136 incidents, mostly involving damage to terrestrial plants. In addition, 337 separate aggregate incident reports were returned which included reports for glyphosate. However, multiple reports of injury to plants, wildlife or other non-target organisms were sometimes contained within each separate report.

4. Listed Species Assessments - Effects Determination

EPA evaluated whether the registration of Enlist One and Enlist Duo poses any reasonable expectation of discernible effects to listed (threatened and endangered) species and designated critical habitats (CH) within the action area in the listed species effects determination. Although EPA did not include candidate species, proposed species and experimental populations in this assessment, if consultation is necessary for this action, these species may be discussed during that process[27]. In making the ESA effects determination, EPA considered direct and indirect effects using the best available scientific information. The term “direct effects” refers to decreases in the survival, growth or reproduction of individuals of a listed species due to exposure to 2,4-D or glyphosate. The term “indirect effects” refers to impacts on individuals of a listed species that may be the result of the effects of 2,4-D or glyphosate on organisms on which the listed species depends for prey, pollination, habitat and/or dispersal (PPHD).

In this analysis, EPA determined whether the use of the Enlist products will have “no effect” (NE) on a given listed species or designated CH or “may affect” (MA) the species or designated CH. The Services’ regulations provide that the consultation obligation is triggered when an agency action MA one or more listed species or designated CH. For those species and designated CH for which EPA makes a MA determination, EPA goes on to determine whether the action: "may affect, but is not likely to adversely affect" (NLAA) the listed species or designated CH; or "may affect and is likely to adversely affect" (LAA) the listed species or designated CH. An LAA determination means that there is a discernible adverse effect to one or more individuals of a listed species or their designated CH. It is EPA’s obligation under the Endangered Species Act to ensure that the registration of the Enlist products does not jeopardize the continued existence of endangered or threatened species or destroy or adversely modify designated CH. Therefore, for species or designated CH with LAA determinations, this analysis considered whether the action could potentially lead to the likelihood of jeopardy to listed species, or destruction or adverse modification to designated CH.

Section 4.1.1 provides a broad overview of the methodology used in this listed species effects determination. **Section 4.1.2** describes the action area for the registration of the Enlist products. **Section 4.1.3** summarizes the species-specific NE and MA determination methodology. **Section 4.1.4** summarizes the NLAA and LAA determination methodology. **Section 4.1.5** summarizes the potential to lead to jeopardy and the potential to destroy or adversely modify designated CH determination methodology. **Section 4.2.1** summarizes the species-specific NE and MA determination results. **Section 4.2.2** summarizes the NLAA and LAA determination results. **Section 4.2.3** summarizes the species that EPA concluded are likely to be jeopardized by this action and those designated CH that may be adversely modified.

[27] 50 CFR 402.40(b) states: Effects determination is a written determination by the U.S. Environmental Protection Agency (EPA) addressing the effects of a FIFRA action on listed species or critical habitat. The contents of an effects determination will depend on the nature of the action. An effects determination submitted under § 402.46 or § 402.47 shall contain the information described in § 402.14(c) and a summary of the information on which the determination is based, detailing how the FIFRA action affects the listed species or critical habitat.

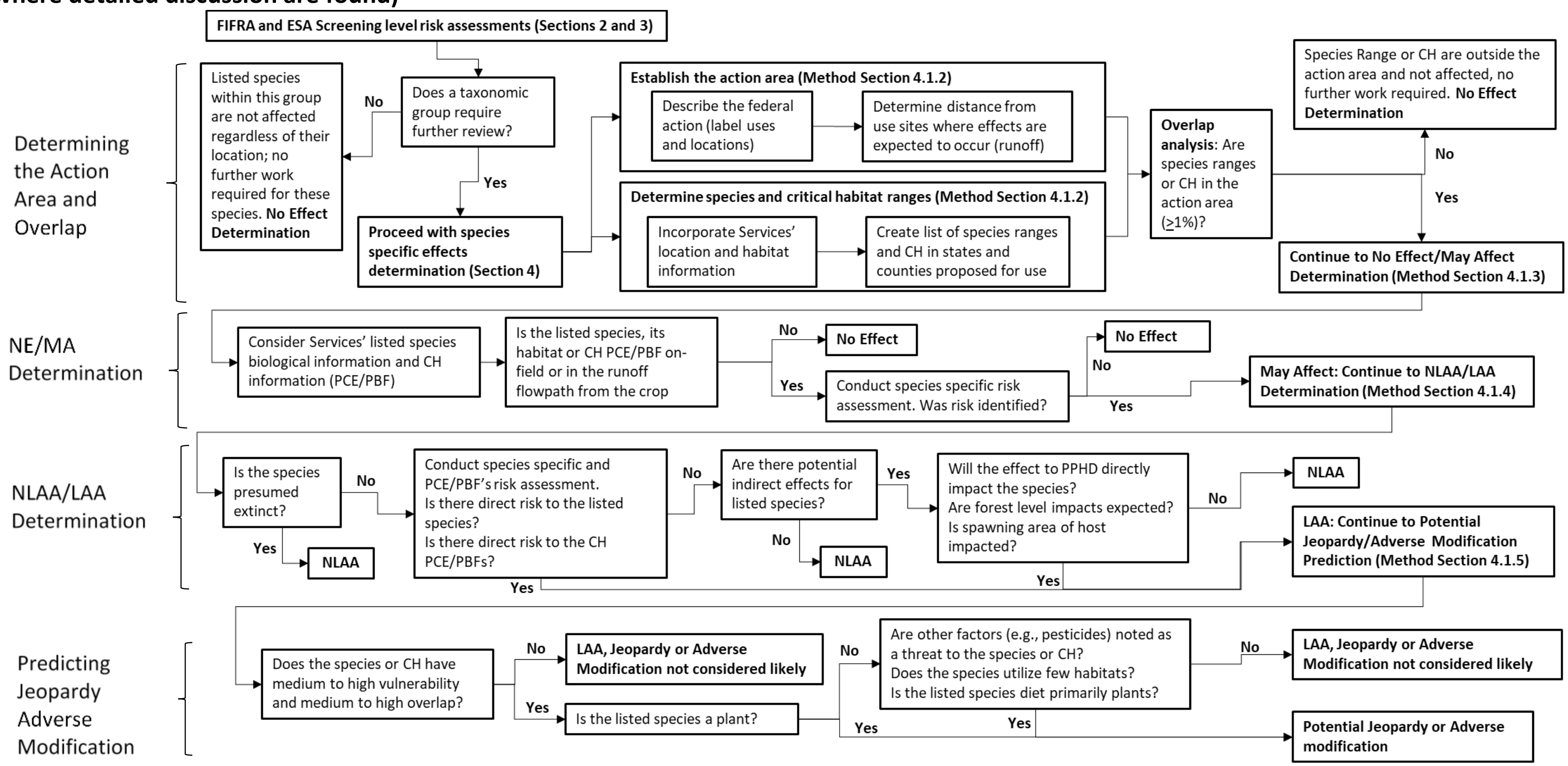
4.1. Methodology

4.1.1. Overview

As summarized in reports to Congress,[28] EPA has used a process when assessing risk to listed species from pesticides like 2,4-D and glyphosate that will be used on plants genetically modified to be tolerant to the pesticides. Here, EPA followed that process for the formulated products of Enlist One and Enlist Duo. The overall process and a general description of the major steps in the process are presented in **Figure 4-1**, briefly discussed within this section and more detail is provided in **Sections 4.1.2-4.1.5**.

[28] https://www.epa.gov/endangered-species/reports-congress-improving-consultation-process-under-endangered-species-act; https://www.epa.gov/sites/production/files/2015-07/documents/esareporttocongress.pdf

Figure 4-1. Overview of Effects Determination Process for Evaluating Listed Species and Associated Designated Critical Habitat (sections indicate where detailed discussion are found)



The Agency begins its assessment of risk to listed species with a screening level assessment (see **Sections 2** and **3**) that includes an ecological risk assessment based on its 2004 Overview of the Ecological Risk Assessment Process document. [USEPA, 2004, available at https://www.epa.gov/sites/production/files/2014-11/documents/ecorisk-overview.pdf]. The screening level risk assessment is refined as needed to consider species-specific information and risks.

The current action involves the registration of the Enlist One and Enlist Duo products. As noted previously, Enlist One contains 2,4-D and Enlist Duo contains both 2,4-D and glyphosate. EPA typically conducts risk assessments on an active ingredient basis. Since Enlist Duo contains both 2,4-D and glyphosate, this assessment includes risk assessments for these two active ingredients. EPA separated out the two active ingredients to ensure that the assessment of the product as formulated covered each. The FIFRA risk assessments described in **Section 2** and **Section 3** for 2,4-D and glyphosate, respectively served as the screening level assessment for determining which taxa needed further review at the species-specific level to determine whether the action may affect any listed species or designated CH. EPA determined that some taxa are at risk from either direct or indirect effects and a species-specific assessment may be necessary. The taxa needing further review from direct or indirect effects are summarized in **Table 4-1**.

Table 4-1. Summary of Listed Taxa that Require Further Review from Direct or Indirect Effects of 2,4-D or Glyphosate.

Taxa (Relevant to Listed Species)	Additional Species-Specific Review Needed?			
	2,4-D Choline (Section 2)		Glyphosate (Section 3)	
	Direct Effects	Indirect Effects (Relevant Taxa for PPHD)	Direct Effects	Indirect Effects (Relevant Taxa for PPHD)
Aquatic animals	No	Yes (terrestrial and wetland plants)	No	Yes (terrestrial or wetland plants)
Aquatic plants	Yes (wetland plants only)	No	No	No
Mammals	Yes (on field only)	Yes (terrestrial or wetland plants)	No	Yes (terrestrial or wetland plants)
Birds	Yes (on field only)	Yes (terrestrial or wetland plants)	Yes (on field only)	Yes (terrestrial or wetland plants)
Terrestrial invertebrates	Yes (on field only)	Yes (terrestrial or wetland plants)	No	Yes (terrestrial or wetland plants)
Terrestrial plants	Yes	No	Yes	No
Wetland plants	Yes	No	Yes	No

If taxa-based risk quotients (RQs[29]) representing a listed species and organisms relevant to direct or indirect effects are all below the relevant listed species LOCs, and indirect effects are not expected, a NE determination is made. When risks are not identified to listed species based on RQs, which are considered highly conservative, then EPA is confident that no discernable effects to species will occur. Of the assessed taxa, only aquatic plants were determined NE at this step. While no LOC exceedances were identified for off-field terrestrial or aquatic animals, further consideration of the life

[29] Risk Quotients (RQs) are the ratio of estimated environmental exposure concentration to the toxicity endpoint. RQs for the purposes of identifying potential risk to endangered species are presented in **Sections 2** and **3**.

history, distribution of the species, and effects of 2,4-D or glyphosate on organisms on which the listed species depends for PPHD (i.e., indirect effects) were considered before making an NE/MA determination.

When any given acute or chronic RQ for taxon representing a listed species exceeded the appropriate listed species LOC, or there is the potential for indirect effects to listed species, EPA performed species-specific effects determinations for the taxon. If EPA's screening level assessment shows that an RQ exceeds a listed species LOC, it does not automatically mean that the action may affect a species. Instead, it means further species-specific review is needed to determine whether the action may affect a listed species or its designated CH. This process is as follows:

1. EPA determined the geographical extent of the action area (the area where potential exposure from 2,4-D or glyphosate from Enlist applications could occur; described in **Section 4.1.2**)
2. EPA determined whether a listed species is located within the action area (referred to as "overlap analysis"; described in **Section 4.1.2.6**)
    a. EPA considered a species range to be outside of the action area if the overlap of that species range with the action area was <1%.[30]
3. Depending on the result of the overlap analysis:
    a. For species outside the action area, no further analysis was performed as these species will not be affected and NE determinations are made.
    b. For species within the action area, EPA assigned each species to one or more general environmental exposure compartments (on-field, within 30-m of a field edge - terrestrial, within 1,500 m of a field - wetland or aquatic habitats).
    c. For species within the action area, EPA conducted a species-specific risk assessment for direct effects and an evaluation of indirect effects in order to make effects determinations (i.e., NE, MA-NLAA or MA-LAA; method description in **Sections 4.1.3** and **4.1.4**; determinations provided in **Sections 4.2.1** and **4.2.2**).
    d. Following the effects determinations, EPA conducted additional evaluation of the LAA species to determine whether the action is likely to jeopardize the continued existence of a species (method description in **Section 4.1.5**; determinations provided in **Section 4.2.3**)[31].

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[30] EPA has used this 1% overlap criteria because a known source of error within spatial datasets is positional accuracy and precision. The National Standard for Spatial Data Accuracy outlines the accepted method for calculating the horizontal accuracy of a spatial dataset (FGDC, 1998). To prevent false precision when calculating area and the percent overlap, only two significant digits should be considered for decision purposes given the reported 60 meters of horizontal accuracy for the CDL.
[31] The regulation at 50 CFR 402.40(b) states:
(1) A conclusion whether or not the FIFRA action is likely to jeopardize the continued existence of any listed species or result in the destruction or adverse modification of critical habitat and a description of any reasonable and prudent alternatives that may be available;
(2) A description of the impact of any anticipated incidental taking of such listed species resulting from the FIFRA action, reasonable and prudent measures considered necessary or appropriate to minimize such impact, and terms and conditions necessary to implement such measures; and
(3) A summary of any information or recommendations from an applicant. An effects determination shall be based on the best scientific and commercial data available.

The Agency also follows a similar habitat-specific analysis for designated CH effects determinations according to the following process:

1. EPA determined whether a designated CH is located within the action area (referred to as "overlap analysis"; described in **Section 4.1.2.6**)
   a. EPA considered a designated CH to be outside of the action area if the overlap of its range with the action area was <1%.
2. Depending on the result of the overlap analysis:
   a. For designated CH outside the action area, no further analysis is performed as these designated CH will not be affected and NE determinations were made.
   b. For the overlap, EPA assigned each designated CH to one or more general environmental exposure compartments (on-field, within 30-m of a field edge - terrestrial, within 1,500 m of a field - wetland or aquatic habitats).
   c. For designated CH within the action area:
      - EPA compared the designated CH's principal constituent elements (PCE) or physical/biological factors (PBFs) with the screening level risk assessment conclusions for the environmental compartment (terrestrial, wetland, aquatic) to determine if the use of Enlist One and Enlist Duo potentially affect these attributes. Additionally, EPA determined whether the CH's PCE/PBFs specifically identified row crops (corn, cotton, and soybean) as part of the designated CH.
      - EPA conducted a designated CH-specific assessment for direct effects to the habitat to make effects determinations (i.e., NE, MA-NLAA or MA-LAA; method description in **Sections 4.1.3** and **4.1.4**; determinations provided in **Sections 4.2.1** and **4.2.2**).
      - Following the effects determinations, EPA conducted additional evaluation of the LAA designated CH to determine whether there may be destruction or adverse modification (method description in **Section 4.1.5**; determinations provided in **Section 4.2.3**).

The sections below describe how EPA determined the Action Area (**Section 4.1.2**) and provide details on the methods for the taxa based and the species-specific analyses (**Section 4.1.3** and **Section 4.1.4**).

4.1.2. Determining the Action Area and Overlap Analysis

The action area is the footprint of the federal action plus any additional areas where effects are reasonably expected to occur. To identify this action area, EPA performed three steps:

1. 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.
2. Identified potential use sites of the Enlist products (i.e., areas where corn, cotton and soybean are potentially grown) within the states and counties included on the proposed labels.
3. Considered information from **Section 1** and supporting appendices (**Appendix M**) to determine how far off the site of application effects are reasonably expected to occur.
4. Established a geographical information data layer (GIS layer) that combines the sites of use with the extent of off-site areas where effects are reasonably expected to occur.

4.1.2.1. Describing the Federal Action

The actions considered in this listed species risk assessment are for the proposed amendment to the registration of the following products:

- Enlist One® (active ingredient: 2,4-D choline; registration # 62719-695) and
- Enlist Duo® (active ingredients: 2,4-D choline and glyphosate; registration # 62719-649).

Based on the proposed Enlist labels, EPA assessed 1 application of 1.0 lbs acid equivalent (a.e.)/A 2,4-D as a pre-plant "burndown," pre-plant, at-plant, or preemergence. The labels for Enlist One and Enlist Duo also allow for an additional 2 over-the-top post-emergence applications (in-crop) at 1.0 lbs a.e./A (2,4-D). The maximum annual application from the labeled products is not to exceed an annual maximum of 3.0 lb a.e./acre (2,4-D).

Based on the proposed labels for Enlist Duo, EPA assessed 1 application of 1.0 lbs a.e./A glyphosate per acre as a pre-plant "burndown," pre-plant, at-plant, or preemergence. The Enlist Duo label also allows for an additional 2 over-the-top post-emergence applications (in-crop) at 1.0 lbs a.e./A (glyphosate). The maximum annual application from the labeled products is not to exceed an annual maximum of 3.0 lb a.e./acre (glyphosate).

The products are only for use on 2,4-D- and glyphosate-tolerant corn, soybean, and cotton in the following 34 states (**Figure 4-2**):

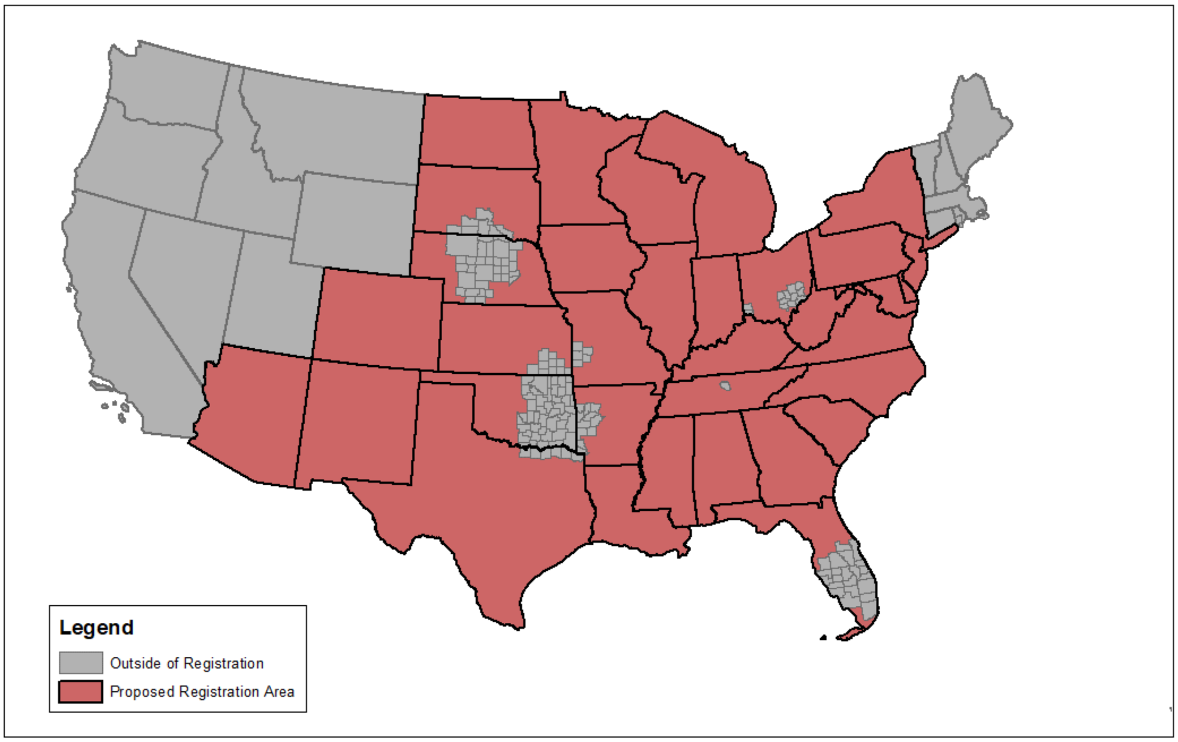
> Alabama, Arizona, Arkansas, Colorado, Delaware, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland, Michigan, Minnesota, Mississippi, Missouri, Nebraska, New Jersey, New Mexico, New York, North Carolina, North Dakota, Ohio, Oklahoma, Pennsylvania, South Carolina, South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

The proposed label contains application prohibitions in the following counties:

- <u>Arizona</u>: Yuma, Pinal or 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 Enlist One or Enlist Duo on land administered by the US Fish and Wildlife Service or National Park Service;
- <u>Arkansas</u>: Crawford, Franklin, Johnson, Little River, Logan, Montgomery, Polk, Scott, Sebastian, Sevier and Yell;
- <u>Florida</u>: 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;
- <u>Kansas</u>: Chautauqua, Cherokee, Cowley, Elk, Greenwood, Labette, Montgomery, Neosho, Wilson, and Woodson;
- <u>Massachusetts</u>: Nantucket;
- <u>Missouri</u>: Barton, Bates, Cedar, St. Clair and Vernon;
- <u>Nebraska</u>: Antelope, Blaine, Boone, Boyd, Brown, Cherry, Custer, Dawson, Frontier, Furnas, Garfield, Gosper, Greeley, Hayes, Holt, Hooker, Howard, Keya Paha, Knox, Lincoln, Logan, Loup, McPherson, Merrick, Nance, Phelps, Red Willow, Rock, Sherman, Thomas, Valley and Wheeler;

- Ohio: Athens, Butler, Fairfield, Guernsey, Hamilton, Hocking, Morgan, Muskingum, Noble, Perry, Vinton and Washington;
- Oklahoma: Adair, Atoka, Bryan, Carter, Cherokee, Choctaw, Cleveland, Coal, Craig, Creek, Delaware, Garvin, Haskell, Hughes, Johnston, Kay, Latimer, Le Flore, Lincoln, Love, Marshall, Mayes, McClain, McCurtain, McIntosh, Murray, Muskogee, Noble, Nowata, Okfuskee, Okmulgee, Osage, Ottawa, Pawnee, Payne, Pittsburg, Pontotoc, Pottawatomie, Pushmataha, Rogers, Seminole, Sequoyah, Tulsa, Wagoner and Washington;
- Rhode Island: Washington
- South Dakota: Bennett, Charles Mix, Gregory, Lyman, Mellette, Todd and Tripp;
- Tennessee: Wilson;
- Texas: Bowie, Cooke, Fannin, Grayson, Lamar and Red River.

Figure 4-2. Map of the Amended Registration Area for Enlist One and Enlist Duo. Subcounty Restriction in Arizona not Presented in this Map



Each of the current product labels include the following application requirements to address spray drift and runoff from the application of the products:

- Spray drift
    - Application equipment must use spray nozzles and pressure settings from an approved equipment list maintained at https://www.corteva.us/content/dam/dpagco/enlist/na/us/en/files/Enlist_Product_Use_Guide.pdf.

  - Enlist products may only be tank-mixed with products that have been tested and found not to adversely affect the spray drift properties of Enlist Duo and Enlist One. A list of those products may be found at EnlistTankmix.com
  - Application is only allowed by ground spray equipment.
  - Application can only occur when wind speed at the boom height is less than 15 miles per hour.
  - DO NOT spray during an inversion
  - Each product label requires a downwind 30-foot in-field spray drift setback (buffer) from "sensitive vegetation" for all application sites
- Runoff
  - DO NOT apply Enlist products when rain is expected within 24 hours
  - DO NOT irrigate treated fields within 24 hours of application of Enlist products

4.1.2.2. Determining How Far Off-Field Effects are Reasonably Expected to Occur

The action area extends from the pesticide use site to the furthest distance at which effects on listed species or designated critical habitat are reasonably expected to occur. EPA determined the action area using the labeling, including any mandatory control measures for use of the product.

EPA's conservative screening-level assessment described in **Section 2** and **Section 3** shows that terrestrial and wetland plants, mammals, birds and terrestrial invertebrates are the most sensitive taxa for direct effects from the use of these products. With the sensitive taxa established, EPA then considered the requirements and restrictions on the proposed labels to determine the action area, including determining how far the 2,4-D or glyphosate could move from the treatment area following applications of Enlist. That distance informs whether there are any discernible effects to listed species or their designated habitat. As discussed in **Sections 2** and **3**, risk from drift is considered low due to the 30 ft in field buffer on the labels. Therefore, off site drift transport of 2,4-D and glyphosate is not part of the action area. Off-site transport of 2,4-D and glyphosate in runoff does pose a risk to terrestrial and wetland plants. For this assessment, EFED estimated that 1,600 meters represents a conservative upper bound of how far runoff could travel between a treated field and a wetland (**Appendix M**). This estimate is based upon geographic information and the National Hydrography Dataset (NHDplus Version 2). EFED calculated a 90th percentile national width value from the estimated widths of nationally delineated watershed catchments. This approach is not intended to represent an actual distance where runoff will travel to wetlands and include concentrations that could result in an effect, but rather to calculate a theoretical maximum travel distance for runoff, beyond which runoff is not expected to travel.

EPA established the action area for the Enlist products using corn, cotton and soybean use data layers (UDLs) established from the USDA's Crop Data Layer (**Appendix O**). All UDLs are buffered in all directions to 1,500 meters, to account for offsite transport, in this assessment that is runoff transport or 1,600 meters from the field. Prior to this assessment spray drift was the only offsite transport considered when setting the action area extent. When considering spray drift, EPA used buffered the UDLs to 1,500 meters, or double the aerial spray drift limit. For this assessment the 1,500 meters was used for the action area extent because the spatial analysis was completed and all species within the were identified at 90 meters or less. The spatial analysis was not updated to reflect the new 1,600 meters because it would not impact the species identified within the action area for this assessment.

4.1.2.3. Developing Geographical Layers for the Action Area

EPA conducted three steps in defining the GIS layer for the action area:

1. EPA established the footprint of corn, cotton, and soybean application sites as ESA Use Data Layers (UDLs) for the 34 states where Enlist One and Enlist Duo is proposed for use on GMO corn, cotton, and soybean (AL, AZ, AR, CO, DE, FL, GA, IL, IA, IN, KS, KY, LA, MD, MI, MN, MS, MO, NE, NM, NJ, NY, NC, ND, OH, OK, PA, SC, SD, TN, TX, VA, WV, WI). These were generated by combining multiple years (2013-2017) of the Cropland Data Layer (CDL) and represent the on-field portions of the action area.

2. EPA then extended the UDLs outwards to 30 m (98 feet, the limit of GIS resolution) in all directions to represent the near-field distance portions of the action area (*i.e.,* risk to terrestrial plants).

3. EPA then extended the UDLs outwards to 1,500 m in all directions to represent the portions of the action area in the runoff flow path (i.e., wetland plants).

4.1.2.4. Determine Listed Species Ranges and Designated CH

EPA conducted three steps in establishing species ranges and designated CH locations:

1. EPA downloaded the listed species and designated CH locations provided by the U.S. Fish and Wildlife Service (USFWS) and the National Marine Fisheries Service (NMFS) in November 2020 (USFWS, 2020).
2. EPA identified those species and designated CH located in the 34 states labeled for use.
3. EPA created a GIS layer of the portions of species ranges and designated CH that reside within the 34 states, focusing on listed terrestrial and aquatic plants, mammals, birds, terrestrial invertebrates, and any additional listed species identified in USFWS Recovery Plans as having an obligate relationship (requiring a specific plant taxon for food, habitat, or to complete its life history,) to terrestrial plants or are generalist species (reliant on plants in general for food, habitat, or to complete its life history) in the runoff flow path.

4.1.2.5. Updates to the List of Federally Threatened and Endangered Species

Since EPA downloaded the list of federally threatened and endangered species in November 2020, several updates to the list have been made. Species and designated CH that have been added to the list since November 2020 are not considered in this assessment. Species and designated CH that were removed from the list (*i.e*., delisted due to recovery or extinction) since November 2020 were removed from further consideration in this assessment (**Table 4-2**). EPA is aware that several new species were added since November 2020; however, because spatial files for these species are not available for this assessment, those species will be considered while in consultation with USFWS.

Table 4-2. Species Delisted from the List of Federally Threatened and Endangered Species that Overlap with Action Area

Entity ID	Taxa Group	Scientific Name	Common Name	Date Delisted
11	Mammals	*Canis lupus*	Gray wolf	11/3/2020
12	Mammals	*Canis lupus*	Gray wolf	11/3/2020
134	Birds	*Sterna antillarum*	California least tern	1/13/2021
1041	Flowering Plants	*Trifolium stoloniferum*	Running buffalo clover	08/06/2021

4.1.2.6. Overlap Analysis of Action Area and Listed Species Ranges/Designated CH

The overlap analysis phase consists of a comparison of the GIS layers for the action area and the species range and designated CH locations. First, entire species ranges and designated CH with greater than 1% overlap[32] with the action area are identified (on-field and near field). For entire species ranges and designated CH with greater than 1% overlap with the action area, counties with greater than 1% overlap are identified. Finally, all species and designated CH within the action area and in the runoff flow path from the field are identified. For species ranges and CH within the action area and in the runoff flow path with greater than 1% overlap with the action area, counties with greater than 1% overlap are identified. See **Appendix N** for the results of the overlap analysis.

4.1.2.7. Uncertainties and Conservative Assumptions Associated with the Overlap Analysis

EPA based the overlap analysis on the species locations provided by USFWS (USFWS, 2020). Species range is defined as the geographical area where a species could be found in its lifetime. Produced and managed by the species experts in the agencies responsible for implementing the ESA, these data are the best available information for species range. EPA acknowledges that even though these are the best available data, there are several uncertainties. The range information is not sub-divided into additional qualifiers such as current/historical locations or temporal information to account for distribution variations relating to timing such as seasons. Without additional distribution information, EPA applies certain additional conservatisms: specifically, a uniform distribution within the range is assumed, meaning the species is assumed to be present in all sections of the range at all times of the year. This assumption is an additional conservatism because this distribution is unlikely to occur based a species life history.

Other commonly known and related sources of uncertainty for GIS data generally relate to accuracy and precision. Accuracy can be defined as how well information on a map matches the values in the real world. Precision relates to how well the description of the data used for mapping matches reality, based on closeness of repeated sets of measurements. The more precise the data, the more likely additional measurement or calculation will show the same result. Some sources of inaccuracy and imprecision in

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[32] EPA has used this 1% overlap criteria because a known source of error within spatial datasets is positional accuracy and precision. The National Standard for Spatial Data Accuracy outlines the accepted method for calculating the horizontal accuracy of a spatial dataset (FGDC, 1998). To prevent false precision when calculating area and the percent overlap, only two significant digits should be considered for decision purposes given the reported 60 meters of horizontal accuracy for the CDL.

GIS data are obvious while others are difficult to identify. It is important to consider these sources of error as GIS software can make it appear that data are accurate and precise beyond the limits of the data. When conducting this spatial analysis to assess the relationship between the species range and agricultural location, EPA made conservative assumptions related to the accuracy and precision of the available data (*e.g.,* using a 30 m resolution for the overlap process). These assumptions impact the uncertainty of the relationship, and generally overestimate the overlap between of species range and agricultural locations.

To address classification accuracy and positional accuracy of the agricultural GIS data used, EPA combined multiple years into a Use Data Layer (UDL) for each crop to represent anywhere the crop could be found. This is likely an overestimate of where a crop is found in any given year due to common agricultural practices such as rotation. Data resolution, or the smallest difference between features that could be recorded, is related to accuracy. The raster land cover data used to identify agricultural land, the Cropland data layer (CDL) produced by United States Department of Agriculture (USDA), has a resolution of 30 meters. A raster data set can be re-sampled into smaller increments, but this does not improve the resolution or accuracy of the dataset. For this reason, values cannot be established with a higher level of resolution than 30 meters, values that are not multiples of 30 cannot be determined (*e.g.,* 30, 60, 90 are distance in the dataset; 50 is not).

Precision errors can be introduced when formatting data for processing. Formatting changes can include changes to scale, reprojections of data, and data format conversions (raster to vector or vice versa). Sources of errors that are not as obvious can include those originating from the initial measurements, digitizing of data, and using different versions of a dataset. These types of precision error may introduce edge effect, or misaligned dataset when conducting the spatial analysis. Borders following the general shape of the county boundaries but do not align exactly with range information used could be result of this type of precision error.

These uncertainties impact the relationship between the agricultural areas and species locations. EPA's spatial analysis makes conservative assumptions to err on the side of overestimating the potential for species exposure when assessing the relationship of the species range to agricultural land. EPA uses five years of crop information in constructing the UDLs representing the agricultural land, so that the UDLs include every location where the crop was grown during those five years. Due to normal agricultural practices (*e.g.,* crop rotations), this is more land than expected in a given year. The relationship between the species and the agricultural land may be overestimated when the range is larger than the actual area occupied, and the additional area includes agricultural use or where edge effects were introduced. When considering the species location data, all areas may be occupied at the time the pesticide is used. County or state boundaries can be used as a conservative estimate for species range but species and natural habitats are not expected to follow man-made boundaries. When the species locations have not been refined beyond these man-made boundaries, underestimates of the relationship between species range and agricultural use can occur. While this underestimation is possible, EPA makes several conservative assumptions for agricultural land and species life history to account for this possibility. For agricultural land, use of the UDLs representing multiple years of agriculture, expands the agricultural footprint beyond what is expected in a given year. In addition to these assumptions, EPA uses the best available species location information from the species experts at USFWS and NMFS, minimizing this possibility.

4.1.3. NE and MA Determination Methodology

4.1.3.1. NE/MA Determination Methodology for Listed Species

For purposes of making the NE and MA determinations for listed species, EPA considered:

1. Listed species biological, life history and habitat information.
2. The environment of the species, habitats and exposure (*i.e.,* on-field, within 30 m of the field (terrestrial, TPEZ), within 1,500 m of the field (wetland, WPEZ or aquatic, non-wetland, APEZ).
3. If there is a direct or indirect effect on the species.

As part of the effect determinations, EPA reviewed USFWS and NMFS documents to establish the life history and habitat information for each species. Life history information is critical to determining which exposures and risks are relevant to each specific species. The life history and habitat descriptions were used to inform which environmental exposure compartment a species may be likely to occur. EPA assigned species as on-field, within 30 m of the field (terrestrial habitats, also referred to as TPEZ), within 1,500 m of the field (wetland habitats, also referred to as WPEZ), and non-wetland aquatic environments (aquatic habitats, also referred to as APEZ), such that a species may be represented by all of these modeling compartments or only one. A species was determined as being on-field based on the USFWS descriptions of the species habitat and life history. Further refinements were made with consideration of the overlap analysis. Species with less than 1% overlap were considered off-field (**Section 4.1.2; Appendix N**). All listed species that had less than 1% overlap with the field would have no risk in the on-field environmental compartment. For plants, the species that had less than 1% overlap on-field may have been captured in the TPEZ and WPEZ environmental compartments (**Appendix K**). As noted in **Table 4-1**, species that are on-field, in adjacent terrestrial habitats or in wetland habitats need additional consideration for either direct or indirect effects. Species with habitat descriptions described as 3rd order streams or greater, ponds, lakes, rivers, reservoirs, or tidal estuaries (APEZ) are not at risk because all RQs are below the LOC for listed and non-listed aquatic plants. Therefore, NE was determined for all animals, plants and habitats that were only found within these aquatic (non-wetland) habitats.

Because 2,4-D and glyphosate are herbicides, there could be a variety of effects on listed species' forage base and other resources (e.g., host species; habitat) on which they depend. As described in **Sections 2** and **3**, the toxicity data for 2,4-D and glyphosate show that both monocots and dicots are sensitive to these active ingredients, therefore overall risk to plants did not differ across products or for monocots and dicots. Neither 2,4-D nor glyphosate were determined to affect aquatic (non-wetland) habitats.

EPA further considered the habitat descriptions and determined if a species or habitat was in a location on the landscape that would not receive runoff from row-crop agricultural fields (such as corn, cotton or soybean). Extreme examples of this are represented by cliff, cave, beach dune, rocky island, and mountaintop outcrop habitats. If species or critical habitats were limited to these environments, a NE determination was made because it is assumed that these habitats will not receive runoff from corn, cotton or soybean fields (in other words, these species are not in the runoff path).

EPA considered whether the pathway to pesticide exposure is complete for an individual of a listed species or the taxa upon which it depends (*i.e.,* PPHD). In general, exposures to non-target animals and plants may occur through contact, consumption, or inhalation. The pathways of exposure that are relevant to a given pesticide are dependent upon the application parameters and fate properties of a pesticide. An exposure pathway is considered incomplete when there is no reasonable expectation of continuity between the source of pesticide exposure and an individual organism of a listed species. In other words, the exposure pathway is considered incomplete if an individual of a listed species or organisms upon which it depends are not expected to be exposed through contact, consumption, or inhalation. When the exposure pathway is incomplete, effects are not reasonably expected to occur. Therefore, a NE determination was made for a species for which exposure pathways are incomplete.

Terrestrial and Wetland Plants

As described in **Sections 2** and **3**, the toxicity data for 2,4-D and glyphosate show that both monocots and dicots are sensitive, therefore overall risk to plants did not differ across products or for monocots and dicots. The habitat and life history descriptions of each animal species were considered and placed in either on-field, terrestrial (TPEZ), within a broad definition of wetlands (WPEZ) or within fully aquatic (non-wetland) habitats (APEZ). The potential effects to plants relied upon the species sensitivity distributions (SSDs) for plant effects and was considered high when the tenth percentile IC_{25} from the SSD (HC_{10}) was exceeded. The HC_{10} was selected from the SSD because it represents the point where 90% of plants would have an IC_{25} greater than that value, and thus the endpoint would be protective of these 90%. Listed plants that were only found within the aquatic non-wetland environment were given NE determinations.

Terrestrial Animals

There could be a variety of effects on listed animal species found on-field, depending on the considerations listed above as well as the overall toxicity of Enlist One and Enlist Duo to individuals. As outlined in **Sections 2 and 3**, the toxicity of 2,4-D and glyphosate to animals varies, as well as their diet and exposure potential; however, all direct risks to animals were determined to be limited to the on-field environment where the species may occur during an application of the pesticide product. When determining the potential for effects to on-field animals, both lethal (*i.e.,* mortality) and sublethal (*i.e.,* growth, behavior and reproduction) effects to a given species were considered. For on-field animals, the level of potential adverse effect from each of the two active ingredients in the Enlist products differs, therefore, product-based determinations were made.

There may be indirect effects (*e.g.,* forage items or host species) on listed animal species with obligate relationships to plants from the use of these products. USFWS documents were used to identify the obligates considered in this assessment (see **Appendix K**). In addition to the considerations listed above, EPA considered the obligate plant species using the same process for evaluating direct risks to plants, and the obligate plant species were placed in either on-field, terrestrial (TPEZ), within a broad definition of wetlands (WPEZ) or within fully aquatic (non-wetland) habitats (APEZ).

For the majority of listed animal species where indirect effects were considered in this assessment, species did not have obligate relationships; however, they rely upon plants for habitat and/or diet (referred to as “generalists”). Similar to the process for evaluating direct risks to plants, the habitat and life history descriptions of each animal species were considered and placed in either on-field, terrestrial (TPEZ), within a broad definition of wetlands (WPEZ) or within fully aquatic (non-wetland) habitats (APEZ). The potential effects to plants relied upon the species sensitivity distributions (SSDs) for plant effects and was considered high when the tenth percentile IC_{25} from the SSD (HC_{10}) was exceeded.

Appendix K provides EPA's assignments to each of the environmental compartments and the designation of species and habitats outside of a runoff route of exposure from agriculture.

4.1.3.2. NE/MA Determination Methodology for Designated CH

EPA followed a similar habitat-specific analysis for designated CH effects determinations. As part of the effect determinations, EPA reviewed USFWS and NMFS (the Services) documents to establish the habitat information for each designated CH. The designated CH analysis is based on an assessment of how Enlist One and Enlist Duo products used on corn, cotton and soybean would affect the PCE/PBF's of the designated CH, as well as how direct species effects outcomes would impact designated CH present and future utility for promoting the conservation of a particular listed species. These PCE/PBF's are established by the USFWS or NMFS. For purposes of making the NE and MA determinations for designated CH, EPA considered:

1. If the designated CH is in the adjacent areas that receive runoff.
2. If the Services' description of designated CH characteristics indicates that the action area contains any of the Services' established PCE/PBFs that could be impacted by the use of Enlist One or Enlist Duo.
3. Consideration of the environment of the species, habitats, and exposure (*i.e.,* on-field, within 30 m of the field (Terrestrial, TPEZ), within 1,500 m of the field (Wetland, WPEZ) or Aquatic (non-wetland, APEZ).

As described above to listed species, designated CH that were described as 3rd order streams or greater, ponds, lakes, rivers, reservoirs, or tidal estuaries (APEZ) are not at risk because all RQs are below the LOC for listed and non-listed aquatic plants. Therefore, EPA made NE determinations for all designated CH that were only found within these aquatic (non-wetland) habitats. Additionally, EPA made NE determinations for designated CH that were not in the runoff flow path or had incomplete exposure pathways. **Appendix K** provides EPA's assignments to each of the environmental compartments and the designation of species and habitats outside of a runoff route of exposure from agriculture.

4.1.3.3. Refinements for the Listed Species that Overlap with the Action Area

EPA determined that there are several species of plants, birds, mammals, terrestrial-phase amphibians and reptiles that may be located on agricultural fields. For all plants potentially located on treated field, EPA made MA determinations. For all listed vertebrate species that may be located on treated fields, EPA evaluated additional lines of evidence for the determination.

For terrestrial vertebrates, EPA determined Enlist One and Enlist Duo exposure values from the upper bound of the modeled T-REX run for exposures following spray applications based on the Kenaga nomogram modified by Fletcher *et al* (1984), which is based on a large set of actual field residue data. EPA's refined assessment investigated the impacts of more specific-species data related to:

1. Estimated food consumption based on:
    a. The body weight of the species
    b. The species' diet and
    c. Species taxonomy
2. Toxicity endpoints were scaled from the weight of the tested surrogate species to reflect the size of the listed animal, where appropriate.

Full details of this approach can be found in **Appendix L**.

4.1.4. NLAA and LAA Determination Methodology

This method builds upon those described in **Sections 4.1.3**.

4.1.4.1. NLAA/LAA Determination Methodology for Listed Species

For purposes of making the NLAA and LAA determinations, EPA considered:

1. Whether a species is presumed extinct by USFWS.
2. The effects of the action in the context of the status of the listed species, including lethal (*i.e.,* mortality) and sublethal effects (*i.e.,* growth and reproduction).
3. The effects to other resources that the listed species rely upon (*i.e.,* for prey, pollination, habitat and/or dispersal).
4. The general habitat descriptions for the listed species (**Appendix K**).

Species Presumed Extinct

Species recommended for delisting due to extinction by the Services are presumed extinct and receive a NLAA determination. EPA made NLAA determinations for six species as exposure from the action is not reasonably certain to occur, and, therefore, effects on the species are not anticipated. Species are only presumed extinct after a recommendation to delist is made by the Services in a review document (*e.g.*, Recovery plan, 5-year review).

Terrestrial and Wetland Plants

Because of the direct toxicity to terrestrial and wetland plants, EPA assigned a species an LAA determination if it is likely present in the flow path. Since no direct risks to animals were identified in off-field environments, there are no identified indirect effects to plants.

Terrestrial Animals

For on-field animals, EPA made a LAA determination where the refined RQs exceeded the LOC. For obligate animals, EPA made a LAA determination for the listed animal if the obligate plant was present within terrestrial or wetland habitats potentially receiving runoff from corn, cotton and/or soybean fields. For generalist animals, EPA made a LAA determination if there was potential for indirect effects based on direct effect to plants within terrestrial or wetland habitats potentially receiving runoff from corn, cotton and/or soybean fields. Additionally, EPA considered that some species require other species to complete their life cycle, such as freshwater mussels that require host fish for survival and development of glochidia (juvenile life stage), and that these fish may rely upon plant habitats. In these cases, EPA determined that effects on plants were not reasonably expected to translate through the ecosystem to impact non-plant dependent taxa, therefore these non-plant dependent species were designated as NLAA.

4.1.4.2. NLAA/LAA Determination Methodology for Designated CH

In this assessment, EPA identified numerous listed designated CH that may be affected by the action (**Appendix K**). For purposes of making the NLAA and LAA determinations, EPA considered:

1. The effects of the action in the context of the status of the critical habitat, which describes the range-wide condition of the designated CH in terms of the key components (i.e., PCEs) that provide for the conservation of the listed species, the factors responsible for that condition, and the intended value of the designated CH overall for the conservation/recovery of the listed species.
2. The general designated CH descriptions (**Appendix K**).
3. The potential effects to designated CH based upon the species sensitivity distributions for plant effects and was considered high when EECs exceeded the HC_{10}.
4. Consideration of the habitat description and the exposure (*i.e.,* on-field, within 30 m of the field (terrestrial, TPEZ), within 1,500 m of the field (wetland, WPEZ) or outside of the action area (aquatic non-wetland, APEZ)).

For designated CH that had plant based PCEs, EPA made a LAA determination if there was potential for indirect effects based on direct effect to plants within terrestrial or wetland habitats potentially receiving runoff from corn, cotton and/or soybean fields. For designated CH that did not include PCE descriptions of integral plant portions of the habitat, EPA made a NLAA was determination. Lastly, EPA made a NLAA determination if the PCEs did not describe the habitats of larval (glochidia) hosts.

Some designated CH had descriptions and/or PCEs that include agricultural land. For the portion of the designated CH that was agriculture, EPA designated these as NLAA, as those habitats are maintained by the agricultural practice including the use of herbicides and would not be expected altered by the action. No designated CH only had agricultural fields listed, so EPA used information for the other off-field habitats to determine NLAA/LAA.

4.1.5. Methodology Used to Determine Likelihood of Jeopardy

For listed species and designated CH with LAA determinations, EPA evaluated whether the effects are likely to lead to jeopardy or adverse modification. To accomplish this, EPA based its approach on the information contained in FWS's draft Biological Opinion for Malathion[33]. The primary goal of performing this analysis was to identify listed species and designated CH for which EPA would need additional mitigations in order to avoid a potential likelihood of Jeopardy or adverse modification. While EPA needs meaningful and effective mitigations to reduce take for all LAA species and designated CH, EPA

[33] https://www.epa.gov/endangered-species/biological-opinions-available-public-comment

recognizes that some species and designated CH may require more restrictive or special mitigations in order to avoid jeopardy.

4.1.5.1. Likelihood of Jeopardy Determination Methodology for Listed Species

EPAs approach builds upon those described in **Sections 4.1.3 and 4.1.4**, by classifying the LAA species based on their adverse effect potential. There were no MA determinations for NMFS species. Since all NLAA and LAA determinations were for species for which USFWS is responsible, USFWS's methodology for evaluating whether a listed species is likely to rise to jeopardy is most relevant. Therefore, EPA considered the elements and factors presented in the USFWS Draft Malathion Biological Opinion (2021)[34] to evaluate likely J/AM from the Enlist products. For purposes of determining whether the action is likely to jeopardize the continued existence of a species, EPA evaluated:

1. The overall vulnerability (i.e., low, medium or high)[35] of the listed species as outlined in the USFWS Draft Malathion Biological Opinion (2021)[36].
2. The overlap of the listed species with the action area (i.e., low = < 5% overlap; medium = >%5 but below 10% overlap; high = >10% overlap; USFWS Draft Malathion Biological Opinion 2021).
3. Additional risk modifiers including whether pesticides are noted as contributing to the overall decline of the listed species (USFWS Draft Malathion Biological Opinion 2021), whether a listed species occupies diverse habitats and the importance of plants in the life history.

Terrestrial and Wetland Animals

As discussed previously, the proposed action may have direct effects on on-field animals and indirect effects (*e.g.,* forage items or host species) on listed animal species with obligate or generalist relationships to plants. In addition to the considerations discussed in **Sections 4.1.3** and **4.1.4**, the potential for rising to the level of jeopardy was generally ranked higher where the USFWS draft malathion BiOp listed the vulnerability of the species as being high or medium, and the overlap with the on-field use area was high (≥10%) or medium (≥5%). EPA determined LAA, but not rising to the level of the likelihood of jeopardy for listed species with low vulnerability or low (<5%) overlap. For on-field animals, the level of potential adverse effect from each of the two active ingredients in the Enlist products differs, therefore, EPA made product-based likelihood of jeopardy determinations.

For terrestrial and wetland animals, EPA also considered risk modifiers. For on-field, obligate and generalist animals, EPA considered the documentation of pesticides being a threat. If a listed species had low vulnerability or low (<5%) overlap, but pesticides were identified as a concern for the species in the USFWS draft malathion BiOp (**Appendix K**), EPA determined the listed species had the potential for a jeopardy finding. For generalist animals, EPA also considered whether the generalist animal species used a variety of habitat types or food sources (e.g., plants are not the primary diet). If a listed generalist animal had high or medium vulnerability and the overlap with the use area was high (≥10%) or medium

[34] https://www.epa.gov/endangered-species/biological-opinions-available-public-comment
[35] High vulnerability was assumed when this information was not available.
[36] https://www.epa.gov/endangered-species/biological-opinions-available-public-comment

(≥5%), but the listed animal utilizes multiple terrestrial and wetland habitat types or does not depend on plants for the primary diet, EPA determined it would not likely lead to jeopardy.

Terrestrial and Wetland Plants

EPA concluded that all terrestrial and wetland plant species that had a vulnerability of medium or high, and an overlap of medium (≥5%) to high (≥10%) would have the potential to rise to the level of jeopardy.

4.1.5.2. Likelihood of Adverse Modification Determination Methodology for Designated CH

EPA followed a similar habitat-specific analysis for designated CH. For purposes of evaluating whether there was likelihood for destruction or adverse modification of designated CH, EPA evaluated:

1. The overall vulnerability (i.e., low, medium or high)[37] of the listed species associated with the designated CH as outlined in the USFWS Draft Malathion Biological Opinion (2021)[38].
2. The overlap of the designated CH with the action area (i.e., low = < 5% overlap; medium = >%5 but below 10% overlap; high = >10% overlap; USFWS Draft Malathion Biological Opinion 2021).
3. Additional risk modifiers including whether pesticides are noted as contributing to the overall decline of the listed species associated with the designated CH (USFWS Draft Malathion Biological Opinion 2021) and whether a designated CH consists of diverse habitats.

EPA evaluated the likelihood of destruction or adverse modification for designated CH primarily upon the direct risk to the plant component of the PCEs and the degree of overlap. All plant based PCEs that were associated with terrestrial or wetland plants were determined to be at high risk based on the exceedance of the species sensitivity distribution HC_{10}. All designated CH with medium (≥5%) and high (≥10%) overlaps were determined to have a likelihood of destruction or adverse modification. For designated CH that had low (<5%) overlap with the action area, EPA concluded that destruction or adverse modification is not likely. EPA also concluded that destruction or adverse modification was not likely for designated CH PCEs identified as agricultural fields as these are maintained by the agricultural practice and likely include the use of pesticides.

4.2. Determination Results

4.2.1. NE and MA Determination Results

EPA identified listed species within the action area that could be affected in the 34 states from Enlist One and Enlist Duo product use (**Section 2**, **3** and **4**). EPA identified 24 listed animals that could experience on-field direct effects, 108 listed plants that could experience direct effects, 5 listed species that have an obligate relationship with terrestrial plants, and 291 generalist species (relying on plants; **Table 4-3**). EPA then followed the methodology outlined in **Section 4.1.3** and made MA or NE

[37] High vulnerability was assumed when this information was not available.
[38] https://www.epa.gov/endangered-species/biological-opinions-available-public-comment

determinations. Further information regarding the species considerations supporting these determinations is provided in **Appendix K**.

Table 4-3. List of Listed Species of Concern

Entity ID	Common Name (Refinements Section Number)	Scientific Name
On-field Animals: Birds (Sections 4.2.1.2)		
83	Attwater's greater prairie-chicken	*Tympanuchus cupido attwateri*
91	Eskimo curlew	*Numenius borealis*
139	Golden-cheeked warbler (=wood)	*Dendroica chrysoparia*
126	Northern aplomado falcon	*Falco femoralis septentrionalis*
84	Yuma Ridgways (clapper) rail	*Rallus obsoletus [=longirostris] yumanensis*
On-field Animals: Reptiles and Amphibians (Section 4.2.1.3)		
199	Frosted Flatwoods salamander	*Ambystoma cingulatum*
190	Houston toad	*Bufo houstonensis*
192	Red Hills salamander	*Phaeognathus hubrichti*
9943	Reticulated flatwoods salamander	*Ambystoma bishopi*
168	Alabama red-belly turtle	*Pseudemys alabamensis*
182	Bog turtle	*Clemmys muhlenbergii*
180	Copperbelly water snake	*Nerodia erythrogaster neglecta*
7800	Eastern Massasauga (=rattlesnake)	*Sistrurus catenatus*
3722	Louisiana pine snake	*Pituophis ruthveni*
171	Ringed map turtle	*Graptemys oculifera*
On-field Animals: Mammals (Section 4.2.1.4)		
60	Florida salt marsh vole	*Microtus pennsylvanicus dukecampbelli*
22	Gulf Coast jaguarundi	*Herpailurus (=Felis) yagouaroundi cacomitli*
30	Ocelot	*Leopardus (=Felis) pardalis*
25	Ozark big-eared bat	*Corynorhinus (=Plecotus) townsendii ingens*
35	Perdido Key beach mouse	*Peromyscus polionotus trissyllepsis*
52	Preble's meadow jumping mouse	*Zapus hudsonius preblei*
9	Sonoran pronghorn	*Antilocapra americana sonoriensis*
27	Virginia big-eared bat	*Corynorhinus (=Plecotus) townsendii virginianus*
Plants: Ferns and Allies (Section 4.2.1.6)		
1195	American hart's-tongue fern	*Asplenium scolopendrium var. americanum*
1203	Black spored quillwort	*Isoetes melanospora*
1199	Louisiana quillwort	*Isoetes louisianensis*
1204	Mat-forming quillwort	*Isoetes tegetiformans*
Plants: Conifers and Cycads (Section 4.2.1.6)		
1191	Florida torreya	*Torreya taxifolia*
Plants: Monocots (Section 4.2.1.6)		
818	Bunched arrowhead	*Sagittaria fasciculata*
1172	Canelo Hills ladies'-tresses	*Spiranthes delitescens*
930	Clay-Loving wild buckwheat	*Eriogonum pelinophilum*
950	Dwarf lake iris	*Iris lacustris*
984	Eastern prairie fringed orchid	*Platanthera leucophaea*
1189	Golden sedge	*Carex lutea*
723	Harper's beauty	*Harperocallis flava*
1228	Knieskern's Beaked-rush	*Rhynchospora knieskernii*
1064	Kral's water-plantain	*Sagittaria secundifolia*
807	Little Aguja (=Creek) Pondweed	*Potamogeton clystocarpus*

Entity ID	Common Name (Refinements Section Number)	Scientific Name
935	Minnesota dwarf trout lily	*Erythronium propullans*
656	Navajo sedge	*Carex specuicola*
837	Navasota ladies'-tresses	*Spiranthes parksii*
823	Northeastern bulrush	*Scirpus ancistrochaetus*
857	Persistent trillium	*Trillium persistens*
946	Swamp pink	*Helonias bullata*
1017	Tennessee yellow-eyed grass	*Xyris tennesseensis*
870	Texas wild-rice	*Zizania texana*
1073	Ute ladies'-tresses	*Spiranthes diluvialis*
1080	Western prairie fringed Orchid	*Platanthera praeclara*
1415	White fringeless orchid	*Platanthera integrilabia*
Plants: Dicots (Section 4.2.1.6)		
994	Alabama canebrake pitcher-plant	*Sarracenia rubra ssp. alabamensis*
1048	Alabama leather flower	*Clematis socialis*
702	Black lace cactus	*Echinocereus reichenbachii var. albertii*
1004	Blue Ridge goldenrod	*Solidago spithamaea*
630	Braun's rock-cress	*Arabis perstellata*
653	Brooksville bellflower	*Campanula robinsiae*
976	Canby's dropwort	*Oxypolis canbyi*
816	Chapman rhododendron	*Rhododendron chapmanii*
925	Chisos Mountain hedgehog Cactus	*Echinocereus chisoensis var. chisoensis*
824	Colorado hookless Cactus	*Sclerocactus glaucus*
852	Cooley's meadowrue	*Thalictrum cooleyi*
677	Cumberland rosemary	*Conradina verticillata*
891	Decurrent false aster	*Boltonia decurrens*
734	Dwarf-flowered heartleaf	*Hexastylis naniflora*
977	Fassett's locoweed	*Oxytropis campestris var. chartacea*
1710	Fleshy-fruit gladecress	*Leavenworthia crassa*
997	Florida skullcap	*Scutellaria floridana*
831	Fringed campion	*Silene polypetala*
836	Gentian pinkroot	*Spigelia gentianoides*
982	Godfrey's butterwort	*Pinguicula ionantha*
819	Green pitcher-plant	*Sarracenia oreophila*
1087	Guthrie's (=Pyne's) ground-plum	*Astragalus bibullatus*
991	Harperella	*Ptilimnium nodosum*
959	Heller's blazingstar	*Liatris helleri*
1003	Houghton's goldenrod	*Solidago houghtonii*
1030	Huachuca water-umbel	*Lilaeopsis schaffneriana var. recurva*
7167	Kentucky glade cress	*Leavenworthia exigua laciniata*
1059	Lakeside daisy	*Hymenoxys herbacea*
998	Large-flowered skullcap	*Scutellaria montana*
872	Large-fruited sand-verbena	*Abronia macrocarpa*
920	Leafy prairie-clover	*Dalea foliosa*
1150	Leedy's roseroot	*Rhodiola integrifolia ssp. leedyi*
625	Little amphianthus	*Amphianthus pusillus*
750	Lyrate bladderpod	*Lesquerella lyrata*
636	Mead's milkweed	*Asclepias meadii*
817	Miccosukee gooseberry	*Ribes echinellum*
992	Michaux's sumac	*Rhus michauxii*

Entity ID	Common Name (Refinements Section Number)	Scientific Name
969	Michigan monkey-flower	*Mimulus michiganensis*
764	Mohr's Barbara's buttons	*Marshallia mohrii*
1096	Morefield's leather flower	*Clematis morefieldii*
1058	Mountain golden heather	*Hudsonia montana*
995	Mountain sweet pitcher-plant	*Sarracenia rubra ssp. jonesii*
6617	Neches River rose-mallow	*Hibiscus dasycalyx*
716	No common name	*Geocarpon minimum*
797	North Park phacelia	*Phacelia formosula*
620	Northern wild monkshood	*Aconitum noveboracense*
914	Okeechobee gourd	*Cucurbita okeechobeensis ssp. okeechobeensis*
558	Pecos (=puzzle =paradox) sunflower	*Helianthus paradoxus*
949	Peter's Mountain mallow	*Iliamna corei*
905	Pitcher's thistle	*Cirsium pitcheri*
960	Pondberry	*Lindera melissifolia*
943	Roan Mountain bluet	*Hedyotis purpurea var. montana*
967	Rough-leaved loosestrife	*Lysimachia asperulaefolia*
1036	Ruth's golden aster	*Pityopsis ruthii*
906	Sacramento Mountains thistle	*Cirsium vinaceum*
945	Schweinitz's sunflower	*Helianthus schweinitzii*
1019	Seabeach amaranth	*Amaranthus pumilus*
875	Sensitive joint-vetch	*Aeschynomene virginica*
1831	Short's bladderpod	*Physaria globosa*
835	Short's goldenrod	*Solidago shortii*
739	Slender rush-pea	*Hoffmannseggia tenella*
655	Small-anthered bittercress	*Cardamine micranthera*
924	Smooth coneflower	*Echinacea laevigata*
624	South Texas ambrosia	*Ambrosia cheiranthifolia*
718	Spreading avens	*Geum radiatum*
568	Spring Creek bladderpod	*Lesquerella perforata*
513	Star cactus	*Astrophytum asterias*
937	Telephus spurge	*Euphorbia telephioides*
1077	Texas ayenia	*Ayenia limitaris*
1400	Texas golden Gladecress	*Leavenworthia texana*
651	Texas poppy-mallow	*Callirhoe scabriuscula*
1045	Texas prairie dawn-flower	*Hymenoxys texana*
627	Tobusch fishhook cactus	*Sclerocactus brevihamatus ssp. tobuschii*
644	Virginia round-leaf birch	*Betula uber*
1028	Virginia sneezeweed	*Helenium virginicum*
1039	Virginia spiraea	*Spiraea virginiana*
763	Walker's manioc	*Manihot walkerae*
884	Welsh's milkweed	*Asclepias welshii*
761	White birds-in-a-nest	*Macbridea alba*
1153	White irisette	*Sisyrinchium dichotomum*
1881	Whorled Sunflower	*Helianthus verticillatus*
569	Zapata bladderpod	*Lesquerella thamnophila*
Obligate Animals: Birds (Section 4.2.1.7)		
139	Golden-cheeked warbler (=wood)	*Dendroica chrysoparia*
107	Red-cockaded woodpecker	*Picoides borealis*
Obligate Animals: Terrestrial Invertebrates (Section 4.2.1.7)		

Entity ID	Common Name (Refinements Section Number)	Scientific Name
420	Karner blue butterfly	*Lycaeides melissa samuelis*
424	Mitchell's satyr Butterfly	*Neonympha mitchellii mitchellii*
455	Saint Francis' satyr butterfly	*Neonympha mitchellii francisci*
Generalist Species: Amphibians (Section 4.2.1.8)		
6346	Austin blind Salamander	*Eurycea waterlooensis*
197	Barton Springs salamander	*Eurycea sosorum*
198	Cheat Mountain salamander	*Plethodon nettingi*
206	Chiricahua leopard frog	*Rana chiricahuensis*
208	Dusky gopher frog	*Rana sevosa*
199	Frosted Flatwoods salamander	*Ambystoma cingulatum*
5434	Georgetown Salamander	*Eurycea naufragia*
190	Houston toad	*Bufo houstonensis*
3849	Jemez Mountains salamander	*Plethodon neomexicanus*
8231	Jollyville Plateau Salamander	*Eurycea tonkawae*
192	Red Hills salamander	*Phaeognathus hubrichti*
9943	Reticulated flatwoods salamander	*Ambystoma bishopi*
7610	Salado Salamander	*Eurycea chisholmensis*
194	San Marcos salamander	*Eurycea nana*
201	Sonora tiger Salamander	*Ambystoma tigrinum stebbinsi*
189	Texas blind salamander	*Typhlomolge rathbuni*
Generalist Species: Birds (Section 4.2.1.8)		
83	Attwater's greater prairie-chicken	*Tympanuchus cupido attwateri*
93	Bachman's warbler (=wood)	*Vermivora bachmanii*
66	California condor	*Gymnogyps californianus*
96	California least tern	*Sterna antillarum browni*
11319	Eastern Black rail	*Laterallus jamaicensis ssp. jamaicensis*
1221	Everglade snail kite	*Rostrhamus sociabilis plumbeus*
140	Florida scrub-jay	*Aphelocoma coerulescens*
139	Golden-cheeked warbler (=wood)	*Dendroica chrysoparia*
4064	Gunnison sage-grouse	*Centrocercus minimus*
89	Masked bobwhite (quail)	*Colinus virginianus ridgwayi*
129	Mexican spotted owl	*Strix occidentalis lucida*
110	Mississippi sandhill crane	*Grus canadensis pulla*
126	Northern aplomado falcon	*Falco femoralis septentrionalis*
131	Piping Plover	*Charadrius melodus*
130	Piping Plover	*Charadrius melodus*
8621	Red knot	*Calidris canutus rufa*
107	Red-cockaded woodpecker	*Picoides borealis*
135	Roseate tern	*Sterna dougallii dougallii*
136	Roseate tern	*Sterna dougallii dougallii*
149	Southwestern willow flycatcher	*Empidonax traillii extimus*
67	Whooping crane	*Grus americana*
124	Wood stork	*Mycteria americana*
6901	Yellow-billed Cuckoo	*Coccyzus americanus*
Generalist Species: Clams (Section 4.2.1.8)		
356	Alabama (=inflated) heelsplitter	*Potamilus inflatus*
326	Alabama lampmussel	*Lampsilis virescens*
380	Alabama moccasinshell	*Medionidus acutissimus*
4411	Alabama pearlshell	*Margaritifera marrianae*

Entity ID	Common Name (Refinements Section Number)	Scientific Name
354	Appalachian elktoe	*Alasmidonta raveneliana*
329	Appalachian monkeyface (pearlymussel)	*Quadrula sparsa*
369	Arkansas fatmucket	*Lampsilis powellii*
332	Birdwing pearlymussel	*Lemiox rimosus*
370	Carolina heelsplitter	*Lasmigona decorata*
386	Chipola slabshell	*Elliptio chipolaensis*
352	Clubshell	*Pleurobema clava*
381	Coosa moccasinshell	*Medionidus parvulus*
317	Cumberland bean (pearlymussel)	*Villosa trabalis*
355	Cumberland elktoe	*Alasmidonta atropurpurea*
330	Cumberland monkeyface (pearlymussel)	*Quadrula intermedia*
376	Cumberland pigtoe	*Pleurobema gibberum*
353	Cumberlandian combshell	*Epioblasma brevidens*
333	Curtis pearlymussel	*Epioblasma florentina curtisii*
382	Dark pigtoe	*Pleurobema furvum*
334	Dromedary pearlymussel	*Dromus dromas*
363	Dwarf wedgemussel	*Alasmidonta heterodon*
368	Fanshell	*Cyprogenia stegaria*
342	Fat pocketbook	*Potamilus capax*
375	Fat threeridge (mussel)	*Amblema neislerii*
372	Finelined pocketbook	*Lampsilis altilis*
337	Finerayed pigtoe	*Fusconaia cuneolus*
1559	Fluted kidneyshell	*Ptychobranchus subtentus*
1369	Fuzzy pigtoe	*Pleurobema strodeanum*
384	Gulf moccasinshell	*Medionidus penicillatus*
350	Heavy pigtoe	*Pleurobema taitianum*
325	Higgins eye (pearlymussel)	*Lampsilis higginsii*
361	James spinymussel	*Pleurobema collina*
335	Littlewing pearlymussel	*Pegias fabula*
364	Louisiana pearlshell	*Margaritifera hembeli*
7177	Narrow pigtoe	*Fusconaia escambia*
4086	Neosho Mucket	*Lampsilis rafinesqueana*
374	Northern riffleshell	*Epioblasma torulosa rangiana*
340	Orangefoot pimpleback (pearlymussel)	*Plethobasus cooperianus*
357	Orangenacre mucket	*Lampsilis perovalis*
343	Ouachita rock pocketbook	*Arkansia wheeleri*
371	Oval pigtoe	*Pleurobema pyriforme*
358	Oyster mussel	*Epioblasma capsaeformis*
327	Pale lilliput (pearlymussel)	*Toxolasma cylindrellus*
331	Pink mucket (pearlymussel)	*Lampsilis abrupta*
366	Purple bankclimber (mussel)	*Elliptoideus sloatianus*
318	Purple bean	*Villosa perpurpurea*
323	Purple Cat's paw (=Purple Cat's paw pearlymussel)	*Epioblasma obliquata obliquata*
3645	Rabbitsfoot	*Quadrula cylindrica cylindrica*
6062	Rayed Bean	*Villosa fabalis*
344	Rough rabbitsfoot	*Quadrula cylindrica strigillata*
345	Scaleshell mussel	*Leptodea leptodon*
7816	Sheepnose Mussel	*Plethobasus cyphyus*

Entity ID	Common Name (Refinements Section Number)	Scientific Name
339	Shiny pigtoe	*Fusconaia cor*
373	Shinyrayed pocketbook	*Lampsilis subangulata*
6841	Slabside Pearlymussel	*Pleuronaia dolabelloides*
5281	Snuffbox mussel	*Epioblasma triquetra*
365	Southern acornshell	*Epioblasma othcaloogensis*
378	Southern clubshell	*Pleurobema decisum*
348	Southern combshell	*Epioblasma penita*
7949	Southern kidneyshell	*Ptychobranchus jonesi*
383	Southern pigtoe	*Pleurobema georgianum*
7349	Southern sandshell	*Hamiota australis*
360	Speckled pocketbook	*Lampsilis streckeri*
4490	Spectaclecase (mussel)	*Cumberlandia monodonta*
362	Stirrupshell	*Quadrula stapes*
7372	Suwannee moccasinshell	*Medionidus walkeri*
346	Tan riffleshell	*Epioblasma florentina walkeri (=E. walkeri)*
6534	Tapered pigtoe	*Fusconaia burkei*
351	Tar River spinymussel	*Elliptio steinstansana*
2917	Texas Hornshell	*Popenaias popeii*
379	Triangular Kidneyshell	*Ptychobranchus greenii*
367	Upland combshell	*Epioblasma metastriata*
324	White catspaw (pearlymussel)	*Epioblasma obliquata perobliqua*
336	White wartyback (pearlymussel)	*Plethobasus cicatricosus*
328	Winged Mapleleaf	*Quadrula fragosa*
4074	Yellow lance	*Elliptio lanceolata*
Generalist Species: Crustaceans (Section 4.2.1.8)		
480	Alabama cave shrimp	*Palaemonias alabamae*
489	Benton County cave crayfish	*Cambarus aculabrum*
475	Hay's Spring amphipod	*Stygobromus hayi*
488	Hell Creek Cave crayfish	*Cambarus zophonastes*
484	Illinois cave amphipod	*Gammarus acherondytes*
482	Kentucky cave shrimp	*Palaemonias ganteri*
486	Lee County cave isopod	*Lirceus usdagalun*
476	Madison Cave isopod	*Antrolana lira*
477	Peck's cave amphipod	*Stygobromus (=Stygonectes) pecki*
6596	Pecos amphipod	*Gammarus pecos*
483	Socorro isopod	*Thermosphaeroma thermophilus*
487	Squirrel Chimney Cave shrimp	*Palaemonetes cummingi*
Generalist Species: Fishes (Section 4.2.1.8)		
236	Alabama cavefish	*Speoplatyrhinus poulsoni*
220	Apache trout	*Oncorhynchus apache*
299	Arkansas River shiner	*Notropis girardi*
10297	Atlantic sturgeon	*Acipenser oxyrinchus oxyrinchus*
10298	Atlantic sturgeon	*Acipenser oxyrinchus oxyrinchus*
10299	Atlantic sturgeon	*Acipenser oxyrinchus oxyrinchus*
10300	Atlantic sturgeon	*Acipenser oxyrinchus oxyrinchus*
10301	Atlantic sturgeon	*Acipenser oxyrinchus oxyrinchus*
286	Atlantic sturgeon (Gulf subspecies)	*Acipenser oxyrinchus (=oxyrhynchus) desotoi*
244	Bayou darter	*Etheostoma rubrum*
276	Beautiful shiner	*Cyprinella formosa*

Entity ID	Common Name (Refinements Section Number)	Scientific Name
213	Big Bend gambusia	*Gambusia gaigei*
295	Blackside dace	*Phoxinus cumberlandensis*
300	Blue shiner	*Cyprinella caerulea*
307	Bluemask darter	*Etheostoma akatulo*
277	Cahaba shiner	*Notropis cahabae*
8352	Candy darter	*Etheostoma osburni*
242	Cape Fear shiner	*Notropis mekistocholas*
269	Cherokee darter	*Etheostoma scotti*
254	Chihuahua chub	*Gila nigrescens*
7150	Chucky Madtom	*Noturus crypticus*
214	Clear Creek gambusia	*Gambusia heterochir*
215	Colorado pikeminnow (=squawfish)	*Ptychocheilus lucius*
216	Comanche Springs pupfish	*Cyprinodon elegans*
5719	Cumberland darter	*Etheostoma susanae*
275	Desert pupfish	*Cyprinodon macularius*
272	Devils River minnow	*Dionda diaboli*
6557	Diamond Darter	*Crystallaria cincotta*
315	Etowah darter	*Etheostoma etowahae*
228	Fountain darter	*Etheostoma fonticola*
NMFS174	Giant manta ray	*Manta birostris*
6297	Gila chub	*Gila intermedia*
219	Gila topminnow (incl. Yaqui)	*Poeciliopsis occidentalis*
221	Gila trout	*Oncorhynchus gilae*
298	Goldline darter	*Percina aurolineata*
222	Greenback Cutthroat trout	*Oncorhynchus clarki stomias*
4248	Grotto Sculpin	*Cottus specus*
209	Humpback chub	*Gila cypha*
10060	Kentucky arrow darter	*Etheostoma spilotum*
233	Lahontan cutthroat trout	*Oncorhynchus clarkii henshawi*
9220	Laurel dace	*Chrosomus saylori*
251	Leon Springs pupfish	*Cyprinodon bovinus*
281	Little Colorado spinedace	*Lepidomeda vittata*
273	Loach minnow	*Tiaroga cobitis*
212	Maryland darter	*Etheostoma sellare*
211	Moapa dace	*Moapa coriacea*
257	Niangua darter	*Etheostoma nianguae*
224	Okaloosa darter	*Etheostoma okaloosae*
260	Ozark cavefish	*Amblyopsis rosae*
8389	Pahrump poolfish	*Empetrichthys latos*
278	Palezone shiner	*Notropis albizonatus*
303	Pallid sturgeon	*Scaphirhynchus albus*
279	Pecos bluntnose shiner	*Notropis simus pecosensis*
230	Pecos gambusia	*Gambusia nobilis*
241	Pygmy Sculpin	*Cottus paulus (=pygmaeus)*
290	Razorback sucker	*Xyrauchen texanus*
313	Relict darter	*Etheostoma chienense*
3525	Rush Darter	*Etheostoma phytophilum*
250	San Marcos gambusia	*Gambusia georgei*
4330	Shortnose sturgeon	*Acipenser brevirostrum*

Entity ID	Common Name (Refinements Section Number)	Scientific Name
239	Slackwater darter	*Etheostoma boschungi*
4881	Smalltooth sawfish	*Pristis pectinata*
258	Smoky madtom	*Noturus baileyi*
235	Snail darter	*Percina tanasi*
255	Sonora chub	*Gila ditaenia*
296	Spikedace	*Meda fulgida*
7332	Spring pygmy sunfish	*Elassoma alabamae*
311	Topeka shiner	*Notropis topeka (=tristis)*
3069	Trispot darter	*Etheostoma trisella*
316	Vermilion darter	*Etheostoma chermocki*
256	Virgin River Chub	*Gila seminuda (=robusta)*
243	Waccamaw silverside	*Menidia extensa*
229	Watercress darter	*Etheostoma nuchale*
234	Woundfin	*Plagopterus argentissimus*
259	Yaqui catfish	*Ictalurus pricei*
263	Yaqui chub	*Gila purpurea*
6662	Yellowcheek Darter	*Etheostoma moorei*
247	Yellowfin madtom	*Noturus flavipinnis*
3280	Zuni bluehead Sucker	*Catostomus discobolus yarrowi*
Generalist Species: Insects (Section 4.2.1.8)		
440	American burying beetle	*Nicrophorus americanus*
454	Comal Springs dryopid beetle	*Stygoparnus comalensis*
453	Comal Springs riffle beetle	*Heterelmis comalensis*
3412	Dakota Skipper	*Hesperia dacotae*
445	Hine's emerald dragonfly	*Somatochlora hineana*
441	Hungerford's crawling water Beetle	*Brychius hungerfordi*
420	Karner blue butterfly	*Lycaeides melissa samuelis*
10909	Miami tiger beetle	*Cicindelidia floridana*
424	Mitchell's satyr Butterfly	*Neonympha mitchellii mitchellii*
442	Northeastern beach tiger beetle	*Cicindela dorsalis dorsalis*
434	Pawnee montane skipper	*Hesperia leonardus montana*
10147	Poweshiek skipperling	*Oarisma poweshiek*
443	Puritan tiger beetle	*Cicindela puritana*
10383	Rusty patched bumble bee	*Bombus affinis*
455	Saint Francis' satyr butterfly	*Neonympha mitchellii francisci*
4910	Salt Creek Tiger beetle	*Cicindela nevadica lincolniana*
437	Uncompahgre fritillary butterfly	*Boloria acrocnema*
Generalist Species: Lichens (Section 4.2.1.8)		
1219	Florida perforate cladonia	*Cladonia perforata*
1220	Rock gnome lichen	*Gymnoderma lineare*
Generalist Species: Mammals (Section 4.2.1.8)		
41	Alabama beach mouse	*Peromyscus polionotus ammobates*
50	Anastasia Island beach mouse	*Peromyscus polionotus phasma*
5	Black-footed ferret	*Mustela nigripes*
24	Canada Lynx	*Lynx canadensis*
42	Carolina northern flying squirrel	*Glaucomys sabrinus coloratus*
34	Choctawhatchee beach mouse	*Peromyscus polionotus allophrys*
21	Gray bat	*Myotis grisescens*
22	Gulf Coast jaguarundi	*Herpailurus (=Felis) yagouaroundi cacomitli*

Entity ID	Common Name (Refinements Section Number)	Scientific Name
1	Indiana bat	*Myotis sodalis*
18	Jaguar	*Panthera onca*
48	Mexican long-nosed bat	*Leptonycteris nivalis*
13	Mexican wolf	*Canis lupus baileyi*
43	Mount Graham red squirrel	*Tamiasciurus hudsonicus grahamensis*
5210	New Mexico meadow jumping mouse	*Zapus hudsonius luteus*
10043	Northern Long-Eared Bat	*Myotis septentrionalis*
30	Ocelot	*Leopardus (=Felis) pardalis*
52	Preble's meadow jumping mouse	*Zapus hudsonius preblei*
14	Red wolf	*Canis rufus*
53	Southeastern beach mouse	*Peromyscus polionotus niveiventris*
54	St. Andrew beach mouse	*Peromyscus polionotus peninsularis*
Generalist Species: Reptiles (Section 4.2.1.8)		
176	American crocodile	*Crocodylus acutus*
167	Atlantic salt marsh snake	*Nerodia clarkii taeniata*
6097	Black pine snake	*Pituophis melanoleucus lodingi*
182	Bog turtle	*Clemmys muhlenbergii*
185	Desert tortoise	*Gopherus agassizii*
173	Eastern indigo snake	*Drymarchon corais couperi*
7800	Eastern Massasauga (=rattlesnake)	*Sistrurus catenatus*
169	Flattened musk turtle	*Sternotherus depressus*
181	Gopher tortoise	*Gopherus polyphemus*
3722	Louisiana pine snake	*Pituophis ruthveni*
3271	Narrow-headed gartersnake	*Thamnophis rufipunctatus*
166	New Mexican ridge-nosed rattlesnake	*Crotalus willardi obscurus*
1783	Northern Mexican gartersnake	*Thamnophis eques megalops*
171	Ringed map turtle	*Graptemys oculifera*
179	Sand skink	*Neoseps reynoldsi*
172	Yellow-blotched map turtle	*Graptemys flavimaculata*
Generalist Species: Snails (Section 4.2.1.8)		
403	Alamosa springsnail	*Tryonia alamosae*
396	Anthony's riversnail	*Athearnia anthonyi*
402	Armored snail	*Pyrgulopsis (=Marstonia) pachyta*
4162	Chupadera springsnail	*Pyrgulopsis chupaderae*
412	Cylindrical lioplax (snail)	*Lioplax cyclostomaformis*
4437	Diamond Tryonia	*Pseudotryonia adamantina*
413	Flat pebblesnail	*Lepyrium showalteri*
390	Flat-spired three-toothed Snail	*Triodopsis platysayoides*
5362	Gonzales tryonia	*Tryonia circumstriata (=stocktonensis)*
2561	Interrupted (=Georgia) Rocksnail	*Leptoxis foremani*
391	Iowa Pleistocene snail	*Discus macclintocki*
1247	Koster's springsnail	*Juturnia kosteri*
411	Lacy elimia (snail)	*Elimia crenatella*
392	Noonday snail	*Mesodon clarki nantahala*
414	Painted rocksnail	*Leptoxis taeniata*
393	Painted snake coiled forest snail	*Anguispira picta*
1245	Pecos assiminea snail	*Assiminea pecos*
4479	Phantom Springsnail	*Pyrgulopsis texana*
6138	Phantom Tryonia	*Tryonia cheatumi*

Entity ID	Common Name (Refinements Section Number)	Scientific Name
415	Plicate rocksnail	*Leptoxis plicata*
1246	Roswell springsnail	*Pyrgulopsis roswellensis*
416	Round rocksnail	*Leptoxis ampla*
401	Royal marstonia (snail)	*Pyrgulopsis ogmorhaphe*
1380	San Bernardino springsnail	*Pyrgulopsis bernardina*
417	Slender campeloma	*Campeloma decampi*
408	Socorro springsnail	*Pyrgulopsis neomexicana*
4766	Three Forks Springsnail	*Pyrgulopsis trivialis*
407	Tulotoma snail	*Tulotoma magnifica*
406	Tumbling Creek cavesnail	*Antrobia culveri*
395	Virginia fringed mountain snail	*Polygyriscus virginianus*

All other species within the 34 states of Enlist One and Enlist Duo product use are outside the action area and are therefore, not affected by this federal action (no effect or NE).

EPA identified designated CH overlapping the action area that could be affected in the 34 states of Enlist One and Enlist Duo product use (**Table 4-4**). EPA identified 9 listed animal designated CH that could experience on-field direct effects, 6 listed plant designated CH that could experience direct effects, and 127 generalist designated CH. EPA then followed the methodology outlined in **Section 4.1.3** and made MA or NE determinations. Further information regarding the species considerations supporting these determinations is provided in **Appendix K**.

Table 4-4. List of Listed Species Designated Critical Habitats of Concern

Entity ID	Common Name	Scientific Name
On-Field: Birds (Sections 4.2.1.9)		
149	Southwestern willow flycatcher	*Empidonax traillii extimus*
67	Whooping crane	*Grus americana*
On-Field: Amphibians (Section 4.2.1.9)		
190	Houston toad	*Bufo houstonensis*
On-Field: Mammals (Section 4.2.1.9)		
41	Alabama beach mouse	*Peromyscus polionotus ammobates*
1	Indiana bat	*Myotis sodalis*
On-Field: Terrestrial Invertebrates (4.2.1.9)		
3412	Dakota Skipper	*Hesperia dacotae*
445	Hine's emerald dragonfly	*Somatochlora hineana*
10147	Poweshiek skipperling	*Oarisma poweshiek*
4910	Salt Creek Tiger beetle	*Cicindela nevadica lincolniana*
Plants: Dicots (Section 4.2.1.10)		
630	Braun's rock-cress	*Arabis perstellata*
1710	Fleshy-fruit gladecress	*Leavenworthia crassa*
6672	Georgia rockcress	*Arabis georgiana*
7167	Kentucky glade cress	*Leavenworthia exigua laciniata*
1831	Short's bladderpod	*Physaria globosa*
569	Zapata bladderpod	*Lesquerella thamnophila*
Generalist Species: Amphibians (Section 4.2.1.11)		
6346	Austin blind Salamander	*Eurycea waterlooensis*
206	Chiricahua leopard frog	*Rana chiricahuensis*
208	dusky gopher frog	*Rana sevosa*
199	Frosted Flatwoods salamander	*Ambystoma cingulatum*

Entity ID	Common Name	Scientific Name
5434	Georgetown Salamander	*Eurycea naufragia*
190	Houston toad	*Bufo houstonensis*
8231	Jollyville Plateau Salamander	*Eurycea tonkawae*
9943	Reticulated flatwoods salamander	*Ambystoma bishopi*
7610	Salado Salamander	*Eurycea chisholmensis*
194	San Marcos salamander	*Eurycea nana*
Generalist Species: Birds (Section 4.2.1.11)		
4064	Gunnison sage-grouse	*Centrocercus minimus*
129	Mexican spotted owl	*Strix occidentalis lucida*
110	Mississippi sandhill crane	*Grus canadensis pulla*
130	Piping Plover	*Charadrius melodus*
131	Piping Plover	*Charadrius melodus*
149	Southwestern willow flycatcher	*Empidonax traillii extimus*
67	Whooping crane	*Grus americana*
6901	Yellow-billed Cuckoo	*Coccyzus americanus*
Generalist Species: Clams (Section 4.2.1.11)		
380	Alabama moccasinshell	*Medionidus acutissimus*
4411	Alabama pearlshell	*Margaritifera marrianae*
354	Appalachian elktoe	*Alasmidonta raveneliana*
370	Carolina heelsplitter	*Lasmigona decorata*
386	Chipola slabshell	*Elliptio chipolaensis*
381	Coosa moccasinshell	*Medionidus parvulus*
355	Cumberland elktoe	*Alasmidonta atropurpurea*
353	Cumberlandian combshell	*Epioblasma brevidens*
382	Dark pigtoe	*Pleurobema furvum*
375	Fat threeridge (mussel)	*Amblema neislerii*
372	Finelined pocketbook	*Lampsilis altilis*
1559	Fluted kidneyshell	*Ptychobranchus subtentus*
1369	Fuzzy pigtoe	*Pleurobema strodeanum*
384	Gulf moccasinshell	*Medionidus penicillatus*
7177	Narrow pigtoe	*Fusconaia escambia*
4086	Neosho Mucket	*Lampsilis rafinesqueana*
357	Orangenacre mucket	*Lampsilis perovalis*
371	Oval pigtoe	*Pleurobema pyriforme*
358	Oyster mussel	*Epioblasma capsaeformis*
366	Purple bankclimber (mussel)	*Elliptoideus sloatianus*
318	Purple bean	*Villosa perpurpurea*
3645	Rabbitsfoot	*Quadrula cylindrica cylindrica*
344	Rough rabbitsfoot	*Quadrula cylindrica strigillata*
373	Shinyrayed pocketbook	*Lampsilis subangulata*
6841	Slabside Pearlymussel	*Pleuronaia dolabelloides*
365	Southern acornshell	*Epioblasma othcaloogensis*
378	Southern clubshell	*Pleurobema decisum*
7949	Southern kidneyshell	*Ptychobranchus jonesi*
383	Southern pigtoe	*Pleurobema georgianum*
7349	Southern sandshell	*Hamiota australis*
7372	Suwannee moccasinshell	*Medionidus walkeri*
6534	Tapered pigtoe	*Fusconaia burkei*
379	Triangular Kidneyshell	*Ptychobranchus greenii*

Entity ID	Common Name	Scientific Name
367	Upland combshell	*Epioblasma metastriata*
4074	Yellow lance	*Elliptio lanceolata*
Generalist Species: Crustaceans (Section 4.2.1.11)		
477	Peck's cave amphipod	*Stygobromus (=Stygonectes) pecki*
6596	Pecos amphipod	*Gammarus pecos*
Generalist Species: Fishes (Section 4.2.1.11)		
236	Alabama cavefish	*Speoplatyrhinus poulsoni*
299	Arkansas River shiner	*Notropis girardi*
286	Atlantic sturgeon (Gulf subspecies)	*Acipenser oxyrinchus (=oxyrhynchus) desotoi*
276	Beautiful shiner	*Cyprinella formosa*
277	Cahaba shiner	*Notropis cahabae*
8352	Candy darter	*Etheostoma osburni*
242	Cape Fear shiner	*Notropis mekistocholas*
7150	Chucky Madtom	*Noturus crypticus*
215	Colorado pikeminnow (=squawfish)	*Ptychocheilus lucius*
5719	Cumberland darter	*Etheostoma susanae*
272	Devils River minnow	*Dionda diaboli*
6557	diamond Darter	*Crystallaria cincotta*
228	Fountain darter	*Etheostoma fonticola*
6297	Gila chub	*Gila intermedia*
209	Humpback chub	*Gila cypha*
10060	Kentucky arrow darter	*Etheostoma spilotum*
9220	Laurel dace	*Chrosomus saylori*
251	Leon Springs pupfish	*Cyprinodon bovinus*
273	Loach minnow	*Tiaroga cobitis*
212	Maryland darter	*Etheostoma sellare*
257	Niangua darter	*Etheostoma nianguae*
279	Pecos bluntnose shiner	*Notropis simus pecosensis*
241	Pygmy Sculpin	*Cottus paulus (=pygmaeus)*
290	Razorback sucker	*Xyrauchen texanus*
3525	Rush Darter	*Etheostoma phytophilum*
250	San Marcos gambusia	*Gambusia georgei*
239	Slackwater darter	*Etheostoma boschungi*
4881	Smalltooth sawfish	*Pristis pectinata*
258	Smoky madtom	*Noturus baileyi*
235	Snail darter	*Percina tanasi*
296	Spikedace	*Meda fulgida*
7332	Spring pygmy sunfish	*Elassoma alabamae*
311	Topeka shiner	*Notropis topeka (=tristis)*
316	Vermilion darter	*Etheostoma chermocki*
256	Virgin River Chub	*Gila seminuda (=robusta)*
243	Waccamaw silverside	*Menidia extensa*
234	Woundfin	*Plagopterus argentissimus*
259	Yaqui catfish	*Ictalurus pricei*
263	Yaqui chub	*Gila purpurea*
6662	Yellowcheek Darter	*Etheostoma moorei*
247	Yellowfin madtom	*Noturus flavipinnis*
3280	Zuni bluehead Sucker	*Catostomus discobolus yarrowi*
Generalist Species: Insects (Section 4.2.1.11)		

Entity ID	Common Name	Scientific Name
454	Comal Springs dryopid beetle	*Stygoparnus comalensis*
453	Comal Springs riffle beetle	*Heterelmis comalensis*
3412	Dakota Skipper	*Hesperia dacotae*
445	Hine's emerald dragonfly	*Somatochlora hineana*
10147	Poweshiek skipperling	*Oarisma poweshiek*
4910	Salt Creek Tiger beetle	*Cicindela nevadica lincolniana*
Generalist Species: Mammals (Section 4.2.1.11)		
41	Alabama beach mouse	*Peromyscus polionotus ammobates*
24	Canada Lynx	*Lynx canadensis*
34	Choctawhatchee beach mouse	*Peromyscus polionotus allophrys*
1	Indiana bat	*Myotis sodalis*
18	Jaguar	*Panthera onca*
43	Mount Graham red squirrel	*Tamiasciurus hudsonicus grahamensis*
5210	New Mexico meadow jumping mouse	*Zapus hudsonius luteus*
35	Perdido Key beach mouse	*Peromyscus polionotus trissyllepsis*
52	Preble's meadow jumping mouse	*Zapus hudsonius preblei*
54	St. Andrew beach mouse	*Peromyscus polionotus peninsularis*
27	Virginia big-eared bat	*Corynorhinus (=Plecotus) townsendii virginianus*
Generalist Species: Reptiles (Section 4.2.1.11)		
6097	Black pine snake	*Pituophis melanoleucus lodingi*
3271	Narrow-headed gartersnake	*Thamnophis rufipunctatus*
1783	Northern Mexican gartersnake	*Thamnophis eques megalops*
Generalist Species: Snails (Section 4.2.1.11)		
4437	Diamond Tryonia	*Pseudotryonia adamantina*
5362	Gonzales tryonia	*Tryonia circumstriata (=stocktonensis)*
2561	Interrupted (=Georgia) Rocksnail	*Leptoxis foremani*
1247	Koster's springsnail	*Juturnia kosteri*
1245	Pecos assiminea snail	*Assiminea pecos*
4479	Phantom Springsnail	*Pyrgulopsis texana*
6138	Phantom Tryonia	*Tryonia cheatumi*
1246	Roswell springsnail	*Pyrgulopsis roswellensis*
1380	San Bernardino springsnail	*Pyrgulopsis bernardina*
406	Tumbling Creek cavesnail	*Antrobia culveri*

All other designated CH within the 34 states of Enlist One and Enlist Duo product use are outside the action area and are therefore, not affected by this federal action (NE).

4.2.1.1. On-Field Animals: Risk-Based Species-Specific Analysis

EPA evaluated species-specific biological information (e.g., body size, dietary requirements, and seasonality) for listed species and the labeled Enlist One and Enlist Duo use patterns to further refine a species-specific assessment for the listed animal species found within the action area. EPA followed the species-specific diet refinement methodology discussed in **Section 4.1.3.3** and **Appendix L**. EPA made off-field effects determinations separately from the on-field effects determinations for taxa discussed in **Section 4.2**.

4.2.1.2. Listed Bird Species on Treated-Fields

The screening-level assessment indicated that birds could be at risk of mortality from acute exposures to both Enlist One and Enlist Duo and chronic exposure to Enlist Duo on treated fields (**Section 2 and 3**). The species-specific assessment and effects determinations examined where potential effects could reasonably be expected to occur on treated corn, soybean, and cotton fields. EPA conducted this species-specific diet refinement for acute (Enlist One and Enlist Duo) and chronic (Enlist Duo) exposures to make NE/MA effects determinations for these listed species. EPA identified five listed bird species as overlapping with the action area. EPA evaluated species-specific biological information in more depth to further refine the assessment as applicable and NE/MA effects determinations for the species (**Table 4-5; Appendix K**). The refined acute RQs for three of the species still exceeded the listed species LOC of 0.1. See **Appendix K** for details on the species-specific NE/MA determinations.

Table 4-5. Refined Risk and NE/MA Effects Determinations for On-Field Listed Bird Species Exposed to Enlist One and Enlist Duo

Species	2,4-D Refined acute RQ	Glyphosate Refined chronic RQ[1]	Enlist One NE/MA Effects determination	Enlist Duo NE/MA Effects determination
Attwater's greater prairie chicken	0.04	**Assumed on-field risk**	NE	MA
Eskimo curlew	**0.14**	**Assumed on-field risk**	MA	MA
Northern aplomado falcon	**0.30**	**Assumed on-field risk**	MA	MA
Golden-cheeked warbler	**1.02**	**Assumed on-field risk**	MA	MA
Yuma Ridgways (clapper) rail	N/A	N/A	NE	NE

NE = No effect; MA = May affect; N/A = Not applicable
Bolded values exceed the LOC for acute risk to listed species of 0.1 or the chronic risk LOC of 1.0.
[1] Effects observed at all test concentrations (see **Section 3** for details)

4.2.1.3. Listed Reptiles and Amphibians on the Treated Field

As described in the screening level assessment, EPA uses birds as a surrogate for reptiles and terrestrial-phase amphibians. The screening-level assessment indicated that reptiles and terrestrial-phase amphibians could be at risk of mortality from acute exposures to both Enlist One and Enlist Duo and chronic exposure to Enlist Duo on treated fields (**Section 2 and 3**). The species-specific assessment and effects determinations examined where potential effects could reasonably be expected to occur on treated corn, soybean, and cotton fields. EPA conducted this species-specific diet refinement for acute (Enlist One and Enlist Duo) and chronic (Enlist Duo) exposures to make NE/MA effects determinations for these listed species. EPA identified four listed amphibian species and six listed reptiles as overlapping with the action area. EPA evaluated species-specific biological information in more depth to further refine the assessment and effects determinations, as applicable, for the species listed below (**Table 4-6**). Chronic risk for glyphosate could not be further refined. See **Appendix K** for details on the species-specific NE/MA determinations.

Table 4-6. Refined Risk and NE/MA Effects Determinations for On-Field Listed Reptiles and Terrestrial-Phase Amphibian Species Exposed to Enlist One and Enlist Duo

Species	2,4-D	Glyphosate	Enlist One	Enlist Duo
	Refined acute RQ	**Refined chronic RQ[1]**	**NE/MA Effects determination**	**NE/MA Effects determination**
Frosted Flatwoods Salamander	0.05	**Assumed on-field risk**	NE	MA
Houston Toad	0.03	**Assumed on-field risk**	NE	MA
Red Hills Salamander	0.04	**Assumed on-field risk**	NE	MA
Reticulated Flatwoods Salamander	0.05	**Assumed on-field risk**	NE	MA
Bog Turtle	0.07	**Assumed on-field risk**	NE	MA
Eastern Massasauga (=rattlesnake)	0.02	**Assumed on-field risk**	NE	MA
Louisiana pine snake	0.02	**Assumed on-field risk**	NE	MA
Alabama red-belly turtle	N/A	N/A	NE	NE
Copperbelly water snake	N/A	N/A	NE	NE
Ringed map turtle	N/A	N/A	NE	NE

NE = No effect; MA = May affect; N/A = Not applicable

Bolded values exceed the LOC for acute risk to listed species of 0.1 or the chronic risk LOC of 1.0.

[1] Effects observed at all test concentrations (see **Section 3** for details)

4.2.1.4. Listed Mammal Species on Treated-Field

The screening-level assessment indicated that mammals could be at risk of mortality from acute and chronic exposure to Enlist One and Enlist Duo on treated fields (**Section 2 and 3**). The species-specific assessment and effects determinations examined where potential effects could reasonably be expected to occur on treated corn, soybean, and cotton fields (from 2,4-D exposure). EPA conducted this species-specific diet refinement for acute and chronic (Enlist One and Enlist Duo) exposures to make NE/MA effects determinations for these listed species. EPA identified eight listed mammal species as overlapping with the action area (**Table 4-7**). EPA evaluated species-specific biological information in more depth to further refine the assessment and effects determinations, as applicable, for the species. See **Appendix K** for details on the species-specific NE/MA determinations.

Table 4-7. Refined Risk and NE/MA Effects Determinations for On-Field Listed Mammal Species Exposed to Enlist One and Enlist Duo

Species	2,4-D		Enlist One	Enlist Duo
	Refined acute RQ	Refined chronic RQ	NE/MA Effects determination	NE/MA Effects determination
Gulf Coast Jaguarundi	**0.22**	**1.78**	MA	MA
Ocelot	**0.22**	**1.72**	MA	MA
Preble's Meadow Jumping Mouse	**0.44**	0.47	MA	MA
Virginia Big-eared Bat	0.04	0.30	NE	NE
Florida salt marsh vole	N/A	N/A	NE	NE
Perdido Key beach mouse	N/A	N/A	NE	NE
Sonoran Pronghorn	N/A	N/A	NE	NE
Ozark Big-eared Bat	N/A	N/A	NE	NE

NE = No effect; MA = May affect; N/A = Not applicable
Bolded values exceed the LOC for acute risk to listed species of 0.1 or the chronic risk LOC of 1.0.

4.2.1.5. On-Field Listed Terrestrial Invertebrate Species

The screening-level assessment indicated that terrestrial invertebrates could be at risk of mortality from acute and chronic exposure to Enlist One and Enlist Duo on treated fields (**Section 2 and 3**). EPA identified no listed terrestrial invertebrate species as overlapping with the action area that are reasonably expected to occur on treated corn, soybean, and cotton fields.

4.2.1.6. Listed Plant Species

The screening-level assessment indicated that terrestrial plants could be at risk from exposure to Enlist One and Enlist Duo on treated fields, up to 30 m from the treated fields (TPEZ) and up to 1,500 m from the treated fields (WPEZ; **Section 2 and 3**). EPA used the species-specific assessment and effects determinations to examine where potential effects could reasonably be expected to occur on treated corn, soybean and cotton fields, TPEZ or WPEZ environmental compartments. EPA conducted this refined assessment for (Enlist One and Enlist Duo) exposures to make effects determinations for these listed species. EPA identified 108 listed plant species as overlapping with the action (**Table 4-8**). See **Appendix K** for details on the species-specific NE/MA determinations.

Table 4-8. NE Effects Determinations for Listed Plants Exposed to Enlist One and Enlist Duo

Entity ID	Common Name	Environmental Compartment
Conifers and Cycads		
1191	Florida torreya	WPEZ
Dicots		
702	Black lace cactus	TPEZ
1004	Blue Ridge goldenrod	TPEZ
630	Braun's rock-cress	TPEZ
816	Chapman rhododendron	On-Field, TPEZ
925	Chisos Mountain hedgehog Cactus	WPEZ
824	Colorado hookless Cactus	TPEZ
677	Cumberland rosemary	On-Field, TPEZ, WPEZ
977	Fassett's locoweed	WPEZ
991	Harperella	WPEZ
959	Heller's blazingstar	TPEZ
1003	Houghton's goldenrod	WPEZ
1030	Huachuca water-umbel	WPEZ
1059	Lakeside daisy	TPEZ
998	Large-flowered skullcap	On-Field, TPEZ
872	Large-fruited sand-verbena	TPEZ
1150	Leedy's roseroot	TPEZ
625	Little amphianthus	WPEZ
817	Miccosukee gooseberry	TPEZ
992	Michaux's sumac	TPEZ
1096	Morefield's leather flower	TPEZ
1058	Mountain golden heather	TPEZ
995	Mountain sweet pitcher-plant	WPEZ
797	North Park phacelia	TPEZ
620	Northern wild monkshood	WPEZ
949	Peter's Mountain mallow	TPEZ
905	Pitcher's thistle	TPEZ
943	Roan Mountain bluet	TPEZ
1036	Ruth's golden aster	TPEZ
906	Sacramento Mountains thistle	WPEZ
1019	Seabeach amaranth	TPEZ
1831	Short's bladderpod	TPEZ
739	Slender rush-pea	WPEZ
924	Smooth coneflower	On-Field, TPEZ
718	Spreading avens	On-Field, TPEZ
513	Star cactus	TPEZ
937	Telephus spurge	WPEZ
1077	Texas ayenia	TPEZ
1400	Texas golden Gladecress	WPEZ
627	Tobusch fishhook cactus	WPEZ
644	Virginia round-leaf birch	TPEZ
1039	Virginia spiraea	WPEZ
763	Walker's manioc	On-Field, TPEZ
884	Welsh's milkweed	WPEZ
761	White birds-in-a-nest	WPEZ

Entity ID	Common Name	Environmental Compartment
1153	White irisette	TPEZ
569	Zapata bladderpod	TPEZ
Ferns and Allies		
1195	American hart's-tongue fern	TPEZ
1203	Black spored quillwort	WPEZ
1204	Mat-forming quillwort	WPEZ
Monocots		
818	Bunched arrowhead	WPEZ
1172	Canelo Hills ladies'-tresses	WPEZ
930	Clay-Loving wild buckwheat	WPEZ
950	Dwarf lake iris	TPEZ
723	Harper's beauty	WPEZ
807	Little Aguja (=Creek) Pondweed	WPEZ
935	Minnesota dwarf trout lily	WPEZ
656	Navajo sedge	WPEZ
857	Persistent trillium	TPEZ

Table 4-9. MA Effects Determinations for Listed Plants Exposed to Enlist One and Enlist Duo

Entity ID	Common Name	Environmental Compartment
Dicots		
994	Alabama canebrake pitcher-plant	WPEZ
1048	Alabama leather flower	TPEZ
653	Brooksville bellflower	WPEZ
976	Canby's dropwort	WPEZ
852	Cooley's meadowrue	WPEZ
891	Decurrent false aster	WPEZ
734	Dwarf-flowered heartleaf	TPEZ, WPEZ
1710	Fleshy-fruit gladecress	TPEZ
997	Florida skullcap	WPEZ
831	Fringed campion	TPEZ
836	Gentian pinkroot	TPEZ
982	Godfrey's butterwort	WPEZ
819	Green pitcher-plant	WPEZ
1087	Guthrie's (=Pyne's) ground-plum	TPEZ
7167	Kentucky glade cress	TPEZ, WPEZ
920	Leafy prairie-clover	TPEZ
750	Lyrate bladderpod	TPEZ, WPEZ
636	Mead's milkweed	TPEZ
969	Michigan monkey-flower	WPEZ
764	Mohr's Barbara's buttons	WPEZ
6617	Neches River rose-mallow	WPEZ
716	No common name	TPEZ
914	Okeechobee gourd	WPEZ
558	Pecos (=puzzle =paradox) sunflower	WPEZ
960	Pondberry	WPEZ
967	Rough-leaved loosestrife	WPEZ
945	Schweinitz's sunflower	TPEZ
875	Sensitive joint-vetch	WPEZ
835	Short's goldenrod	TPEZ

Entity ID	Common Name	Environmental Compartment
655	Small-anthered bittercress	WPEZ
624	South Texas ambrosia	TPEZ
568	Spring Creek bladderpod	WPEZ
651	Texas poppy-mallow	TPEZ
1045	Texas prairie dawn-flower	TPEZ
1028	Virginia sneezeweed	WPEZ
1881	Whorled Sunflower	WPEZ
Ferns and Allies		
1199	Louisiana quillwort	WPEZ
Monocots		
984	Eastern prairie fringed orchid	WPEZ
1189	Golden sedge	WPEZ
1228	Knieskern's Beaked-rush	TPEZ, WPEZ
1064	Kral's water-plantain	WPEZ
837	Navasota ladies'-tresses	WPEZ
823	Northeastern bulrush	TPEZ, WPEZ
946	Swamp pink	WPEZ
1017	Tennessee yellow-eyed grass	WPEZ
870	Texas wild-rice	WPEZ
1073	Ute ladies'-tresses	WPEZ
1080	Western prairie fringed Orchid	WPEZ
1415	White fringeless orchid	WPEZ

4.2.1.7. Animals with an Obligate Relationship to Plants

The screening-level assessment indicated that species with an obligate relationship to plants could be at risk from exposure to Enlist and Enlist Duo on and adjacent to treated fields, as well as in the flow path of the products (**Section 2 and 3**). EPA used the species-specific assessment and effects determinations to examine where potential effects could reasonably be expected to occur. EPA conducted this refined assessment for (Enlist One and Enlist Duo) exposures to make NE/MA effects determinations for these listed species. EPA identified five listed species with obligate relationships to plants as overlapping with the action area (**Table 4-10**) and made a MA determination for all obligate animals. See **Appendix K** for details on the species-specific MA determinations.

Table 4-10. MA Effects Determinations for Listed Animals with Obligate Relationships to Plants Exposed to Enlist One and Enlist Duo

Entity ID	Common Name	Obligate Plant
Birds		
107	Red-cockaded woodpecker	Pine trees
139	Golden-cheeked warbler (=wood)	Ashe juniper
Insects		
420	Karner blue butterfly	Wild lupine
424	Mitchell's satyr Butterfly	Almost certainly sedges, and *Carex stricta* is probably the primary hostplant
455	Saint Francis' satyr butterfly	*Carex mitchelliana*

4.2.1.8. Species with a Generalist Relationship to Plants

The screening-level assessment indicated that species with a generalist relationship to plants could be at risk from exposure to Enlist One and Enlist Duo on and adjacent to treated fields, as well as in the runoff flow path of the products (**Section 2 and 3**). EPA used the species-specific assessment and effects determinations to examine where potential discernible effects could reasonably be expected to occur. EPA conducted this refined assessment for (Enlist One and Enlist Duo) exposures to make NE/MA effects determinations for these listed species. EPA identified 290 listed species as overlapping with the action area that are reasonably expected to occur on or within the terrestrial compartment of the action area (TPEZ), as well as within the runoff flow path of the product (WPEZ; **Table 4-11**). See **Appendix K** for details on the species-specific NE/MA determinations.

Table 4-11. NE Effects Determinations for Listed Species with Generalist Relationships to Plants Exposed to Enlist One and Enlist Duo

Entity ID	Common Name	Environmental Compartment
Amphibians		
6346	Austin blind Salamander	APEZ
197	Barton Springs salamander	WPEZ, APEZ
198	Cheat Mountain salamander	TPEZ
5434	Georgetown Salamander	APEZ
3849	Jemez Mountains salamander	TPEZ
8231	Jollyville Plateau Salamander	APEZ
7610	Salado Salamander	APEZ
194	San Marcos salamander	APEZ
201	Sonora tiger Salamander	TPEZ, Not Revelant
189	Texas blind salamander	Not Relevant
Birds		
66	California condor	TPEZ
96	California least tern	APEZ
89	Masked bobwhite (quail)	TPEZ
130	Piping Plover	TPEZ, Not Revelant
8621	Red knot	APEZ
135	Roseate tern	Not Relevant
136	Roseate tern	Not Relevant
Clams		
4411	Alabama pearlshell	APEZ
386	Chipola slabshell	APEZ
317	Cumberland bean (pearlymussel)	APEZ
375	Fat threeridge (mussel)	APEZ
1559	Fluted kidneyshell	APEZ
1369	Fuzzy pigtoe	APEZ
325	Higgins eye (pearlymussel)	APEZ
7177	Narrow pigtoe	APEZ
4086	Neosho Mucket	APEZ
366	Purple bankclimber (mussel)	APEZ
318	Purple bean	APEZ
323	Purple Cat's paw (=Purple Cat's paw pearlymussel)	APEZ
3645	Rabbitsfoot	APEZ
6841	Slabside Pearlymussel	APEZ
7949	Southern kidneyshell	APEZ

Entity ID	Common Name	Environmental Compartment
7349	Southern sandshell	APEZ
7372	Suwannee moccasinshell	APEZ
6534	Tapered pigtoe	APEZ
351	Tar River spinymussel	APEZ
324	White catspaw (pearlymussel)	APEZ
328	Winged Mapleleaf	APEZ
Crustaceans		
480	Alabama cave shrimp	Not Relevant
475	Hay's Spring amphipod	Not Relevant
482	Kentucky cave shrimp	Not Relevant
486	Lee County cave isopod	Not Relevant
476	Madison Cave isopod	Not Relevant
477	Peck's cave amphipod	Not Relevant
483	Socorro isopod	Not Relevant
487	Squirrel Chimney Cave shrimp	Not Relevant
Fishes		
236	Alabama cavefish	Not Relevant
220	Apache trout	APEZ
299	Arkansas River shiner	APEZ
10297	Atlantic sturgeon	APEZ
10298	Atlantic sturgeon	APEZ
10299	Atlantic sturgeon	APEZ
10300	Atlantic sturgeon	APEZ
10301	Atlantic sturgeon	APEZ
286	Atlantic sturgeon (Gulf subspecies)	APEZ
244	Bayou darter	APEZ
276	Beautiful shiner	APEZ
213	Big Bend gambusia	APEZ
295	Blackside dace	APEZ
300	Blue shiner	APEZ
307	Bluemask darter	APEZ
277	Cahaba shiner	APEZ
8352	Candy darter	WPEZ, APEZ
7150	Chucky Madtom	APEZ
214	Clear Creek gambusia	WPEZ, APEZ
215	Colorado pikeminnow (=squawfish)	APEZ
275	Desert pupfish	APEZ
6557	Diamond Darter	APEZ
315	Etowah darter	APEZ
NMFS174	Giant manta ray	APEZ
221	Gila trout	WPEZ, APEZ
298	Goldline darter	APEZ
222	Greenback Cutthroat trout	WPEZ, APEZ
4248	Grotto Sculpin	APEZ
209	Humpback chub	WPEZ, APEZ
233	Lahontan cutthroat trout	APEZ
9220	Laurel dace	WPEZ, APEZ
251	Leon Springs pupfish	WPEZ, APEZ
281	Little Colorado spinedace	APEZ

Entity ID	Common Name	Environmental Compartment
273	Loach minnow	APEZ
211	Moapa dace	WPEZ, APEZ
224	Okaloosa darter	APEZ
260	Ozark cavefish	Not Relevant
8389	Pahrump poolfish	APEZ
278	Palezone shiner	APEZ
303	Pallid sturgeon	APEZ
279	Pecos bluntnose shiner	APEZ
230	Pecos gambusia	WPEZ, APEZ
313	Relict darter	APEZ
4330	Shortnose sturgeon	APEZ
239	Slackwater darter	APEZ
4881	Smalltooth sawfish	APEZ
235	Snail darter	APEZ
3069	Trispot darter	WPEZ, APEZ
316	Vermilion darter	APEZ
243	Waccamaw silverside	APEZ
234	Woundfin	APEZ
259	Yaqui catfish	WPEZ, APEZ
6662	Yellowcheek Darter	APEZ
247	Yellowfin madtom	APEZ
3280	Zuni bluehead Sucker	APEZ
Insects		
454	Comal Springs dryopid beetle	APEZ
453	Comal Springs riffle beetle	APEZ
442	Northeastern beach tiger beetle	TPEZ
434	Pawnee montane skipper	TPEZ
437	Uncompahgre fritillary butterfly	TPEZ
Lichens		
1219	Florida perforate cladonia	TPEZ
1220	Rock gnome lichen	Not Relevant
Mammals		
41	Alabama beach mouse	Not Relevant
50	Anastasia Island beach mouse	TPEZ
34	Choctawhatchee beach mouse	Not Relevant
18	Jaguar	TPEZ
43	Mount Graham red squirrel	TPEZ
10043	Northern Long-Eared Bat	TPEZ, Not Revelant
53	Southeastern beach mouse	TPEZ, Not Revelant
54	St. Andrew beach mouse	Not Relevant
Reptiles		
167	Atlantic salt marsh snake	APEZ
185	Desert tortoise	TPEZ
181	Gopher tortoise	TPEZ
166	New Mexican ridge-nosed rattlesnake	TPEZ
171	Ringed map turtle	APEZ
179	Sand skink	TPEZ
172	Yellow-blotched map turtle	APEZ
Snails		

Entity ID	Common Name	Environmental Compartment
396	Anthony's riversnail	APEZ
4162	Chupadera springsnail	Not Relevant
412	Cylindrical lioplax (snail)	WPEZ, APEZ
413	Flat pebblesnail	WPEZ, APEZ
390	Flat-spired three-toothed Snail	TPEZ
391	Iowa Pleistocene snail	Not Relevant
411	Lacy elimia (snail)	WPEZ, APEZ
414	Painted rocksnail	WPEZ, APEZ
393	Painted snake coiled forest snail	TPEZ
415	Plicate rocksnail	WPEZ, APEZ
416	Round rocksnail	WPEZ, APEZ
407	Tulotoma snail	WPEZ, APEZ
406	Tumbling Creek cavesnail	Not Relevant

Table 4-12. MA Effects Determinations for Listed Species with Generalist Relationships to Plants Exposed to Enlist One and Enlist Duo

Entity ID	Common Name	Environmental Compartment
Amphibians		
206	Chiricahua leopard frog	WPEZ, APEZ
208	Dusky gopher frog	TPEZ, WPEZ
199	Frosted Flatwoods salamander	TPEZ, WPEZ
190	Houston toad	TPEZ
192	Red Hills salamander	TPEZ, WPEZ
9943	Reticulated flatwoods salamander	TPEZ, WPEZ
Birds		
83	Attwater's greater prairie-chicken	TPEZ
93	Bachman's warbler (=wood)	TPEZ, WPEZ
11319	Eastern Black rail	TPEZ, WPEZ, APEZ
1221	Everglade snail kite	WPEZ, APEZ
140	Florida scrub-jay	TPEZ
139	Golden-cheeked warbler (=wood)	TPEZ
4064	Gunnison sage-grouse	TPEZ, WPEZ
129	Mexican spotted owl	TPEZ
110	Mississippi sandhill crane	TPEZ, WPEZ
126	Northern aplomado falcon	TPEZ
131	Piping Plover	TPEZ, WPEZ
107	Red-cockaded woodpecker	TPEZ
149	Southwestern willow flycatcher	TPEZ
67	Whooping crane	WPEZ
124	Wood stork	WPEZ
6901	Yellow-billed Cuckoo	WPEZ
Clams		
356	Alabama (=inflated) heelsplitter	WPEZ, APEZ
326	Alabama lampmussel	WPEZ, APEZ
380	Alabama moccasinshell	WPEZ, APEZ
354	Appalachian elktoe	WPEZ, APEZ
329	Appalachian monkeyface (pearlymussel)	WPEZ, APEZ
369	Arkansas fatmucket	WPEZ, APEZ
332	Birdwing pearlymussel	WPEZ, APEZ

Entity ID	Common Name	Environmental Compartment
370	Carolina heelsplitter	WPEZ, APEZ
352	Clubshell	WPEZ, APEZ
381	Coosa moccasinshell	WPEZ, APEZ
355	Cumberland elktoe	WPEZ, APEZ
330	Cumberland monkeyface (pearlymussel)	WPEZ, APEZ
376	Cumberland pigtoe	WPEZ, APEZ
353	Cumberlandian combshell	WPEZ, APEZ
333	Curtis pearlymussel	WPEZ, APEZ
382	Dark pigtoe	WPEZ, APEZ
334	Dromedary pearlymussel	WPEZ, APEZ
363	Dwarf wedgemussel	WPEZ, APEZ
368	Fanshell	WPEZ, APEZ
342	Fat pocketbook	WPEZ, APEZ
372	Finelined pocketbook	WPEZ, APEZ
337	Finerayed pigtoe	WPEZ, APEZ
384	Gulf moccasinshell	WPEZ, APEZ
350	Heavy pigtoe	WPEZ, APEZ
361	James spinymussel	WPEZ, APEZ
335	Littlewing pearlymussel	WPEZ, APEZ
364	Louisiana pearlshell	WPEZ, APEZ
374	Northern riffleshell	WPEZ, APEZ
340	Orangefoot pimpleback (pearlymussel)	WPEZ, APEZ
357	Orangenacre mucket	WPEZ, APEZ
343	Ouachita rock pocketbook	WPEZ, APEZ
371	Oval pigtoe	WPEZ, APEZ
358	Oyster mussel	WPEZ, APEZ
327	Pale lilliput (pearlymussel)	WPEZ, APEZ
331	Pink mucket (pearlymussel)	WPEZ, APEZ
6062	Rayed Bean	WPEZ, APEZ
344	Rough rabbitsfoot	WPEZ, APEZ
345	Scaleshell mussel	WPEZ, APEZ
7816	Sheepnose Mussel	WPEZ, APEZ
339	Shiny pigtoe	WPEZ, APEZ
373	Shinyrayed pocketbook	WPEZ, APEZ
5281	Snuffbox mussel	WPEZ, APEZ
365	Southern acornshell	WPEZ, APEZ
378	Southern clubshell	WPEZ, APEZ
348	Southern combshell	WPEZ, APEZ
383	Southern pigtoe	WPEZ, APEZ
360	Speckled pocketbook	WPEZ, APEZ
4490	Spectaclecase (mussel)	WPEZ, APEZ
362	Stirrupshell	WPEZ, APEZ
346	Tan riffleshell	WPEZ, APEZ
2917	Texas Hornshell	WPEZ, APEZ
379	Triangular Kidneyshell	WPEZ, APEZ
367	Upland combshell	WPEZ, APEZ
336	White wartyback (pearlymussel)	WPEZ, APEZ
4074	Yellow lance	WPEZ, APEZ
Crustaceans		

Entity ID	Common Name	Environmental Compartment
489	Benton County cave crayfish	WPEZ, APEZ
488	Hell Creek Cave crayfish	WPEZ, APEZ
484	Illinois cave amphipod	WPEZ, APEZ
6596	Pecos amphipod	WPEZ, APEZ
Fishes		
242	Cape Fear shiner	WPEZ, APEZ
269	Cherokee darter	WPEZ, APEZ
254	Chihuahua chub	WPEZ, APEZ
216	Comanche Springs pupfish	WPEZ
5719	Cumberland darter	WPEZ, APEZ
272	Devils River minnow	WPEZ, APEZ
228	Fountain darter	WPEZ, APEZ
6297	Gila chub	WPEZ, APEZ
219	Gila topminnow (incl. Yaqui)	WPEZ, APEZ
10060	Kentucky arrow darter	WPEZ, APEZ
212	Maryland darter	WPEZ, APEZ
257	Niangua darter	WPEZ, APEZ
241	Pygmy Sculpin	WPEZ, APEZ
290	Razorback sucker	WPEZ, APEZ
3525	Rush Darter	WPEZ, APEZ
250	San Marcos gambusia	WPEZ, APEZ
258	Smoky madtom	WPEZ, APEZ
255	Sonora chub	WPEZ
296	Spikedace	WPEZ, APEZ
7332	Spring pygmy sunfish	WPEZ, APEZ
311	Topeka shiner	WPEZ, APEZ
256	Virgin River Chub	WPEZ, APEZ
229	Watercress darter	WPEZ
263	Yaqui chub	WPEZ, APEZ
Insects		
440	American burying beetle	TPEZ
3412	Dakota Skipper	TPEZ, WPEZ
445	Hine's emerald dragonfly	WPEZ
441	Hungerford's crawling water Beetle	WPEZ, APEZ
420	Karner blue butterfly	TPEZ
10909	Miami tiger beetle	TPEZ
424	Mitchell's satyr Butterfly	WPEZ
10147	Poweshiek skipperling	TPEZ, WPEZ
443	Puritan tiger beetle	APEZ, TPEZ
10383	Rusty patched bumble bee	TPEZ
455	Saint Francis' satyr butterfly	TPEZ, WPEZ
4910	Salt Creek Tiger beetle	WPEZ
Mammals		
5	Black-footed ferret	TPEZ
24	Canada Lynx	TPEZ
42	Carolina northern flying squirrel	TPEZ
21	Gray bat	TPEZ, WPEZ, APEZ
22	Gulf Coast jaguarundi	TPEZ, WPEZ
1	Indiana bat	TPEZ, WPEZ

Entity ID	Common Name	Environmental Compartment
48	Mexican long-nosed bat	TPEZ
13	Mexican wolf	TPEZ
5210	New Mexico meadow jumping mouse	WPEZ
30	Ocelot	TPEZ
52	Preble's meadow jumping mouse	TPEZ, WPEZ, APEZ
14	Red wolf	TPEZ, WPEZ
Reptiles		
176	American crocodile	WPEZ, APEZ
6097	Black pine snake	TPEZ
182	Bog turtle	WPEZ
173	Eastern indigo snake	TPEZ, WPEZ
7800	Eastern Massasauga (=rattlesnake)	TPEZ, WPEZ
169	Flattened musk turtle	WPEZ, APEZ
3722	Louisiana pine snake	TPEZ
3271	Narrow-headed gartersnake	WPEZ
1783	Northern Mexican gartersnake	WPEZ
Snails		
403	Alamosa springsnail	WPEZ
402	Armored snail	WPEZ, APEZ
4437	Diamond Tryonia	WPEZ
5362	Gonzales tryonia	WPEZ
2561	Interrupted (=Georgia) Rocksnail	WPEZ, APEZ
1247	Koster's springsnail	WPEZ, APEZ
392	Noonday snail	TPEZ
1245	Pecos assiminea snail	WPEZ, APEZ
4479	Phantom Springsnail	WPEZ, APEZ
6138	Phantom Tryonia	WPEZ
1246	Roswell springsnail	WPEZ, APEZ
401	Royal marstonia (snail)	WPEZ, APEZ
1380	San Bernardino springsnail	WPEZ, APEZ
417	Slender campeloma	WPEZ, APEZ
408	Socorro springsnail	WPEZ
4766	Three Forks Springsnail	WPEZ, APEZ
395	Virginia fringed mountain snail	TPEZ

4.2.1.9. On-Field Designated CH Specific Analysis

In addition to the species-specific effects determinations, EPA also conducted the same overlap analysis to any designated CH location information and identified any designated CH within the expanded action area for listed terrestrial species (USFWS, 2020).

EPA identified two listed bird species, one listed amphibian, four listed terrestrial invertebrates and two listed mammals with designated CH with PCE/PBF's overlapping the action area (**Table 4-13** and **Table 4-14**). See **Appendix K** for notes on relevant primary constituent elements (PCE) for the listed species with designated CH on-field.

Table 4-13. NE Effects Determinations for On-Field Listed Species Designated Critical Habitat Exposed to Enlist One and Enlist Duo

Entity ID	Species with Designated CH	Environmental Compartment
Birds		
149	Southwestern willow flycatcher	On-field
Mammals		
41	Alabama beach mouse	On-field
1	Indiana bat	On-field
Insects		
3412	Dakota Skipper	On-field
445	Hine's emerald dragonfly	On-field
10147	Poweshiek skipperling	On-field
4910	Salt Creek Tiger beetle	On-field

Table 4-14. MA Effects Determinations for On-Field Listed Species Designated Critical Habitat Exposed to Enlist One and Enlist Duo

Entity ID	Species with Designated CH	Environmental Compartment
Birds		
67	Whooping crane	On-field
Amphibians		
190	Houston toad	On-field

4.2.1.10. Listed Plant Species with Designated CH

EPA identified six listed plant species with designated CH with PCE/PBF's overlapping the action area (**Table 4-15** and **Table 4-16**). See **Appendix K** for notes on relevant primary constituent elements (PCE) for the species.

Table 4-15. NE Effects Determinations for Listed Terrestrial Invertebrate Designated Critical Habitat Exposed to Enlist One and Enlist Duo

Entity ID	Species with Designated CH	Environmental Compartment
Dicots		
630	Braun's rock-cress	TPEZ
1831	Short's bladderpod	TPEZ
569	Zapata bladderpod	On-field, TPEZ

Table 4-16. MA Effects Determinations for Listed Terrestrial Invertebrate Designated Critical Habitat Exposed to Enlist One and Enlist Duo

Entity ID	Species with Designated CH	Environmental Compartment
Dicots		
1710	Fleshy-fruit gladecress	TPEZ
6672	Georgia rockcress	TPEZ
7167	Kentucky glade cress	TPEZ, WPEZ

4.2.1.11. Generalist Species with Designated CH

EPA identified 127 listed generalist species with designated CH within the action area (**Table 4-17** and **Table 4-18**). See **Appendix K** for a full list of designated CH impacted.

Table 4-17. NE Effects Determinations for Listed Terrestrial Invertebrate Designated Critical Habitat Exposed to Enlist One and Enlist Duo

Entity ID	Species with Designated CH	Environmental Compartment
Amphibians		
6346	Austin blind Salamander	APEZ
5434	Georgetown Salamander	APEZ
8231	Jollyville Plateau Salamander	APEZ
7610	Salado Salamander	APEZ
194	San Marcos salamander	APEZ
Birds		
131	Piping Plover	APEZ, Not Relevant
Clams		
4411	Alabama pearlshell	APEZ
386	Chipola slabshell	APEZ
375	Fat threeridge (mussel)	APEZ
1559	Fluted kidneyshell	APEZ
1369	Fuzzy pigtoe	APEZ
384	Gulf moccasinshell	APEZ
7177	Narrow pigtoe	APEZ
4086	Neosho Mucket	APEZ
371	Oval pigtoe	APEZ
366	Purple bankclimber (mussel)	APEZ
3645	Rabbitsfoot	APEZ
373	Shinyrayed pocketbook	APEZ
6841	Slabside Pearlymussel	APEZ
7949	Southern kidneyshell	APEZ
7349	Southern sandshell	APEZ
7372	Suwannee moccasinshell	APEZ
6534	Tapered pigtoe	APEZ
4074	Yellow lance	APEZ
Crustaceans		
477	Peck's cave amphipod	APEZ
Fishes		
236	Alabama cavefish	Not Relevant
299	Arkansas River shiner	APEZ
286	Atlantic sturgeon (Gulf subspecies)	APEZ
276	Beautiful shiner	APEZ
277	Cahaba shiner	APEZ
8352	Candy darter	APEZ
242	Cape Fear shiner	APEZ
7150	Chucky Madtom	APEZ
6557	diamond Darter	APEZ
9220	Laurel dace	WPEZ, APEZ

Entity ID	Species with Designated CH	Environmental Compartment
251	Leon Springs pupfish	APEZ
279	Pecos bluntnose shiner	APEZ
241	Pygmy Sculpin	APEZ
250	San Marcos gambusia	APEZ
239	Slackwater darter	APEZ
4881	Smalltooth sawfish	APEZ
258	Smoky madtom	APEZ
235	Snail darter	APEZ
296	Spikedace	APEZ
316	Vermilion darter	APEZ
256	Virgin River Chub	APEZ
243	Waccamaw silverside	APEZ
234	Woundfin	APEZ
259	Yaqui catfish	APEZ
6662	Yellowcheek Darter	APEZ
247	Yellowfin madtom	APEZ
3280	Zuni bluehead Sucker	APEZ
Insects		
454	Comal Springs dryopid beetle	APEZ
453	Comal Springs riffle beetle	APEZ
Mammals		
41	Alabama beach mouse	TPEZ, Not Relevant
34	Choctawhatchee beach mouse	TPEZ, Not Relevant
1	Indiana bat	Not Relevant
18	Jaguar	TPEZ
43	Mount Graham red squirrel	TPEZ
35	Perdido Key beach mouse	TPEZ, Not Relevant
54	St. Andrew beach mouse	TPEZ, Not Relevant
27	Virginia big-eared bat	Not Relevant
Snails		
406	Tumbling Creek cavesnail	Not Relevant

Table 4-18. MA Effects Determinations for Listed Terrestrial Invertebrate Designated Critical Habitat Exposed to Enlist One and Enlist Duo

Entity ID	Species with Designated CH	Environmental Compartment
Amphibians		
206	Chiricahua leopard frog	WPEZ, APEZ
208	dusky gopher frog	TPEZ, WPEZ
199	Frosted Flatwoods salamander	TPEZ, WPEZ
190	Houston toad	TPEZ
9943	Reticulated flatwoods salamander	TPEZ, APEZ
Birds		
4064	Gunnison sage-grouse	On-field, TPEZ, WPEZ
129	Mexican spotted owl	TPEZ
110	Mississippi sandhill crane	TPEZ, WPEZ
130	Piping Plover	TPEZ, WPEZ
149	Southwestern willow flycatcher	TPEZ, WPEZ, APEZ
67	Whooping crane	WPEZ, APEZ
6901	Yellow-billed Cuckoo	TPEZ, WPEZ

Entity ID	Species with Designated CH	Environmental Compartment
Clams		
380	Alabama moccasinshell	WPEZ, APEZ
354	Appalachian elktoe	WPEZ, APEZ
370	Carolina heelsplitter	WPEZ, APEZ
381	Coosa moccasinshell	WPEZ, APEZ
355	Cumberland elktoe	WPEZ, APEZ
353	Cumberlandian combshell	WPEZ, APEZ
382	Dark pigtoe	WPEZ, APEZ
372	Finelined pocketbook	WPEZ, APEZ
357	Orangenacre mucket	WPEZ, APEZ
358	Oyster mussel	WPEZ, APEZ
318	Purple bean	WPEZ, APEZ
344	Rough rabbitsfoot	WPEZ, APEZ
365	Southern acornshell	WPEZ, APEZ
378	Southern clubshell	WPEZ, APEZ
383	Southern pigtoe	WPEZ, APEZ
379	Triangular Kidneyshell	WPEZ, APEZ
367	Upland combshell	WPEZ, APEZ
Crustaceans		
6596	Pecos amphipod	WPEZ, APEZ
Fishes		
215	Colorado pikeminnow (=squawfish)	WPEZ, APEZ
5719	Cumberland darter	WPEZ, APEZ
272	Devils River minnow	WPEZ
228	Fountain darter	WPEZ, APEZ
6297	Gila chub	WPEZ, APEZ
209	Humpback chub	WPEZ, APEZ
10060	Kentucky arrow darter	WPEZ, APEZ
273	Loach minnow	WPEZ
212	Maryland darter	WPEZ, APEZ
257	Niangua darter	WPEZ, APEZ
290	Razorback sucker	WPEZ, APEZ
3525	Rush Darter	WPEZ, APEZ
7332	Spring pygmy sunfish	WPEZ, APEZ
311	Topeka shiner	WPEZ, APEZ
263	Yaqui chub	WPEZ, APEZ
Insects		
3412	Dakota Skipper	TPEZ, WPEZ
445	Hine's emerald dragonfly	WPEZ
10147	Poweshiek skipperling	TPEZ, WPEZ
4910	Salt Creek Tiger beetle	WPEZ
Mammals		
24	Canada Lynx	TPEZ
5210	New Mexico meadow jumping mouse	WPEZ, APEZ
52	Preble's meadow jumping mouse	TPEZ, WPEZ, APEZ
Reptiles		
6097	Black pine snake	TPEZ
3271	Narrow-headed gartersnake	TPEZ, WPEZ, APEZ
1783	Northern Mexican gartersnake	TPEZ, WPEZ, APEZ

Entity ID	Species with Designated CH	Environmental Compartment
Snails		
4437	Diamond Tryonia	WPEZ, APEZ
5362	Gonzales tryonia	WPEZ, APEZ
2561	Interrupted (=Georgia) Rocksnail	WPEZ, APEZ
1247	Koster's springsnail	WPEZ, APEZ
1245	Pecos assiminea snail	WPEZ, APEZ
4479	Phantom Springsnail	WPEZ, APEZ
6138	Phantom Tryonia	WPEZ
1246	Roswell springsnail	WPEZ, APEZ
1380	San Bernardino springsnail	WPEZ, APEZ

4.2.1.12. Summary of NE and MA Determination Results

Based on the overlap analysis, inclusive of all current and proposed labeled measures to address runoff, EPA determined that 24 listed animals on-field, 108 listed plants, 5 listed obligate animals, and 291 generalist species are within the action area of this Federal Action. EPA conducted a refined risk-based analysis incorporating best available information on the biology of the species and determined NE for 18 on-field animals for Enlist One, 10 on-field animals for Enlist Duo, 59 plants and 136 generalist species for both products as direct or indirect effects are not anticipated. EPA determined MA for 6 on-field animals for Enlist One, 14 on-field animals for Enlist Duo, 49 plants, 5 obligates species and 155 generalist species for both products. See **Appendix K** for a full list of species determinations.

The overlap analysis, based on the proposed use area on Enlist One and Enlist Duo labels (dated 5/14/2021), EPA determined that 9 listed animal designated CH on-field, 6 listed plant designated CH, and 127 generalist designated CH were within the action area of this Federal Action. EPA conducted a refined risk-based analysis incorporating best available information on the habitat of the species, where applicable, and determined NE for seven on-field animal designated CH, three plant designated CH and 63 generalist designated CH for Enlist One and Enlist Duo as no direct or indirect effects are anticipated. EPA determined MA for two on-field animal designated CH, three plant designated CH and 64 generalist designated CH for Enlist One and Enlist Duo. See **Appendix K** for a full list of designated CH impacted.

4.2.2. NLAA and LAA Determination Results

EPA used the methodology discussed in **Section 4.1.4** to determine whether uses of Enlist products are 'Not Likely to Adversely Affect' (NLAA) or 'Likely to Adversely Affect' (LAA) each species or designated CH with a MA determination. An LAA determination means that there is a discernible adverse effect to one or more individuals of a listed species or their designated CH. As noted above, there are 214 listed species for Enlist One, 222 listed species for Enlist Duo and 69 designated CH identified to be within the action area for both products where EPA made MA determinations. Further information regarding the species and designated CH considerations supporting these determinations is provided in **Appendix K**.

4.2.2.1. NLAA Determination Results

EPA made NLAA determinations for one on-field species, 95 generalist species, one animal on field designated CH and 27 generalist species designated CH as summarized in **Sections 4.2.2.2** and **4.2.2.3** Further information regarding the species and designated CH considerations supporting these determinations is provided in **Appendix K**.

4.2.2.2. NLAA Determination Results for Listed Species

EPA followed the methodology discussed in **Section 4.1.4** and made NLAA determinations for one animal on field (**Table 4-19**) and 124 generalist species (**Table 4-20**) for Enlist One and Enlist Duo. Further information regarding the species considerations supporting these determinations is provided in **Appendix K**.

Table 4-19. Summary of NLAA Determinations for On-Field Federally Listed Threatened or Endangered Species within the Action Area

Entity ID	Species	Exposure Compartment
Birds		
91	Eskimo curlew	On-field

Table 4-20. Summary of NLAA Determinations for Federally Listed Threatened or Endangered Generalist Species within the Action Area

Entity ID	Species	Exposure Compartment
Amphibians		
206	Chiricahua leopard frog	WPEZ, APEZ
199	Frosted Flatwoods salamander	TPEZ, WPEZ
9943	Reticulated flatwoods salamander	TPEZ, WPEZ
Birds		
93	Bachman's warbler (=wood)	TPEZ, WPEZ
1221	Everglade snail kite	WPEZ, APEZ
139	Golden-cheeked warbler (=wood)	TPEZ
4064	Gunnison sage-grouse	TPEZ, WPEZ
131	Piping Plover	TPEZ, WPEZ
67	Whooping crane	WPEZ
Clams		
356	Alabama (=inflated) heelsplitter	WPEZ, APEZ
326	Alabama lampmussel	WPEZ, APEZ
380	Alabama moccasinshell	WPEZ, APEZ
354	Appalachian elktoe	WPEZ, APEZ
329	Appalachian monkeyface (pearlymussel)	WPEZ, APEZ
369	Arkansas fatmucket	WPEZ, APEZ
332	Birdwing pearlymussel	WPEZ, APEZ
370	Carolina heelsplitter	WPEZ, APEZ
352	Clubshell	WPEZ, APEZ
381	Coosa moccasinshell	WPEZ, APEZ
355	Cumberland elktoe	WPEZ, APEZ

Entity ID	Species	Exposure Compartment
330	Cumberland monkeyface (pearlymussel)	WPEZ, APEZ
376	Cumberland pigtoe	WPEZ, APEZ
353	Cumberlandian combshell	WPEZ, APEZ
333	Curtis pearlymussel	WPEZ, APEZ
382	Dark pigtoe	WPEZ, APEZ
334	Dromedary pearlymussel	WPEZ, APEZ
363	Dwarf wedgemussel	WPEZ, APEZ
368	Fanshell	WPEZ, APEZ
342	Fat pocketbook	WPEZ, APEZ
372	Finelined pocketbook	WPEZ, APEZ
337	Finerayed pigtoe	WPEZ, APEZ
384	Gulf moccasinshell	WPEZ, APEZ
350	Heavy pigtoe	WPEZ, APEZ
361	James spinymussel	WPEZ, APEZ
335	Littlewing pearlymussel	WPEZ, APEZ
364	Louisiana pearlshell	WPEZ, APEZ
374	Northern riffleshell	WPEZ, APEZ
340	Orangefoot pimpleback (pearlymussel)	WPEZ, APEZ
357	Orangenacre mucket	WPEZ, APEZ
343	Ouachita rock pocketbook	WPEZ, APEZ
371	Oval pigtoe	WPEZ, APEZ
358	Oyster mussel	WPEZ, APEZ
327	Pale lilliput (pearlymussel)	WPEZ, APEZ
331	Pink mucket (pearlymussel)	WPEZ, APEZ
6062	Rayed Bean	WPEZ, APEZ
344	Rough rabbitsfoot	WPEZ, APEZ
345	Scaleshell mussel	WPEZ, APEZ
7816	Sheepnose Mussel	WPEZ, APEZ
339	Shiny pigtoe	WPEZ, APEZ
373	Shinyrayed pocketbook	WPEZ, APEZ
5281	Snuffbox mussel	WPEZ, APEZ
365	Southern acornshell	WPEZ, APEZ
378	Southern clubshell	WPEZ, APEZ
348	Southern combshell	WPEZ, APEZ
383	Southern pigtoe	WPEZ, APEZ
360	Speckled pocketbook	WPEZ, APEZ
4490	Spectaclecase (mussel)	WPEZ, APEZ
362	Stirrupshell	WPEZ, APEZ
346	Tan riffleshell	WPEZ, APEZ
2917	Texas Hornshell	WPEZ, APEZ
379	Triangular Kidneyshell	WPEZ, APEZ
367	Upland combshell	WPEZ, APEZ
336	White wartyback (pearlymussel)	WPEZ, APEZ
4074	Yellow lance	WPEZ, APEZ
Crustaceans		
489	Benton County cave crayfish	WPEZ, APEZ
488	Hell Creek Cave crayfish	WPEZ, APEZ
484	Illinois cave amphipod	WPEZ, APEZ
6596	Pecos amphipod	WPEZ, APEZ

Entity ID	Species	Exposure Compartment
Fishes		
254	Chihuahua chub	WPEZ, APEZ
216	Comanche Springs pupfish	WPEZ
5719	Cumberland darter	WPEZ, APEZ
228	Fountain darter	WPEZ, APEZ
219	Gila topminnow (incl. Yaqui)	WPEZ, APEZ
10060	Kentucky arrow darter	WPEZ, APEZ
290	Razorback sucker	WPEZ, APEZ
3525	Rush Darter	WPEZ, APEZ
250	San Marcos gambusia	WPEZ, APEZ
255	Sonora chub	WPEZ
296	Spikedace	WPEZ, APEZ
256	Virgin River Chub	WPEZ, APEZ
263	Yaqui chub	WPEZ, APEZ
Insects		
440	American burying beetle	TPEZ
441	Hungerford's crawling water Beetle	WPEZ, APEZ
10909	Miami tiger beetle	TPEZ
443	Puritan tiger beetle	APEZ, TPEZ
4910	Salt Creek Tiger beetle	WPEZ
Mammals		
5	Black-footed ferret	TPEZ
24	Canada Lynx	TPEZ
21	Gray bat	TPEZ, WPEZ, APEZ
1	Indiana bat	TPEZ, WPEZ
13	Mexican wolf	TPEZ
14	Red wolf	TPEZ, WPEZ
Reptiles		
176	American crocodile	WPEZ, APEZ
173	Eastern indigo snake	TPEZ, WPEZ
7800	Eastern Massasauga (=rattlesnake)	TPEZ, WPEZ

4.2.2.3. NLAA Determination Results for Designated CH

EPA followed the methodology discussed in **Section 4.1.4** and made NLAA determinations for one animal on field designated CH (**Table 4-21**) and 27 generalist species designated CH (**Table 4-22**) for Enlist One and Enlist Duo. Further information regarding the designated CH considerations supporting these determinations is provided in **Appendix K**.

Table 4-21. Summary of NLAA Determinations for On-Field Federally Listed Threatened or Endangered Designated Critical Habitat within the Action Area

Entity ID	Species with Designated CH	Exposure Compartment
Birds		
67	Whooping crane	On-field

Table 4-22. Summary of NLAA Determinations for Federally Listed Threatened or Endangered Generalist Species Designated Critical Habitat within the Action Area

Entity ID	Species with Designated CH	Exposure Compartment
Birds		
129	Mexican spotted owl	TPEZ
6901	Yellow-billed Cuckoo	WPEZ
Clams		
380	Alabama moccasinshell	WPEZ, APEZ
354	Appalachian elktoe	WPEZ, APEZ
370	Carolina heelsplitter	WPEZ, APEZ
381	Coosa moccasinshell	WPEZ, APEZ
355	Cumberland elktoe	WPEZ, APEZ
353	Cumberlandian combshell	WPEZ, APEZ
382	Dark pigtoe	WPEZ, APEZ
372	Finelined pocketbook	WPEZ, APEZ
357	Orangenacre mucket	WPEZ, APEZ
358	Oyster mussel	WPEZ, APEZ
318	Purple bean	APEZ
344	Rough rabbitsfoot	WPEZ, APEZ
365	Southern acornshell	WPEZ, APEZ
378	Southern clubshell	WPEZ, APEZ
383	Southern pigtoe	WPEZ, APEZ
379	Triangular Kidneyshell	WPEZ, APEZ
367	Upland combshell	WPEZ, APEZ
Fishes		
215	Colorado pikeminnow (=squawfish)	APEZ
5719	Cumberland darter	WPEZ, APEZ
209	Humpback chub	WPEZ, APEZ
10060	Kentucky arrow darter	WPEZ, APEZ
273	Loach minnow	APEZ
257	Niangua darter	WPEZ, APEZ
Reptiles		
6097	Black pine snake	TPEZ
Snails		
2561	Interrupted (=Georgia) Rocksnail	WPEZ, APEZ

4.2.2.4. LAA Determination Results

EPA made LAA determinations for 5 on-field species for Enlist One, 13 on-field species for Enlist Duo, 49 plants, five obligate animals, 60 generalist animals, one animal on field designated CH, three plant designated CH and 35 generalist animal designated CH as summarized in **Section 4.2.2.5** and **4.2.2.6.**

Further information regarding the species and designated CH considerations supporting these determinations is provided in **Appendix K**.

4.2.2.5. LAA Determination Results for Listed Species

EPA followed the methodology discussed in **Section 4.1.4** and made LAA determinations for 5 on-field species for Enlist One, 13 on-field species for Enlist Duo (**Table 4-23**), 49 plants (**Table 4-24**), five obligate animals (**Table 4-25**) and 60 generalist species (**Table 4-26**) for Enlist One and Enlist Duo. Further information regarding the species considerations supporting these determinations is provided in **Appendix K**.

Table 4-23. Summary of LAA Determinations for On-Field Federally Listed Threatened or Endangered Animal Species within the Action Area

Entity ID	Species	Exposure Compartment
Birds		
83	Attwater's greater prairie-chicken	On-field
139	Golden-cheeked warbler (=wood)	On-field
126	Northern aplomado falcon	On-field
Amphibians and Reptiles		
199	Frosted Flatwoods salamander	On-field
190	Houston toad	On-field
192	Red Hills salamander	On-field
9943	Reticulated flatwoods salamander	On-field
182	Bog turtle	On-field
7800	Eastern Massasauga (=rattlesnake)	On-field
3722	Louisiana pine snake	On-field
Mammals		
22	Gulf Coast jaguarundi	On-field
30	Ocelot	On-field
52	Preble's meadow jumping mouse	On-field

Table 4-24. Summary of LAA Determinations for Federally Listed Threatened or Endangered Plant Species within the Action Area

Entity ID	Species	Exposure Compartment
Dicots		
994	Alabama canebrake pitcher-plant	WPEZ
1048	Alabama leather flower	TPEZ
653	Brooksville bellflower	WPEZ
976	Canby's dropwort	WPEZ
852	Cooley's meadowrue	WPEZ
891	Decurrent false aster	WPEZ
734	Dwarf-flowered heartleaf	TPEZ, WPEZ
1710	Fleshy-fruit gladecress	TPEZ
997	Florida skullcap	WPEZ
831	Fringed campion	TPEZ
836	Gentian pinkroot	TPEZ
982	Godfrey's butterwort	WPEZ
819	Green pitcher-plant	WPEZ
1087	Guthrie's (=Pyne's) ground-plum	TPEZ
7167	Kentucky glade cress	TPEZ, WPEZ

Entity ID	Species	Exposure Compartment
920	Leafy prairie-clover	TPEZ
750	Lyrate bladderpod	TPEZ, WPEZ
636	Mead's milkweed	TPEZ
969	Michigan monkey-flower	WPEZ
764	Mohr's Barbara's buttons	WPEZ
6617	Neches River rose-mallow	WPEZ
716	Geocarpon minimum	TPEZ
914	Okeechobee gourd	WPEZ
558	Pecos (=puzzle =paradox) sunflower	WPEZ
960	Pondberry	WPEZ
967	Rough-leaved loosestrife	WPEZ
945	Schweinitz's sunflower	TPEZ
875	Sensitive joint-vetch	WPEZ
835	Short's goldenrod	TPEZ
655	Small-anthered bittercress	WPEZ
624	South Texas ambrosia	TPEZ
568	Spring Creek bladderpod	WPEZ
651	Texas poppy-mallow	TPEZ
1045	Texas prairie dawn-flower	TPEZ
1028	Virginia sneezeweed	WPEZ
1881	Whorled Sunflower	WPEZ
Ferns and Allies		
1199	Louisiana quillwort	WPEZ
Monocots		
984	Eastern prairie fringed orchid	WPEZ
1189	Golden sedge	WPEZ
1228	Knieskern's Beaked-rush	TPEZ, WPEZ
1064	Kral's water-plantain	WPEZ
837	Navasota ladies'-tresses	WPEZ
823	Northeastern bulrush	TPEZ, WPEZ
946	Swamp pink	WPEZ
1017	Tennessee yellow-eyed grass	WPEZ
870	Texas wild-rice	WPEZ
1073	Ute ladies'-tresses	WPEZ
1080	Western prairie fringed Orchid	WPEZ
1415	White fringeless orchid	WPEZ

Table 4-25. Summary of LAA Determinations for Federally Listed Threatened or Endangered Obligate Animal Species within the Action Area

Entity ID	Species	Species Exposure Compartment
Terrestrial Invertebrates		
420	Karner blue butterfly	On-field, TPEZ, WPEZ
424	Mitchell's satyr Butterfly	On-field, TPEZ, WPEZ
455	Saint Francis' satyr butterfly	On-field, TPEZ, WPEZ
Birds		
139	Golden-cheeked warbler (=wood)	On-field, TPEZ
107	Red-cockaded woodpecker	On-field, TPEZ

Table 4-26. Summary of LAA Determinations for Federally Listed Threatened or Endangered Generalist Species within the Action Area

Entity ID	Species	Exposure Compartment
Amphibians		
208	Dusky gopher frog	TPEZ, WPEZ
190	Houston toad	TPEZ
192	Red Hills salamander	TPEZ, WPEZ
Birds		
83	Attwater's greater prairie-chicken	TPEZ
11319	Eastern Black rail	TPEZ, WPEZ, APEZ
140	Florida scrub-jay	TPEZ
129	Mexican spotted owl	TPEZ
110	Mississippi sandhill crane	TPEZ, WPEZ
126	Northern aplomado falcon	TPEZ
107	Red-cockaded woodpecker	TPEZ
149	Southwestern willow flycatcher	TPEZ
124	Wood stork	WPEZ
6901	Yellow-billed Cuckoo	WPEZ
Fishes		
242	Cape Fear shiner	WPEZ, APEZ
269	Cherokee darter	WPEZ, APEZ
272	Devils River minnow	WPEZ, APEZ
6297	Gila chub	WPEZ, APEZ
212	Maryland darter	WPEZ, APEZ
257	Niangua darter	WPEZ, APEZ
241	Pygmy Sculpin	WPEZ, APEZ
258	Smoky madtom	WPEZ, APEZ
7332	Spring pygmy sunfish	WPEZ, APEZ
311	Topeka shiner	WPEZ, APEZ
229	Watercress darter	WPEZ
Insects		
3412	Dakota Skipper	TPEZ, WPEZ
445	Hine's emerald dragonfly	WPEZ
420	Karner blue butterfly	TPEZ
424	Mitchell's satyr Butterfly	WPEZ
10147	Poweshiek skipperling	TPEZ, WPEZ
10383	Rusty patched bumble bee	TPEZ
455	Saint Francis' satyr butterfly	TPEZ, WPEZ
Mammals		
42	Carolina northern flying squirrel	TPEZ
22	Gulf Coast jaguarundi	TPEZ, WPEZ
48	Mexican long-nosed bat	TPEZ
5210	New Mexico meadow jumping mouse	WPEZ
30	Ocelot	TPEZ
52	Preble’s meadow jumping mouse	TPEZ, WPEZ, APEZ
Reptiles		
6097	Black pine snake	TPEZ
182	Bog turtle	WPEZ
169	Flattened musk turtle	WPEZ, APEZ
3722	Louisiana pine snake	TPEZ

Entity ID	Species	Exposure Compartment
3271	Narrow-headed gartersnake	WPEZ
1783	Northern Mexican gartersnake	WPEZ
Snails		
403	Alamosa springsnail	WPEZ
402	Armored snail	WPEZ, APEZ
4437	Diamond Tryonia	WPEZ
5362	Gonzales tryonia	WPEZ
2561	Interrupted (=Georgia) Rocksnail	WPEZ, APEZ
1247	Koster's springsnail	WPEZ, APEZ
392	Noonday snail	TPEZ
1245	Pecos assiminea snail	WPEZ, APEZ
4479	Phantom Springsnail	WPEZ, APEZ
6138	Phantom Tryonia	WPEZ
1246	Roswell springsnail	WPEZ, APEZ
401	Royal marstonia (snail)	WPEZ, APEZ
1380	San Bernardino springsnail	WPEZ, APEZ
417	Slender campeloma	WPEZ, APEZ
408	Socorro springsnail	WPEZ
4766	Three Forks Springsnail	WPEZ, APEZ
395	Virginia fringed mountain snail	TPEZ

4.2.2.6. LAA Determination Results for Designated CH

EPA followed the methodology discussed in **Section 4.1.4** and made LAA determinations for one animal on field designated CH (**Table 4-27**), three plant designated CH (**Table 4-28**) and 35 generalist designated CH (**Table 4-29**) for Enlist One and Enlist Duo. Further information regarding the designated CH considerations supporting these determinations is provided in **Appendix K**.

Table 4-27. Summary of LAA Determinations for On-Field Federally Listed Threatened or Endangered Animal Designated Critical Habitat within the Action Area

Entity ID	Species with Designated CH	Exposure Compartment
Amphibians		
190	Houston toad	On-field

Table 4-28. Summary of LAA Determinations for Federally Listed Threatened or Endangered Plant Designated Critical Habitat within the Action Area

Entity ID	Species with Designated CH	Exposure Compartment
Dicots		
1710	Fleshy-fruit gladecress	TPEZ
6672	Georgia rockcress	TPEZ
7167	Kentucky glade cress	TPEZ, WPEZ

Table 4-29. Summary of LAA Determinations for Federally Listed Threatened or Endangered Generalist Species Designated Critical Habitat within the Action Area

Entity ID	Species with Designated CH	Exposure Compartment
Amphibians		
206	Chiricahua leopard frog	WPEZ, APEZ
208	dusky gopher frog	TPEZ, WPEZ
199	Frosted Flatwoods salamander	TPEZ, WPEZ
190	Houston toad	TPEZ
9943	Reticulated flatwoods salamander	TPEZ, APEZ
Birds		
4064	Gunnison sage-grouse	On-field, TPEZ, WPEZ
110	Mississippi sandhill crane	TPEZ, WPEZ
130	Piping Plover	TPEZ, WPEZ
149	Southwestern willow flycatcher	TPEZ, WPEZ, APEZ
67	Whooping crane	WPEZ, APEZ
Crustaceans		
6596	Pecos amphipod	WPEZ, APEZ
Fishes		
272	Devils River minnow	WPEZ
6297	Gila chub	WPEZ, APEZ
212	Maryland darter	WPEZ, APEZ
290	Razorback sucker	WPEZ, APEZ
3525	Rush Darter	WPEZ, APEZ
7332	Spring pygmy sunfish	WPEZ, APEZ
311	Topeka shiner	WPEZ, APEZ
263	Yaqui chub	WPEZ, APEZ
Insects		
3412	Dakota Skipper	TPEZ, WPEZ
445	Hine's emerald dragonfly	WPEZ
10147	Poweshiek skipperling	TPEZ, WPEZ
Mammals		
24	Canada Lynx	TPEZ
5210	New Mexico meadow jumping mouse	WPEZ, APEZ
52	Preble's meadow jumping mouse	TPEZ, WPEZ, APEZ
Reptiles		
3271	Narrow-headed gartersnake	TPEZ, WPEZ, APEZ
1783	Northern Mexican gartersnake	TPEZ, WPEZ, APEZ
Snails		
4437	Diamond Tryonia	WPEZ, APEZ
5362	Gonzales tryonia	WPEZ, APEZ
1247	Koster's springsnail	WPEZ, APEZ
1245	Pecos assiminea snail	WPEZ, APEZ
4479	Phantom Springsnail	WPEZ, APEZ
6138	Phantom Tryonia	WPEZ
1246	Roswell springsnail	WPEZ, APEZ
1380	San Bernardino springsnail	WPEZ, APEZ

4.2.3. Identification of Species and Designated CH that EPA Determined Likely to be Jeopardized or Adversely Modified by the Proposed Action

As discussed in **Section 4.2.2.5** and **4.2.2.6**, EPA made LAA determinations for 5 on-field species for Enlist One, 13 on-field species for Enlist Duo, 49 plants, five obligates, 60 generalists, one animal on field designated CH, three plant designated CH and 35 generalist designated CH. All of these species and designated CH are under the authority of FWS. EPA further evaluated the species and designated CH with LAA determinations in order to evaluate whether the action is likely to rise to jeopardy or adverse modification. Further information regarding the species and designated CH considerations supporting these determinations is provided in **Appendix K**.

4.2.3.1. Listed Species that are Likely to be Jeopardized

EPA followed the methodology discussed in **Section 4.1.5** and concluded likely jeopardy determinations for two animals on-field for Enlist One and seven animals on-field for Enlist Duo (**Table 4-30**), 45 plants (**Table 4-31**), four obligates (**Table 4-32**) and 39 generalists (**Table 4-33**) for Enlist One and Enlist Duo. Further information regarding the species considerations supporting these determinations is provided in **Appendix K**.

Table 4-30. Summary of Likely Jeopardy Determinations for On-Field Federally Listed Threatened or Endangered Animal Species within the Action Area

Entity ID	Species	Exposure Compartment
Birds		
83	Attwater's greater prairie-chicken	On-field
Amphibians and Reptiles		
199	Frosted Flatwoods salamander	On-field
9943	Reticulated flatwoods salamander	On-field
182	Bog turtle	On-field
7800	Eastern Massasauga (=rattlesnake)	On-field
Mammals		
30	Ocelot	On-field
22	Gulf Coast jaguarundi	On-field

Table 4-31. Summary of Likely Jeopardy Determinations for Federally Listed Threatened or Endangered Plant Species within the Action Area

Entity ID	Species	Exposure Compartment
Dicots		
994	Alabama canebrake pitcher-plant	WPEZ
1048	Alabama leather flower	TPEZ
653	Brooksville bellflower	WPEZ
976	Canby's dropwort	WPEZ
852	Cooley's meadowrue	WPEZ
891	Decurrent false aster	WPEZ
1710	Fleshy-fruit gladecress	TPEZ

Entity ID	Species	Exposure Compartment
997	Florida skullcap	WPEZ
831	Fringed campion	TPEZ
836	Gentian pinkroot	TPEZ
819	Green pitcher-plant	WPEZ
1087	Guthrie's (=Pyne's) ground-plum	TPEZ
7167	Kentucky glade cress	TPEZ, WPEZ
920	Leafy prairie-clover	TPEZ
750	Lyrate bladderpod	TPEZ, WPEZ
636	Mead's milkweed	TPEZ
969	Michigan monkey-flower	WPEZ
764	Mohr's Barbara's buttons	WPEZ
6617	Neches River rose-mallow	WPEZ
914	Okeechobee gourd	WPEZ
558	Pecos (=puzzle =paradox) sunflower	WPEZ
960	Pondberry	WPEZ
967	Rough-leaved loosestrife	WPEZ
945	Schweinitz's sunflower	TPEZ
875	Sensitive joint-vetch	WPEZ
835	Short's goldenrod	TPEZ
655	Small-anthered bittercress	WPEZ
624	South Texas ambrosia	TPEZ
568	Spring Creek bladderpod	WPEZ
651	Texas poppy-mallow	TPEZ
1045	Texas prairie dawn-flower	TPEZ
1028	Virginia sneezeweed	WPEZ
1881	Whorled Sunflower	WPEZ
Ferns and Allies		
1199	Louisiana quillwort	WPEZ
Monocots		
984	Eastern prairie fringed orchid	WPEZ
1189	Golden sedge	WPEZ
1064	Kral's water-plantain	WPEZ
837	Navasota ladies'-tresses	WPEZ
823	Northeastern bulrush	TPEZ, WPEZ
946	Swamp pink	WPEZ
1017	Tennessee yellow-eyed grass	WPEZ
870	Texas wild-rice	WPEZ
1073	Ute ladies'-tresses	WPEZ
1080	Western prairie fringed Orchid	WPEZ
1415	White fringeless orchid	WPEZ

Table 4-32. Summary of Likely Jeopardy Determinations for Federally Listed Threatened or Endangered Obligate Animal Species within the Action Area

Species	Entity ID	Species Exposure Compartment
Terrestrial Invertebrates		
420	Karner blue butterfly	On-field, TPEZ, WPEZ
424	Mitchell's satyr Butterfly	On-field, TPEZ, WPEZ
455	Saint Francis' satyr butterfly	On-field, TPEZ, WPEZ
Birds		
107	Red-cockaded woodpecker	On-field, TPEZ

Table 4-33. Summary of Likely Jeopardy Determinations for Federally Listed Threatened or Endangered Generalist Species within the Action Area

Entity ID	Species	Exposure Compartment
Amphibians		
208	Dusky gopher frog	TPEZ, WPEZ
192	Red Hills salamander	TPEZ, WPEZ
Birds		
83	Attwater's greater prairie-chicken	TPEZ
11319	Eastern Black rail	TPEZ, WPEZ, APEZ
110	Mississippi sandhill crane	TPEZ, WPEZ
124	Wood stork	WPEZ
Fishes		
242	Cape Fear shiner	WPEZ, APEZ
269	Cherokee darter	WPEZ, APEZ
272	Devils River minnow	WPEZ, APEZ
6297	Gila chub	WPEZ, APEZ
212	Maryland darter	WPEZ, APEZ
241	Pygmy Sculpin	WPEZ, APEZ
258	Smoky madtom	WPEZ, APEZ
7332	Spring pygmy sunfish	WPEZ, APEZ
311	Topeka shiner	WPEZ, APEZ
229	Watercress darter	WPEZ
Insects		
3412	Dakota Skipper	TPEZ, WPEZ
445	Hine's emerald dragonfly	WPEZ
420	Karner blue butterfly	TPEZ
424	Mitchell's satyr Butterfly	WPEZ
10147	Poweshiek skipperling	TPEZ, WPEZ
10383	Rusty patched bumble bee	TPEZ
455	Saint Francis' satyr butterfly	TPEZ, WPEZ
Mammals		
22	Gulf Coast jaguarundi	TPEZ, WPEZ
52	Preble’s meadow jumping mouse	TPEZ, WPEZ, APEZ
Reptiles		
182	Bog turtle	WPEZ
169	Flattened musk turtle	WPEZ, APEZ
Snails		
402	Armored snail	WPEZ, APEZ
4437	Diamond Tryonia	WPEZ

Entity ID	Species	Exposure Compartment
5362	Gonzales tryonia	WPEZ
2561	Interrupted (=Georgia) Rocksnail	WPEZ, APEZ
1247	Koster's springsnail	WPEZ, APEZ
1245	Pecos assiminea snail	WPEZ, APEZ
4479	Phantom Springsnail	WPEZ, APEZ
6138	Phantom Tryonia	WPEZ
1246	Roswell springsnail	WPEZ, APEZ
401	Royal marstonia (snail)	WPEZ, APEZ
1380	San Bernardino springsnail	WPEZ, APEZ
417	Slender campeloma	WPEZ, APEZ

4.2.3.1. Designated CH that are Likely to be Adversely Modified

EPA followed the methodology discussed in **Section 4.1.5** and made likely adverse modification determinations for one plant designated CH (**Table 4-34**) and 33 generalist species designated CH (**Table 4-35)** for Enlist One and Enlist Duo. Further information regarding the species considerations supporting these determinations is provided in **Appendix K**.

Table 4-34. Summary of Likely Adverse Modification Determinations for Federally Listed Threatened or Endangered Plant Designated Critical Habitat within the Action Area

Entity ID	Species with Designated CH	Exposure Compartment
Plants: Dicots		
7167	Kentucky glade cress	On-field, TPEZ, WPEZ

Table 4-35. Summary of Likely Adverse Modification Determinations for Federally Listed Threatened or Endangered Generalist Species Designated Critical Habitat within the Action Area

Entity ID	Species with Designated CH	Exposure Compartment
Amphibians		
206	Chiricahua leopard frog	WPEZ, APEZ
208	dusky gopher frog	TPEZ, WPEZ
199	Frosted Flatwoods salamander	TPEZ, WPEZ
9943	Reticulated flatwoods salamander	TPEZ, APEZ
Birds		
4064	Gunnison sage-grouse	On-field, TPEZ, WPEZ
110	Mississippi sandhill crane	TPEZ, WPEZ
130	Piping Plover	TPEZ, WPEZ
149	Southwestern willow flycatcher	TPEZ, WPEZ, APEZ
67	Whooping crane	WPEZ, APEZ
Crustaceans		
6596	Pecos amphipod	WPEZ, APEZ
Fishes		
272	Devils River minnow	WPEZ
6297	Gila chub	WPEZ, APEZ
212	Maryland darter	WPEZ, APEZ
290	Razorback sucker	WPEZ, APEZ
3525	Rush Darter	WPEZ, APEZ
7332	Spring pygmy sunfish	WPEZ, APEZ
311	Topeka shiner	WPEZ, APEZ

Entity ID	Species with Designated CH	Exposure Compartment
263	Yaqui chub	WPEZ, APEZ
Insects		
3412	Dakota Skipper	TPEZ, WPEZ
445	Hine's emerald dragonfly	WPEZ
10147	Poweshiek skipperling	TPEZ, WPEZ
Mammals		
5210	New Mexico meadow jumping mouse	WPEZ, APEZ
52	Preble's meadow jumping mouse	TPEZ, WPEZ, APEZ
Reptiles		
3271	Narrow-headed gartersnake	TPEZ, WPEZ, APEZ
1783	Northern Mexican gartersnake	TPEZ, WPEZ, APEZ
Snails		
4437	Diamond Tryonia	WPEZ, APEZ
5362	Gonzales tryonia	WPEZ, APEZ
1247	Koster's springsnail	WPEZ, APEZ
1245	Pecos assiminea snail	WPEZ, APEZ
4479	Phantom Springsnail	WPEZ, APEZ
6138	Phantom Tryonia	WPEZ
1246	Roswell springsnail	WPEZ, APEZ
1380	San Bernardino springsnail	WPEZ, APEZ

4.3. Effects Determination Conclusions

As summarized in **Section 4.2**, EPA identified 24 listed animals on-field, 108 listed plants, five listed species with an obligate relationship with terrestrial plants, and 291 generalist species (relying on plants) as being in the action area. EPA refined its analysis by incorporating best available information on the life history of the species. This refinement led EPA to determine NE for 18 on-field animals for Enlist One, 10 on-field animals for Enlist Duo, 59 plants and 136 generalist species for both products as direct or indirect effects are anticipated. Similar analysis led to a MA determination for six listed animals on-field for Enlist One, 14 listed animals on-field for Enlist Duo, 49 listed plants, five listed species with an obligate relationship with terrestrial plants, and 155 generalist species for Enlist One and Enlist Duo. Additionally, EPA identified nine listed animal designated CH on-field, six listed plant designated CH, and 127 generalist animal designated CH within the action area of this Federal Action. Refined risk-based analysis incorporating best available information on the PCEs of the habitat led to a determination of NE for seven on-field animal designated CH, three plant designated CH and 65 generalist designated CH for Enlist One and Enlist Duo as no direct or indirect effects are anticipated. Similar analysis led to a MA determination for two on-field animal designated CH, three plant designated CH and 62 generalist designated CH for Enlist One and Enlist Duo. All species and designated CH where MA determinations were made are under the authority of USFWS. NE determinations are made for all species and designated CH under the authority of NMFS.

For those species and designated CH that EPA made MA determinations, EPA then determined whether the action "may affect, but is not likely to adversely affect" the listed species or designated CH (NLAA); or "may affect and is likely to adversely affect" the listed species or designated CH (LAA). Of the MA species, refined risk-based analysis incorporating best available information on the biology of the species led to a conclusion of NLAA for one on-field species, and 95 generalist species for Enlist One and Enlist Duo. Similar analysis led to a LAA determination for five on-field species for Enlist One, 13 on-field species for Enlist Duo, 49 plants, five obligate animals and 60 generalist species for Enlist One and Enlist Duo. Of the MA designated CH, refined risk-based analysis incorporating best available information on the PCEs of the habitat led to a conclusion of NLAA for one animal on field designated CH and 27 generalist designated CH for Enlist One and Enlist Duo. Similar analysis led to a LAA determination for one animal on field designated CH, three plant designated CH and 35 generalist species designated CH for Enlist One and Enlist Duo.

EPA further prioritized species by evaluating their likelihood to rise to Jeopardy or Adverse Modification when in consultation with FWS (using the method of FWS' draft malathion Biological Opinion). Of the LAA species, EPA made Likely Jeopardy determinations for two animals on-field for Enlist One and seven animals on-field for Enlist Duo, 45 plants, four obligate animals and 39 generalist species and Likely Adverse Modification determinations for one plant designated CH and 33 generalist species designated CH. These results are summarized in **Table 4-36** by group and determination, and by USFWS Regions in **Table 4-37**.

Table 4-36. Summary of Effects Determinations and Likely Jeopardy and Adverse Modification.

Taxon (of Listed Species)	Number of Species or Designated CH with Determination[1]			
	NE	MA - NLAA	MA – LAA Not Likely J/AM	Likely J/AM
On-field Animals (Enlist One; Enlist Duo)	18; 10	1; 1	3; 6	2; 7
Plants	59	0	4	45
Obligate Species	0	0	1	4
Generalist Species	136	95	21	39
On-field designated CH	7	1	1	0
Plant designated CH	3	0	2	1
Obligate designated CH	0	0	0	0
Generalist designated CH	65	27	2	33

[1] Some species and designated CH have determinations in on- and off-field environmental compartments.

Table 4-37. Summary of Effects Determinations by USFWS Region.

USFWS Region Lead	Total LAA Species	Likely Jeopardy	Total LAA Designated CH	Likely Adverse Modification
Region 2	35	20	19	18
Region 3	12	11	4	4
Region 4	59	49	9	7
Region 5	8	6	1	1
Region 6	3	3	5	4

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6. List of Acronyms

2,4-DCP	2,4-dichlorophenol
ACR	Acute-to-Chronic Ratio
AIC	Akaike Information Criterion
AIMS	Avian Incident Monitoring System
AMPA	Aminomethylphosphonic acid
APEZ	Aquatic plant exposure zone
ASABE	American Society of Agricultural and Biological Engineers
BE	Biological Evaluation
BEAD	Biological and Economic Analysis Division
Bee-REX	Bee Residue EXposure model
BiOp	Biological Opinion
CDL	Cropland Data Layer
CH	Critical Habitat
CHQ	Chlorohydroquinone
DT_{50}	Dissipation time required for the concentration to decline to half of the initial value
EC_{25}	Concentration leading to 25% effect
EC_{50}	Concentration leading to 50% effect
EDDP	Exponential Decay, Double, 4 Parameter
EEC	Estimated Environmental Concentration
EFED	Environmental Fate and Effects Division
EIIS	Ecological Incident Information System
EPA	Environmental Protection Agency
ESA	Endangered Species Act
FGDC	Federal Geospatial Data Committee
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GIS	Geographic Information System
GLEAMS	Groundwater Loading Effects of Agricultural Management Systems
GMO	Genetically Modified Organism
HC_{05}	5^{th} percentile Hazard Criterion
HC_{10}	10^{th} percentile Hazard Criterion
HC_{25}	25^{th} percentile Hazard Criterion
HED	Health Effects Division
HUC	Hydrologic Unit Code
IC_{25}	Concentration leading to 25% inhibition
IDS	Incident Data System
IPA	Isopropylamine salt of glyphosate
LAA	Likely to Adversely Affect
lbs a.e./A	Pounds of Acid Equivalent per Acre
LC_{50}	Concentration leading to 50% mortality
LD_{50}	Dose leading to 50% mortality
LOAEC	Lowest Observed Adverse Effect Concentration
LOAEL	Lowest Observed Adverse Effect Level
LOC	Level of Concern
MA	May Affect
ML	Maximum likelihood

MOA	Mechanism of Action
MRID	Master Record Identification
MSDD	Minimum Statistically Detectible Difference
NC	Not Calculated
NE	No Effect
NHD	National Hydrography Dataset
NLAA	Not Likely to Adversely Affect
NMFS	National Marine Fisheries Service
NOAEC	No Observed Adverse Effect Concentration
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
NOEL	No Observed Effect Level
NRC	National Research Council
NWIS	National Water Information System
PAT	Plant Assessment Tool
PCE/PBF	Principal Constituent Elements / Physical or Biological Features
PERFUM	Probabilistic Exposure and Risk Model for Fumigants
PGR	Plant Growth Regulator
PPHD	Prey, Pollination, Habitat and/or Dispersal
PRN	Prairie Rivers Network
PRZM	Pesticide Root Zone Model
PWC	Pesticide in Water Calculator
RNA	Ribonucleic Acid
RQ	Risk Quotient
SAP	Scientific Advisory Panel
SE	Seedling Emergence
SSD	Species Sensitivity Distribution
STIR	Screening Tool for Inhalation Risk
STORET	STOrage and RETrieval Data Warehouse
TGAI	Technical Grade Active Ingredient
TPEZ	Terrestrial plant exposure zone
T-REX	Terrestrial Residue EXposure model
UDL	Use Data Layer
USDA	United States Department of Agriculture
USFWS	United States Fish and Wildlife Service
USGS	United States Geological Survey
VSI	Visual Signs of Injury
VV	Vegetative Vigor
VVWM	Variable Volume Water Body Model
WPEZ	Wetland plant exposure zone
WQP	Water Quality Portal

Appendix A. Spray Drift Studies

To address the limitations of AgDRIFT in the case of Enlist products, the Agency determined buffer distances using the label-required droplet spectrum, combined with the results of a registrant submitted spray-drift field study (MRID 48844001). This study empirically measured spray drift deposition with various drift reduction nozzles and formulations. The Agency considered this specific combination of nozzle and formulation as measured in the study to evaluate more realistic spray drift buffer requirements than were achievable using AgDRIFT modeling. The technical explanation for this finding is that the empirical deposition data show a biphasic distribution (Figure J-1, Appendix J of USEPA, 2016, DP 428301) with a bias toward near field deposition. To determine far-field deposition, the Agency truncated the deposition data from 0 to10 feet and used 90th percentile data from 25 to 400 feet to determine buffer distances (Figure A-1). The deposition curve was developed using Exponential Decay, Double, 4 Parameter equation using SigmaPlot version 10.0. The Exponential Decay, Double, 4 Parameter (EDDP) and various coefficients are listed as below follows with fractions of applied Table A-1.

f= 25(ft) + a*exp(-b*x)+c*exp(-d*x)
Where: 25 ft represents 0 distance for the deposition curve
a = 0.0013, b = 0.0388, c = 0.0006, and d = 0.0012

Figure A-1. 90th Percentile Deposition Curve of AIXR Nozzle for GF-2726 2,4-D Choline Formulation Distance

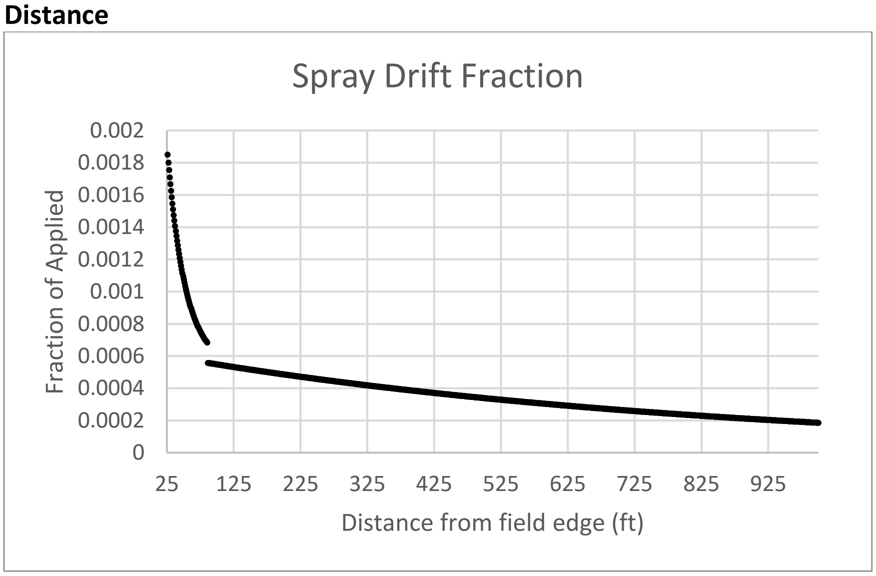


Table A-1. Fractions of Applied GF-2726 (Enlist Duo) at Various Distances

Distance (ft)	Fraction of applied	Distance (ft)	Fraction of applied	Distance (ft)	Fraction of applied
26	0.00185	54	0.001001	80	0.000716
27	0.001801	53	0.001019	75	0.000752
28	0.001755	55	0.000985	76	0.000744
29	0.00171	56	0.000969	77	0.000737
30	0.001667	57	0.000953	78	0.000729

Distance (ft)	Fraction of applied	Distance (ft)	Fraction of applied	Distance (ft)	Fraction of applied
31	0.001626	58	0.000938	79	0.000722
32	0.001586	59	0.000924	80	0.000716
33	0.001547	60	0.00091	81	0.000709
34	0.00151	61	0.000896	82	0.000703
35	0.001475	62	0.000883	83	0.000697
36	0.001441	63	0.000871	84	0.000691
37	0.001408	64	0.000859	85	0.000685
38	0.001376	65	0.000847	86	0.000558
39	0.001345	66	0.000836	87	0.000557
40	0.001316	67	0.000825	88	0.000556
41	0.001287	68	0.000815	89	0.000556
42	0.00126	69	0.000805	90	0.000555
43	0.001234	70	0.000795	91	0.000554
44	0.001208	71	0.000786	92	0.000554
45	0.001184	72	0.000777	93	0.000553
46	0.001161	73	0.000768	94	0.000552
47	0.001138	74	0.00076	95	0.000552
48	0.001116	75	0.000752	96	0.000551
49	0.001095	76	0.000744	97	0.00055
50	0.001075	77	0.000737	98	0.00055
51	0.001056	78	0.000729	99	0.000549
52	0.001037	79	0.000722	100	0.000548

Appendix B. Animal and Plant Toxicity Data

2,4-D Studies

This appendix provides a full list of the studies and additional study details on the 2,4-D studies EPA evaluated when selecting the most sensitive 2,4-D endpoints for the FIFRA (**Section 2**) and ESA (**Section 4**) portions of the risk assessment. See **Section 2.3** for list of selected endpoints.

Table B.1. 2,4-D Aquatic Toxicity Endpoints

Exposure Duration and Taxon	Species	2,4-D Form Tested	Endpoint (μg a.e./L)	Study Classification	MRID
Freshwater Fish					
Acute Freshwater Fish	Bluegill sunfish	2,4-D IPA (47.8 %, in water)	96-h LC_{50} = 1,338,583	Acceptable	13869
Acute Freshwater Fish	Bluegill sunfish	2,4-D 2-EHE (92%)	96-h LC_{50} = 11,921	Acceptable	226397
Acute Freshwater Fish	Bluegill sunfish	2,4-D IPE 98.2%)	96-h LC_{50} = 261	Acceptable	43930701
Acute Freshwater Fish	Bluegill sunfish	2,4-D BEE (97.4%)	96-h LC50 = 428	Acceptable	41353801
Acute Freshwater Fish	Bluegill sunfish	2,4-D DMA (67.3 %)	96-h LC_{50} = 436,667	Acceptable	41158311
Acute Freshwater Fish	Bluegill sunfish	2,4-D DEA (73.1%)	96-h LC_{50} > 81,757	Acceptable	41975104
Acute Freshwater Fish	Bluegill sunfish	2,4-D DMA (Formulation)	96-h LC_{50} > 833,333	supplemental	34697
Acute Freshwater Fish	Bluegill sunfish	2,4-D sodium salt (Formulation)	96-h LC_{50} > 90,909	Acceptable	41158301
Acute Freshwater Fish	Cutthroat trout (*Oncorhynchus clarkii*)	2,4-D acid	96-h LC_{50} = 64,000	Acceptable	116622
Acute Freshwater Fish	Fathead minnow (*Pimphales promelas*)	2,4-D BEE (97.4%)	96-h LC50 = 1,793	Acceptable	41353801
Acute Freshwater Fish	Fathead minnow (*Pimphales promelas*)	2,4-D DMA (67.3 %)	96-h LC_{50} = 265,000	Acceptable	41158311
Acute Freshwater Fish	Fathead minnow (*Pimphales promelas*)	2,4-D IPA (47.8 %, in water)	96-h LC_{50} = 459,055	Acceptable	13869
Acute Freshwater Fish	Fathead minnow (*Pimphales promelas*)	2,4-D acid	96-h LC_{50} 320,000	Acceptable	41158301
Acute Freshwater Fish	Lake trout	2,4-D acid	96-h LC_{50} = 45,000	Acceptable	116622
Acute Freshwater Fish	Rainbow trout (*Oncorhynchus mykiss*)	2,4-D BEE (97.4%)	96-h LC_{50} = 1,441	Acceptable	41353801

Exposure Duration and Taxon	Species	2,4-D Form Tested	Endpoint (µg a.e./L)	Study Classification	MRID
Acute Freshwater Fish	Rainbow trout (*Oncorhynchus mykiss*)	2,4-D 2-EHE (92%)	96-h LC_{50} = 14,570	Acceptable	226397
Acute Freshwater Fish	Rainbow trout (*Oncorhynchus mykiss*)	2,4-D IPA (47.8 %, in water)	96-h LC_{50} = 2,236,220	Acceptable	13869
Acute Freshwater Fish	Rainbow trout (*Oncorhynchus mykiss*)	2,4-D DMA (67.3 %)	96-h LC_{50} = 208,333	Acceptable	41158311
Acute Freshwater Fish	Rainbow trout (*Oncorhynchus mykiss*)	2,4-D IPE 98.2%)	96-h LC_{50} = 580	Acceptable	43933101
Acute Freshwater Fish	Rainbow trout (*Oncorhynchus mykiss*)	2,4-D TIPA (69.2%)	96-h LC_{50} > 160,428	Acceptable	41353803
Acute Freshwater Fish	Rainbow trout (*Oncorhynchus mykiss*)	2,4-D Choline Salt	96-h LC_{50} > 32,653	Acceptable	48892401
Acute Freshwater Fish	Rainbow trout (*Oncorhynchus mykiss*)	2,4-D DEA (73.1%)	96-h LC_{50} > 81,082	Acceptable	41975105
Acute Freshwater Fish	Rainbow trout (*Oncorhynchus mykiss*)	2,4-D DMA (Formulation)	96-h LC_{50} > 833,333	Supplemental	36927
Acute Freshwater Fish	Rainbow trout (*Oncorhynchus mykiss*)	2,4-D sodium salt (formulation (80%)	96-h LC_{50} > 90,909	Supplemental	McCann 1973[39]
Chronic Freshwater Fish (early life cycle) [1]	Fathead minnow (*Pimphales promelas*)	2,4-D DMA	NOAEC = 11,833 LOAEC = 31,333 Based on Length	Acceptable	41767701
Chronic Freshwater Fish (early life cycle) [1]	Fathead minnow (*Pimphales promelas*)	2,4-D DEA (73.1%)	NOAEC = 19,662 LOAEC = 66,284 Based on survival	Acceptable	42018304
Chronic Freshwater Fish (early life cycle) [1]	Fathead minnow (*Pimphales promelas*)	2,4-D BEE (96%)	NOAEC = 55.5 LOAEC = 79.0 Based on survival	Acceptable	41345701
Estuarine/Marine Fish					
Acute Estuarine/Marine Fish	Sheepshead minnow (*Cyprinodon variegatus*)	2,4-D DMA (Formulation)	96-h LC_{50} > 466,667	Supplemental	36924
Acute Estuarine/Marine Fish	Tidewater silverside (Menidid beryllina)	2,4-D DMA	96-h LC_{50} = 145,833	Acceptable	41737307
Acute Estuarine/Marine Fish	Tidewater silverside (*Menidid beryllina*)	2,4-D IPA (50.3%) formulation	96-h LC_{50} = 186,614	Supplemental	41429001
Acute Estuarine/Marine Fish	Tidewater silverside (*Menidid beryllina*)	2,4-D TIPA (70.4%)	96-h LC_{50} = 201,070	Acceptable	41429004

[39] McCann, J.A., Pitcher, F. 1973. Aquacide: Rainbow Trout (Salmo gairdneri); Test No. 546. U.S. Environmental Protection Agency, Pesticides Regulation Div., Animal Biology Laboratory, unpublished study; CDL:128584-A. MRID 00053986

Exposure Duration and Taxon	Species	2,4-D Form Tested	Endpoint (µg a.e./L)	Study Classification	MRID
Acute Estuarine/Marine Fish	Tidewater silverside (*Menidid beryllina*)	2,4-D DMA (66.8%)	96-h LC_{50} = 390,833	Supplemental	41835209
Acute Estuarine/Marine Fish	Tidewater silverside (*Menidid beryllina*)	2,4-D 2-EHE (95.4%)	96-h LC_{50} > 1258	Supplemental	41835205
Acute Estuarine/Marine Fish	Tidewater silverside (*Menidid beryllina*)	2,4-D DMA	96-h LC_{50} > 66,867	Acceptable	42018301
Freshwater Amphibians					
Acute Freshwater Amphibians	Leopard frog tadpoles (*Rana pipiens*)	2,4-D DMA	96-H LC_{50} = 231,667	Acceptable	44517306
Acute Freshwater Amphibians	Leopard frog tadpoles (*Rana pipiens*)	2,4-D acid	96-H LC_{50} = 349,000	Supplemental	44517307
Acute Freshwater Amphibians	Leopard frog tadpoles (*Rana pipiens*)	2,4-D 2-EHE (99%)	96-H LC_{50} > 507	Supplemental	44517305
Freshwater Invertebrates					
Acute Freshwater Invertebrates	Water flea (*Daphnia magna*)	2,4-D Choline Salt	48-h EC_{50} > 27,687	Acceptable	48892402
Acute Freshwater Invertebrates	Water flea (*Daphnia magna*)	2,4-D 2-EHE (92%)	48-h LC_{50} = 12.5	Acceptable	67328
Acute Freshwater Invertebrates	Water flea (*Daphnia magna*)	2,4-D IPE (98.2%)	48-h LC_{50} = 2,185	Acceptable	43930601
Acute Freshwater Invertebrates	Water flea (*Daphnia magna*)	2,4-D Acid	48-h LC_{50} = 25,000	Acceptable	41158301
Acute Freshwater Invertebrates	Water flea (*Daphnia magna*)	2,4-D IPA (47.8 %, in water)	48-h LC_{50} = 459,055	Acceptable	13869
Acute Freshwater Invertebrates	Water flea (*Daphnia magna*)	2,4-D BEE	48-h LC_{50} = 4966	Acceptable	41353801
Acute Freshwater Invertebrates	Water flea (*Daphnia magna*)	2,4-D DMA (51% formulation)	48-h LC_{50} = 645,416	Supplemental	25388
Acute Freshwater Invertebrates	Water flea (*Daphnia magna*)	2,4-D DMA (66.8%)	48-h LC_{50} = 76,000	Supplemental	41835210
Acute Freshwater Invertebrates	Water flea (*Daphnia magna*)	2,4-D DMA (51% formulation)	48-h LC_{50} > 150,000	Supplemental	36926
Acute Freshwater Invertebrates	Water flea (*Daphnia magna*)	2,4-D DMA (67.3%)	48-h LC_{50} > 153,333	Acceptable	41158311

Exposure Duration and Taxon	Species	2,4-D Form Tested	Endpoint (µg a.e./L)	Study Classification	MRID
Acute Freshwater Invertebrates	Water flea (*Daphnia magna*)	2,4-D DEA (73.1%)	48-h LC_{50}> 67,568	Acceptable	41975106
Acute Freshwater Invertebrates	Water flea (*Daphnia magna*)	2,4-D EMPE (96.21%)	48-h LC_{50} > 5,000	Acceptable	41158306
Chronic Freshwater Invertebrates	Water flea (*Daphnia magna*)	2,4-D DMA	NOAEC = 13,375 Based on survival and reproduction	Acceptable	42018303
Chronic Freshwater Invertebrates	Water flea (*Daphnia magna*)	2,4-D BEE (96,0%)	NOAEC = 200 LOAEC = 483 Based on survival and reproduction	Acceptable	41353802
Chronic Freshwater Invertebrates	Water flea (*Daphnia magna*)	2,4-D Acid	NOAEC = 79,000 LOAEC = 151,000 Based on survival and reproduction	Acceptable	41835211
Estuarine/Marine Invertebrates					
Acute Estuarine/Marine Invertebrates	Eastern oyster (*Crassostrea virginica*)	2,4-D IPA	96-h EC_{50} = 39,055	Acceptable	41429003
Acute Estuarine/Marine Invertebrates	Eastern oyster (*Crassostrea virginica*)	2,4-D IPA (50.3%) formulation	96-h EC_{50} = 49,449	Acceptable	41429003
Acute Estuarine/Marine Invertebrates	Eastern oyster (*Crassostrea virginica*)	2,4-D acid	96-h EC_{50} = 57,000	Acceptable	42979701
Acute Estuarine/Marine Invertebrates	Eastern oyster (*Crassostrea virginica*)	2,4-D DEA (73.1%)	96-h EC_{50} = 75,676	Acceptable	42018302
Acute Estuarine/Marine Invertebrates	Eastern oyster (*Crassostrea virginica*)	2,4-D DMA (66.8)	96-h EC_{50} = 85,208	Acceptable	41973401
Acute Estuarine/Marine Invertebrates	Eastern oyster (*Crassostrea virginica*)	2,4-D TIPA (70.4%)	96-h EC_{50} = 88,235	Supplemental	41429006
Acute Estuarine/Marine Invertebrates	Eastern oyster (*Crassostrea virginica*)	2,4-D 2-EHE (95.4%)	96-h EC_{50} > 1258	Supplemental	41835204
Acute Estuarine/Marine Invertebrates	Fiddler Crab (*Uca pugilator*)	2,4-D DMA (51% formulation)	96-h LC_{50} = 83,333	Supplemental	25389
Acute Estuarine/Marine Invertebrates	Grass Shrimp (*Palaemonetes pugio*)	2,4-D DMA (51% formulation)	96-h LC_{50} = 104,917	Supplemental	34700

Exposure Duration and Taxon	Species	2,4-D Form Tested	Endpoint (µg a.e./L)	Study Classification	MRID
Acute Estuarine/Marine Invertebrates	Grass Shrimp (*Palaemonetes pugio*)	2,4-D 2-EHE (95.4%)	96-h LC_{50} > 1258	Supplemental	41835206
Acute Estuarine/Marine Invertebrates	Pink shrimp (*Penaeus duorarum*)	2,4-D DMA (66.8)	96-h LC_{50} = 150,833	Acceptable	41835208
Acute Estuarine/Marine Invertebrates	Pink shrimp (*Penaeus duorarum*)	2,4-D TIPA (70.4%)	96-h LC_{50} = 397,861	Acceptable	41429005
Acute Estuarine/Marine Invertebrates	Pink shrimp (*Penaeus duorarum*)	2,4-D Acid	96-h LC_{50} = 467,000	Acceptable	41737306
Acute Estuarine/Marine Invertebrates	Pink shrimp (*Penaeus duorarum*)	2,4-D IPA (50.2%)	96-h LC_{50} > 476,378	Acceptable	41429002
Acute Estuarine/Marine Invertebrates	Pink shrimp (*Penaeus duorarum*)	2,4-D DEA (73.1%)	96-h LC_{50} > 67,297	Acceptable	41975107
Chronic Estuarine/Marine Invertebrates	Eastern oyster (*Crassostrea virginica*)	ACR	NOAEC = 31,800	ACR	ACR
Aquatic Non-Vascular Plants					
Non-vascular aquatic plant	Anabaena	2,4-D DMA (66.7%)	EC_{50} = 157,083 NOAEC = 56,550	Acceptable	41505902
Non-vascular aquatic plant	Anabaena	2,4-D BEE (96,0%)	EC_{50} = 4,393	Acceptable	42068401
Non-vascular aquatic plant	Anabaena	2,4-D TIPA (70.9%)	EC_{50} = 71,123 NOAEC = 25,615	Acceptable	43488604
Non-vascular aquatic plant	Anabaena	2,4-D acid	EC_{50} > 2,020	Acceptable	43307901
Non-vascular aquatic plant	Anabaena	2,4-D 2-EHE (94.7%)	EC_{50} > 212 NOAEC= 320	Acceptable	41735202
Non-vascular aquatic plant	Anabaena	2,4-D DEA (73.1%)	EC_{50} > 64,865	Acceptable	42712203
Non-vascular aquatic plant	Freshwater diatom (*Navicula pelliculosa*)	2,4-D BEE (96%)	EC_{50} = 1,283 NOAEC = 593	Acceptable	42068403
Non-vascular aquatic plant	Freshwater diatom (*Navicula pelliculosa*)	2,4-D 2-EHE (94.7%)	EC_{50} = 2,715 NOAEC = 1,242	Acceptable	41735205
Non-vascular aquatic plant	Freshwater diatom (*Navicula pelliculosa*)	2,4-D DMA (66.7%)	EC_{50} = 3,880 NOAEC = 1,410	Acceptable	41505903
Non-vascular aquatic plant	Freshwater diatom (*Navicula pelliculosa*)	2,4-D TIPA (70.9%)	EC_{50} = 50,481	Acceptable	43488601

Exposure Duration and Taxon	Species	2,4-D Form Tested	Endpoint (µg a.e./L)	Study Classification	MRID
Non-vascular aquatic plant	Freshwater diatom (*Navicula pelliculosa*)	2,4-D acid	EC_{50} > 2,130 NOAEC < 2,130	Acceptable	43307902
Non-vascular aquatic plant	Freshwater diatom (*Navicula pelliculosa*)	2,4-D DEA (73.1%)	EC_{50} > 65,541 NOAEC = 65,541	Acceptable	42712202
Non-vascular aquatic plant	Green algae (*Pseudokirchneriella subcapitata*)	2,4-D Choline Salt	IC_{50} > 31.2 NOAEC = 15.9	Acceptable	48892405
Non-vascular aquatic plant	*Selenastrum capricornutum*	2,4-D IPA (51.3%)	EC_{50} = 34,173 NOAEC = 10,945	Acceptable	41732102
Non-vascular aquatic plant	*Selenastrum capricornutum*	2,4-D DMA (66.7%)	EC_{50} = 42,667 NOAEC = 16,000	Acceptable	41420002
Non-vascular aquatic plant	*Selenastrum capricornutum*	2,4-D TIPA (70.9%)	EC_{50} = 5,882 NOAEC = 267 LOAEC = 524	Acceptable	42712205
Non-vascular aquatic plant	*Selenastrum capricornutum*	2,4-D DEA (73.1%)	EC_{50} = 7,432 NOAEC = 338 LOAEC = 662	Acceptable	42712205
Non-vascular aquatic plant	*Selenastrum capricornutum*	2,4-D IPE (98.2%)	EC_{50} > 109 NOAEC = 109	Acceptable	43768001
Non-vascular aquatic plant	*Selenastrum capricornutum*	2,4-D 2-EHE (94.7%)	EC_{50} > 19,868 NOAEC = 2,483	Acceptable	41735206
Non-vascular aquatic plant	*Skeletonema costatum*	2,4-D DMA (66.7%)	EC_{50} = 123,750 NOAEC = 80,208	Acceptable	41505901
Non-vascular aquatic plant	*Skeletonema costatum*	2,4-D 2-EHE (94.7%)	EC_{50} = 152 NOAEC = 62	Acceptable	41735204
Non-vascular aquatic plant	*Skeletonema costatum*	2,4-D TIPA (70.9%)	EC_{50} = 42,620 NOAEC = 26952	Acceptable	43488603
Non-vascular aquatic plant	*Skeletonema costatum*	2,4-D acid	EC_{50} > 2,080 NOAEC = 2,080	Acceptable	43307903
Non-vascular aquatic plant	*Skeletonema costatum*	2,4-D BEE (96%)	EC_{50} 1021 NOAEC = 537	Acceptable	42068404
Aquatic Vascular Plants					

Exposure Duration and Taxon	Species	2,4-D Form Tested	Endpoint (µg a.e./L)	Study Classification	MRID
Vascular aquatic plant	Duckweed (*Lemna gibba*)	2,4-D DMA	EC_{50} = 249 NOAEC = 39.2	Acceptable	42712204
Vascular aquatic plant	Duckweed (*Lemna gibba*)	2,4-D TIPA (70.9%)	EC_{50} = 1,267 NOAEC = 189	Acceptable	43488602
Vascular aquatic plant	Duckweed (*Lemna gibba*)	2,4-D 2-EHE (94.7%)	EC_{50} = 331 NOAEC < 62	Acceptable	41735203
Vascular aquatic plant	Duckweed (*Lemna gibba*)	2,4-D BEE (96,0%)	EC_{50} = 397 NOAEC = 141	Acceptable	42068402
Vascular aquatic plant	Duckweed (*Lemna gibba*)	2,4-D DMA (66.7%)	EC_{50} = 483 NOAEC = 225	Acceptable	41505904
Vascular aquatic plant	Duckweed (*Lemna minor*)	2,4-D acid	EC_{50} < 1,910 NOAEC <1,910 74% decrease in frond count	Acceptable Tier 1	43307904
Vascular aquatic plant	Duckweed (*Lemna minor*)	2,4-D DEA (73.1%)	EC_{50} = 297 NOAEC = 47 LOAEC = 88	Acceptable	42712204
Vascular aquatic plant	Duckweed (*Lemna minor*)	2,4-D acid	EC_{50} = 695 NOAEC = 58.1 12% reduction in frond count at 122 µg/L	Acceptable	44295101
Vascular aquatic plant	Duckweed (*Lemna minor*)	2,4-D acid	EC_{50} = 7,080	Supplemental	51610901
Vascular aquatic plant	*Myriophyllum aquaticum*	2,4-D acid	EC_{50} = 270	Supplemental	51610901

Table B.2. 2,4-D Avian and Terrestrial Invertebrate Data

Test	Species	2,4-D Form Tested	Endpoint (mg a.e./L)	Study Classification	MRID	Comments
Birds						
Acute oral toxicity	Mallard Duck (*Anas platyrhynchos*)	2,4-D 2-EHE (92%%) Formulation	LD50 >3073 mg a.e./kg-diet	Acceptable	72742	
Acute oral toxicity	Mallard Duck (*Anas platyrhynchos*)	2,4-D DMA (51.0%) Formulation	LD50 > 3867 mg a.e./kg-diet	Supplemental	36923	No mortality.
Acute oral toxicity	Mallard Duck (*Anas platyrhynchos*)	2,4-D EMPE (96.2%)	LD50 = 663 mg a.i./kg-bw	Acceptable	41158303	
Acute oral toxicity	Northern bobwhite quail (*Colinus virginianus*)	2,4-D BEE (96.0%)	LD50 > 1379 mg a.i./kg-bw	Acceptable	41454101	Significant body weight reduction at all dose levels; highest no mortality dose level 292 mg a.i./kg-bw
Acute oral toxicity	Northern bobwhite quail (*Colinus virginianus*)	2,4-D DEA (73.1%)	LD50 = 402 mg a.i./kg-bw	Supplemental	41975101	Significant body weight reduction at all dose levels; highest no mortality dose level 292 mg a.i./kg-bw
Acute oral toxicity	Northern bobwhite quail (*Colinus virginianus*)	2,4-D DMA (66.8%)	LD50 = 3867 mg a.e./kg-bw	Acceptable	41546201	No mortality level was 250 mg a.i./kg-bw; reduced body weight at all doses NOAEL <125 mg a.i./kg-bw
Acute oral toxicity	Northern bobwhite quail (*Colinus virginianus*)	2,4-D IPA (48.7% Technical)	LD50 = 297 mg a.e./kg-bw	Acceptable	44275701	
Acute oral toxicity	Northern bobwhite quail (*Colinus virginianus*)	2,4-D IPE (98.2%)	LD50 = 1579 mg a.i./kg-bw	Acceptable	43935001	
Acute oral toxicity	Northern bobwhite quail (*Colinus virginianus*)	2,4-D TIPA	LD50 = 117 mg a.e./kg-bw	Acceptable	41644401	
Acute dietary toxicity	Canary (*Serinus canaria*)	2,4-D acid	LC50 > 4790 mg a.e./kg-diet	Acceptable	49472501	

Test	Species	2,4-D Form Tested	Endpoint (mg a.e./L)	Study Classification	MRID	Comments
Acute dietary toxicity	Mallard Duck (*Anas platyrhynchos*)	2,4-D acid	LC50 >5620 mg a.e./kg-diet	Acceptable	41546202	
Acute dietary toxicity	Mallard Duck (*Anas platyrhynchos*)	2,4-D BEE (96.0%)	LC50 > 3876 mg a.e./kg-diet	Acceptable	41448401	
Acute dietary toxicity	Mallard Duck (*Anas platyrhynchos*)	2,4-D BEE (96.0%)	LC50 >3876 mg a.e./kg-diet	Acceptable	41429007	
Acute dietary toxicity	Mallard Duck (*Anas platyrhynchos*)	2,4-D DEA (57.9%) Formulation	LC50 > 3135 mg a.e./kg-diet	Supplemental	7309005	No mortality.
Acute dietary toxicity	Mallard Duck (*Anas platyrhynchos*)	2,4-D DEA (73.1%)	LC50 >3797 mg a.e./kg-diet	Acceptable	41975103	No mortality.
Acute dietary toxicity	Mallard Duck (*Anas platyrhynchos*)	2,4-D DMA (66.8%)	LC50 > 4683 mg a.e./kg-diet	Acceptable	41749501	No mortality, reduced body weight gain, NOAEC 562 mg a.e./kg-diet
Acute dietary toxicity	Mallard Duck (*Anas platyrhynchos*)	2,4-D IPA (48.7% Technical)	LC50 > 4425 mg a.e./kg-diet	Acceptable	138872	
Acute dietary toxicity	Mallard Duck (*Anas platyrhynchos*)	2,4-D IPE (98.2%)	LC50 > 4385 mg a.e./kg-diet	Acceptable	43935201	
Acute dietary toxicity	Mallard Duck (*Anas platyrhynchos*)	2,4-D sodium salt	LC50 > 1841 mg a.i./kg-diet	Supplemental	Citation: Hudson 1984[40]	
Acute dietary toxicity	Mallard Duck (*Anas platyrhynchos*)	2,4-D TIPA	LC50 > 1623 mg a.e./kg-diet	Acceptable	41644403	No mortality, reduced bodyweight >1780 mg a.i./kg-diet
Acute dietary toxicity	Mallard Duck (*Anas platyrhynchos*)	2,4-D EMPE (96.2%)	LC50 > 5,620 mg a.i./kg-diet	Acceptable	41158304	Reduced body weight gain, NOAEC 1,780 mg a.i./kg-diet
Acute dietary toxicity	Northern bobwhite quail (*Colinus virginianus*)	2,4-D 2-EHE (92%)	LC50 = 4760 mg a.e./kg-diet	Acceptable	45701	

[40] Hudson, R.H., Tucker, R.K., & Haegele, M.A. 1984. Handbook of toxicity of pesticides to wildlife, 2nd edition, Washington, DC, US Department of the Interior, Fish and Wildlife Service, pp. 90 (Resource Publication 153).

Test	Species	2,4-D Form Tested	Endpoint (mg a.e./L)	Study Classification	MRID	Comments
Acute dietary toxicity	Northern bobwhite quail (*Colinus virginianus*)	2,4-D acid	LC50 >5620 mg a.e./kg-diet	Acceptable	41586101	
Acute dietary toxicity	Northern bobwhite quail (*Colinus virginianus*)	2,4-D DEA (57.9%) Formulation	LC50 > 6757 mg a.e./kg-diet	Supplemental	28285	No mortality.
Acute dietary toxicity	Northern bobwhite quail (*Colinus virginianus*)	2,4-D DEA (73.1%)	LC50 >3797 mg a.e./kg-diet	Acceptable	41975102	No mortality.
Acute dietary toxicity	Northern bobwhite quail (*Colinus virginianus*)	2,4-D DMA (51.0%) Formulation	LC50 > 8333 mg a.e./kg-diet	Supplemental	36925	No mortality.
Acute dietary toxicity	Northern bobwhite quail (*Colinus virginianus*)	2,4-D DMA (51.0%) Formulation	LC50 > 3867 mg a.e./kg-diet	Supplemental	240143214	No mortality.
Acute dietary toxicity	Northern bobwhite quail (*Colinus virginianus*)	2,4-D DMA (66.8%)	LC50 > 4683 mg a.e./kg-diet	Acceptable	41749501	No mortality, reduced body weight, NOAEC 3160 mg a.e./kg-diet
Acute dietary toxicity	Northern bobwhite quail (*Colinus virginianus*)	2,4-D IPA (48.7% Technical)	LC50 > 4425 mg a.e./kg-diet	Acceptable	13870	
Acute dietary toxicity	Northern bobwhite quail (*Colinus virginianus*)	2,4-D IPE (98.2%)	LC50 > 4585 mg a.e./kg-diet	Acceptable	43934901	
Acute dietary toxicity	Northern bobwhite quail (*Colinus virginianus*)	2,4-D TIPA	LC50 > 1623 mg a.e./kg-diet	Acceptable	41644402	No mortality, reduced bodyweight >1780 mg a.i./kg-diet
Acute dietary toxicity	Northern bobwhite quail (*Colinus virginianus*)	2,4-D EMPE (96.2%)	LC50 > 5,620 mg a.i./kg-diet	Acceptable	41158305	Reduced body weight, NOAEC 3,160 mg a.i./kg-diet
Invertebrates						
Acute contact	Ladybird Beetle (*Coccinella septempunctata* L.)	2,4-D acid TGAI 99.1% a.i.	NA	Supplemental	51049401	Determined not useful for endpoint or risk assessment

Test	Species	2,4-D Form Tested	Endpoint (mg a.e./L)	Study Classification	MRID	Comments
Acute contact (adult)	Honey bee (*Apis mellifera* L.)	2,4-D DMA (67.3%)	LD_{50} > 83.3 µg a.e./bee	Acceptable	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	Acceptable	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	Acceptable	48892404	24% mortality at highest dose.
Acute oral (adult)	Honey bee (*Apis mellifera* L.)	2,4-D DMA (67.3%)	LD_{50} > 83.3 µg a.e./bee	Supplemental	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	Supplemental	44517302	
Acute oral (larval)	Honey bee (*Apis mellifera* L.)	2,4-D acid TGAI 98.8% a.i.	LD_{50} = 6 µg a.e./larva LC50 = 156 mg a.e./kg-diet	Supplemental	50282701	Day 8 larval mortality endpoints from this 22-day chronic study were determined suitable for quantitative use in risk assessment
Chronic oral (adult)	Honey bee (*Apis mellifera* L.)	2,4-D acid TGAI 97.5% a.i.	NOAEL = 5.3 LOAEL = 8.2 µg a.e./bee NOAEC = 276 LOAEC = 668 mg a.e./kg-diet	Acceptable	50751201	71% mortality at LOAEC
Chronic oral (larval)	Honey bee (*Apis mellifera* L.)	2,4-D acid TGAI 97.5% a.i.	NOAEL <0.459 LOAEL = 0.459 µg a.e./larvae NOAEC <11.9 LOAEC = 11.9 mg a.e./kg-diet Day 22 LD15 = 0.039 ug a.e./larvae (2.91 mg a.e./kg-diet)	Supplemental	50751301	Day 8 Larval mortality 24% at highest dose. Estimated larval acute LD50 > 2.93 ug a.e./larvae (respectively, 34% and 33% difference from controls) Day 15 Mortality and Adult Emergence were both significantly different from control

Test	Species	2,4-D Form Tested	Endpoint (mg a.e./L)	Study Classification	MRID	Comments
Chronic reproduction	Northern bobwhite quail (*Colinus virginianus*)	2,4-D EHE TGAI 99% a.i.	LOAEC > 637 mg a.e./kg-diet NOAEC = 637 mg a.e./kg-diet	Acceptable	45336401	
Honey bee Semi-Field Colony Study	Honey bee (*Apis mellifera* L.)	2,4-D acid TGAI 99.1% a.i.	NA	Supplemental	49270401	Determined not useful for endpoint or risk assessment

Table B.3.a. 2,4-D Vegetative Vigor (850.4150) Toxicity Data

Test Species	Test Chemical	Endpoint	Endpoint ug a.e./A		MRID
			NOEC	EC_{25}/IC_{25}	
Buckwheat	2,4-D 2-EHE	Fresh weight	0.015	0.021	42343902
Buckwheat	2,4-D acid	Fresh weight	0.0075	0.023	42416801
Buckwheat	2,4-D DEA	Fresh weight	0.0025	0.039	42609102
Buckwheat	Enlist Duo	Dry Weight	0.0075	0.02	49903202
Cabbage	2,4-D 2-EHE	Dry Weight	0.00167	0.027	47106004
Cabbage	2,4-D DMA	Dry Weight	0.0981	0.18	47106002
Cabbage	Enlist Duo	Dry Weight	0.0018	0.03	49903202
Corn	2,4-D 2-EHE	Fresh weight	0.96	>0.96	42343902
Corn	2,4-D 2-EHE	Dry Weight	0.0289	0.17	47106004
Corn	2,4-D acid	Fresh weight	4.2	>4.2	42416801
Corn	2,4-D DEA	Fresh weight	0.75	>1,5	42609102
Corn	2,4-D DMA	Dry Weight	0.136	0.77	47106002
Corn	Enlist Duo	Dry Weight	0.12	0.11	49903202
Cucumber	2,4-D 2-EHE	Fresh weight	0.015	0.192	42343902
Cucumber	2,4-D acid	Fresh weight	<0.0075	0.015	42416801
Cucumber	2,4-D DEA	Fresh weight	<0.0025	0.008	42609102
Cucumber	Enlist Duo	Dry Weight	0.0038	0.035	49903202
Horseweed	Enlist Duo	Dry Weight	0.0075	0.019	49903204
Lambsquarters	Enlist Duo	Height	0.03	0.024	49903204
Lettuce	2,4-D 2-EHE	Dry Weight	0.00167	0.0021	47106004
Lettuce	2,4-D DMA	Dry Weight	0.0017	0.0038	47106002
Mustard	2,4-D 2-EHE	Fresh weight	0.03	<0.06	42343902
Mustard	2,4-D acid	Fresh weight	<0.0075	0.011	42416801
Mustard	2,4-D DEA	Fresh weight	0.005	0.01	42609102
Mustard	Enlist Duo	Height	0.0038	0.011	49903202
Oat	2,4-D 2-EHE	Fresh weight	0.96	>0.96	42343902
Oat	2,4-D acid	Fresh weight	4.2	>4.2	42416801

Test Species	Test Chemical	Endpoint	Endpoint ug a.e./A		MRID
			NOEC	EC_{25}/IC_{25}	
Oat	2,4-D DEA	Fresh weight	>1.5	>1.5	42609102
Oat	Enlist Duo	Dry Weight	0.06	0.21	49903202
Oilseed Rape	Enlist Duo	Dry Weight	0.015	0.038	49903202
Onion	2,4-D 2-EHE	Fresh weight	0.24	>0.24	42343902
Onion	2,4-D 2-EHE	Dry Weight	0.0254	0.088	47106004
Onion	2,4-D acid	Fresh weight	<0.0075	<0.0075	42416801
Onion	2,4-D DEA	Fresh weight	0.01	0.037	42609102
Onion	2,4-D DMA	Dry Weight	0.135	0.14	47106002
Onion	Enlist Duo	Dry Weight	0.25	0.21	49903202
Quackgrass	Enlist Duo	Dry Weight	0.057	0.075	49903204
Radish	2,4-D 2-EHE	Fresh weight	0.015	0.03	42343902
Radish	2,4-D 2-EHE	Survival	0.00527	0.0068	47106004
Radish	2,4-D acid	Fresh weight	0.0075	0.016	42416801
Radish	2,4-D DEA	Fresh weight	0.0025	0.01	42609102
Radish	2,4-D DMA	Dry Weight	0.0016	0.012	47106002
Radish	Enlist Duo	Dry Weight	0.0075	0.016	49903202
Ryegrass	2,4-D 2-EHE	no effects	2.03	>2.03	47106004
Ryegrass	2,4-D DMA	Dry Weight	2.07	>2.07	47106002
Sorghum	2,4-D 2-EHE	Fresh weight	0.06	0.218	42343902
Sorghum	2,4-D acid	Fresh weight	0.53	1.34	42416801
Sorghum	2,4-D DEA	Fresh weight	0.19	0.23	42609102
Sorghum	Enlist Duo	Height	0.12	0.21	49903202
Soybean	2,4-D 2-EHE	Fresh weight	0.0075	0.02	42343902
Soybean	2,4-D 2-EHE	Dry Weight	0.0259	0.058	47106004
Soybean	2,4-D acid	Fresh weight	0.015	0.008	42416801
Soybean	2,4-D DEA	Fresh weight	0.005	0.045	42609102
Soybean	2,4-D DMA	Dry Weight	0.0072	0.039	47106002
Soybean	Enlist Duo	Dry Weight	0.015	0.02	49903202
Sugarbeet	Enlist Duo	Dry Weight	0.0075	0.022	49903202

Test Species	Test Chemical	Endpoint	Endpoint ug a.e./A		MRID
			NOEC	EC_{25}/IC_{25}	
Sunflower	Enlist Duo	Dry Weight	0.0075	0.022	49903202
Tomato	2,4-D 2-EHE	Fresh weight	0.006	0.01	42343902
Tomato	2,4-D 2-EHE	Dry Weight	<0.00134	0.0044	47106004
Tomato	2,4-D acid	Fresh weight	<0.0075	0.0075	42416801
Tomato	2,4-D DEA	Fresh weight	<0.0025	0.005	42609102
Tomato	2,4-D DMA	Dry Weight	0.0016	0.0074	47106002
Tomato	Enlist Duo	Dry Weight	0.0075	0.0099	49903202
Turnip	2,4-D 2-EHE	Dry Weight	<0.00134	0.0021	47106004
Turnip	2,4-D DMA	Dry Weight	0.0015	0.011	47106002
Wheat	2,4-D 2-EHE	Dry Weight	0.0356	0.34	47106004
Wheat	2,4-D DMA	Dry Weight	0.133	1.1	47106002
Wheat	Enlist Duo	Dry Weight	0.03	0.048	49903202

Table B.3.b. 2,4-D Seedling Emergence (850.4100) Toxicity Data

Test Species	Test chemical	Endpoint	Endpoint ug a.e./A		MRID
			NOEC	EC_{25}/IC_{25}	
Buckwheat	2,4-D acid	Weight	1.05	1.29	42416802
Buckwheat	Enlist Duo	Height	0.48	>0.96	49903201
Cabbage	2,4-D 2-EHE	Dry Weight	0.0015	0.021	47106003
Cabbage	2,4-D DMA	Dry Weight	0.02	0.043	47106001
Cabbage	2,4-D IPE	Emergence	0.0011	0.00219	43982101
Cabbage	Enlist Duo	Dry Weight	0.12	0.082	49903201
Corn	2,4-D 2-EHE	Dry Weight	1.9	3.4	47106003
Corn	2,4-D acid	Weight	2.1	4.2	42416802
Corn	2,4-D DMA	Height	4	>4.0	47106001
Corn	2,4-D IPE	Phytotoxicity	0.0064	0.071	43982101
Corn	Enlist Duo	No Effects	0.96	>0.96	49903201
Cucumber	2,4-D acid	Weight	0.13	0.53	42416802
Cucumber	2,4-D IPE	Length	0.28	0.044	43982101
Cucumber	Enlist Duo	No Effects	0.96	>0.96	49903201
Lambsquarters	Enlist Duo	Dry Weight	0.06	0.069	49903203
Lettuce	2,4-D 2-EHE	Dry Weight	0.0058	0.018	47106003
Lettuce	2,4-D DMA	Dry Weight	0.02	0.026	47106001
Lettuce	2,4-D IPE	Length	0.00056	0.00079	43982101
Mustard	2,4-D acid	Weight	0.015	0.033	42416802
Mustard	Enlist Duo	Dry Weight	0.12	0.16	49903201
Oat	2,4-D acid	Weight	2.1	>4.2	42416802
Oat	2,4-D IPE	No Effects	0.11	>0.11	43982101
Oat	Enlist Duo	Height	0.48	>0.96	49903201
Oilseed Rape	Enlist Duo	Dry Weight	0.12	0.42	49903201
Onion	2,4-D 2-EHE	Height	0.019	0.17	47106003
Onion	2,4-D acid	Weight	2.1	>2.1	42416802
Onion	2,4-D DEA	Dry Weight	0.188	0.262	44275601
Onion	2,4-D DMA	Dry Weight	0.091	0.097	47106001

Test Species	Test chemical	Endpoint	Endpoint ug a.e./A		MRID
			NOEC	EC_{25}/IC_{25}	
Onion	2,4-D IPE	Length	0.0067	0.0099	43982101
Onion	Enlist Duo	Dry Weight	0.245	0.254	49903201
Radish	2,4-D 2-EHE	Dry Weight	0.0058	0.0061	47106003
Radish	2,4-D acid	Weight	0.015	0.03	42416802
Radish	2,4-D DMA	Dry Weight	0.02	0.033	47106001
Radish	Enlist Duo	Survival	0.25	0.25	49903201
Ryegrass	2,4-D 2-EHE	Dry Weight	0.26	0.27	47106003
Ryegrass	2,4-D DMA	Height	4	>4.0	47106001
Ryegrass	2,4-D IPE	No Effects	0.11	0.011	43982101
Sorghum	2,4-D acid	Weight	2.1	>2.1	42416802
Sorghum	Enlist Duo	No Effects	0.96	>0.96	49903201
Soybean	2,4-D 2-EHE	Dry Weight	0.47	0.85	47106003
Soybean	2,4-D acid	Weight	0.015	1.71	42416802
Soybean	2,4-D DMA	Dry Weight	0.26	0.37	47106001
Soybean	2,4-D IPE	Phytotoxicity	0.0064	0.0082	43982101
Soybean	Enlist Duo	Dry Weight	0.96	>0.96	49903201
Sugarbeet	Enlist Duo	No Effects	0.49	>0.49	49955101
Sunflower	Enlist Duo	Dry Weight	0.96	>0.96	49903201
Tomato	2,4-D 2-EHE	Dry Weight	0.0058	0.012	47106003
Tomato	2,4-D acid	Weight	4.2	>4.2	42416802
Tomato	2,4-D DMA	Dry Weight	0.34	>0.34	47106001
Tomato	2,4-D IPE	Length	0.013	0.024	43982101
Tomato	Enlist Duo	Dry Weight	0.49	>0.96	49903201
Turnip	2,4-D 2-EHE	Dry Weight	0.0058	0.062	47106003
Turnip	2,4-D DMA	Dry Weight	0.02	0.13	47106001
Turnip	2,4-D IPE	Length	0.007	0.009	43982101
Wheat	2,4-D 2-EHE	No Effects	4	>4.0	47106003
Wheat	2,4-D DMA	Dry Weight	0.35	0.2	47106001
Wheat	Enlist Duo	No Effects	0.96	>0.96	49903201

Open Literature Plant Reproduction studies for 2,4-D

Marple et al. 2008.

Marple, M.E., K. Al-Khatib, and D.E. Peterson. 2008. Cotton injury and yield as affected by simulated drift of 2,4-D and dicamba. Weed Technology, 22:609-614.

Study Classification: Supplemental, suitable for qualitative use in risk characterization.

The authors report four separate cotton field studies conducted in Manhattan, KS in 2005 and 2006. In the first study, applications of 2,4-D were made at 0.0025 and 0.00125 lbs a.i./A at four different growth stages. In the second study, applications were made at 0.00125, 0.000625, and 0.000417 lbs a.i./A and were repeated up to three times in some trials. Visual signs of injury (VSI) and yield were reported.

Results from the first study show that VSI is highest at the younger growth stages and generally increases in percent effect as time progresses. Results from both years are similar. In terms of impact to yield in the first study, there were significant reductions in yield at the 0.0025 lbs a.i./A rate for all growth stages (>10%) with the youngest stage having nearly 35% reductions in yield on average across the years. This corresponded to an average VSI of 57% (range 25-80%) for the 14-56 days after observation in these two years.

In the second study, multiple exposures resulted in significantly higher VSI than a single exposure. The results from 2005 show significant impacts to yield (approximately 40% reduction) for the 2 and 3 applications at 0.00125 lbs a.i./A rate. VSI was observed as high as 84% after 3 applications, but generally reflect >60% VSI for the 2 and 3 application scenarios at this rate. In 2006, the impact to yield rose to nearly 90% reductions for the 2 and 3 applications of 0.00125 lbs a.i./A rate, but also showed approximately 40% reductions in yield for the 0.000625 lbs a.i./A rate. Again, similar or greater impact to VSI was observed for these trials with VSI generally greater than 45% but rose to 93%.

Everitt and Keeling 2009

Everitt, J.D. and J.W. Keeling. 2009. Cotton growth and yield response to simulated 2,4-D and dicamba drift. Weed Technology, 23:503-506.

Study Classification: Supplemental, suitable for qualitative use in risk characterization.

The field study tested the effects (visual signs of injury (VSI) and yield) of 2,4-D amine and dicamba when applied at different growth stages of cotton development and at different rates. This evaluation focuses only on the results pertaining to 2,4-D. The application rates of 2,4-D were 0.00025, 0.0025, 0.025, and 0.25 lbs a.i./A.

The results indicate that there were generally greater effects in terms of VSI when plants were younger and at all rates. As plants aged, VSI became less significant at the two lower rates, with the latest applications only having significant impact to VSI at the two highest rates. The impacts to yield reflected a similar but less defined pattern. The highest two test concentrations significantly reduced yields for all ages (15-97% reductions); however, the latest time of exposure had the lower end of the impacts. The relationship of VSI to yield varied with regard to the time of observation of VSI, but maximum observed VSI was similar to the reductions observed in yield.

Table B.4. Summary of results for and relationship between VSI and yield.

Time of Application	Application Rate (lbs a.e./A)	Day 14 VSI	Maximum VSI	% Reduction in Yield	Ratio of 14DAT VSI:Percent Yield	Ratio of Max VSI:Percent Yield
Cotyledon - 2lf	Control	0	0	-		
	0.00025	12	12	1.0		
	0.0025	50	50	2.7		
	0.025	66	66	**49.7**	1.3	1.3
	0.25	92	92	**81.7**	1.1	1.1
4-5 leaf						
	0.00025	3	4	-19.8		
	0.0025	35	35	6.5		
	0.025	62	62	**44.2**	1.4	1.4
	0.25	84	84	**84.5**	1.0	1.0
Pinhead Square						
	0.00025	3	18	-24.4		
	0.0025	10	44	-6.8		
	0.025	38	57	**67.9**	0.6	0.8
	0.25	77	78	**96.7**	0.8	0.8
First Bloom						
	0.00025	0	3	-12.0		
	0.0025	3	3	4.7		
	0.025	8	32	**15.2**	0.5	2.1
	0.25	45	58	**60.3**	0.7	1.0

Robinson et al. 2013

Robinson, A.P., V.M. Davis, D.M. Simpson, and W.G. Johnson. 2013. Response of soybean yield components to 2,4-D. Weed Science, 61: 68-76.

Study Classification: Supplemental, suitable for qualitative use in risk characterization.

The authors conducted field-based direct spray toxicity studies in 2009 and 2010 with 2,4-D DMA (“Weedar 64”) on soybean in Lafayette IN, Urbana IL, and Fowler, IN. VSI and yield were reported. The design was a complete randomized block layout, with treatments at 0, 0.000089, 0.00098, 0.010, 0.031, 0.064, 0.12, 0.25, 0.50, and 2.0 lbs DMA/A (0, 0.1, 1.1, 11.2, 35, 70, 140, 280, 560, and 2240 g/ha). Applications were made at the V2, V5 or R2 growth stages. Visual signs of injury (VSI) were reported for days 14 and 28, plant height was sampled at the R8 growth stage, and several yield endpoints were collected.

The authors describe the relationships of the varied components of yield measures. Of these, the most relevant to the risk assessment is total seed-yield loss (most sensitive at V5 exposure ED_{20} = 0.026 lbs DMA/A; range up to 0.22 lbs DMA/A). The authors reported VSI was a good indicator of impacts to plant yield. They report that 10% seed-yield loss was observed when approximately 35% VSI was observed on day 14, but at 28 days after treatment (DAT) for 10% yield loss VSI ranged from 15 to 40%. When combining their studies (Figure 4 in publication), a 25% reduction in yield (i.e., 3 Mg/ha) had corresponding VSI that ranged from ~40-60%.

Andersen et al. 2004

Andersen, S.M., S.A. Clay, L.J. Wrage, and D. Matthees. 2004. Soybean foliage residues of dicamba and 2,4-D and correlation to application rates and yield. Agronomy Journal, 96:750-760.

Study Classification: Supplemental, suitable for qualitative use in risk characterization.

The authors conducted two field-based soybean studies in Beresford (planted soybean: Prairie Brand PB1901RR) and Brookings (planted soybean: Asgrow AG1301RR), SD in 2001 and 2002, respectively. Herbicides were used for preparing the fields and controlling weeds during early development, prior to application of the test chemicals, 2,4-D (dimethylamine salt) and dicamba (diglycolamine salt). The review here pertains only to the test and results for 2,4-D.

A treatment design was set up as a randomized complete block design with 4 replications and untreated controls. Application rates for 2,4-D were 0.01, 0.05, and 0.1 lbs a.e./A (0.0112, 0.056 and 0.112 kg a.e./ha) at the V3 growth stage of the soybean crop. Plants were observed for height, biomass, visual signs of injury (VSI), and yield. Six total plants (from outermost rows) were sampled for height and biomass per plot. A plant residue analysis was also completed, but residue data results will not be discussed here. However, results indicate that 2,4-D residue data are not reflective of the injury or reductions in height or weight effects observed.

The authors point to Andersen 2003 (Master's Thesis) for the plant height results but do not provide the data for plant height in this publication. They reported a maximum impact on plant height at 6 and 12 days after treatment. They also report that the apical meristems in the treatment plots died or were significantly damaged, and as a result, development from lateral buds was prevalent.

The presented results suggest that VSI increased with a dose response manner, and that injury was generally stable and observed across the time periods. Recovery was potentially reflected in the 0.05 lbs a.e./A rate. Plant weight results were more variable, however, fairly stable from the 6 to 48 day period of observation. Plant weights were similarly impacted (~30%) at the 0.05 and 0.1 lb a.e./A rates. Yield displayed a clear dose response pattern with 7% reduction at the 0.05 lb a.e./A rate and 25 – 32 % reductions at 0.1 lb a.e./A. Slightly greater impacts to plant weight (19-35%) were observed as compared to percent reductions in yield (0-32%) at respective doses.

The ratio of VSI to weight for each time period and application rate suggests that the recovery of VSI over time results in greater observed effect to weight than seen in VSI, and that under some conditions, the impacts on plant weight may surpass those of VSI. For plant yield, the ratio of the maximum observed VSI to yield effect ranged from 1.1 to 3.5 (average = 2.2), suggesting that maximum VSI observations after an exposure is equivalent or protective of plant yield observations. The lack of availability of the plant height data limit the utility of the study for comparisons across studies for plant height; however, the VSI to yield data suggest that VSI is generally two times greater than the impacts to yield.

Kelley et al. 2005.

Kelley, K.B., L.M. Wax, A.G. Hager, and D.E. Riechers. 2005. Soybean response to plant growth regulator herbicides is affected by other postemergence herbicides. Weed Science, 53:101-112.

Study Classification: Supplemental, suitable for qualitative use in risk characterization.

The authors conducted two field-based soybean studies in Urbana, IL in 2001, 2002, and 2003. A glyphosate tolerant variety of soybean (Pioneer 94B01RR) was planted in the spring, and fields were maintained with applications of metolachlor, chlorimuron-ethyl and metribuzin.

The experiment sought answers regarding the response of this soybean variety to exposures of dicamba (dichloamine salt); dicamba sodium salt + diflufenzopyr sodium salt, monoethanolamine salt of clopyralid, isooctyl ester formation of 2,4-D, imazethapyr, and the isopropylamine salt of glyphosate and the sodium salt of fomesafen. The review provided here will only discuss the results for 2,4-D. 2,4-D was sprayed over the crop at 0.05 or 0.16 lbs a.e./A at V3, V7 and R2 soybean growth stages and observed at 2 and 4-6 weeks after treatment.

Following the 0.05 lbs a.e./A application, visual signs of injury ranged from 8, 22, and 19% injury for the 2-week observation for V3, V7 and R2 stages, respectively. Observations of VSI after 4-6 weeks showed lower injury across all treatments (3-7%), suggesting vegetative recovery from the exposure. The application at 0.16 lbs a.e./A resulted in greater injury (49, 52 and 37% after 2 weeks, and maintained 30, 20, and 25% injury after 4-6 weeks). This suggests recovery was slower following a more significant impact. The authors also reported significant impacts on plant height and yield. EPA has summarized the injury, yield and height results in **Table B.5**. Notably the percent injury observed was a larger percentage than observed reductions for height or yield. Also, plant height was more sensitive (higher percent reductions) than yield at V7 and R2 growth stages, suggesting that it is protective of reproduction at these later potential exposure stages. Because recovery was observed in visual signs of injury (new growth didn't show as much or little damage) EPA makes comparison of plant height and yield against the earlier observation of VSI (2 weeks after treatments).

Table B.5. Summary of soybean injury, yield and height after exposure to 2,4-D

Application Rate (lbs a.e./A)	**Time of Application**		
	V3	**V7**	**R2**
Percent Injury (2 weeks after treatment)			
0.05	8	22	19
0.16	49	52	37
Yield Percent Reduction			
0.05	6	4	2
0.16	25	17	15
Height Percent Reduction			
0.05	5	12	13
0.16	18	25	21

Comparisons between the effects observed for each of these endpoints are useful for understanding the relationship of VSI and reproduction to the typical threshold endpoint of plant height. Comparisons of the percent effect can be made using ratios. **Table B.6** provides the ratios for VSI to yield, VSI to height, and yield to height. Notably the results for early growth stage exposure (V3) have ratios for VSI to height and yield at approximately 1.4-2.7. Furthermore, the ratio of VSI to height is approximately 1. These results suggest that VSI in excess of 25% would be needed to have a 25% reduction in either of these endpoints at this growth stage of soybeans. The ratios for later growth stages show similar or wider ratios, supporting the conclusion that percent VSI at the regulatory threshold of plant height is much greater than the observed effect on height. The results for VSI to yield were similar across all growth stages but continue to support the conclusion. Yield to height ratios at the later growth stages show that plant height-based endpoints for older plants are protective of yield at later growth stages of exposure in the study. The V3 growth stage had slightly higher observed effects in yield than height at the time of observation, but the differences are not biologically significant. The typical vegetative vigor endpoints are selected for growth stages similar to V3 and thus EPA considers the 2,4-D's vegetative vigor-based endpoints to be representative of the reproductive endpoints for soybeans.

Table B.6. Ratios illustrating the relationships between the observed effects of VSI, height and yield for different aged exposures of soybean.

Application Rate (lbs a.e./A)	Time of Application		
	V3	V7	R2
VSI:Yield			
0.05	1.4	5.1	11.5
0.16	2.0	3.1	2.5
VSI:Height			
0.05	1.6	1.8	1.5
0.16	2.7	2.1	1.8
Yield:Height			
0.05	1.1	0.4	0.1
0.16	1.4	0.7	0.7

This study provides evidence that the endpoints selected for soybean in this study will be protective of reproduction-based endpoints. The study also provides evidence that supports a threshold of VSI 1.4 times higher than that of plant height or reproduction. Assuming that this relationship is maintained over the entire exposure-response curve, this would translate to 35% VSI at the IC_{25} for plant height.

Ogg et al. 1991.

Ogg, A.G. Jr., M.A. Ahmedullah, and G.M. Wright. 1991. Influence of repeated applications of 2,4-D on yield and juice quality of concord grapes (Vitis labruscana). Weed Science, 39: 284-295.

Study Classification: Supplemental, suitable for qualitative use in risk characterization.

The authors applied 2,4-D dimethylamine salt between planted rows of mature grapes. Care was taken to avoid direct spray, including a low boom hooded sprayer system and applying in the morning when the air was "calm". Sprays were applied at 0.00074, 0.0038, and 0.0074 lbs a.e./A (0.001, 0.005, and

0.10 kg a.i./ha) at single or multiple growth stages of grape development (e.g., 50% bud burst; 3rd leaf; 5th leaf; full bloom) in different combinations. There were 12 treatment combinations (rates x four number-timings of application) and replicated six times. Two nearly identical studies were conducted through 1979 and 1982.

A defined system for determining percent visual signs of injury (VSI) was provided. In addition to VSI, yield (total weight and number of clusters per plot, number of berries per cluster) and juice quality endpoints were also collected. 2,4-D residues in fruit were analyzed and provided.

Negative controls showed “low background” of visual symptomology as a result of 2,4-D exposure. The authors suggest that the air within the Yakima Valley was likely related to these exposures. VSI in July was similar to controls for single applications at bud break for each treatment concentration. VSI following two or more applications at 0.0074 lbs a.e./A were significantly different controls in July. In 1979, there were also significant reductions in the number of berries per cluster and weight of clusters was reported following two or more applications at 0.0074 lbs a.e./A. The authors report that in 1980, two applications of 0.00074 lbs a.e./A or higher resulted in significant reductions of yield.

The VSI scales the authors used changed from 1979 to 1980 to account for more descriptions of the symptoms that were being observed the first year. In terms of percent VSI categories in 1979, category 2 represented effects up to 50%, whereas in 1980 category 2 represented effects up to 20% and 3 represented from 20% to 50%. These two categories are relevant to the targeted effect levels in the assessment. The authors provide a polynomial regression of yield vs plant injury (Figure 3 in publication). Based on the provided regression, there is an equivalent yield loss of 30% at approximately 20-50% VSI.

Table B.7. Predicted yield losses based upon regression equation in Figure 3. Treatment included single or multiple applications.

Injury Rating	Percent VSI	Percent Yield Loss
2	<20	8
3	20-50	29
4	50-75	67

Glyphosate Studies

This appendix provides a full list of the registrant-submitted studies and additional study details on the studies EPA evaluated when selecting the most sensitive glyphosate endpoints for the FIFRA (**Section 3**) and ESA (**Section 4**) portions of the risk assessment. See **Section 3.3** for list of selected endpoints. Open literature studies are discussed in the Draft Glyphosate BE (USEPA, 2020b), with references therein.

Note: These tables are in large part extracted from the USEPA, 2008 California red-legged frog risk assessment.

Table B-8. Freshwater Fish Acute Toxicity for Technical Glyphosate and Its Salts

Species	% Active Ingredient*	96-hour LC_{50}/ NOAEC (mg a.e./L)*/ Slope	Toxicity Category[2]	MRID #/Year	Study Classification
Bluegill sunfish (*Lepomis macrochirus*)	83	LC_{50}: 99.6 (92.1 - 107.9)[1] NOAEC: 83 Slope: Not available	Slightly toxic	00108205/1978	Acceptable
Rainbow trout (*Oncorhynchus mykiss*)	83	LC_{50}: 71.4 (58.1-84.8) NOAEC: 34.9 Slope: Not available	Slightly toxic	00136339/1978	Acceptable
Rainbow trout (*Oncorhynchus mykiss*)	95.6	LC_{50}: 128.1 (95.6 - 172.1) NOAEC: 30.6 Slope: Not available	Practically nontoxic	44320629/1995	Acceptable
Bluegill sunfish (*Lepomis macrochirus*)	95.6	LC_{50}: 43 (30.6 - 53.5) NOAEC: 30.6 Slope: Not available	Slightly toxic	44320630/1995	Acceptable
Fathead minnow (*Pimephales promelas*)	96.7	LC_{50}: 69.4 (56.5 - 85.9)[3] NOAEC not reported Slope: Not available	Slightly toxic	00162296/1979	Acceptable
Channel catfish (*Ictalurus punctatus*)	96.7	LC_{50}: 93 (78.7 - 114.5)[3] NOAEC not reported Slope: Not available	Slightly toxic	00162296/1979	Acceptable

Species	% Active Ingredient*	96-hour LC_{50}/ NOAEC (mg a.e./L)*/ Slope	Toxicity Category[2]	MRID #/Year	Study Classification
Rainbow trout (*Oncorhynchus mykiss*)	96.7	LC_{50}: 100.2 (85.9 - 121.6)[3] NOAEC not reported Slope: Not available	Practically nontoxic	00162296/1979	Acceptable
Bluegill sunfish (*Lepomis macrochirus*)	96.7	LC_{50}: 100.2 (78.7 - 114.5)[3] NOAEC not reported Slope: Not available	Practically nontoxic	00162296/1979	Acceptable

* a.i. = active ingredient; a.e. = acid equivalent
[1] Range is 95% confidence interval for endpoint
[2]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[3] Study conducted with the isopropylamine salt

Table B-9. Freshwater Fish Acute Toxicity for Glyphosate Formulations

Chemical	Species	% a.i.*	96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope	Toxicity Category[1]	MRID #/Year	Study Classification
Glyphosate IPA*	Rainbow trout (*Oncorhynchus mykiss*)	30	LC_{50}: 1 (0.8 - 1.2)[2] (3.17 mg formulation/L) NOAEC: N.R.* Slope:N.R.	Highly toxic	40098001/1986	Supplemental
Glyphosate IPA (MON 77360)	Rainbow trout (*Oncorhynchus mykiss*)	30	LC_{50}: 1.6 (1.3 - 2.1) NOAEC: 1.3 Slope:NA*	Moderately toxic	45365003/2000	Supplemental
Glyphosate IPA	Fathead minnow (*Pimephales promelas*)	30	LC_{50}: 1.7 (1.4 - 2.1) NOAEC: N.R. Slope:N.R.	Moderately toxic	00162296/1979	Supplemental
Glyphosate IPA (Roundup)	Bluegill sunfish (*Lepomis macrochirus*)	31	LC_{50}: 1.8 (1.4 - 2.6) NOAEC: 0.7 Slope:N.R.	Moderately toxic	00124760/1982	Acceptable

Chemical	Species	% a.i.*	96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope	Toxicity Category[1]	MRID #/Year	Study Classification
Glyphosate monoammonium salt (MON78568)	Rainbow trout (*Oncorhynchus mykiss*)	66	LC_{50}: 1.9 (1.04 - 2.31) NOAEC: 1.04 Slope:N.R.	Moderately toxic	45767101/2002	Not classified
Glyphosate IPA (MON 77360)	Bluegill sunfish (*Lepomis macrochirus*)	30	LC_{50}: 2.2 (1.3 - 3.3) NOAEC: 1.3 Slope:NA	Moderately toxic	45365002/2000	Acceptable
Glyphosate IPA (MON65005)	Bluegill sunfish (*Lepomis macrochirus*)	31	LC_{50}: 2.4 (2.0 - 3.5) NOAEC: 1.2 Slope:N.R	Moderately toxic	44538203/1998	Acceptable
Glyphosate IPA (Roundup)	Rainbow trout (*Oncorhynchus mykiss*)	31	LC_{50}: 2.5 (2.0 - 3.1) NOAEC: 1.8 Slope:N.R.	Moderately toxic	00124761/1982	Supplemental
Glyphosate IPA (MON65005)	Rainbow trout (*Oncorhynchus mykiss*)	31	LC_{50}: 2.5 (1.9 - 3.1) NOAEC: 1.9 Slope:N.R	Moderately toxic	44538202/1998	Acceptable
Glyphosate IPA (Roundup)	Fathead minnow (*Pimephales promelas*)	41	LC_{50}: 2.9 (1.7 - 4.9) NOAEC: 1.7 Slope:N.R.	Moderately toxic	00070896/1980	Acceptable
Glyphosate IPA	Bluegill sunfish (*Lepomis macrochirus*)	30	LC_{50}: 3 (2.4 - 3.7) NOAEC: N.R. Slope:N.R.	Moderately toxic	40098001/1986	Supplemental
Glyphosate IPA	Rainbow trout (*Oncorhynchus mykiss*)	30	LC_{50}: 3.4 (5.2 - 7.3) NOAEC: N.R. Slope:N.R.	Moderately toxic	00162296/1979	Supplemental
Glyphosate IPA (MON 2139, Roundup)	Rainbow trout (*Oncorhynchus mykiss*)	41	LC_{50}: 3.4 (2.7 - 4.3) NOAEC: 2.7 Slope:N.R.	Moderately toxic	00070895/1980	Acceptable

Chemical	Species	% a.i.*	96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope	Toxicity Category[1]	MRID #/Year	Study Classification
Glyphosate IPA	Bluegill sunfish (*Lepomis macrochirus*)	30	LC_{50}: 3.7 (2.8 - 4.9) NOAEC: N.R. Slope:N.R.	Moderately toxic	00162296/1979	Supplemental
Glyphosate IPA (Roundup)	Bluegill sunfish (*Lepomis macrochirus*)	41	LC_{50}: 4.3 (2.7 - 7.3) NOAEC: 2.7 Slope:N.R.	Moderately toxic	00070897/1980	Acceptable
Glyphosate IPA (Roundup)	Channel catfish (*Ictalurus punctatus*)	41	LC_{50}: 4.9 (2.9 - 8.0) NOAEC: 2.9 Slope:N.R.	Moderately toxic	00070894/1980	Supplemental
Glyphosate IPA (Roundup)	Rainbow trout ((*Salmo gairdneri*)	36	LC_{50}: 5.5 - 9.2 (4.2 - 13) NOAEC: 4.2 Slope:N.R.	Moderately toxic	40579203/1986	Supplemental
Glyphosate IPA (Roundup)	Chinook Salmon (*Oncorhynchus tschawytscha*)	36	LC_{50}: 7.0 (5.4 - 9.9) NOAEC: <1.3 Slope:N.R.	Moderately toxic	40579201/1986	Not classified
Glyphosate IPA (Roundup)	Coho Salmon (*Oncorhynchus kisutch*)	36	LC_{50}: 8.2 (4.2 – 13.4) NOAEC: 3.42 Slope:N.R.	Moderately toxic	40579202/1986	Not classified
Glyphosate IPA with 0.5% "X-77"	Rainbow trout (*Oncorhynchus mykiss*)	5	LC_{50}: 9.4 (7.0 - 12.4) NOAEC: 7 Slope:N.R.	Moderately toxic	00078664/1980	Acceptable
Glyphosate IPA	Channel catfish (*Ictalurus punctatus*)	30	LC_{50}: 9.6 (8.1 - 11.8) NOAEC: N.R. Slope:N.R.	Moderately toxic	00162296/1979	Acceptable
Glyphosate IPA (Roundup with 15 % "W")	Bluegill sunfish (*Lepomis macrochirus*)	41	LC_{50}: >30 (30 - 96.4.) NOAEC: 30 Slope:N.R.	Slightly toxic	00078656/1980	Supplemental

Chemical	Species	% a.i.*	96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope	Toxicity Category[1]	MRID #/Year	Study Classification
Glyphosate IPA with 0.5% "X-77"	Bluegill sunfish (*Lepomis macrochirus*)	5.3	LC_{50}: 32.4 (24.2 - 62.4) NOAEC: 7.1 Slope:4.2	Slightly toxic	00078665/1980	Acceptable
Glyphosate IPA (Roundup with 15.3 % "AA")	Rainbow trout (*Oncorhynchus mykiss*)	41	LC_{50}: 36.6 (17.1 - 54.9) NOAEC: N.R. Slope:N.R.	Slightly toxic	00078658/1980	Acceptable
Glyphosate IPA (Roundup with 15 % "W")	Rainbow trout (*Oncorhynchus mykiss*)	41	LC_{50}: 45.2 (30.1 - 96.4) NOAEC: 30.1 Slope:N.R.	Slightly toxic	00078655/1980	Acceptable
Glyphosate (360 g/L SL)	Bluegill sunfish (*Lepomis macrochirus*)	28	LC_{50}: >52 (N.A.) NOAEC: 52 Slope:N.A.	Slightly toxic	45374002/2000	Supplemental
Glyphosate(80WDG)	Fathead minnow (*Pimephales promelas*)	79	LC_{50}: 54.3 (47.3 - 79.1) NOAEC: 28.7 Slope:N.R.	Slightly toxic	44125704/1996	Acceptable
Glyphosate(80WDG)	Rainbow trout (*Oncorhynchus mykiss*)	80	LC_{50}: 62.1 (48.2 - 80.0) NOAEC: 28.7 Slope:N.R.	Slightly toxic	44125705/1996	Acceptable
Glyphosate IPA (Rodeo/X-77)	Rainbow trout (*Salmo gairdneri*)	41	LC_{50}: 96.4 (89.0 - 118.7) NOAEC: 37.5 Slope:N.R.	Slightly toxic	40579301/1985	Not classified
Glyphosate IPA (Rodeo/X-77)	Chinook salmon (*Oncorhynchus tschawytscha*)	41	LC_{50}: 103.8 (89.0 - 148.3) NOAEC: 47.5 Slope:N.R.	Practically non-toxic	40579303/1985	Not classified
Glyphosate IPA (Rodeo/X-77)	Rainbow trout ((*Salmo gairdneri*)	41	LC_{50}: 134 (75 - 240) NOAEC: 43 Slope:N.R.	Practically non-toxic	40579306/1987	Not classified

Chemical	Species	% a.i.*	96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope	Toxicity Category[1]	MRID #/Year	Study Classification
Glyphosate IPA (Rodeo/X-77)	Coho Salmon (*Oncorhynchus kisutch*)	41	LC_{50}: 148.3 (89.0 - 274.4) NOAEC: 88.5 Slope:N.R.	Practically non-toxic	40579302/1985	Not classified
Glyphosate IPA (Rodeo/X-77)	Chinook salmon (*Oncorhynchus tschawytscha*)	40	LC_{50}: 180.2 (133.5 - 240.3) NOAEC: 74.8 Slope:N.R.	Practically non-toxic	40579305/1987	Not classified
Glyphosate (360 g/L SL)	Rainbow trout (*Oncorhynchus mykiss*)	27	LC_{50}: 224.5 (160.1 - 280.0) (824 mg formulation/L) NOAEC: 160 Slope:N.R.	Practically non-toxic	45374001/1999	Supplemental
Trisodium diglyphosate/Urea (Polado formula) - MON 8000	Bluegill sunfish (*Lepomis macrochirus*)	75	LC_{50}:>315 (N.R.) NOAEC: 315 Slope:N.R.	Practically non-toxic	00079146/1980	Supplemental
Trisodium diglyphosate/Urea (Polado formula) - MON 8000	Rainbow trout (*Oncorhynchus mykiss*)	75	LC_{50}: >315 (N.R.) NOAEC: 315 Slope:N.R.	Practically non-toxic	00085637/1980	Supplemental
Glyphosate IPA (Rodeo)	Rainbow trout ((*Salmo gairdneri*)	41	LC_{50}: 430.1 (341 - 541) NOAEC: 157 Slope:N.R.	Practically non-toxic	40579301/1985	Not classified
Glyphosate IPA (No surfactant)	Rainbow trout (*Oncorhynchus mykiss*)	62	LC_{50}: >461.8 (N.R.) NOAEC: N.R. Slope:N.R.	Practically non-toxic	00078661/1980	Acceptable
Glyphosate IPA (No surfactant)	Bluegill sunfish (*Lepomis macrochirus*)	62	LC_{50}: >461.8 (N.R.) NOAEC: N.R. Slope:N.R.	Practically non-toxic	00078662/1981	Supplemental
Glyphosate IPA (MON77945 Manufacturing concentrate)	Rainbow trout (*Oncorhynchus mykiss*)	46	LC_{50}: >977 (N.A.) mg formulation/L NOAEC: 591 Slope:N.A.	Practically non-toxic	44715409/1998	Not classified

Chemical	Species	% a.i.*	96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope	Toxicity Category[1]	MRID #/Year	Study Classification
Glyphosate IPA with Geronol CF/AR	Rainbow trout (*Oncorhynchus mykiss*)	10	LC_{50}: > 450 (N.A.) mg a.e./L or > 1000 mg formulation/L NOAEC: 1000 mg formulation/L Slope:N.A.	Practically non-toxic	44738201/1996	Not classified
Glyphosate IPA with Geronol CF/AR	Rainbow trout (*Oncorhynchus mykiss*)	36	LC_{50}: >1000 (N.A.) mg formulation/L NOAEC: 800 Slope:N.A.	Practically non-toxic	44738201/1996	Not classified
Glyphosate IPA (Roundup Biactive)	Rainbow trout (*Oncorhynchus mykiss*)	36	LC_{50}: >1000 (N.A.) mg formulation/L NOAEC: 800 Slope:N.A.	Practically non-toxic	44738201/1996	Not classified
Glyphosate IPA with Geronol CF/AR	Rainbow trout (*Oncorhynchus mykiss*)	45	LC_{50}: >1000 (N.A.) mg formulation/L NOAEC: 1000 Slope:N.A.	Practically non-toxic	44738201/1996	Not classified

* a.i. = active ingredient; a.e. = acid equivalent; IPA = isopropylamine salt; NR = not reported; NA = not available
[1]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[2] Range is 95% confidence interval for endpoint

Table B-10. Freshwater Fish Acute Toxicity for Surfactants Used with Glyphosate Formulations

Chemical	Species	% a.i.[1]	LC/EC_{50} (mg/L)	Toxicity Category[2]	MRID #/Year; comment	Study Classification
Polyoxy ethylene fatty amine (POEA) (MON 0818)	Fathead minnow (*Pimephales promelas*)	70[4]	96-hr LC_{50}: 0.7 (0.84 - 1.19)[3] NOAEC and slope not reported	Highly toxic	00162296/1979; MON 0818 LC_{50}: 1 mg/L	Acceptable

Chemical	Species	% a.i.[1]	LC/EC_{50} (mg/L)	Toxicity Category[2]	MRID #/Year; comment	Study Classification
Polyoxy ethylene fatty amine (POEA) (MON 0818)	Rainbow trout (*Oncorhynchus mykiss*)	70[4]	96-hr LC_{50}: 1.4 (1.05 – 1.89) NOAEC and slope not reported	Moderately toxic	00162296/1979; MON 0818 LC_{50}: 2mg/L[5]	Acceptable
Polyoxy ethylene fatty amine (POEA) (MON 0818)	Bluegill sunfish (*Lepomis macrochirus*)	70[4]	96-hr LC_{50}: 2.1 (1.75 – 2.59) NOAEC and slope not reported	Moderately toxic	00162296/1979; MON 0818 LC_{50}: 3 mg/L	Acceptable
Polyoxy ethylene fatty amine (POEA) (MON 0818)	Channel catfish (*Ictalurus punctatus*)	70[4]	96-hr LC_{50}: 9.1 (7.0 – 11.9) NOAEC and slope not reported	Slightly toxic	00162296/1979; MON 0818 LC_{50}: 13 mg/L	Acceptable
Surfactant Geronol CF/AR (alkyl polyoxy ethylene phosphoric acid ester)	Zebra fish (*Brachydanio rerio*)	100	96-hr LC_{50}: >100 (N.A.) NOAEC and slope not reported	Practically non-toxic	44738201/ Summary from another study	Not Classified

[1] a.i. = active ingredient, assumed 100% for technical material
[2]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[3] Range is 95% confidence interval for endpoint.
[4] Based on information provided by Registrant, the test material, MON 0818, contains 70% POEA; (comment from Monsanto Co. September 21, 2009; http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2009-0361-0013)
[5] In 00162296 (Folmer 1979), for MON 0818, a reported acute 96-hr LC50 toxicity value of 0.65 mg/L was reported for rainbow trout, however, this study was conducted at a pH of 9.5 and therefore, was not used quantitatively

Table B-11. Freshwater Fish Acute Toxicity for Aminomethyl Phosphonic Acid (AMPA) Degradate of Glyphosate

Chemical	Species	% a.i.[1]	96-hour LC_{50}/NOAEC (mg/L)/Slope	Toxicity Category[2]	MRID #/Year	Study Classification
AMPA	Rainbow trout (Oncorhynchus mykiss)	94.38	LC50: 499 (391 - 647) NOAEC: 174 Slope: 6.42	Practically nontoxic	43334713/1991	Acceptable

[1] a.i. = active ingredient, assumed 100% for technical material
[2]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[3] Range is 95% confidence interval for endpoint.

Table B-12. Estuarine/marine Fish Acute Toxicity for Technical Glyphosate and Its Salts

Species	% Active Ingredient*	96-hour LC_{50}/ NOAEC (mg a.e./L)*/ Slope	Toxicity Category[2]	MRID #/Year	Study Classification
Sheepshead minnow (*Cyprinodon variegatus*)	96	96-hr. LC_{50}: 240 (180-320) NOAEC: 100 Slope: NA	Practically nontoxic	44320632/1996	Acceptable

* a.i. = active ingredient; a.e. = acid equivalent; N.R. = not reported
[1] Range is 95% confidence interval for endpoint
[2]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic

Table B-13. Estuarine/Marine Fish Acute Toxicity for Glyphosate Formulations

Chemical	Species	% Active Ingredient*	96-hour LC_{50} NOAEC (mg a.e./L)*/ Slope[1]	Toxicity Category[2]	MRID #/ Year	Study Classification
Glyphosate (MON 2139)	Sheepshead minnow (*Cyprinodon variegatus*)	30.75	96-hr LC_{50} = 2.7 mg a.e./L 96-hr LC_{50}: 8.8 mg formulation/L Slope: NA NOAEC: 6.8	Moderately toxic	48934205/2012	Acceptable
Glyphosate SL formulation	Sheepshead minnow (*Cyprinodon variegatus*)	28.3	96-hr. LC_{50}: >50.9 mg a.e./L >180.2 ppm formulation	Practically nontoxic	45374005 /2000	Supplemental

* a.i. = active ingredient; a.e. = acid equivalent; N.R. = not reported
[1] Range is 95% confidence interval for endpoint
[2]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic

Table B-14. Aquatic-Phase Amphibian Acute Toxicity for Technical Glyphosate and Its Salts

Species	% Active Ingredient*	96-hour LC_{50}/ NOAEC (mg a.e./L)*/ Slope	Toxicity Category[2]	MRID #/Year	Study Classification
Australian tree frog (*Litoria moorei*) Tadpole	96	LC50: 103.2 (43.2 - 172.8)[1] NOAEL: N.R.* Slope: N.R.	Practically nontoxic	43839601/1995	Supplemental
Australian frog (*Crinia insignifera*) Adult	96	LC50: 75 (60.4-92.7) NOAEL: N.R. Slope: N.R.	Slightly toxic	43839601/1995	Supplemental
Green Frog (*Rana clamitans*) Gosner Stg 25	Tech[3]	LC50: >17.9 (NR) NOAEL: NR Slope: NR	Slightly toxic	46650501/2001	Supplemental

* a.i. = active ingredient; a.e. = acid equivalent; N.R. = not reported
[1] Range is 95% confidence interval for endpoint
[2]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[3] Study conducted with the isopropylamine salt

Table B-15. Aquatic-Phase Amphibian Acute Toxicity for Glyphosate Formulations

Chemical	Species	% a.i.*	96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope	Toxicity Category[1]	MRID #/Year	Study Classification
Glyphosate- IPA (Cosmo Flux Coca mix)	Africian clawed frog (*Xenopus laevis*) Larvae	18	LC50: 1.1 (0.56 - 2.3) or 10 mg/L formulation NOAEL: 0.14 Slope: 4.92	Moderately toxic	46873601/2006	Supplemental
Glyphosate IPA (Cosmo Flux Poppy mix)	Africian clawed frog (*Xenopus laevis*) Larvae	0.0205	LC50: 1.3 (0.92 - 1.8) or 16 mg/L formulation NOAEL: 0.43 Slope: NA*	Moderately toxic	46873602/2006	Supplemental

Chemical	Species	% a.i.*	96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope	Toxicity Category[1]	MRID #/Year	Study Classification
Glyphosate IPA (Roundup Original with 15% POEA)	Green Frog (*Rana clamitans*) Gosner Stg 25	NR	LC50: 2 (1.9-2.2) or 6.5 mg/L formulation NOAEL: NR* Slope: NR	Moderately toxic	46650501/2001	Supplemental
Glyphosate IPA (Roundup Transorb with 15% POEA)	Green Frog (*Rana clamitans*) Gosner Stg 25	NR	LC50: 2.2 (2.1-2.4) or 7.2 mg/L formulation NOAEL: NR Slope: NA	Moderately toxic	46650501/2001	Supplemental
Glyphosate IPA (Roundup Original with 15% POEA)	Leopard Frog (*Rana pipiens*) Gosner Stg 25	NR	LC50: 2.9 (NR) or 9.2 mg/L formulation NOAEL: NR Slope: NR	Moderately toxic	46650501/2000	Supplemental
Glyphosate IPA (Roundup Original with 15% POEA)	American toad (*Bufo americanus*) Gosner Stg 25	NR	LC50: <4.0 (NR) or < 12.9 mg/L formulation NOAEL: NR Slope: NR	Moderately toxic	46650501/1994	Supplemental
Glyphosate IPA (Roundup with 15% POEA)	Wood Frog (*Rana sylvatica*) Gosner Stg 25	NR	LC50: 5.1 (4.9-5.4) or 16.5 mg/L formulation NOAEL: NR Slope: NR	Moderately toxic	46650501/1994	Supplemental
Glyphosate IPA (Roundup 360)	Australian tree frog (*Litoria moorei*) Tadpole	30.3	LC50: 5.6 (4.4 - 7.1) or 18.5 mg/L formulation NOAEL: N.R. Slope: N.R.	Moderately toxic	43839601/1995	Supplemental
Glyphosate IPA (Roundup Original with 15% POEA)	Leopard Frog (*Rana pipiens*) Gosner Stg 20	NR	LC50: 6.5 (6.1-6.8) or 20.9 mg/L formulation NOAEL: NR Slope: NA	Moderately toxic	46650501/1994	Supplemental

Chemical	Species	% a.i.*	96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope	Toxicity Category[1]	MRID #/Year	Study Classification
Glyphosate IPA (Roundup Original with 15% POEA)	Green frog (*Rana clamitans*) Gosner Stg 20	NR	LC50: 7.1 (6.6-7.6) or 22.8 mg/L formulation NOAEL: NR Slope: NR	Moderately toxic	46650501/1994	Supplemental
Glyphosate IPA (Roundup Original with 15% POEA)	American toad (*Bufo americanus*) Gosner Stg 20	NR	LC50: 8 (NR) or 25.8 mg/L formulation NOAEL: NR Slope: NR	Moderately toxic	46650501/1994	Supplemental
Glyphosate IPA (Roundup Original with 15% POEA)	Wood Frog (*Rana sylvatica*) Gosner Stg 20	NR	LC50: > 8 (NR) or > 25.8 mg/L formulation NOAEL: NR Slope: NR	Moderately toxic	46650501/1994	Supplemental
Glyphosate IPA (Glyphos AU with 3-7% POEA)	Green Frog (*Rana clamitans*) Gosner Stg 25	NR	LC50: 8.9 (8.6-9.2) or 28.6 mg/L formulation NOAEL: NR Slope: NR	Moderately toxic	46650501/2001	Supplemental
Glyphosate IPA (Roundup Biactive with 10-20% unspecified surfactant)	Green frog (*Rana clamitans*) Gosner Stg 25	NR	LC50: >17.9 (NR) or > 57.7 mg/L formulation NOAEL: NR Slope: NR	Slightly toxic	46650501/2001	Supplemental
Glyphosate IPA (Glyphos BIO with 3-7% POEA)	Green Frog (*Rana clamitans*) Gosner Stg 25	NR	LC50: >17.9 (NR) or >57.7 mg/L formulation NOAEL: NR Slope: NR	Slightly toxic	46650501/2001	Supplemental
Glyphosate IPA (Roundup 360)	Australian frog (*Crinia insignifera*) Adult	30.3	LC50: 30.4 (0-infinity) or 100.2 mg/L formulation NOAEL: N.R. Slope: N.R.	Slightly toxic	43839601/1995	Supplemental

Chemical	Species	% a.i.*	96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope	Toxicity Category[1]	MRID #/Year	Study Classification
Glyphosate IPA (Roundup 360)	Australian frog (*Crinia insignifera*) Tadpole	30.3	48 hr LC50: 38.2 (30.2 - 48.8) or 125.9 mg/L formulation NOAEL: N.R. Slope: N.R.	Slightly toxic	43839601/1995	Supplemental
Glyphosate IPA (with surfactant Geronol CF/AR	Common froglet (*Ranidella signifera*) Tadpole	45	LC50: >450 (N.A.) or >1000 mg/L formulation NOAEL: 1000 Slope: N.A.	Practically nontoxic	44738201/1996	Supplemental
Glyphosate IPA (Roundup Biactive)	Common froglet (*Ranidella signifera*) Tadpole	36	LC50: >360 (N.A) or >1000 mg/L formulation NOAEL: <800 Slope: N.A.	Practically nontoxic	44738201/1996	Supplemental
Glyphosate IPA (with surfactant Geronol CF/AR)	Common froglet (*Ranidella signifera*) Tadpole	36	LC50: >360 (N.A) or >1000 mg/L formulation NOAEL: 1000 Slope: N.A.	Practically nontoxic	44738201/1996	Supplemental
Glyphosate IPA (with surfactant Geronol CF/AR)	Common froglet (*Ranidella signifera*) Tadpole	10	LC50: >100 (N.A.) or >1000 mg/L formulation NOAEL: 1000 Slope: N.A.	Practically nontoxic	44738201/1996	Supplemental

* a.i. = active ingredient; a.e. = acid equivalent; IPA = isopropylamine salt, N.A. = not available, N.R. = not reported
[1]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[2] Range is 95% confidence interval for endpoint

Table B-16. Aquatic-Phase Amphibian Acute Toxicity for POEA Surfactant Used with Glyphosate Formulations

Chemical	Species	% a.i.[1]	96-hour LC_{50}/NOAEC (mg/L)/Slope	Toxicity Category[2]	MRID #/Year	Study Classification
Polyoxy ethylene fatty amine (POEA or MON 0818)	Green Frog (*Rana clamitans*) Gosner Stg 25	69-73	LC50: 2.2 (2.1-2.4) NOAEC: NR* Slope: NR	Moderately toxic	46650501/2001	Supplemental

* NR = not reported
[1] a.i. = active ingredient, assumed 100% for technical material
[2]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[3] Range is 95% confidence interval for endpoint.

Table B-17. Freshwater Fish Chronic Toxicity for Technical Glyphosate and Its Salts

Species	% Active Ingredient	NOAEC/LOAEC (mg acid equivalent/L)	MRID #/Year	Study Classification
Fathead minnow (*Pimephales promelas*)	87.3	25.7/>25.7[1]	00108171/1975	Acceptable

Table B-18. Aquatic Phase Amphibian Chronic Toxicity for Technical Glyphosate IPA Salt and IPA Salt Formulations

Species	% Active Ingredient	NOAEC/LOAEC (mg acid equivalent/L)	MRID #/Year	Study Classification
Leopard Frog (*Rana pipiens*)	Tech (assumed 100%)	NOAEC/LOAEC: 1.8/>1.8	46650501/2004	Supplemental
Leopard Frog (*Rana pipiens*)	Roundup Original & Transorb 15% POEA	NOAEC/LOAEC: <0.6/0.6	46650501/2004	Supplemental

Table B-19. Aquatic-Phase Amphibian Chronic Toxicity for POEA Surfactant Used with Glyphosate Formulations

Chemical	Species	% a.i.[1]	NOAEC/ LOAEC (mg a.i./L)	MRID #/Year	Study Classification
Polyoxy ethylene fatty amine (POEA or MON 0818)	Leopard Frog (*Rana pipiens*) Larvae	Tech	NOAEC/ LOAEC: <0.6/0.6 [reported in units of mg acid equivalents/L]	46650501/2004	Supplemental
[1] a.i. = active ingredient, assumed 100% for technical material					

Table B-20. Other Fish Effects

Species	Chemical	NOAEC	LOAEC:Effects	MRID/ECOTOX Reference No.
Rainbow trout (*O. mykiss*)	Technical glyphosate 95.6%	30.6 ppm	53.6 ppm: dark coloration	MRID 44320629

Table B-21. Freshwater Invertebrates Acute Toxicity for Technical Glyphosate*

Species	% a.i.*	48-hour EC_{50} - LC_{50}/ NOAEC (mg a.e./L)*/ Slope	Toxicity Category[1]	MRID #/Year	Study Classification
Midge (*Chironomus plumosus*)	96.7	LC_{50}: **53.2 (30.0 - 93.8)**[2] NOAEC: N.R. Slope: N.R.	Slightly toxic	00162296/1979	Acceptable
Water flea (*Daphnia magna*)	95.6	EC_{50}: 128.1 (95.6 - 172.1) NOAEC: 95.6 Slope: N.R.	Practically nontoxic	44320631/1995	Acceptable
Water flea (*Daphnia magna*)	83	EC_{50}: 647.4 (577.7 - 725.4) NOAEC: 464.8 Slope: N.R.	Practically nontoxic	00108172/1978	Acceptable

* No technical glyphosate salts were tested; a.i. = active ingredient; a.e. = acid equivalent, N.R. = not reported
[1]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[2] Range is 95% confidence interval for endpoint

Table B-22. Freshwater Invertebrates Acute Toxicity for Glyphosate Formulations

Chemical	Species	% a.i.*	48-hour EC_{50} - LC_{50}/ NOAEC (mg a.e./L)*/ Slope	Toxicity Category[1]	MRID #/Year	Study Classification
Glyphosate IPA - Roundup	Water flea (*Daphnia magna*)	41.36	EC50: 1.6 (1.4 - 1.9)[2] NOAEC: 0.6 Slope: 5.4	Moderately toxic	00070893/1980	Acceptable
Glyphosate IPA	Water flea (*Daphnia magna*)	30.3	EC50: 2.2 (1.9 - 2.5) NOAEC: N.R. Slope: N.R.	Moderately toxic	00162296/1979	Acceptable
Glyphosate IPA (MON65005)	Water flea (*Daphnia magna*)	31.32	EC50: 2.7 (2.3 - 3.1) NOAEC: 1.3 Slope: 6.2	Moderately toxic	44538201/1998	Acceptable
Glyphosate IPA (MON 77360)	Waterflea (*Daphnia magna*)	30.0	EC50: 3.2 (2.9 - 3.7) NOAEC: 0.8 Slope: NA	Moderately toxic	45365004/2000	Acceptable
Glyphosate IPA	Crayfish (*Orconectes nais*)	30.3	LC50: 5.2 (4.1 - 6.4) NOAEC: N.R. Slope: N.R.	Moderately toxic	40098001/1986	Supplemental
Glyphosate IPA (Roundup)	Water flea (*Daphnia pulex*)	30.3	EC50: 5.8 (5.3 - 6.4) NOAEC: N.R. Slope: N.R.	Moderately toxic	44125714/1984	Supplemental
Glyphosate IPA (Roundup)	Scud (*Gammarus pseudolimnaeus*)	31	LC50: 13 (9.6 - 19.2) NOAEC: 1.4 Slope: 2.33	Slightly toxic	00124762/1982	Supplemental
Glyphosate IPA	Midge (*Chironomus plumosus*)	30.3	LC50: 13.3 (7.0 - 23.7) NOAEC: N.R. Slope: N.R.	Slightly toxic	00162296/1979	Acceptable

Chemical	Species	% a.i.*	48-hour EC_{50} - LC_{50}/ NOAEC (mg a.e./L)*/ Slope	Toxicity Category[1]	MRID #/Year	Study Classification
Glyphosate (80WDG formulation)	Water flea (*Daphnia magna*)	80	EC50: >17.6 (N.A.) NOAEC: 17.6 Slope: N.A.	Slightly toxic	44125706/1996	Supplemental
Glyphosate IPA (Roundup with "W" surfactant)	Water flea (*Daphnia magna*)	40.7	EC50: 21.7 (18.7 - 25.0) NOAEC: N.R. Slope: N.R.	Slightly toxic	00078657/1980	Acceptable
Glyphosate monoammonium salt (MON 14420)	Daphnia (*Daphnia magna*)	68.5	EC50: 28.8 (12.3 - 48.5) NOAEC: 12.3 Slope: N.R.	Slightly toxic	45777401/1999	Acceptable
Glyphosate IPA	Scud (*Gammarus pseudolimnaeus*)	30.3	LC50: 31.8 (20.7 - 48.8) NOAEC: N.R. Slope: N.R.	Slightly toxic	00162296/1979	Acceptable
Glyphosate IPA (X-77 surfactant)	Water flea (*Daphnia magna*)	5.27	EC50: >39 (N.R.) NOAEC: 21.8 Slope: N.A.	Slightly toxic	00078666/1980	Supplemental
Glyphosate (360 g/L SL formulation)	Water flea (*Daphnia magna*)	27.25	EC50: 44.8 (38.0 - 52.0) NOAEC: 26 Slope: 7.6	Slightly toxic	45374003/1999	Acceptable
Glyphosate IPA	Water flea (*Daphnia pulex*)	48	EC50: 68.3 (64.3 - 72.8.) NOAEC: <21.3 Slope: 3.9	Slightly toxic	00108109/1973	Supplemental
Glyphosate (Roundup with "AA" surfactant)	Water flea (*Daphnia magna*)	41.2	EC50: 94.5 (76.3 - 122.0) NOAEC: 17.1 Slope: 3.5	Slightly toxic	00078660/1980	Acceptable
Glyphosate IPA (Roundup Biactive)	Water flea (*Daphnia carinata*)	36	EC50: 150 (151 - 179) NOAEC: 45 Slope: N.R.	Practically nontoxic	44738201/1996	Not classified

Chemical	Species	% a.i.*	48-hour EC_{50} - LC_{50}/ NOAEC (mg a.e./L)*/ Slope	Toxicity Category[1]	MRID #/Year	Study Classification
Trisodium diglyphosate/Urea (Polado formula - MON 8000)	Water flea (*Daphnia magna*)	75	LC50: >315 (N.R.) NOAEC: 315 Slope: N.R.	Practically nontoxic	00079147/1980	Supplemental
Glyphosate IPA with surfactant Geronol CF/AR	Water flea (*Daphnia carinata*)	45	EC50: 365 (315 - 420) NOAEC: 190 Slope: N.R.	Practically nontoxic	44738201/1996	Not classified
Glyphosate IPA (no surfactant)	Water flea (*Daphnia magna*)	62.4	EC50: 401.3 (347.7 - 470.5) NOAEC: 147.8 Slope: 7.6	Practically nontoxic	00078663/1981	Acceptable
Glyphosate IPA with surfactant Geronol CF/AR	Water flea (*Daphnia carinata*)	36	EC50: 610 (540 - 700) NOAEC: 135 Slope: N.R.	Practically nontoxic	44738201/1996	Not classified
Glyphosate IPA with surfactant Geronol CF/AR	Water flea (*Daphnia carinata*)	10	EC50: 810 (700 - 940) NOAEC: 400 Slope: N.R.	Practically nontoxic	44738201/1996	Not classified
Glyphosate IPA (MON77945 Manufacturing concentrate)	Water flea (*Daphnia magna*)	46	EC50: 833 (665 - 1253) NOAEC: 204 Slope: 3.7	Practically nontoxic	44715410/1998	Not classified

* a.i. = active ingredient; a.e. = acid equivalent; IPA = isopropylamine salt
[1]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[2] Range is 95% confidence interval for endpoint

Table B-23. Freshwater Invertebrates Acute Toxicity for Surfactants Used with Glyphosate Formulations

Chemical	Species	% a.i.*	48-hour EC_{50} - LC_{50}/ NOAEC (mg/L)/ Slope	Toxicity Category[1]	MRID #/Year	Study Classification
Surfactant Geronol CF/AR (alkyl polyoxy ethylene phosphoric acid ester)	Daphnia (*Daphnia magna*)	Tech.	EC50: 48 NOAEC: Slope: N.A.	Slightly toxic	44738201/1996	Not classified
MON 0818 (POEA)	Midge (*Chironomus plumosus*)	70[3]	48-hr LC_{50}: 9.1 (4.97-16.8)	Slightly toxic	00162296/1979; MON 0818 LC_{50}: 13 mg/L	Acceptable

* a.i. = active ingredient, assumed 100% for technical.
[1]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[2] Range is 95% confidence interval for endpoint
[3] Based on information provided by Registrant, the test material, MON 0818, contains 70% POEA; (comment from Monsanto Co. September 21, 2009; http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2009-0361-0013)

Table B-24. Freshwater Invertebrates Acute Toxicity for Aminomethyl Phosphonic Acid (AMPA) Degradate of Glyphosate

Chemical	Species	% a.i.[1]	48-hour LC_{50}/NOAEC (mg/L)/Slope	Toxicity Category[2]	MRID #/Year	Study Classification
AMPA	Water flea (*Daphnia magna*)	94.38	EC50: 683 (553 - 1010) NOAEC: 320 Slope: N.A.	Practically nontoxic	43334715/1994	Acceptable

[1] a.i. = active ingredient, assumed 100% for technical material
[2]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[3] Range is 95% confidence interval for endpoint, N.A. = not available

Table B-25. Freshwater Invertebrates Chronic Toxicity for Technical Glyphosate IPA Salt

Species	% Active Ingredient	NOAEC/LOAEC (mg acid equivalent/L)	MRID #/Year	Study Classification
Water flea (*Daphnia magna*)	99.7	49.9/95.7	00124763/1982	Acceptable

Table B-26. Estuarine/marine Invertebrates Acute Toxicity for Technical Glyphosate

Species	% a.i.*	48-hour EC_{50} - LC_{50}/ NOAEC (mg a.e./L)*/ Slope	Toxicity Category[1]	MRID #/Year	Study Classification
Pacific oyster (*Crassostrea gigas*)	96	48-hr EC_{50}: 40 (31-53) NOAEC: 32 Slope: NA	Slightly toxic	44320634 /1996	Acceptable
Mysid shrimp (*Americamysis bahia*)	96	96-hr LC50: 79 (63-99) NOAEC: 32 Slope: 8.5	Slightly toxic	44320633 /1996	Acceptable

a.i. = active ingredient; a.e. = acid equivalent, N.R. = not reported
[1]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[2] Range is 95% confidence interval for endpoint

Table B-27. Estuarine/marine Invertebrates Acute Toxicity for Glyphosate Formulations

Chemical	Species	% Active Ingredient*	96-hour LC_{50} NOAEC (mg a.e./L)*/ Slope[1]	Toxicity Category[2]	MRID #/ Year	Study Classification
MON 2139	Eastern oyster (*Crassostrea virginica*)	30.75 ae	96-hr IC50: 1.06 mg a.e./L (0.846-1.33); 3.45 mg formulation/L (2.75-4.33)[2] NOAEC: 0.51 Slope: NA	Moderately toxic	48934201 / 2012	Acceptable
MON 2139	Mysid shrimp (*Americamysis bahia*)	30.75 a.e.	96-hr LC50: 0.765 mg a.e./L (0.601-0.909); 2.45 mg formulation/L (1.95-2.96)[2] NOAEC: 0.51 Slope: 10.4 (5.46-15.3)	Highly toxic	(48934202 / 2012	Acceptable
MON 2139	White shrimp (*Litopenaeous vannamei*)	30.87 a.e.	96-hr LC50: 54 mg a.e./L (33-134); 175 mg formulation/L (107-434)[2] NOAEC: 5.2 Slope: 1.33	Slightly toxic	48934203/ 2012	Acceptable
MON 2139	Eastern oyster (*Crassostrea virginica*)	30.75 ae	48-hr EC50: 0.93 mg a.e./L (0.87-1.0); 3.0 mg formulation/L (2.8-3.3) NOAEC: 0.48	Highly toxic	48934204 / 2012	Acceptable
Glyphosate SL formulation	Pacific oyster (*Crassostrea gigas*)	28.3 a.e.	48-hr LC_{50}: 23.2 mg a.e./L; 82 mg formulation/L Slope: NR NOAEC: 28.6 mg formulation/L	Slightly toxic	45374006/2000	Acceptable

* a.i. = active ingredient; a.e. = acid equivalent; N.R. = not reported
[1] Range is 95% confidence interval for endpoint
[2]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic

Table B-28. Aquatic Vascular and Nonvascular Freshwater Plant Toxicity Studies for Technical Glyphosate

Species	% Active Ingredient*	EC_{50} NOAEC (mg a.e./L)*/ Slope	MRID #/Year	Study Classification
Vascular Plants				
Duckweed (*Lemna gibba*)	95.6	14-day EC50: 11.9 (9.4-14.9) NOAEC: 1.3 Slope: N.R.	44320638/1996	Supplemental
Duckweed (*Lemna gibba*)	96.8	7-day EC50: 23.2 (20.3 - 27.1) NOAEC: 7.3 Slope: 2.91	45773101/2002	Acceptable
Duckweed (*Lemna gibba*)	96.6	14-day EC50: 20.8 (N.R.) NOAEC: <1.8 Slope: N.R.	40236905/1987	Acceptable
Non-vascular Plants				
Green algae (*Selenastrum capricornutum*)	96.6	4-day EC50: 12.1 (11.5 - 12.9) NOAEC: N.R. Slope: 12	40236901/1987	Acceptable
Bluegreen algae (*Anabaena flos-aquae*)	96.6	4-day EC50: 11.4 (10.5 - 12.1) NOAEC: N.R. Slope: 3.53	40236904/1987	Acceptable
Green algae (*Selenastrum capricornutum*)	95.6	5-day EC50: 13.4 (9.6 - 19.1) NOAEC: 9.6 Slope: N.R.	44320637/1995	Acceptable
Bluegreen algae (*Anabaena flos-aquae*)	95.6	5-day EC50: 14.3 (9.3 - 25.8) NOAEC: 11.5 Slope: N.R.	44320639/1996	Acceptable
Freshwater diatom (*Navicula pelliculosa*)	95.6	5-day EC50: 16.3 (11.5 - 22.9) NOAEC: 1.7 Slope: N.R.	44320641/1996	Acceptable

Species	% Active Ingredient*	EC_{50} NOAEC (mg a.e./L)*/ Slope	MRID #/Year	Study Classification
Freshwater diatom (*Navicula pelliculosa*)	96.6	7-day EC50: 37.3 (34.8 - 41.5) NOAEC: 18.5 Slope: 5.87	40236902/1987	Acceptable
* a.i. = active ingredient; a.e. = acid equivalent; N.R. = Not reported [1] Range is 95% confidence interval for endpoint				

Table B-29. Aquatic Vascular and Nonvascular Freshwater Plant Toxicity Studies for Glyphosate Formulations

Chemical	Species	% a.i.*	EC_{50}/ NOAEC (mg a.e.*/L)/ Slope	MRID #/Year	Study Classification
Vascular Plants					
Glyphosate IPA salt* (glyphos (glyphosate product))	Duckweed (*Lemna gibba*)	31.0	7-Day EC50: 7.7 (7.1 - 8.3) [1] NOAEC: 0.29 Slope: 4.76	45666704/2001	Acceptable
Glyphosate IPA salt (Roundup 41%)	Duckweed (*Lemna minor*)	30.3	14-day EC50: 1.5 (N.R.) NOAEC: N.R. Slope: N.R.	44125714/1984	Supplemental
Glyphosate IPA salt (TEP Roundup)	Duckweed (*Lemna minor*)	NR	48 hr. EC50: >16.91 (N.A.) NOAEC: 16.91 Slope: N.A.	44125713/1989	Supplemental
Glyphosate IPA salt	Duckweed (*Lemna minor*)	N.R.	14-day EC50: 2.0 (N.R.) NOAEC: N.R. Slope: N.R.	44125714/1984	Supplemental
Nonvascular Plants					
Glyphosate monoammonium salt (MON 14420)	Green algae (*Selenastrum capricornutum*)	68.5	72-hr EC50: 1.85 (1.3 - 2.3) NOAEC: 0.61 Slope: N.R.	45777403/1999	Supplemental

Chemical	Species	% a.i.*	EC_{50}/ NOAEC (mg a.e.*/L)/ Slope	MRID #/Year	Study Classification
Glyphosate monoammonium salt (MON78568)	Green algae (*Selenastrum capricornutum*)	64.9	72-hr EC50: 11.2 (10 - 12.6) NOAEC: 1.58 Slope: N.R.	45767102/2002	Supplemental
Glyphosate IPA salt with surfactant Geronol CF/AR	Green algae (*Selenastrum capricornutum*)	36	72-hr EC50: 97 (85 - 111) NOAEC: 73 Slope: N.A.	44738201/1996	Supplemental
Glyphosate IPA salt with surfactant Geronol CF/AR	Green algae (*Selenastrum capricornutum*)	36	72-hr EC50: 39 (33 - 45) NOAEC: 16 Slope: N.A.	44738201/1996	Supplemental
Glyphosate (glyphos)	Freshwater diatom (*Navicula pelliculosa*)	31.0	96-hrEC50: 0.12 (0.11 – 0.13) NOAEC: 0.082 Slope: 8.78	45666701/2001	Acceptable
Glyphosate IPA salt (glyphos (glyphosate product))	Green algae (*Selenastrum capricornutum*)	31.0	96-hr EC50: 0.68 (0.57 - 0.81) NOAEC: 0.43 Slope: 4.47	45666702/2001	Acceptable

* a.i. = active ingredient; a.e. = acid equivalent; IPA = isopropylamine salt; NR = not reported; NA = not available
[1] Range is 95% confidence interval for endpoint

Table B-30. Aquatic Nonvascular Freshwater Plant Toxicity Studies on Glyphosate Mixtures

Chemical	Species	% a.i.*	EC_{50}/ NOAEC (mg a.e.*/L)/ Slope	MRID #/Year	Study Classification
Nonvascular Plants					
Glyphosate acid-equivalent (IPA)/Oxyfluorfen mix	Green algae (*Selenastrum capricornutum*)	32	96-hr EC50: 0.0026 (0.0021 - 0.0033)[1] NOAEC: 0.00045 Slope: 3.96	45906008/2001	Acceptable
* a.i. = active ingredient; a.e. = acid equivalent; IPA = isopropylamine salt; [1]Range is 95% confidence interval for endpoint					

Open Literature Data for Aquatic Plants

Table B-31. Open literature glyphosate toxicity studies on aquatic plants

Species	Chemical Form	Endpoint(s)	ECOTOX Ref. No.
Chlorella pyrenoidosa	Tech Glyphosate (95%, unsure if acid or IPA)	96-hr EC_{50}: 3.530 mg/L	E61983/ Ma *et.al* 2001,
Chlorella vulgaris	Tech Glyphosate (95%, assumed to be acid)	96 hr. EC_{50}: 4.70 mg/L	E65938/ Ma *et al*., 2002
Raphidocelis subcapitata (Selenastrum capricornutum)	Tech Glyphosate (95%, assumed to be acid)	96 hr. EC50: 5.56 mg/L	E83543/ Ma *et al*., 2006

Table B-32. Avian Acute Toxicity for Technical Glyphosate

Chemical	Species	% a.i.[1]	LD_{50}/ LC_{50} NOAEL/ NOAEC (mg a.e./kg bw or ppm a.e.)[1]	Toxicity Category[2]	MRID #/ Year	Study Classification
Glyphosate	Bobwhite quail (*Colinus virginianus*)	83	LD50: >3196.3 mg a.e./kg bw	Practically nontoxic	00108204	Acceptable
Glyphosate	Mallard duck (*Anas platyrhynchos*)	98.5	LC50: >4570.4 (N.A.) PPM NOAEC: 4570.4	Slightly toxic	108107/37765/1973	Acceptable

Chemical	Species	% a.i.[1]	LD_{50}/ LC_{50} NOAEL/ NOAEC (mg a.e./kg bw or ppm a.e.)[1]	Toxicity Category[2]	MRID #/ Year	Study Classification
Glyphosate	Bobwhite quail (*Colinus virginianus*)	98.5	LC50: >4570 (N.R.) PPM NOAEC: 4570	Slightly toxic	00076492/1973	Acceptable
Glyphosate	Bobwhite quail (*Colinus virginianus*)	95.6	LD50: >1912 (N.A.) mg/kg bw NOAEL: 1912	Slightly toxic	44320626/1997	Acceptable
Glyphosate	Mallard duck (*Anas platyrhynchos*)	95.6	LC50: >4971.2 (N.A.) PPM NOAEC: 4971.2	Slightly toxic	44320627/1998	Acceptable
Glyphosate	Bobwhite quail (*Colinus virginianus*)	95.6	LC50: >4971.2 (N.A.) PPM NOAEC: 4971.2	Slightly toxic	44320628/1997	Acceptable
Glyphosate	Canary (*Serinus canaria*)	96	LD_{50}: >2,000 ED_{50} (based on regurgitation): 2819 NOAEL: 2000 (visually observed)	Practically nontoxic	4893406 / 2012	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent
[2]Based on LC_{50} (ppm): < 50 very highly toxic; 50 - 500 highly toxic; 501 - 1000 moderately toxic; 1001-5000 slightly toxic; >5000 practically non-toxic; based on LD_{50} (mg/kg bw): < 10 very highly toxic; 10 - 50 highly toxic; 51 - 500 moderately toxic; 501-2000 slightly toxic; >2000 practically non-toxic
[3] Range is 95% confidence interval for endpoint, N.A. = not available, N.R. = not reported

Table B-33. Avian Acute Toxicity for Aminomethyl Phosphonic Acid (AMPA) Degradate of Glyphosate

Chemical	Species	% a.i.[1]	LD_{50}/ LC_{50} NOAEL/ NOAEC (mg a.e./kg bw or ppm a.e.)/ Slope[1]	Toxicity Category[2]	MRID #/Year	Study Classification
AMPA	Bobwhite quail (*Colinus virginianus*)	87.8	LD50: >1976 (N.A.) mg/kg NOAEL: 1185 Slope: N.A.	Slightly toxic	43334709/1991	Acceptable
AMPA	Bobwhite quail (*Colinus virginianus*)	87.8	LC50: >4934 (N.A.) PPM NOAEC: 4934 Slope: N.A.	Slightly toxic	43334710/1994	Acceptable
AMPA	Mallard duck (*Anas platyrhynchos*)	87.8	LC50: >4934 (N.A.) PPM NOAEC: 4934 Slope: N.A.	Slightly toxic	43334711/1994	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent
[2]Based on LC_{50} (ppm): < 50 very highly toxic; 50 - 500 highly toxic; 501 - 1000 moderately toxic; 1001-5000 slightly toxic; >5000 practically non-toxic; based on LD_{50} (mg/kg bw): < 10 very highly toxic; 10 - 50 highly toxic; 51 - 500 moderately toxic; 501-2000 slightly toxic; >2000 practically non-toxic
[4] Range is 95% confidence interval for endpoint, N.A. = not available

Table B-34. Avian Chronic Toxicity for Technical Glyphosate

Chemical	Species	% a.i.[1]	LD_{50}/ LC_{50} NOAEL/ NOAEC (mg a.e./kg bw or ppm a.e.)[1]	Toxicity Category[2]	MRID #/Year	Study Classification
Glyphosate	Mallard duck (*Anas platyrhynchos*)	90.4	LOAEC: >27 (N.A.) PPM NOAEC: 27	N.A	00036328/113457/1975	Supplemental
Glyphosate	Mallard duck (*Anas platyrhynchos*)	83	LOAEC: >830 (N.A.) PPM NOAEC: 830	N.A.	111953/1978	Acceptable
Glyphosate	Bobwhite quail (*Colinus virginianus*)	83	LOAEC: >830 (N.A.) PPM NOAEC: 830	N.A.	108207/1978	Acceptable

Chemical	Species	% a.i.[1]	LD_{50}/ LC_{50} NOAEL/ NOAEC (mg a.e./kg bw or ppm a.e.)[1]	Toxicity Category[2]	MRID #/Year	Study Classification
Glyphosate	Mallard duck (*Anas platyrhynchos*)	96	NOAEC: <501; LOAEC: 501 mg a.e./kg diet (lowest concentration tested), based on effects to male weight gain and offspring weight.	NA	48876602 / 1999	Supplemental

[1] a.i. = active ingredient; a.e. = acid equivalent
[2] Range is 95% confidence interval for endpoint, N.A. = not applicable

Table B-35. Mammalian Acute Toxicity for Technical Glyphosate

Chemical	Species	% a.i.[1]	LD_{50} (mg a.e./kg bw)[1]	Toxicity Category[2]	MRID No.	Study Classification
Glyphosate	Rat (*rattus norvegicus*)	96	>4800 limit test. No mortalities.	Practically non-toxic	43728003	Acceptable
Glyphosate	Rat (*rattus norvegicus*)	95	>4750 – limit test. No mortalities	Practically non-toxic	45058306	Acceptable
Glyphosate	Rat (*rattus norvegicus*)	97.2	>4860 up and down – no mortalities	Practically non-toxic	46760505	Acceptable
Glyphosate	Rat (*rattus norvegicus*)	88	>4400. No mortalities	Practically non-toxic	44320604	Acceptable
Glyphosate	Rat (*rattus norvegicus*)	95	>4750 up and down – no mortalities	Practically non-toxic	46998805	Acceptable
Glyphosate	Rat (*rattus norvegicus*)	76	>3800 – no mortalities	Practically non-toxic	41400601	Acceptable
Glyphosate (IPA 62%)	Rat (*rattus norvegicus*)	96	>1920 – no mortalities	Slightly toxic (when expressed as a.e.)	44142104	Acceptable

Chemical	Species	% a.i.[1]	LD_{50} (mg a.e./kg bw)[1]	Toxicity Category[2]	MRID No.	Study Classification
Glyphosate	Rat (*rattus norvegicus*)	95.4	>4770 up and down – no mortalities	Practically non-toxic	46816107	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent
[2]Based on LD_{50} (mg/kg bw): < 10 very highly toxic; 10 - 50 highly toxic; 51 - 500 moderately toxic; 501-2000 slightly toxic; >2000 practically non-toxic.

Table B-36. Mammalian Chronic Toxicity for Technical Glyphosate

Chemical	Species	% a.i.[1]	NOAEL/ NOAEC (mg a.e./kg bw or ppm a.e.)[1]	MRID #/Year	Study Classification
Glyphosate	Rat (*rattus norvegicus*)	97.67	2-generation reproduction study Parental/Systemic NOAEL: 500 mg/kg/day (10,000 ppm) LOAEL: 1500 mg/kg/day (30,000 ppm) Reproductive NOAEL: 1500 mg/kg/day (HDT) Offspring NOAEL: 500 mg/kg/day (10,000 ppm) LOAEL: 1500 mg/kg/day	41621501/1990	Acceptable
Glyphosate	Rat (*rattus norvegicus*)	100%	3-generation reproduction study Parental/Systemic, Offspring and Reproductive NOAELs: 30 mg/kg/day (highest dose tested).	00081674; 00105995 1981; 1982	Acceptable
Glyphosate	Rabbit (*Oryctolagus cuniculus*)	98.7	Developmental toxicity study Maternal NOAEL = 175 mg/kg/day LOAEL = 350 mg/kg/day based on mortality, diarrhea, soft stools, and nasal discharge. Developmental NOAEL = 350 mg/kg/day (HDT) LOAEL = not established.	00046363/1980	Acceptable

Chemical	Species	% a.i.[1]	NOAEL/ NOAEC (mg a.e./kg bw or ppm a.e.)[1]	MRID #/Year	Study Classification
Glyphosate	Rat (*rattus norvegicus*)		NOAEL: 408/422 mg/kg bw/day (males/females); NOAEC: 5000 mg/kg-diet Reproduction study Offspring toxicity LOAEL: 1234/1273 (males/female) mg/kg bw/day; LOAEC: 15000 ppm (delayed age and increased weight at male sexual development). No observed effects on parental or reproductive toxicity (LOAEL >15000 ppm)	48865101-48865105 /2007 & 2012	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent
[2] Range is 95% confidence interval for endpoint, N.A. = not applicable

Table B-37. Mammalian Chronic Toxicity for Surfactants

Chemical	Species	% a.i.[1]	NOAEL/ NOAEC (mg a.e./kg bw or ppm a.e.)[1]	MRID #/Year	Study Classification
POEA	Rat (*rattus norvegicus*)	100%	Reproduction/developmental screening study temporary endpoints: Parental/Systemic NOAEL: 1000 ppm (52.8 – 56.1 mg/kg bw/day (M) and 64.9 – 66.6 mg/kg bw/day (F) Reproductive NOAEL: 300 ppm (14.9 - 16.6 mg/kg bw/day (M) and 18.9 - 19.5 mg/kg bw/day (F) LOAEL: 1000 ppm (52.8 – 56.1 mg/kg bw/day (M) and 64.9 – 66.6 mg/kg bw/day (F) Based on increased mean number of unaccounted-for sites. Offspring NOAEL: 300 ppm (14.9 - 16.6 mg/kg bw/day (M) and 18.9 - 19.5 mg/kg bw/day (F) LOAEL: 1000 ppm (52.8 – 56.1 mg/kg bw/day (M) and 64.9 – 66.6 mg/kg bw/day (F) Based on litter loss, decreased mean number of pups born live, litter size and postnatal survival from birth to PND 4. Effects not reproducible in second generation; however, this is a definitive NOAEL/LOAEL.	47097401/2006	Not classified

[1] a.i. = active ingredient; a.e. = acid equivalent
[2] Range is 95% confidence interval for endpoint, N.A. = not applicable

Terrestrial Invertebrates

Table B-38. Acute Toxicity Studies on Terrestrial Invertebrates for Technical Glyphosate

Chemical	Species	% a.i.[1]	LD_{50}/ LC_{50} NOAEL/ NOAEC	MRID #/Year	Study Classification
Glyphosate	Honey bee (*Apis mellifera*)	98.5	48 hr LD50 (O): >100 (N.R.)[2] µg/bee NOAEL: N.R. Slope: N.R.	00026489/1972	Acceptable
Glyphosate	Honey bee (*Apis mellifera*)	98.5	48 hr LD50 (C): >100 (N.R.) µg/bee NOAEL: N.R. Slope: N.R.	00026489/1972	Acceptable
Glyphosate	Honey bee (*Apis mellifera*)	97.6	48 hr LD50 (C): >103 (N.R.) µg/bee NOAEL: 103 48 hr LD50 (O): >182 (N.R.) µg/bee NOAEL: 182	48876603	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent
[2] Range is 95% confidence interval for endpoint, N.R. = not reported; O = oral study; C = contact study

Table B-39. Acute Toxicity Studies on Terrestrial Invertebrates for Glyphosate Formulations

Chemical	Species	% a.i.[1]	LD_{50}/ LC_{50} NOAEL/ NOAEC	MRID #/Year	Study Classification
Glyphosate monoammonium salt (MON78568)	Honey bee (*Apis mellifera*)	65.6	48 hr LD50 (C): >100 (N.A.)[2] µg/bee NOAEL: 100 Slope: N.R.	45767104/2001	Not classified
Glyphosate monoammonium salt (MON78568)	Honey bee (*Apis mellifera*)	65.6	48 hr LD50 (O): >76.23 (N.A.) µg a.e./bee NOAEL: <76.23 µg a.e./bee Slope: N.R.	45767104/2001	Not classified

Chemical	Species	% a.i.[1]	LD_{50}/ LC_{50} NOAEL/ NOAEC	MRID #/Year	Study Classification
Glyphosate monoammonium salt (MON78568)	Predatory mite (*Typhlodromus pyri*)	64.9	7 D LD50 (C): 1200 (839-1786) g a.e./ha NOAEL: 216 Slope: N.R.	45767105/2002	Not classified
Glyphosate monoammonium salt (MON78568)	Predatory mite (*Typhlodromus pyri*)	64.9	7 D LD50 (C): >4320 (N.R.) g/ha NOAEL: 216 Slope: N.R.	45767106/2002	Not classified
Glyphosate monoammonium salt (MON78568)	Predatory mite (*Typhlodromus pyri*)	64.9	14 - 21 D LD50 (C): N.A. (N.A.) g/ha NOAEL: 216 or <119 (no dose-response) Slope: N.A.	45767106/2002	Not classified
Glyphosate monoammonium salt (MON78568)	Earthworm (*Eisenia fetida*)	64.9	14 D LD50 (C): >6560 (N.A.) mg/kg soil NOAEL: 6560 Slope: N.R.	45767109/2001	Not classified
Glyphosate monoammonium salt (MON78568)	Parasitic wasp (*Aphidius rhopalosiphi*)	64.9	48 hr - 13 days LD50 (C): >108 (N.R.) g a.e./ha NOAEL: Not established Slope: N.R.	45767107/2002	Not classified
Glyphosate monoammonium salt (MON78568)	Parasitic wasp (*Aphidius rhopalosiphi*)	64.9	48 hr - 13 days LD50 (C): >4320 (N.R.) g/ha NOAEL: 4320 Slope: N.R.	45767107/2002	Not classified
Glyphosate monoammonium salt (MON78568)	Parasitic wasp (*Aphidius rhopalosiphi*)	64.9	48 hr - 13 days LD50 (C): >4320 (N.R.) g a.e./ha NOAEL: 4320 Slope: N.R.	45767108/2002	Not classified
Glyphosate monoammonium salt (MON78568)	Lacewing (*Chrysoperla carnia*)	64.9	Up to 10 days LD50 (C): >4320 (N.R.) g/ha NOAEC: 4320 Slope: N.R.	45767110/2002	Not classified
Glyphosate IPA salt (MON 2139)	Honey bee (*Apis mellifera*)	36	48 hr LD50 (O): >100 (N.R.) µg/bee NOAEL: N.R. Slope: N.R.	00026489/1972	Acceptable

Chemical	Species	% a.i.[1]	LD_{50}/ LC_{50} NOAEL/ NOAEC	MRID #/Year	Study Classification
Glyphosate IPA salt (MON 2139)	Honey bee (*Apis mellifera*)	36	48 hr LD50 (C): >100 (N.R.) µg/bee NOAEL: N.R. Slope: N.R.	00026489/1972	Acceptable
Glyphosate IPA salt (MON65005)	Honey bee (*Apis mellifera*)	31.32	48 hr LD50 (C): >31.3 (N.A.) µg a.e./bee NOAEL: 319 Slope: N.A.	44465703/1997	Acceptable
Glyphosate IPA salt (MON 77360)	Honey bee (*Apis mellifera*)	30.0	48 hr LD50 (C): >30 (NA) µg/bee NOAEL: 30 Slope: NA	45370301/2001	Acceptable
Glyphosate IPA salt (MON 77360)	Honey bee (*Apis mellifera*)	30.0	48 hr LD50 (O): >30 (NA) µg/bee NOAEL: 15 Slope: NA	45370302/2001	Supplemental

[1] a.i. = active ingredient; a.e. = acid equivalent/ IPA = isopropylamine; N.R. = not reported; O = oral study; C = contact study
[2] Range is 95% confidence interval for endpoint,

USEPA. 2008. Risks of Glyphosate Use to Federally Threatened California Red-legged Frog (*Rana aurora draytonii*). Pesticide Effects Determination. Office of Pesticide Programs, Environmental Fate and Effects Division.

Ma, J., Liang, W., Xu, L., Wang, S., Wei, Y., & Lu, J. 2001. Acute Toxicity of 33 Herbicides to the Green Alga *Chlorella pyrenoidosa*. Bulletin of Environmental Contamination and Toxicology, 66(4), 536-541.

Ma, J., Wang, S., Wang, P., Ma, L., Chen, X., & Xu, R. 2006. Toxicity assessment of 40 herbicides to the green alga *Raphidocelis subcapitata*. Ecotoxicol Environ Saf, 63(3), 456-462.

Ma, J., Xu, L., Wang, S., Zheng, R., Jin, S., Huang, S., et al. 2002. Toxicity of 40 herbicides to the green alga *Chlorella vulgaris*. Ecotoxicol Environ Saf, 51(2), 128-132.

Appendix C. List of EFED Assessments on 2,4-D Choline

Date	Assessment Title	DP Bar codes	Comment
January 15, 2013	EFED Environmental Risk Assessment of Proposed Label for Enlist (2,4-D Choline Salt), New Uses on Soybean with DAS 68416-4 (2,4-D Tolerant) and Enlist (2,4-D + Glyphosate Tolerant) Corn and Field Corn.	400223, 400230, 400234,400237, 405028, 405812	This is the 1st S3NU action for corn and soybean
June 13, 2013	Addendum to EFED Environmental Risk Assessment for Enlist (2,4-D Choline Salt), New Uses on Soybean with DAS 68416-4 (2,4-D Tolerant) and Enlist (2,4-D + Glyphosate Tolerant) Corn and Field Corn	411614	Re-evaluated the spray drift buffers
February 12, 2014	Addendum to 2,4-D Choline Salt Section 3 Risk Assessment: Refined Endangered Species Assessment for Proposed New Uses on Herbicide-Tolerant Corn and Soybean	418022	Refined the endangered species risk assessment (1st effects determinations) on the basis of spray drift mitigation language for 6 states (IL. IN, IA, OH, SD and WI)
September 17, 2014	Addendum to 2,4-D Choline salt Section 3 Risk assessment: Refined Endangered Species Assessment, Based on Revised Toxicity Value, for Indiana Bat for Proposed New Uses on 2,4-D Tolerant Corn and Soybean	422717	Revised toxicity value for rat reproduction value and the effects determination for Indiana bat for six states
October 10, 2014	Addendum to 2,4-D Choline Salt Section 3 Risk Assessment: Refined Terrestrial Plant Exposure Estimates and Effects Determination	423309	Terrestrial Plant Exposure Estimates and Effects Determination due to Runoff
February 19, 2015	Screening-Level Ecological Risk Assessment for Section 3 New Use for Ethanaminium (2,4-D choline salt) on Enlist Cotton	423346	S3NU for Cotton inclusion
April 22, 2015	Evaluation of 2,4-D Choline Mitigation Options Proposed by the Registrant to Avoid Effects to the Spring Creek Bladderpod in Tennessee	426524	Evaluated proposed mitigation for the Spring Creek Bladderpod in TN
July 6, 2015	Addendum to 2,4-D Choline Salt Section 3 Risk Assessment: Endangered Species Effects Determinations for 2,4-D Choline Use on Corn and Soybean in 17 U.S. States: AL, CO, DE, FL, GA, KY, MD, MI, NC, NJ, NM, NY, PA, SC, TX, VA and WV	423754, 427392	Revised effects determinations for corn and soybean

August 25, 2015	Addendum to Screening Level Ecological Risk Assessment for Section 3 New Use of Ethanaminium (2,4-D choline salt) on Enlist Cotton: Additional Risk Characterization for Terrestrial Invertebrates	428580	This assessment for cotton expanded to reflect the evolution of pollinator/ terrestrial invertebrate risk characterization methods.
October 19, 2016	2,4-D Choline Salt: EFED Ecological Risk Assessment and Listed Species effects determinations for GF2726 formulation of 2,4-D choline on GE corn, GE cotton, and GE soybean in AL, AR, AZ, CO, DE, FL, GA, IA, IL, IN, KS, KY, LA, MD, MI, MN, MO, MS, NC, ND, NE, NJ, NM, NY, OH, OK, PA, SC, SD, TN, TX, VA, WI, WV	428301	Established terrestrial plant effects endpoints and New Effects Determinations for listed species in all of the states in which Enlist Duo is proposed for use on corn, cotton, and soybeans.
October 31, 2016	2,4-D Choline Salt: Addendum to EFED Ecological Risk Assessment and Listed Species Effects Determinations for GF2726 formulation of 2,4-D choline on GE corn, GE cotton, and GE soybean in AL, AR, AZ, CO, DE, FL, GA, IA, IL, IN, KS, KY, LA, MD, MI, MN, MO, MS, NC, ND, NE, NJ, NM, NY, OH, OK, PA, SC, SD, TN, TX, VA, WI, WV (Additional Species Effects Determinations)	436497	Additional Species Effects Determinations
November 1, 2018	2,4-D Choline Salt: Response to Petition to Allow Use of Enlist and Enlist-Duo within Several Arizona Counties.	445578 and 445579	To expand the use of Enlist and Enlist-Duo within several Arizona counties

Appendix D. Physical Parameters of 2,4-D and Its Forms

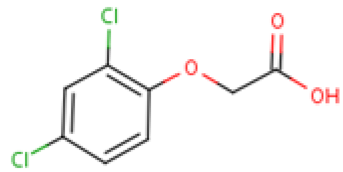
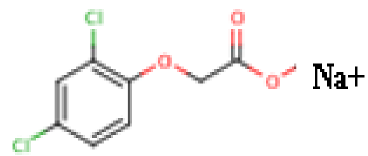
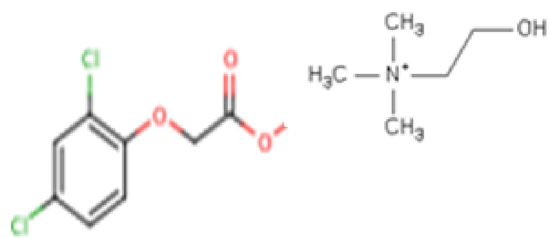
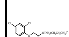
Table D.1. Molecular weight ratio and chemical structure of registered 2,4-D forms		
Active Ingredient and PC Code	**Molecular Weight Ratio**	**Structure**
2,4-D dichlorophenoxyacetic acid 030001 (2,4-D)	1.0	
Sodium Salt of 2,4-D 030004 (2,4-D Na)	1.10	
Choline Salt of 2,4-D 051505 (Choline)	1.47	
Amine Salts		
Diethanolamine Salt of 2,4-D 030016 (DEA)	1.48	

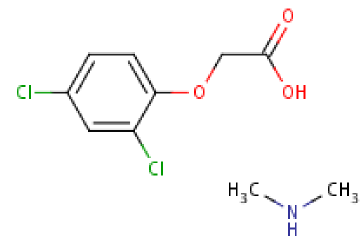
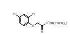
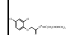
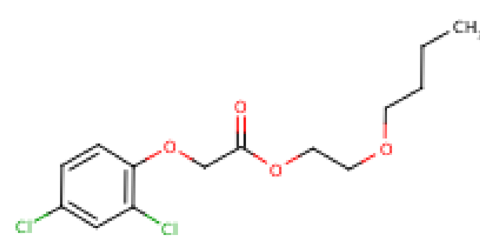
Table D.1. Molecular weight ratio and chemical structure of registered 2,4-D forms		
Active Ingredient and PC Code	**Molecular Weight Ratio**	**Structure**
Dimethylamine Salt of 2,4-D 030019 (DMA)	1.20	
Isopropylamine Salt of 2,4-D 030025 (IPA)	1.27	
Triisopropanolamine Salt of 2,4-D 030035 (TIPA)	1.87	
Esters		
Butoxyethyl Ester of 2,4-D 030053 (BEE)	1.45	

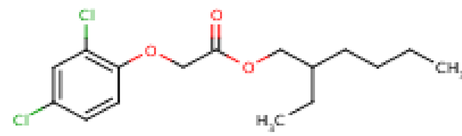
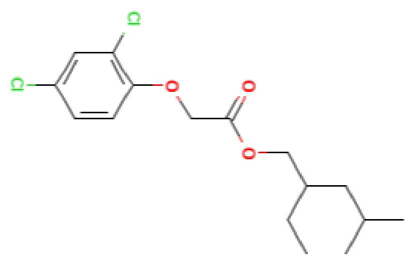
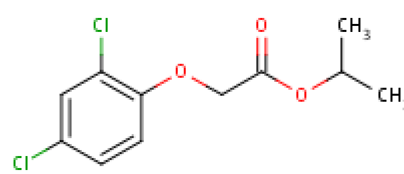
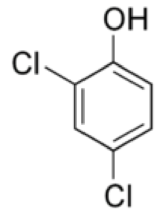
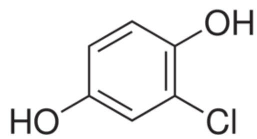
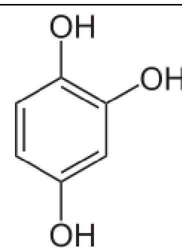
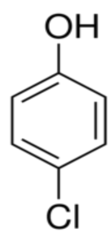
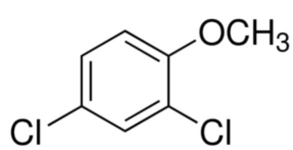
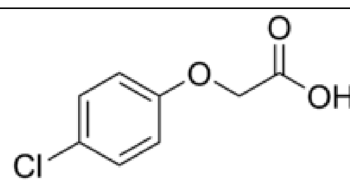
Table D.1. Molecular weight ratio and chemical structure of registered 2,4-D forms		
Active Ingredient and PC Code	**Molecular Weight Ratio**	**Structure**
2-Ethylhexyl Ester of 2,4-D 030063 (2-EHE)	1.51	
2-ethyl-4-methylpentyl ester of 2,4-D 030064 (EMPE)	1.51	
Isopropyl Ester of 2,4-D 030066 (IPE)	1.19	

Table D-2. Major and Minor Degradates Identified in Environmental Fate Studies			
Chemical Name (CAS No.)	**Molecular Formula Molecular wt.: g/mole**	**Chemical Structure**	**Maximum Formed**
2,4-Dichlorophenol [2,4-DCP] (120-83-2)	$C_6H_4Cl_2O$ 163.0		32.6 % of applied in Anaerobic aquatic study
Chlorohydroquinone [CHQ] (615-67-8)	$C_6H_3(OH)_2Cl$ 144.56		16.0 % of applied in aerobic aquatic study
1,2,4-benezenetriol (533-73-3)	$C_6H_6O_3$ 126.11		37.0% formed of applied in aquatic photo-degradation study
4-chlorophenol (106-48-9)	C_6H_5ClO 128.56		<2.0% formed of applied in anaerobic aquatic metabolism study [Intermediate degradate]
2,4-dichloroanisol [2,4-DCA] (553-82-2)	$C_7H_6Cl_2O$ 177.03		<2.0% formed of applied in aerobic soil metabolism study
4-Chlorophenoxyacetic acid [4-CPA] (122-88-3)	C_8H_7ClO 186.59		<2.0% formed of applied in anaerobic aquatic metabolism study

Appendix E. Aquatic Exposure

Example of Summary of PWC 2.0 modeling Output for 2,4-D Choline

Estimated Environmental Concentrations for 2,4-D are presented in **Table E-1** for the USEPA standard pond with the MScornSTD field scenario. A graphical presentation of the year-to-year acute values is presented in **Figure E-1**. These values were generated with the Pesticide Water Calculator (PWC), Version 2.001. Critical input values for the model are summarized in **Table E-2 and Table E-3**.

This model estimates that about 1.2% of 2,4-D applied to the field eventually reaches the water body. The main mechanism of transport from the field to the water body is by runoff (97.2% of the total transport), followed by erosion (2.23%) and spray drift (0.54%).

In the water body, pesticide dissipates with an effective water column half-life of 62.7 days. (This value does not include dissipation by transport to the benthic region; it includes only processes that result in removal of pesticide from the complete system.) The main source of dissipation in the water column is metabolism (effective average half-life = 66.2 days) followed by photolysis (1174.6 days) and volatilization (3216211 days).

In the benthic region, pesticide dissipates very slowly (472.2 days). The main source of dissipation in the benthic region is metabolism (effective average half-life = 472.2 days). The pesticide is about evenly distributed in the benthic region between the pore water and sorbed to sediment.

Table E-1. Estimated Environmental Concentrations (ppb) for 2,4-D

1-day Avg (1-in-10 yr)	32.82
4-day Avg (1-in-10 yr)	32.42
21-day Avg (1-in-10 yr)	30.25
60-day Avg (1-in-10 yr)	26.83
365-day Avg (1-in-10 yr)	8.193
Entire Simulation Mean	4.412

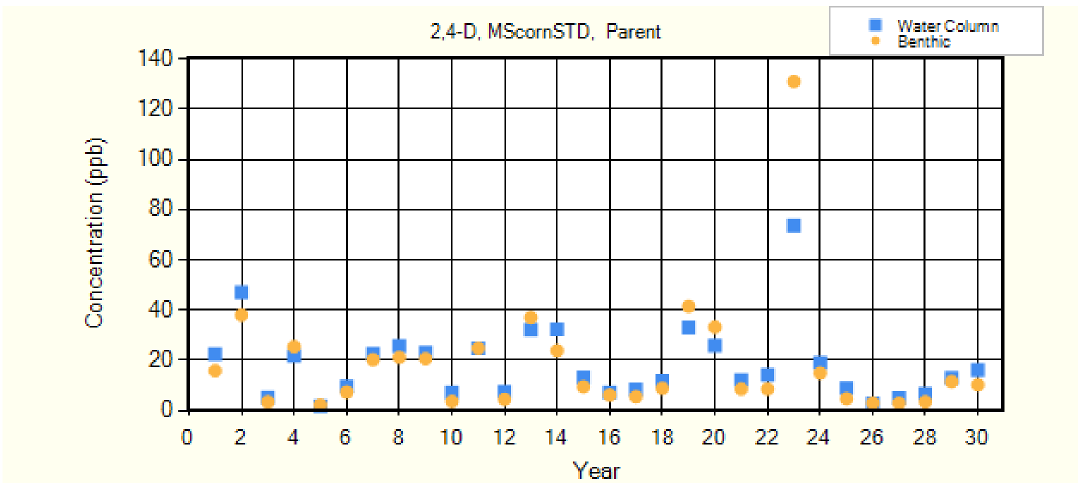
Table E-2. Summary of Model Inputs for 2,4-D

Scenario	MScornSTD
Cropped Area Fraction	1
Kd (ml/g)	0.52
Water Half-Life (days) @ 25 °C	45
Benthic Half-Life (days) @ 25 °C	321
Photolysis Half-Life (days) @ 40 °Lat	12.98
Hydrolysis Half-Life (days)	0
Soil Half-Life (days) @ 25 °C	6.92
Foliar Half-Life (days)	0
Molecular Weight	221.04
Vapor Pressure (torr)	1.4E-07
Solubility (mg/l)	569
Henry's Constant	2.92E-09

Table E-3. Application Schedule for 2,4-D

Date (Days Since Emergence)	Type	Amount (kg/ha)	Eff.	Drift
-12	Above Crop (Foliar)	1.12	0.99	0.00066
0	Above Crop (Foliar)	1.12	0.99	0.00066
12	Above Crop (Foliar)	1.12	0.99	0.00066

Figure E-1. Yearly Highest 1-day Average Concentrations



Example of Summary of PWC 2.0 modeling Output for Glyphosate

Estimated Environmental Concentrations for Glyphosate are presented in **Table E-4** for the USEPA standard pond with the ILCornSTD field scenario. A graphical presentation of the year-to-year acute values is presented in **Figure E-2**. These values were generated with the Pesticide Water Calculator (PWC), Version 2.001. Critical input values for the model are summarized in **Tables E-5** and **E-6**.

This model estimates that about 1.5% of Glyphosate applied to the field eventually reaches the water body. The main mechanism of transport from the field to the water body is by erosion (92.6% of the total transport), followed by runoff (6.99%) and spray drift (0.45%).

In the water body, pesticide dissipates with an effective water column half-life of 834.5 days. (This value does not include dissipation by transport to the benthic region; it includes only processes that result in removal of pesticide from the complete system.) The main source of dissipation in the water column is metabolism (effective average half-life = 834.5 days) followed by volatilization (1.070864E+10 days).

In the benthic region, pesticide dissipates very slowly (455.6 days). The main source of dissipation in the benthic region is metabolism (effective average half-life = 455.6 days). The vast majority of the pesticide in the benthic region (99.79%) is sorbed to sediment rather than in the pore water.

Table E-4. Estimated Environmental Concentrations (ppb) for Glyphosate

1-day Avg (1-in-10 yr)	14.58
4-day Avg (1-in-10 yr)	13.81
21-day Avg (1-in-10 yr)	11.92
60-day Avg (1-in-10 yr)	10.46
365-day Avg (1-in-10 yr)	7.768
Entire Simulation Mean	6.004

Table E-5. Summary of Model Inputs for Glyphosate

Scenario	ILCornSTD
Cropped Area Fraction	1
Kd (ml/g)	175
Water Half-Life (days) @ 25 °C	381
Benthic Half-Life (days) @ 25 °C	208
Photolysis Half-Life (days) @ 40 °Lat	0
Hydrolysis Half-Life (days)	0
Soil Half-Life (days) @ 25 °C	29
Foliar Half-Life (days)	0
Molecular Weight	169.08
Vapor Pressure (torr)	9.75E-10
Solubility (mg/l)	12000
Henry's Constant	7.39E-13

Table E-6. Application Schedule for Glyphosate

Date (Days Since Emergence)	Type	Amount (kg/ha)	Eff.	Drift
-12	Above Crop (Foliar)	1.12	0.99	0.00066
0	Above Crop (Foliar)	1.12	0.99	0.00066
12	Above Crop (Foliar)	1.12	0.99	0.00066

Figure E-2. Yearly Highest 1-day Average Concentrations

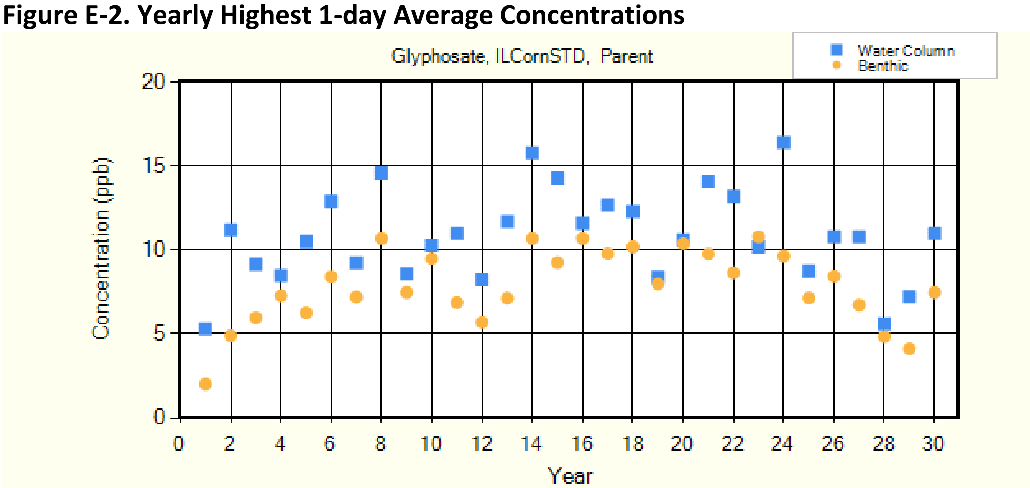
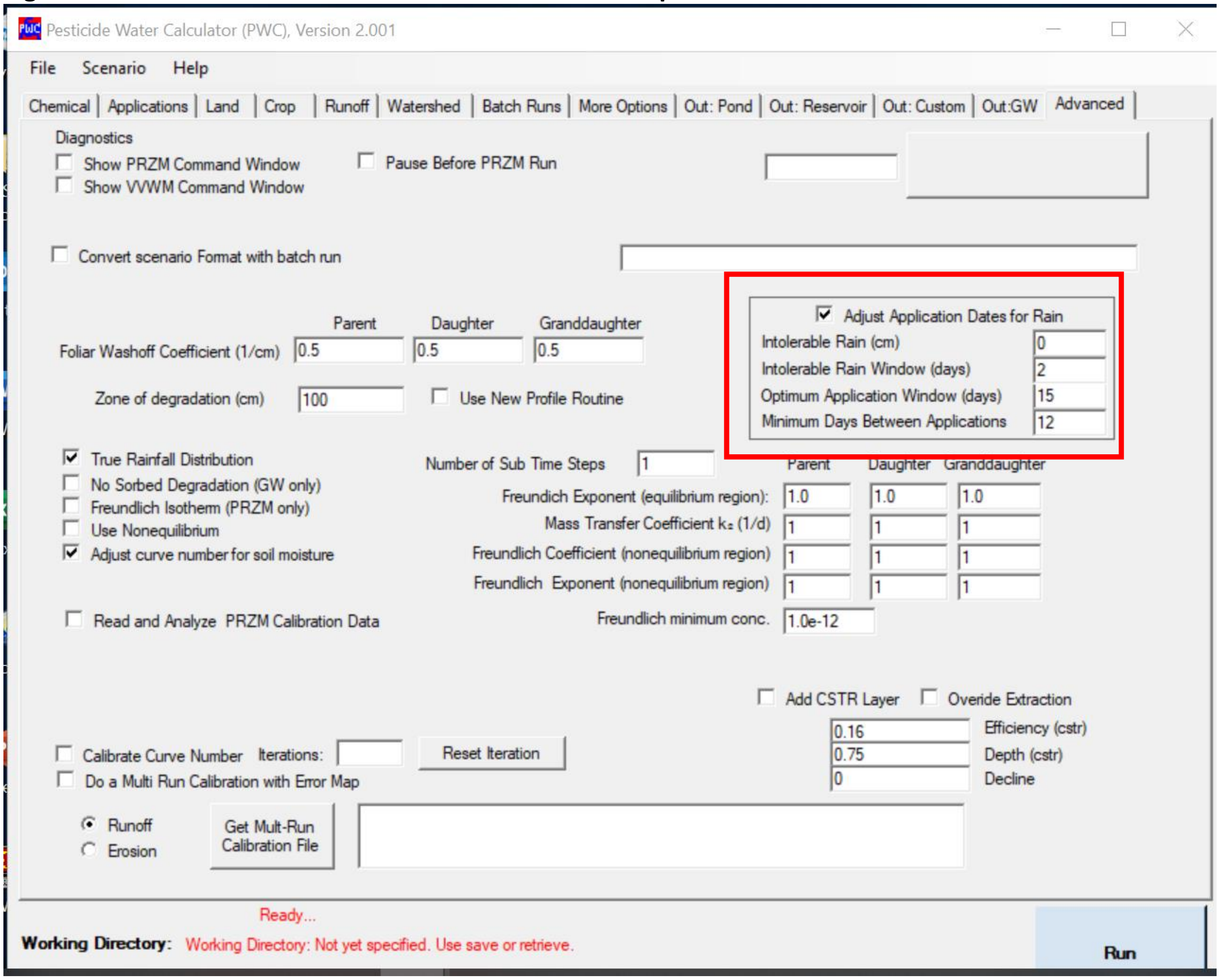


Figure E-3. Screen Shot of PWC's "Advance" Tab with Inputs for Rainfast Simulation



Curve Number Adjustments for Selected PWC Scenarios

Based on the PRZM scenario metadata, the scenario files, and recommendations from the Groundwater Loading Effects of Agricultural Management Systems (GLEAMS) Manual (USDA-NRCS, 2000), the following curve numbers and justifications are provided for the following scenarios, all row crops. For each hydrologic group and cropping practice, the GLEAMS manual provides the average curve number, along with a low- and high-end estimate, to allow users to distinguish differences among soils that are available by series name. The value selected by the scenario developer and its category (low, average, high) are provided.

MS Corn (Hydrologic group C)
Fallow – straight row + conservation tillage/poor (Curve number = 91, low)
Cropping – straight row + conservation tillage/poor (Curve number = 87, low)

PA Corn (Hydrologic group C)
Fallow –Row (contour + conservation tillage/poor (Curve number = 89, low)
Cropping – straight row + conservation tillage/poor (Curve number = 83, low)

IL Corn (Hydrologic group C)
Fallow – straight row + conservation tillage/poor (Curve number =92, low)
Cropping – straight row + conservation tillage/poor (Curve number = 89, low)

IA Corn (Hydrologic group C)
Fallow – straight row + conservation tillage/poor (Curve number =92, low)
Cropping – straight row + conservation tillage/poor (Curve number = 79, low)

MN Corn (Hydrologic group C)
Fallow – straight row + conservation tillage/poor (Curve number = 86, low)
Cropping – straight row + conservation tillage/poor (Curve number = 84, low)

MS Soybean (Hydrologic group C)
Fallow – straight row + conservation tillage/poor (Curve number = 87, low)
Cropping – straight row + conservation tillage/poor (Curve number = 84, low)

MS Cotton (Hydrologic group C)
Fallow – straight row + conservation tillage/poor (Curve number = 89, average)
Cropping – straight row + conservation tillage/poor (Curve number = 86, average)

NC Cotton (Hydrologic group C)
Fallow – straight row + conservation tillage/poor (Curve number = 92, average)
Cropping – straight row + conservation tillage/poor (Curve number = 89, average)

EFED consulted BEAD on the parameters being used in the modeling, and if current agronomic practices had changed. BEAD recommended that EFED should use a "good" classification and the inclusion of good agronomic practices that allow crop residues and pesticide to remain on the field. As a result, EFED assessed using the following curve numbers, based on Table 9-1 in the National Engineering Handbook (USDA, 2004), to refine the analysis (**Table E-7**). It should be noted that, unlike the GLEAMS manual, the National Engineering Handbook only provides the average curve number for each hydrologic group and cropping practice.

Table E-7. Modeling Parameters Based on Agronomic Practices

Scenario	Condition	Original Practice	Curve Number	Current Practice	Curve Number
IAcorn	Fallow	straight row/poor	86	crop residue cover/good	88
	Cropping	straight row + conservation tillage/poor	79	straight row + crop residue cover/good	75
IL Corn (Hydrologic Group C)	Fallow	straight row + conservation tillage/poor	91	crop residue cover/good	88
	Cropping	straight row + conservation tillage/poor	87	straight row + crop residue cover/good	82
MN Corn (Hydrologic Group C)	Fallow	straight row + conservation tillage/poor	91	crop residue cover/good	88

	Cropping	straight row + conservation tillage/poor	84	straight row + crop residue cover/good	82
MS Corn (Hydrologic Group C)	Fallow	straight row + conservation tillage/poor	91	crop residue cover/good	88
	Cropping	straight row + conservation tillage/poor	87	straight row + crop residue cover/good	82
MS Soybean (Hydrologic Group C)	Fallow	straight row + conservation tillage/poor	87	crop residue cover/good	88
	Cropping	straight row + conservation tillage/poor	84	straight row + crop residue cover/good	82
MS Cotton (Hydrologic Group C)	Fallow	straight row + conservation tillage/poor	89	crop residue cover/good	88
	Cropping	straight row + conservation tillage/poor	86	straight row + crop residue cover/good	82
NC Cotton (Hydrologic Group D)	Fallow	straight row + conservation tillage/poor	92	crop residue cover/good	90
	Cropping	straight row + conservation tillage/poor	89	straight row + crop residue cover/good	85
PA Corn (Hydrologic Group C)	Fallow	straight row + conservation tillage/poor	89	crop residue cover/good	88
	Cropping	conservation tillage + contour plowing/poor	83	conservation tillage + contour plowing/good	81

For screening level risk assessments, EPA does not alter standard PWC scenarios. The curve numbers analysis included in this assessment represents an alternative exposure assessment for selected scenarios intended to characterize decreases in runoff exposure due to good agronomic practices. Changes to curve numbers to represent soil types and agronomic practices are intended for characterization purposes and do not represent EPA's standard scenarios used in screening assessments. Given the flexibility of PWC and availability of different curve numbers (USDA 2004; USEPA 2019) use of the model as a characterization tool is considered an appropriate application of PWC.

Contribution of 2,4-D Choline from Runoff and Erosion to Waterbody

The PWC model was used in calculating EECs for 2,4-D Choline for the USEPA standard pond with the MScornSTD and PAcornSTD field scenarios. EFED estimated runoff masses in **Table E-8** based on an Enlist label 3 applications at a single maximum rate of 1.0 lba.e./A. EFED also calculated runoff masses based on a 24-hr rainfast requirement, as recommended in the product labels for 2,4-D Choline. EFED also estimated runoff mass based on a single post-emergence application of 1.0 lba.e./A. The transport of runoff mass from the applied field to a water body ranged from 0.4 to 1.2% of the annual applied mass via runoff and erosion. Average estimated runoff flux rates ranged from 0.004 to 0.012 lbs a.e./A for PACorn and MScorn respectively for 3 annual applications (**Table E-8**). Average estimated runoff flux ranged from 0.001 to 0.003 lbs a.e./A for PACorn and MScorn respectively for 1 post-emergence annual applications (**Table E-8**). In general, the high variable rainfall intensities in MS as compared to PA may have resulted in higher runoff mass in MS.

Table E-8. Percent Losses and Annual Runoff Flux of 2,4-D Choline for Selected Corn Scenarios

PWC Scenario	Total Mass Contribution Based on 30 years (Kg)[1]		Total Mass Over 30-yr Period (Kg)	Total Mass Applied Over 30 -yr (Kg)[2]	Average Annual Mass loss of 2,4-D choline (%)[3]	Average Annual Mass loss of 2,4-D choline per Yr (Kg/HA)[4]	Average Annual Mass loss of 2,4-D choline per HA (Kg/HA)[5]	Average Runoff Flux per application (Kg/HA)[6]	Average Runoff Flux per application (lbs/A)[7]
	Runoff	Erosion							
PWC Standard Scenario 3 applications (Rainfast)									
MScorn	12.06	0.28	12.34	1008	1.2	0.411	0.041	0.014	0.012
PAcron	3.50	0.04	3.54		0.4	0.118	0.012	0.004	0.004
PWC Standard Scenario 1 Application Postemergence (Rainfast)									
MScorn	3.20	0.075	3.275	336	0.3	0.109	0.011	0.004	0.003
PAcorn	0.75	0.006	0.756		0.1	0.025	0.003	0.001	0.001

[1] Derived from PWC model outputs
[2] 1.12 Kg/HA (Application rate) X (# of application/yr) x 10 (Hectares field size) x 30 (years-model simulation)
[3] % annual loss/yr = Total runoff mass over 30-yr period (Kg)/total mass applied over 30-yr period) x100
[4] Annual runoff loss=Total runoff mass/30 yr
[5] Annual runoff loss/HA = (Annual runoff loss/yr)/10 (10 HA watershed size).
[6] Average runoff loss per application/HA = (Annual runoff loss/HA)/3 (# of application per yr).
[7] Kg/HA converted to lbs/A.

A 2,4-D Choline specific field runoff study is not available. Several peer-reviewed 2,4-D runoff studies were considered for relevant crop lands. Two studies (White et al., 1976 and Kenimer et al., 1987) were identified to provide practical information in calculating runoff flux from crop lands for comparison with PWC model generated runoff mass. The maximum runoff concentrations from these studies were used in estimating runoff flux.

White et al. (1976) evaluated off–site transport of 2,4–D through surface runoff from a corn field near Tifton, GA. The study area was a 0.34 ha watershed with a slope of 3.2% on Cowarts loamy sand soil. The 2,4-D application rate for treated plot was 0.56 kg a.e./ha (0.5 lbs a.e./A). Simulated rainfall was applied at a rate of 16.5 cm/hr for 30 minutes for a total of 8.25 cm (3.2 inches) rainfall applied 1 day after 2,4-D application. This applied rainfall event corresponds to a 90th percentile upper-bound 1-in-50-year return frequency based on the precipitation frequency estimates of the National Weather Service [41]. The reported runoff water volume was 3.4 cm (1.34 in) for the applied rainfall event. The maximum 2,4-D concentration was 25.2 µg/L for 1-day after 2,4-D application. The reported concentration was normalized by multiplying by 2 for the maximum 2,4-D Choline application rate of 1.0 lbs a.e./A (1.2 kg a.e./ha). Runoff flux rate was calculated following the sample calculation above presented in the ecological risk assessment of dicamba (USEPA, 2020d, DP 459792+). The estimated runoff flux rate of 0.015 lbs a.e./A is calculated as follows:

$50.4\ \mu g/L \times 1.34\ in \times 1000\ L/m^3 \times 0.025\ m/in = 1688.40\ \mu g/m^2$

$1688.4\ \mu g/m^2 \times 1\ kg/1 \times 10^9\ \mu g \times 10{,}000\ m^2/ha \times 1\ lb/A / 1.12\ kg/HA = 0.015\ lbs\ a.e./A$

[41] https://hdsc.nws.noaa.gov/hdsc/pfds/index.html

Kenimer et al. (1987) evaluated the runoff of atrazine and 2,4-D on twelve unplanted field plots under simulated rainfall in Blacksburg, VA. The plots were located on Groseclose silt loam soil with average slopes of 10.3% for no-till and 10.6% for conventional tillage plots. Both pesticides were applied using a pressure-regulated hand-held sprayer approximately 24 h before rainfall simulation.

Each plot (101 m^2 area) of conventional and no-tillage systems was treated with application rate of 0.56 kg a.e./ha for 2,4-D. The rainfall was applied at a rate of 5.08 cm/hr for a total of 10.16 cm of rainfall over three simulated rainfall events: a 1-hour event 1-day after application followed by 2, 30-minute events 2-days after application. A 1 h duration rainfall of 5.08 cm/h (2 inches/hr) intensity corresponds to a 90^{th} percentile upper-bound 1-in-10-year return frequency based on the precipitation frequency estimates of the National Weather Service[1]. The reported runoff water volume was 1.8 cm (0.71 in) for the applied rainfall event for the conventional tillage plot with maximum residue cover of 1500 Kg/ha. The runoff flux rate was calculated for 2,4-D based on the average maximum 2,4-D concentration of 16 µg/L for same plot with of 1500 Kg/ha residue cover for conventional tillage at an application rate of 0.56 kg a.e./ha. The reported concentration was normalized by multiplying by 2 for the maximum 2,4-D Choline application rate of 1.2 kg/ha to estimate runoff flux rate. The estimated runoff flux rate of 0.005 lbs/A is calculated as follows.

$32\ \mu g/L \times 0.71\ in \times 1000\ L/m^3 \times 0.025\ m/in = 568.40\ \mu g/m^2$
$568\ \mu g/m^2 \times 1\ kg/1\times10^9\ \mu g \times 10{,}000\ m^2/ha \times 1\ lb/A / 1.12\ kg/ha = 0.005\ lbs/A$

The estimated runoff flux rates of 0.015 lbs/A for corn field in Tifton, GA and 0.005 lbs/A for conventional tillage with residue cover in Blacksburg, VA are within the same order of magnitude for the calculated flux rates using PWC model generated runoff mass estimates. However, the runoff fluxes (0.001 to 0.003 lbs a.e./A) estimated using PWC model generated runoff mass based on single post-emergence are lower than estimated runoff fluxes of 0.005-0.015 lbs a.e./A derived from peer-reviewed journal articles.

Vegetative Buffer Evaluations for 2,4-D

A well-maintained vegetative buffer could potentially intercept 2,4-D laden runoff (both soluble and sediment bound) prior to reaching surface waters, including wetlands. Currently, the Agency does not quantitatively assess the effectiveness of these practices in reducing pesticide concentrations in runoff. EFED evaluated currently available scientific literature to determine the reduction of 2,4-D loading in the presence of vegetative buffers.

Cole et al (1997) evaluated the influence of a buffer with varying lengths (8 or 16 ft), mowing heights (1.3 cm or 3.8 cm) and tine aeration for several pesticides and nutrient runoff loss from Bermudagrass turf in Stillwater, OK. Cole *et al*. observed variable results in two different study periods when evaluating effectiveness of turf (bermudagrass) buffers. When antecedent soil moisture and runoff volume was lower, 2,4-D concentration reductions ranged 76-96%. At the same site, reductions in 2,4-D concentrations ranged 8-55% when antecedent soil moisture and runoff volume were higher. This suggests that turf buffers of 5 m or less are not effective at reliably reducing 2,4-D concentrations in runoff. These study results suggest that the effectiveness of vegetative buffers is highly variable for 2,4-D.

Asmussen et al. (1977) evaluated off–site transport of 2,4–D through surface runoff on a grassed waterway with a flow length of 24.4 m for wet and dry antecedent moisture conditions under a simulated rainfall. Study results suggest that 2.5% and 10.3% of 2,4–D was lost under the dry and wet antecedent moisture conditions respectively, and the waterway retained approximately 70% of applied 2,4-D irrespective of antecedent soil moisture conditions.

Vegetative buffers are designed to intercept runoff and minimize soil erosion. Buffers can reduce the amount of sediment and pollutants carried by runoff to adjacent surface water bodies. As described above, the two available 2,4-D specific studies demonstrated a high degree of variability in the effectiveness of the filter strips tested under the conditions of those studies. Reichenberger et al (2007) reviewed 180 publications and evaluated many aspects related to the effectiveness of vegetative buffer in reducing pesticide loads into adjacent water bodies. They concluded that the effectiveness of vegetative buffer to reduce pesticide loading into an adjacent surface water body depended on many factors, such as topography, field conditions, soil types, antecedent moisture conditions, rainfall intensity, properties of the pesticide, application methods, width of the vegetative buffer and types of vegetation within the buffer strip.

However, vegetative buffer maintenance was determined to be critical for their continuing effectiveness in intercepting runoff loads and mitigating pesticide loadings from runoff into water bodies. Long-term effectiveness of a vegetative buffer required regular maintenance including excavation to remove overburdens of sediments, repairing vegetation damage, and removing over-mature vegetation or invasion of noxious weeds (USDA-NRCS, 2000).

Appendix F. Volatility and Area Treated Evaluation

This appendix provides the derived equations for 90th and 95th percentile 1, 4, 8 and 24 hour air concentrations. For more information on this analysis and the utility in risk assessment see **Section 2.5.7.2.** This appendix also provides the output figures for the 90th percentile analyses.

Figure F-1. EPA Equations for Estimating Volatility Driven Air Concentrations as a Function of Number of Treated Acres and Distance from the Application Area

1hr: 90th Percentile

Air Concentration = $e^{-1.07 + 0.04\sqrt{FieldSize} - 0.18\sqrt{Distance}}$

1hr: 95th Percentile

Air Concentration = $e^{-0.83 + 0.06\sqrt{FieldSize} - 0.17\sqrt{Distance}}$

4hr: 90th Percentile

Air Concentration = $e^{-1.63 + 0.04\sqrt{FieldSize} - 0.19\sqrt{Distance}}$

4hr: 95th Percentile

Air Concentration = $e^{-1.45 + 0.03\sqrt{FieldSize} - 0.18\sqrt{Distance}}$

8hr: 90th Percentile

Air Concentration = $e^{-2.03 + 0.04\sqrt{FieldSize} - 0.19\sqrt{Distance}}$

8hr: 95th Percentile

Air Concentration = $e^{-1.84 + 0.04\sqrt{FieldSize} - 0.18\sqrt{Distance}}$

24hr: 90th Percentile

Air Concentration = $e^{-3.04 + 0.04\sqrt{FieldSize} - 0.19\sqrt{Distance}}$

24hr: 95th Percentile

Air Concentration = $e^{-2.84 + 0.04\sqrt{FieldSize} - 0.18\sqrt{Distance}}$

Figure F-2. Model Fit of 1-, 4-, 8- and 24-hr 90th Percentile Air Concentrations

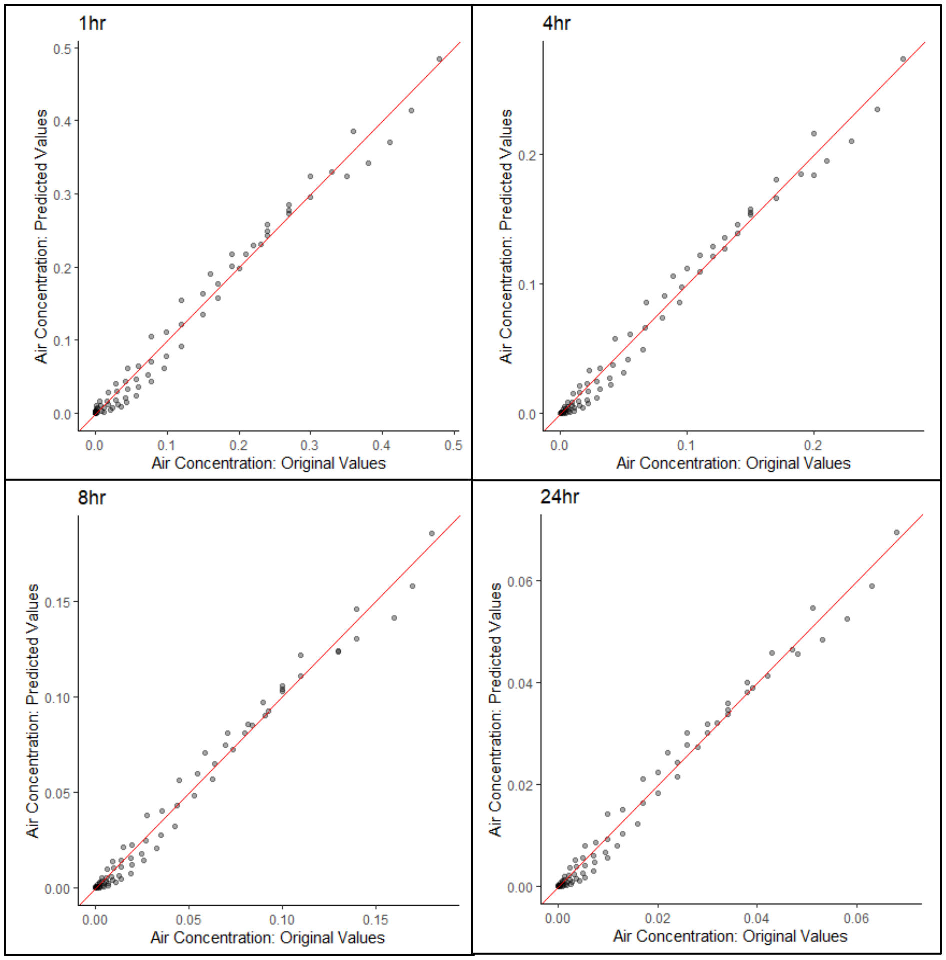


Figure F-3. Estimated 90th Percentile 1-, 4-, 8- and 24-Hr Air Concentrations for Different Field Sizes. Black solid line = IC_{10}. Black dashed line = IC_{20}; Red solid line = mean air concentration (1.527 ng m^3) that showed effects in the greenhouse study.

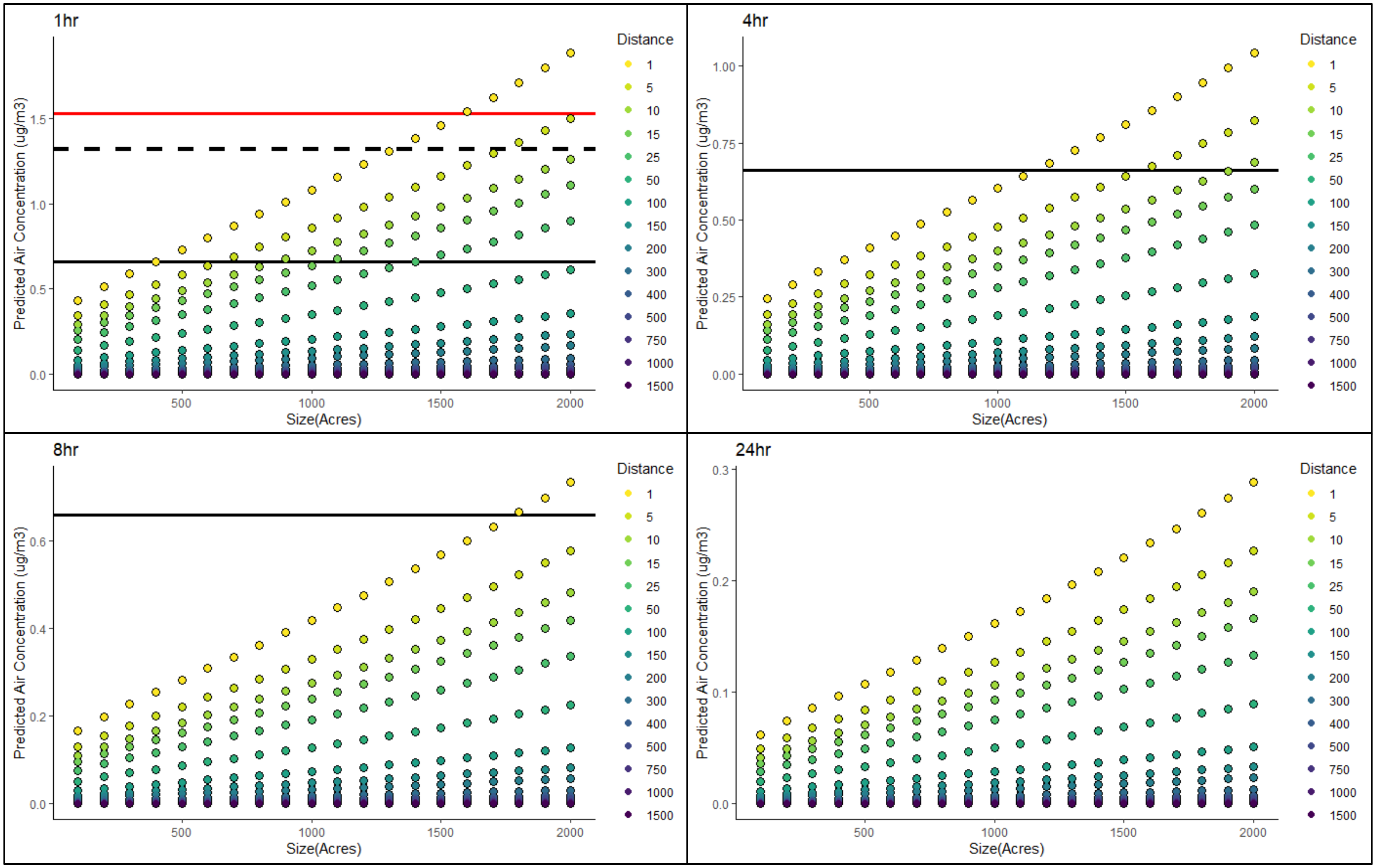
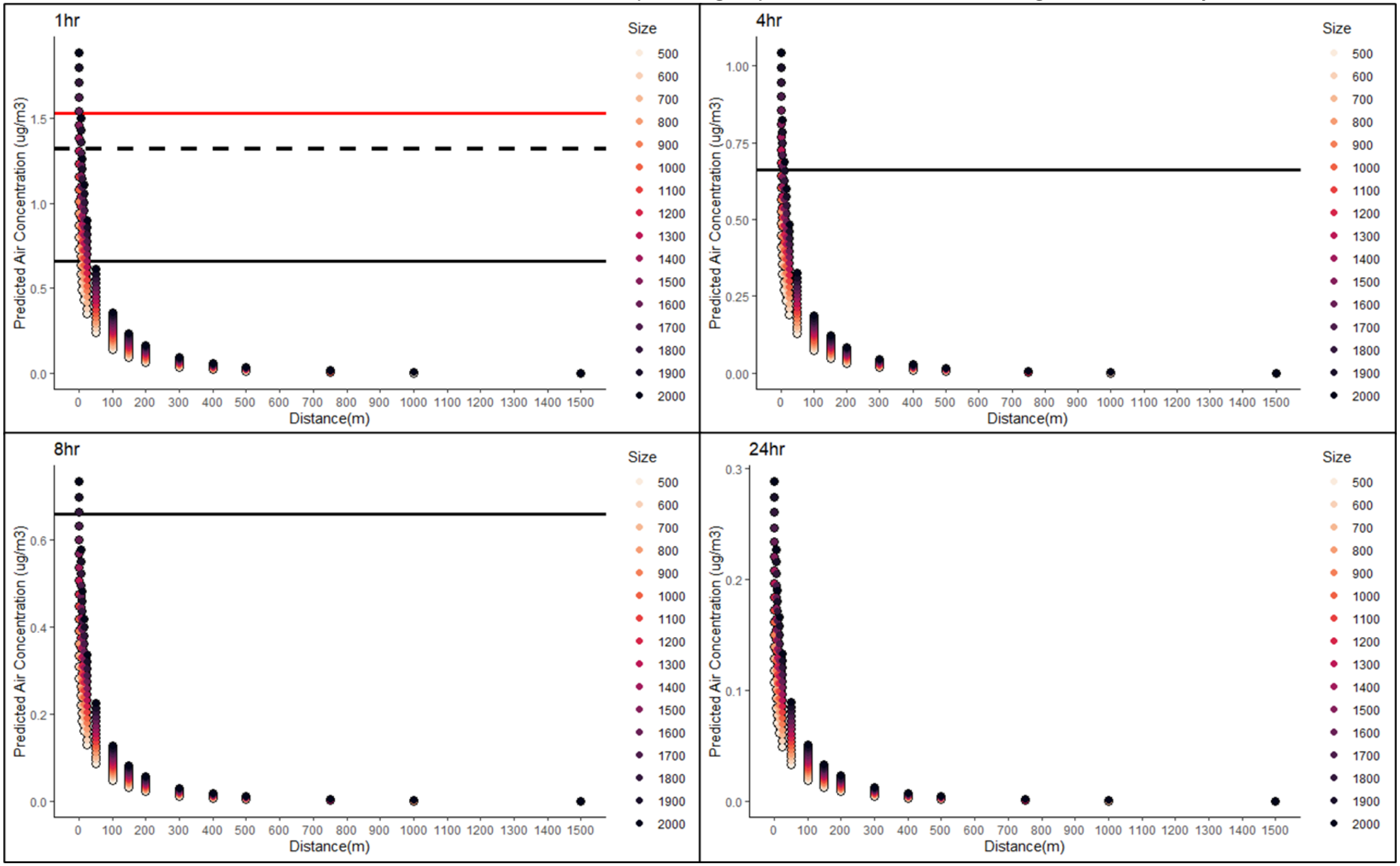


Figure F.4. Estimated 90th Percentile 1-, 4-, 8- and 24-Hr Air Concentrations at Off-Field Distances for Varying Field Sizes. Black solid line = IC_{10}. Black dashed line = IC_{20}; Red solid line = mean air concentration (1.527 ng m^3) that showed effects in the greenhouse study.



Appendix G. Feeding and Depuration Model Example Input and Output

This attachment file “Appendix G – 24-D Mammal and Bird Prey Feeding and Depuration Model.xlsx” provides the calculations to estimate environmental concentrations for terrestrial invertebrate carrion consumers.

Appendix H. Estimation of Concentrations at Thresholds for Comparison to Monitoring Data

All calculations are provided in the attached excel file titled "Appendix H – assumed conc in water at thresholds.xlsx".

Appendix I. SSD Development

Summary

Species Sensitivity Distributions (SSDs) were fit to inhibition concentrations (IC_{25} values) for vegetative vigor (VV) endpoints for plants exposed to 2,4-D choline. Separate SSDs for height and weight were developed.

Six distributions (normal, logistic, triangular, gumbel, weibull and burr) were fit to the available vegetative vigor data for 2,4-D. For weight, the gumbel distribution provided the best fit for the datasets (**Figure I-1**). For height, the normal distribution provided the best fit for the datasets (**Figure I-2**). This decision was based on the Akaike Information Criterion $(AIC)_c$ weight and confidence limits for the different distributions. Summary statistics from the fitted SSD for weight and height are provided in **Table I-1**. The fifth, tenth, twenty-fifth, fiftieth, seventy-fifth, ninetieth and ninety-fifth percentiles of the SSD (abbreviated HC_{05}, HC_{10}, HC_{25}, HC_{50}, HC_{75}, HC_{90}, and HC_{95}, respectively, where "HC" stands for "hazard concentration") are used to calculate endpoints representing effects to listed species of plants associated with height and weight.

Figure I-1. Gumbel SSD for 2,4-D vegetative vigor endpoints for weight.

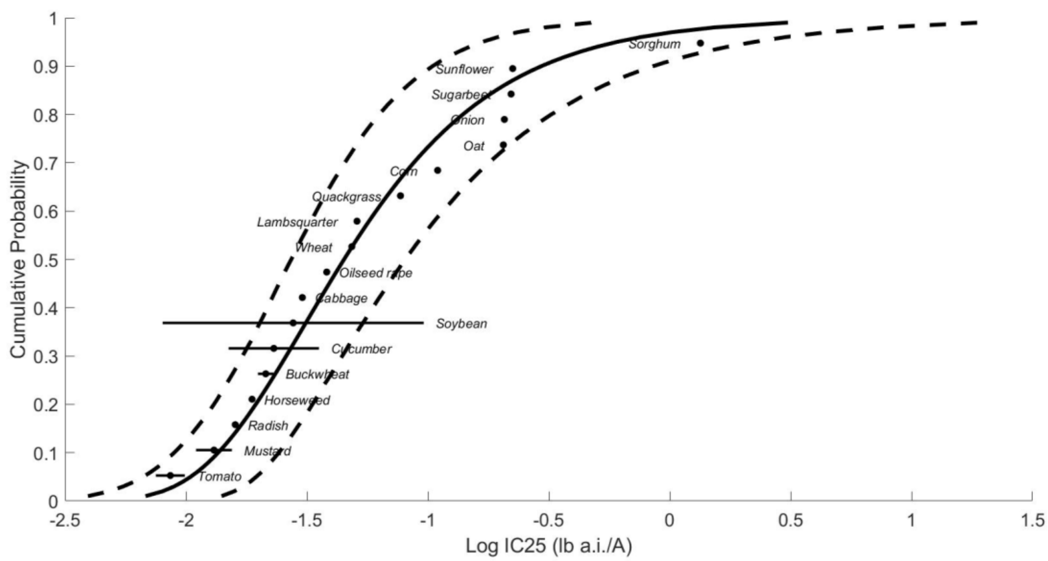


Figure I-2. Normal SSD for 2,4-D vegetative vigor endpoints for height.

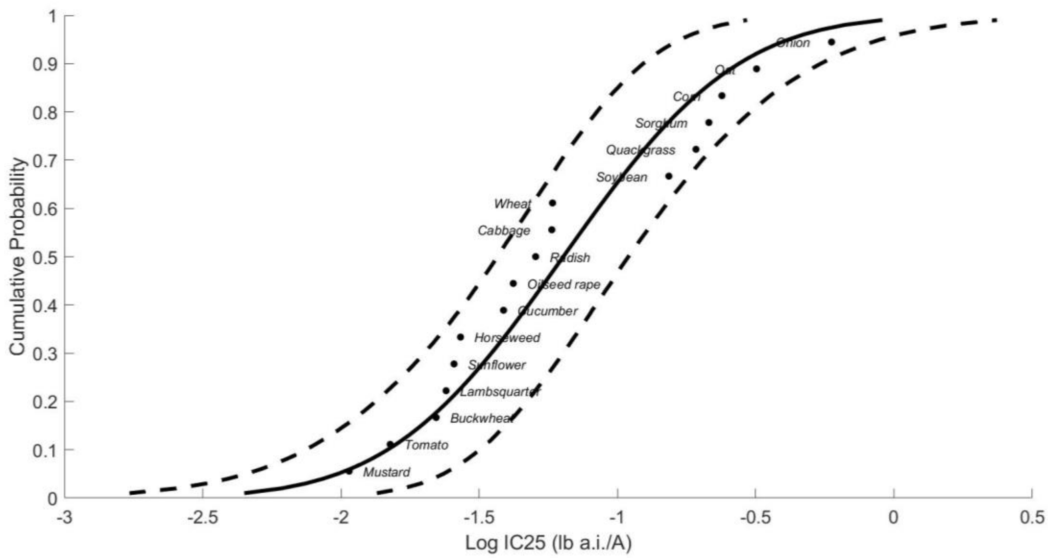


Table I-1. Summary of 2,4-D vegetative vigor IC_{25} endpoints (values in lb a.i./A).

Statistic	VV Weight	VV Height
HC_{05} (95% CI)	0.0104 (0.006-0.0192)	0.0097 (0.0045-0.0235)
HC_{10} (95% CI)	0.0136 (0.0089-0.0242)	0.0147 (0.0075-0.0317)
HC_{25} (95% CI)	0.0225 (0.015-0.0384)	0.0295 (0.0166-0.055)
HC_{50} (95% CI)	0.045 (0.0279-0.0877)	0.0637 (0.0368-0.1095)
HC_{75} (95% CI)	0.1083 (0.0528-0.2343)	0.1376 (0.0723-0.2452)
HC_{90} (95% CI)	0.2954 (0.1047-0.8567)	0.2753 (0.1240-0.5443)
HC_{95} (95% CI)	0.6060 (0.1679-2.2430)	0.4169 (0.1691-0.897)

CI = confidence interval

Toxicity Data

Because an SSD depicts relative sensitivities of different species exposed to the same stressor, it is necessary to standardize the data as much as possible to eliminate variables that would confound the relative sensitivities of species. Such variables can include study exposure duration and other study design factors. All IC_{25} values that were included in the analysis were all height or weight endpoints that followed the OCSPP 850.4150 guideline. Endpoints without definitive endpoints were not used to derive SSDs.

Data used to derive SSDs are from registrant-submitted studies. Those data are included in **Table I-2** and **Table I-3**. There was a total of 18 plant species tested for weight and 17 plant species tested for height (**Table I-4**). In cases where two endpoints were available for the same test species, values were similar (differing by only 1.2-2.4x). The data in **Table I-2** are from 4 different studies, while the data in **Table I-3** from 2 different studies.

Table I-2. Test results used to derive SSDs for 2,4-D for VV weight.

Plant	IC_{25} value (lb a.i./A)	Reference (MRID)
Tomato	0.0075	42416801
Soybean	0.0080	42416801
Tomato	0.0099	49903202
Mustard	0.0110	42416801
Cucumber	0.0150	42416801
Mustard	0.0155	49903202
Radish	0.0160	49903202
Radish	0.0160	42416801
Horseweed	0.0187	49903203
Buckwheat	0.0198	49903202
Buckwheat	0.0230	42416801
Cabbage	0.0303	49903202
Cucumber	0.0355	49903202
Rape	0.0382	49903202
Wheat	0.0485	49903202
Lambsquarter	0.0509	49903204
Quackgrass	0.0770	49903204
Soybean	0.0961	49903202
Corn	0.1098	49903202
Oat	0.2052	49903202
Onion	0.2073	49903202
Beet	0.2209	49903202
Sunflower	0.2243	49903202
Sorghum	1.3400	42416801

Table I-3. Test results used to derive SSDs for 2,4-D for VV height.

Plant	IC_{25} value (lb a.i./A)	Reference (MRID)
Mustard	0.0107	49903202
Tomato	0.0151	49903202
Buckwheat	0.0221	49903202
Lambsquarter	0.0240	49903204
Sunflower	0.0256	49903202
Horseweed	0.0271	49903204
Cucumber	0.0387	49903202
Rape	0.0420	49903202
Radish	0.0506	49903202
Cabbage	0.0579	49903202
Wheat	0.0582	49903202
Soybean	0.1534	49903202

Plant	IC_{25} value (lb a.i./A)	Reference (MRID)
Quackgrass	0.1923	49903204
Sorghum	0.2141	49903202
Corn	0.2386	49903202
Oat	0.3184	49903202
Onion	0.5945	49903202

Table I-4. Distribution of test results available for 2,4-D vegetative vigor endpoints.

Endpoint	Test results	Species
VV Weight	24	18
VV Height	17	17

Determining Distribution with Best Fit

P-values

Six potential distributions for the 2,4-D data were considered (*i.e.,* normal, logistic, triangular, gumbel, weibull and burr). To fit each of the six distributions, the toxicity values were common log (log_{10}) transformed. The SSD toolbox includes four different fitting methods (*i.e.,* maximum likelihood, moment estimators, linearization and metropolis-hastings). All six distributions were fit using the maximum likelihood (ML) method. To test goodness-of-fit, all six distributions were fit to the 2,4-D data and bootstrap goodness-of-fit tests were run with 10,000 replicates. The results of these fitting exercises are presented in **Table I-5**.

Table I-5. P-values calculated for SSDs using vegetative vigor height and weight toxicity data for 2,4-D.

Distribution	VV Weight SSD	VV Height SSD
Normal	0.26	0.12
Logistic	0.20	0.07
Triangular	0.08	0.37
Gumbel	0.62	0.42
Weibull	0.09	0.05
Burr	0.56	0.35

Akaike's Information Criteria Weights

Akaike's Information Criterion corrected for sample size (AIC_c) was used to compare the distributions for plant height and weight at the HC_{05}[2]. For weight, the majority of the weight is attributed to the gumbel distribution (with ≤15% each attributed to logistic, normal, triangular, burr and weibull; **Table I-6**). Based on the AIC weights, the gumbel distribution is used for weight data. For height, the majority of the weight is attributed to the triangular and gumbel distributions (with ≤9% each attributed to logistic, burr and weibull; **Table I-7**). Based on the AIC weights, the fit of the gumbel, triangular and normal distributions are further considered below for plant height data.

Table I-6. Akaike's Information Criteria (AICc) for distributions for VV weight toxicity data for 2,4-D.

Distribution	AICc	Delta AICc	Wt	HC_{05}	SE HC_{05}
Gumbel	-41.71	0	0.52	0.0104	0.0028
Normal	-39.16	2.54	0.15	0.0070	0.0032
Burr	-38.79	2.92	0.12	0.0104	0.0028
Logistic	-38.70	3.00	0.12	0.0059	0.0029
Triangular	-38.26	3.45	0.09	0.0078	0.0045
Weibull	-33.24	8.46	0.01	0.0017	0.0015

Table I-7. Akaike's Information Criteria (AICc) for distributions for VV height toxicity data for 2,4-D.

Distribution	AICc	Delta AICc	Wt	HC_{05}	SE HC_{05}
Triangular	-37.14	0	0.33	0.0104	0.0050
Gumbel	-36.91	0.23	0.29	0.0130	0.0034
Normal	-36.00	1.14	0.18	0.0097	0.0041
Logistic	-34.66	2.48	0.09	0.0079	0.0038
Burr	-33.92	3.22	0.07	0.0130	0.0034
Weibull	-32.97	4.16	0.04	0.0040	0.0031

Distributions

The cumulative distribution functions for vegetative vigor plant height SSDs for the gumbel, triangular and normal distributions are discussed in this section.

Figures I-3 through I-5 depict the three distributions fit to the IC_{25} values for plant species height. **Table I-8** includes the HC_{05}, HC_{10} and HC_{50} values for all three distributions, along with the associated 95% confidence intervals. When comparing the three distributions to the individual toxicity data, all three distributions appear to be good fits for the data (**Figures I-3 through I-5**).

As depicted in the three figures, the lowest available toxicity value (*i.e.,* 0.0107 lb a.i./A for Mustard*;* MRID 49903202) appears to be close to the HC_{05}. Since the HC_{05} is an important threshold used in the assessment, the estimated HC_{05} of the three distributions is used to select the best fit. This value is within the 95% confidence intervals of the HC_{05} for all three distributions (**Table I-8**). The median estimate of the HC_{05} for the gumbel distribution is somewhat higher than 0.0107 lb a.i./A, while the value for the triangular and normal distributions is somewhat lower. Since the HC_{05} for all 3 distributions seem to be similar, the normal distribution is chosen because it generates the most conservative of the HC_{05} values and the one that is closest to the lowest empirical value.

Table I-8. HC_x values (in lb a.i./A) for gumbel, triangular and normal distributions based on vegetative vigor plant height IC_{25} values.

Distribution	HC_{05} (95% CI)	HC_{10} (95% CI)	HC_{25} (95% CI)	HC_{50} (95% CI)	HC_{75} (95% CI)	HC_{90} (95% CI)	HC_{95} (95% CI)
Gumbel	0.0130 (0.0083-0.0237)	0.0167 (0.0111-0.0293)	0.027 (0.0183-0.0456)	0.052 (0.0320-0.0913)	0.1206 (0.0593-0.2532)	0.3135 (0.1125-0.8670)	0.6218 (0.1757-2.1386)

Triangular	0.0104 (0.0069-0.0250)	0.0147 (0.0098-0.0321)	0.029 (0.0193-0.0539)	0.0624 (0.0387-0.1010)	0.1345 (0.0721-0.2012)	0.2657 (0.1195-0.3948)	0.3746 (0.1518-0.5641)
Normal	0.0097 (0.0045-0.0235)	0.0147 (0.0075-0.0317)	0.0295 (0.0166-0.055)	0.0637 (0.0368-0.1095)	0.1376 (0.0723-0.2452)	0.2753 (0.124-0.5443)	0.4169 (0.1691-0.897)

Figure I-3. Gumbel SSD for 2,4-D toxicity values for vegetative vigor height.

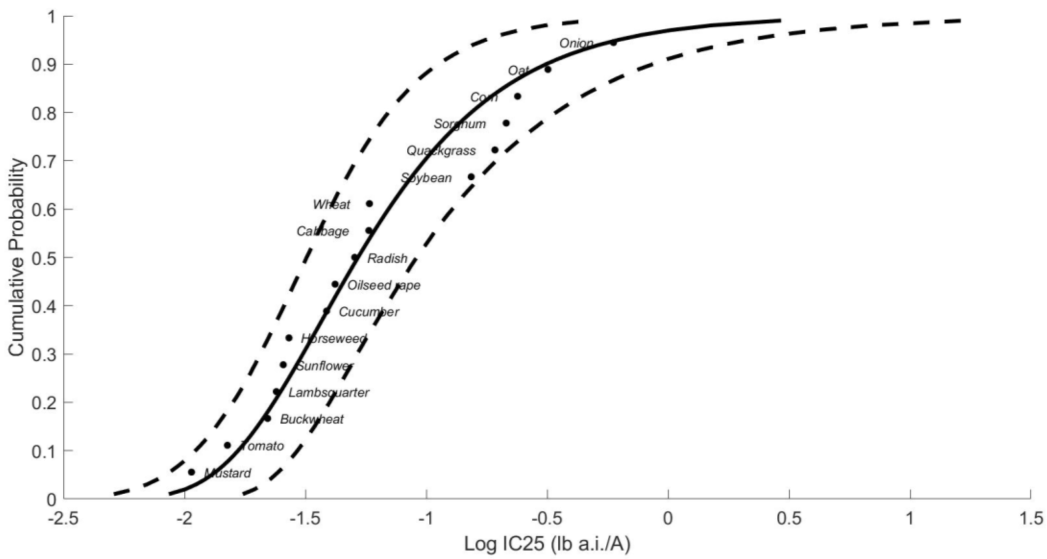


Figure I-4. Triangular SSD for 2,4-D toxicity values for vegetative vigor height.

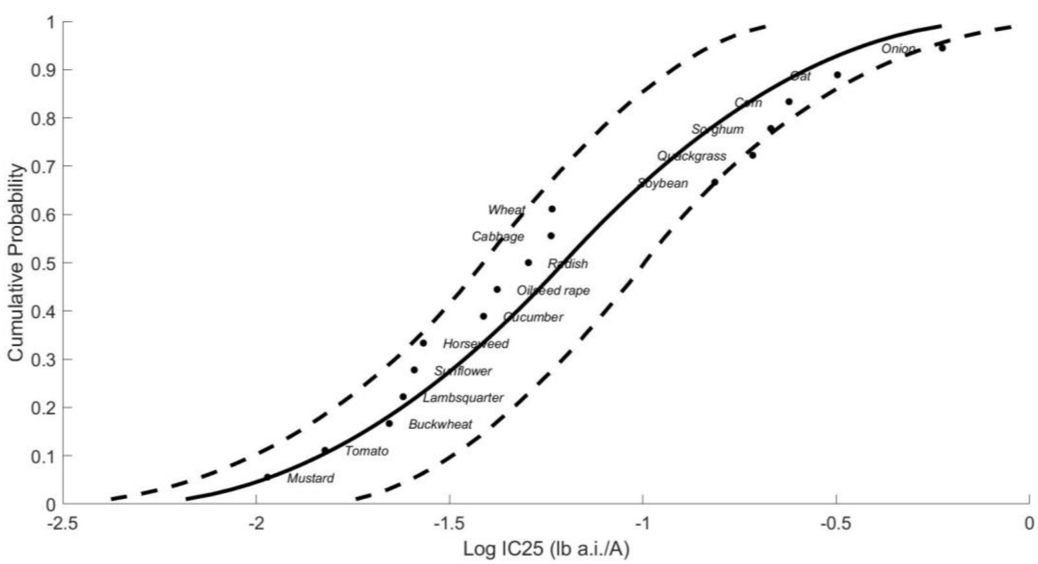
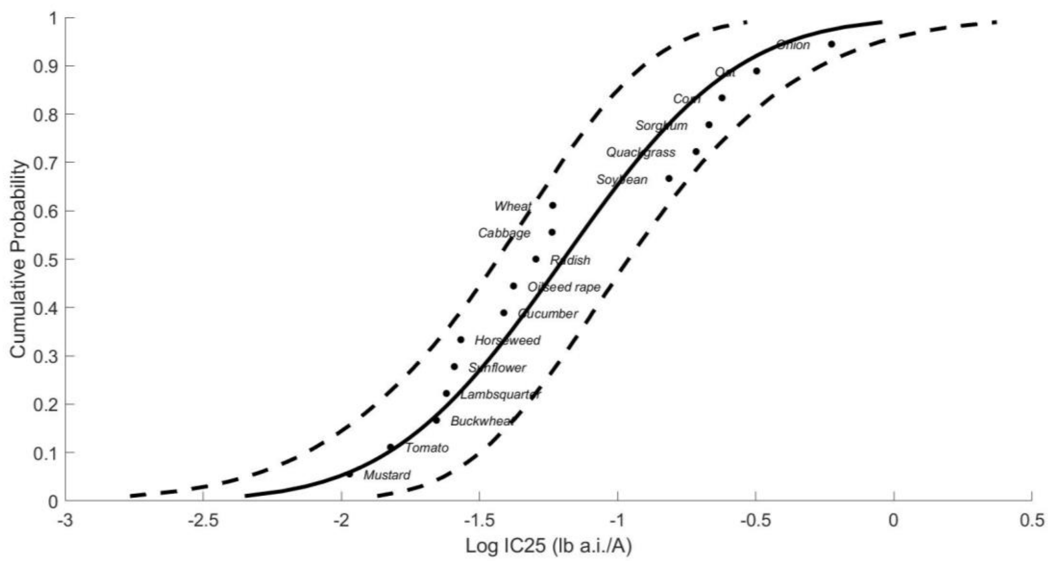


Figure I-5. Normal SSD for 2,4-D toxicity values for vegetative vigor height.



Conclusions

For plant weight, the gumbel distribution provided the best fit for the dataset. This decision was based on the AIC_c weight and confidence limits for the different distributions. For plant height, the normal distribution provided the best fit for the dataset. This decision was based on the AIC_c weight and confidence limits for the different distributions and by visually examining the distributions and their consistency with the toxicity data.

Appendix J. FIFRA Risk Assessment Model Input/Output Examples

Glyphosate Modeling Input and Output -TREX

Chemical Name:	**Glyphosate**	
Use	**Enlist Crops - Corn, Cotton, Soybean**	
Formulation	**Enlist Duo**	
Application Rate	**1**	**lbs a.i./acre**
Half-life	**12**	**days**
Application Interval	**12**	**days**
Maximum # Apps./Year	**3**	
Length of Simulation	**1**	**year**
Variable application rates?	**no**	

Dietary-based EECs (ppm)	**Kenaga Values**
Short Grass	**420.00**
Tall Grass	**192.50**
Broadleaf plants	**236.25**
Fruits/pods/seeds	**26.25**
Arthropods	**164.50**

2,4-D T-REX Inputs and Outputs

Chemical Identity and Application Information

Chemical Name:	2,4-D
Seed Treatment? (Check if yes)	
Use:	corn, all or unspecified 1
Product name and form:	Enlist Duo
% A.I. (leading zero must be entered for formulations <1% a.i.):	100.00%
Application Rate (lb ai/acre)	1
Half-life (days):	8.8 — Willis and McDowell
Application Interval (days):	12
Number of Applications:	3
Are you assessing applications with variable rates or intervals?	no

Dietary-based EECs (ppm)	Kenaga Values
Short Grass	369.51
Tall Grass	169.36
Broadleaf plants	207.85
Fruits/pods/seeds	23.09
Arthropods	144.72

Avian Results

Avian Class	Body Weight (g)	Ingestion (Fdry) (g bw/day)	Ingestion (Fwet) (g/day)	% body wgt consumed	FI (kg-diet/day)
Small	20	5	23	114	2.28E-02
Mid	100	13	65	65	6.49E-02
Large	1000	58	291	29	2.91E-01
Granivores	20	5	5	25	5.06E-03
	100	13	14	14	1.44E-02
	1000	58	65	6	6.46E-02

Avian Body	Adjusted LD50
Weight (g)	(mg/kg-bw)
20	157.56
100	200.58
1000	283.33

Dose-based EECs (mg/kg-bw)	**Avian Classes and Body Weights (grams)**		
	small 20	**mid 100**	**large 1000**
Short Grass	420.83	239.98	107.44
Tall Grass	192.88	109.99	49.24
Broadleaf plants	236.72	134.99	60.44
Fruits/pods	26.30	15.00	6.72
Arthropods	164.83	93.99	42.08

Dose-based RQs (Dose-based EEC/adjusted LD50) Size Class (grams)"	"Avian Acute RQs		
	20	**100**	**1000**
Short Grass	2.67	1.20	0.38
Tall Grass	1.22	0.55	0.17
Broadleaf plants	1.50	0.67	0.21

Fruits/pods	0.17	0.07	0.02
Arthropods	1.05	0.47	0.15

Dietary-based RQs (Dietary-based EEC/LC50 or NOAEC)	RQs	
	Acute	Chronic
Short Grass	0.12	0.38
Tall Grass	0.06	0.18
Broadleaf plants	0.07	0.22
Fruits/pods/seeds	0.01	0.02
Arthropods	0.05	0.15

Mammalian Results

Mammalian Class	Body Weight	Ingestion (Fdry) (g bwt/day)	Ingestion (Fwet) (g/day)	% body wgt consumed	FI (kg-diet/day)
Herbivores/ insectivores	15	3	14	95	1.43E-02
	35	5	23	66	2.31E-02
	1000	31	153	15	1.53E-01
Grainvores	15	3	3	21	3.18E-03
	35	5	5	15	5.13E-03
	1000	31	34	3	3.40E-02

Mammalian Class	Body Weight	Adjusted LD50	Adjusted NOAEL
Herbivores/ insectivores	15	969.24	120.88
	35	784.22	97.81
	1000	339.20	42.30
Granivores	15	969.24	120.88
	35	784.22	97.81
	1000	339.20	42.30

"Dose-Based EECs (mg/kg-bw)"	Mammalian Classes and Body weight (grams)

	15	35	1000
Short Grass	352.30	243.48	56.45
Tall Grass	161.47	111.60	25.87
Broadleaf plants	198.17	136.96	31.75
Fruits/pods	22.02	15.22	3.53
Arthropods	137.98	95.36	22.11

Dose-based RQs (Dose-based EEC/LD50 or NOAEL)	Small mammal 15 grams		Medium mammal 35 grams		Large mammal 1000 grams	
	Acute	Chronic	Acute	Chronic	Acute	Chronic
Short Grass	0.36	2.91	0.31	2.49	0.17	1.33
Tall Grass	0.17	1.34	0.14	1.14	0.08	0.61
Broadleaf plants	0.20	1.64	0.17	1.40	0.09	0.75
Fruits/pods	0.02	0.18	0.02	0.16	0.01	0.08
Arthropods	0.14	1.14	0.12	0.98	0.07	0.52
Seeds	0.01	0.04	0.00	0.03	0.00	0.02

Dietary-based RQs (Dietary-based EEC/LC50 or NOAEC)	Mammal RQs	
	Acute	Chronic
Short Grass	#DIV/0!	0.34
Tall Grass	#DIV/0!	0.15
Broadleaf plants	#DIV/0!	0.19
Fruits/pods/seeds	#DIV/0!	0.02
Arthropods	#DIV/0!	0.13

Upper Bound Kenaga Residues For 2 Pre-emergent Applications only (used for Attwater Prairie Chicken Species Specific Effects Determination in Section 2)

Chemical Name:	Dicamba	
Use	DT-crops	
Formulation	DT-crop products	
Application Rate	0.5	lbs a.i./acre
Half-life	8.4	days
Application Interval	7	days
Maximum # Apps./Year	2	
Length of Simulation	1	year
Variable application rates?	no	

Dietary-based EECs (ppm)	Kenaga Values
Short Grass	187.35
Tall Grass	85.87
Broadleaf plants	105.38
Fruits/pods/seeds	11.71
Arthropods	73.38

Bee-Rex Input and Outputs

Table J.1. User inputs (related to exposure)

Description	Value
Application rate	1
Units of app rate	lb a.i./A
Application method	foliar spray
Are empirical residue data available?	no

Table J.2. Toxicity data

Description	Value (µg a.i./bee)
Adult contact LD50	Non-definitive
Adult oral LD50	Non-definitive
Adult oral NOAEL	5.3
Larval LD50	6
Larval NOAEL	0.459

Table J.3. Estimated concentrations in pollen and nectar

Application method	EECs (mg a.i./kg)	EECs (µg a.i./mg)
foliar spray	110	0.11
soil application	NA	NA
seed treatment	NA	NA
tree trunk	NA	NA

Table J.4. Estimated risk quotients (RQ)

Life stage	Caste or task in hive	Average age (in days)	Jelly (mg/day)	Nectar (mg/day)	Pollen (mg/day)	Total dose (µg a.i./bee)	Acute RQ	Chronic RQ
Larval	Worker	1	1.9	0	0	0.00209	0.00	0.00
		2	9.4	0	0	0.01034	0.00	0.02
		3	19	0	0	0.0209	0.00	0.05
		4	0	60	1.8	6.798	1.13	14.81
		5	0	120	3.6	13.596	2.27	29.62
	Drone	6+	0	130	3.6	14.696	2.45	32.02
	Queen	1	1.9	0	0	0.00209	0.00	0.00
		2	9.4	0	0	0.01034	0.00	0.02
		3	23	0	0	0.0253	0.00	0.06
		4+	141	0	0	0.1551	0.03	0.34
Adult	Worker (cell cleaning and capping)	0-10	0	60	6.65	7.3315	#DIV/0!	1.38
	Worker (brood and queen tending, nurse bees)	6 to 17	0	140	9.6	16.456	#DIV/0!	3.10
	Worker (comb building, cleaning and food handling)	11 to 18	0	60	1.7	6.787	#DIV/0!	1.28
	Worker (foraging for pollen)	>18	0	43.5	0.041	4.78951	#DIV/0!	0.90
	Worker (foraging for nectar)	>18	0	292	0.041	32.12451	#DIV/0!	6.06
	Worker (maintenance of hive in winter)	0-90	0	29	2	3.41	#DIV/0!	0.64
	Drone	>10	0	235	0.0002	25.850022	#DIV/0!	4.88
	Queen (laying 1500 eggs/day)	Entire lifestage	525	0	0	0.5775	#DIV/0!	0.11

Table J.5. Results (highest RQs)

Exposure	Adults	Larvae
Acute contact	#DIV/0!	NA
Acute dietary	#DIV/0!	2.27
Chronic dietary	6.06	29.62

Welcome to the EFED Screening Tool for Inhalation Risk

This tool is designed to provide the risk assessor with a rapid method for determining the potential
significance of the inhalation exposure route to birds and mammals in a risk assessment.

Input

Application and Chemical Information	
Enter Chemical Name	2,4-D
Enter Chemical Use	Soybean
Is the Application a Spray? (enter y or n)	y
If Spray What Type (enter ground or air)	ground
Enter Chemical Molecular Weight (g/mole)	221.04
Enter Chemical Vapor Pressure (mmHg)	1.04E-07
Enter Application Rate (lb a.i./acre)	1
Toxicity Properties	
Bird	
Enter Lowest Bird Oral LD_{50} (mg/kg bw)	218
Enter Mineau Scaling Factor	1.15
Enter Tested Bird Weight (kg)	0.178
Mammal	
Enter Lowest Rat Oral LD_{50} (mg/kg bw)	441
Enter Lowest Rat Inhalation LC_{50} (mg/L)	1.79
Duration of Rat Inhalation Study (hrs)	4
Enter Rat Weight (kg)	0.35

Output

Results Avian (0.020 kg)

Maximum Vapor Concentration in Air at Saturation (mg/m^3)	1.24E-03
Maximum 1-hour Vapor Inhalation Dose (mg/kg)	1.56E-04

Adjusted Inhalation LD_{50}	4.93E+00	
Ratio of Vapor Dose to Adjusted Inhalation LD_{50}	3.15E-05	**Exposure not Likely Significant**
Maximum Post-treatment Spray Inhalation Dose (mg/kg)	1.06E-01	
Ratio of Droplet Inhalation Dose to Adjusted Inhalation LD_{50}	2.14E-02	**Exposure not Likely Significant**
Results Mammalian (0.015 kg)		
Maximum Vapor Concentration in Air at Saturation (mg/m^3)	1.24E-03	
Maximum 1-hour Vapor Inhalation Dose (mg/kg)	1.95E-04	
Adjusted Inhalation LD_{50}	1.07E+02	
Ratio of Vapor Dose to Adjusted Inhalation LD_{50}	1.83E-06	**Exposure not Likely Significant**
Maximum Post-treatment Spray Inhalation Dose (mg/kg)	1.33E-01	
Ratio of Droplet Inhalation Dose to Adjusted Inhalation LD_{50}	1.25E-03	**Exposure not Likely Significant**

Appendix K. Listed Species and Critical Habitat Determinations

The attachment "Appendix K – 1-10-22.xlsx" contains all information relevant to the listed species and critical habitat effects determinations. See **Section 4** for full details on how determinations were made.

Appendix L. Estimating Pesticide Exposure Through Diet

Pesticide exposure through diet was estimated for birds, amphibians, reptiles and mammals at risk from 2,4-D[42]. Refinements were not calculated for glyphosate given that chronic risk is assumed for birds, amphibians and reptiles, and risks are low for mammals. Total daily food intake rate (TDIR) is calculated using **Equation 1.** The total daily intake rate (TDIR; g food/animal-day) for food is calculated by considering the field metabolic rate (FMR; kcal/animal-day), the total metabolizable energy (ME_{total}; kcal/g food) of the food consumed by the animal. **Equations 2-9** are used to derive TDIR. **Equations 10-13** are used to derive refined risk quotients. **Table L-1** provides descriptions of the variables used to calculate TDIR.

Table L-1. Parameters Used for Equations to Estimate Dietary Exposure Concentrations for terrestrial vertebrates

Symbol	Definition	Units
AE_k	Assimilation efficiency	none
BW	Body weight	g/animal
DF_k	Fraction of diet attributed to food item k	none
EEC	Estimated environmental concentration	mg/kg-food
FMR	Field metabolic rate	kcal/animal-day
GE_k	Gross energy	Kcal/g food (wet wt)
LD_{50}	Median lethal dose	mg/kg-bw
ME_k	Metabolizable energy of food item k	kcal/animal-day
ME_{total}	Total metabolizable energy	kcal/g food
NOAEC	No observed adverse effect concentration	mg/kg-bw
RQ	Risk quotient	
TBW	Test species body weight	g/animal
TDIR	Total daily intake rate (for food)	g food/animal-day

Equation 1. $TDIR = \frac{FMR}{ME_{total}}$

Food ingestion rates are based on relating body weight to the free-living metabolic rate (FMR units are expressed in kcal/animal-day).

Equation 2. $FMR = 2.123 * BW^{0.749}$ (Adult passerines)

Equation 3. $FMR = 1.146 * BW^{0.749}$ (Adult non-passerines)

Equation 4. $FMR = 1.419 * BW^{0.727}$ (Mammal; herbivore)

---

[42] Pesticide exposure through diet was estimated using information from the Wildlife Exposure Factors Handbook (USEPA, 1993).

Equation 5. $FMR = 0.6167 * BW^{0.862}$ (Mammal; non-herbivore)

Equation 6. $FMR = 2.514 * BW^{0.507}$ (Mammal; rodent)

Equation 7. $FMR = 0.0535 * BW^{0.799}$ (Amphibians and reptiles)

The total amount of metabolizable energy in the food of an animal is determined by considering the fractions of the different food items (DF_k is unitless) that make up the animal's diet and the amount of metabolizable energy in each food item (ME_k in kcal/animal-day) (**Equation 8**). The fraction of each item in the total diet (DF_k) is dependent on the species being modeled. The DF_k is either one of the generic species for which dietary fractions are preset or a defined custom species with an assigned dietary fraction for each food category.

Equation 8. $ME_{total} = \Sigma(DF_k * ME_k)$

The metabolizable energy of food item k (ME_k) is estimated based on values for the gross energy (GE_k) and assimilation efficiency (AE_k) for that food item (**Equation 9** from USEPA (1993)). GE_k (kcal/g food (wet weight)) is based on individual fresh food items and is independent of the organism consuming the food. AE_k (unitless) of fresh food items is that portion of gross energy that can be assimilated by the animal. The AE_k values are based on assimilation efficiencies of individual food items by animals.

Equation 9. $ME_k = GE_k * AE_k$

As stated above, the total daily intake rate (TDIR; g/day) is estimated based on the field metabolic rate (FMR) and the metabolizable energy of food item k (ME_k; **Equation 1**).

The species-specific dose (mg/kg-bw) received through the diet is calculated by multiplying the upper bound estimate of the pesticide on each dietary item (dietary based estimated environmental concentration (EEC; mg/kg-food) by the total daily intake rate (TDIR; g food/animal-day), divided by the body weight (g/animal; USFWS 2019; **Equation 10**). The mass of the chemical on food items (mg/kg-food) is estimated using T-REX and can be found in **Appendix L** attachment.

Equation 10. $Dose = EEC * TDIR/BW$

Adjusted LD_{50} and NOAEC values (mg/kg-bw) can then be estimated for birds and mammals using the calculated LD_{50}, the test species body weight (TBW) and the body weight of the species of concern (BW; **Equations 11** and **12)**. For amphibians and reptiles, toxicity endpoints are not adjusted for body weights of test and assessed species because no scaling factors are available.

Equation 11. $\text{Adjusted LD50 or NOAEC } = LD50\ or\ NOAEC * (BW/TBW)^{0.15}$ (Birds)

Equation 12. $\text{Adjusted LD50 or NOAEC } = LD50\ or\ NOAEC * (TBW/BW)^{0.25}$ (Mammals)

Finally, the refined risk quotient is estimated using the refined LD_{50} or NOAEC values and the dose (**Equation 13**).

Equation 13. $RQ = Dose\ /adjusted\ LD50\ or\ NOAEC$

All calculations are provided within this attached file “Appendix L_11-3-21.xlsx”:

Appendix M: Upper Bound Distance Representing Runoff Transport to Wetlands for use in Establishing Action Area for Listed Species

The following EFED scientists and Branch Chiefs were involved in the development and review of this approach: Jennifer Connolly, Nelson Thurman, Chuck Peck, Frank Farruggia, Jerrett Fowler, Katrina White, Kris Garber, William Eckel, Joshua Antoline, Stephen Muela, Sumathy Sinnathamby, Mark Corbin, Dana Spatz, and Sujatha Sankula

Summary

The Environmental Fate and Effects Division (EFED) has developed a method to quantitatively determine a distance beyond which there is no expectation of runoff, referred to in this document as the upper bound distance. This distance is used to establish the action area in the federally listed endangered and threatened species ("listed species") risk assessment for Enlist products (**Section 4**).

In listed species assessments, the action area represents the area where discernable effects from pesticide exposure could potentially occur through direct application or off-site transport (e.g., through spray drift or runoff) and is based on the locations of registered label uses. Previously, EPA only quantitatively included off-site transport from spray drift when establishing the action area. The risk assessment on Enlist products did not identify risks from spray drift, but runoff concerns extending beyond the area directly adjacent to the treated field were identified for terrestrial and wetland habitats. A standard risk assessment tool does not currently exist to estimate runoff distances or estimates concentrations found in runoff at different distances. For this reason, EPA established an upper bound runoff distance of 1600 m (equivalent to 1.6 km or 1.0 mi) from the edge of a treated field to a wetland. This was based on analyses of 1st and 2nd order catchments (watershed) sizes from the NHDplus, as a purposefully conservative distance beyond which there is no expectation of runoff to wetlands.

The upper bound runoff distance estimate of 1600 m presented in this appendix is a conservative screen and may not represent real world conditions as it assumes the treated field is found on one side of the catchment and the runoff from the treated field travels to the far side of the catchment without being impacted by the geographic factors (e.g., locations of treated areas, stream channels, and species or habitat locations within the catchment), physical factors (e.g., infiltration, surface roughness, micro-scale landscape changes that affect retention), and chemical degradation and sorption properties of the chemical. However, the purpose of this distance is to set the maximum extent of the Enlist action area for runoff to wetland habitats to enable EFED determine which species and critical habitats are found within the action area. EFED acknowledges that this action area is protective because it represents a maximum distance between the treated field and wetlands and maximum width of the entire NHDplus catchment.

Because this is considered a conservative approach for the overlap evaluation, EFED considered whether the use of the 1600 m upper bound distance (actually quantified in the GIS analysis as 1500 m) influenced the effects determinations or evaluations of the likelihood of jeopardy or adverse modification. EFED compared the number of species where jeopardy or adverse modification was likely using the overlap with a buffer of 1500 m to a buffer of 150 m. Percent overlap of the potential footprint of the action area with a species' range increased as the assumption of runoff distance increased, but their overlap category (high, medium, or low) remains the same for 150 m and 1500 m buffers. Therefore, the conservative nature of this runoff distance does not have an impact on conclusions related to potential jeopardy or adverse modification.

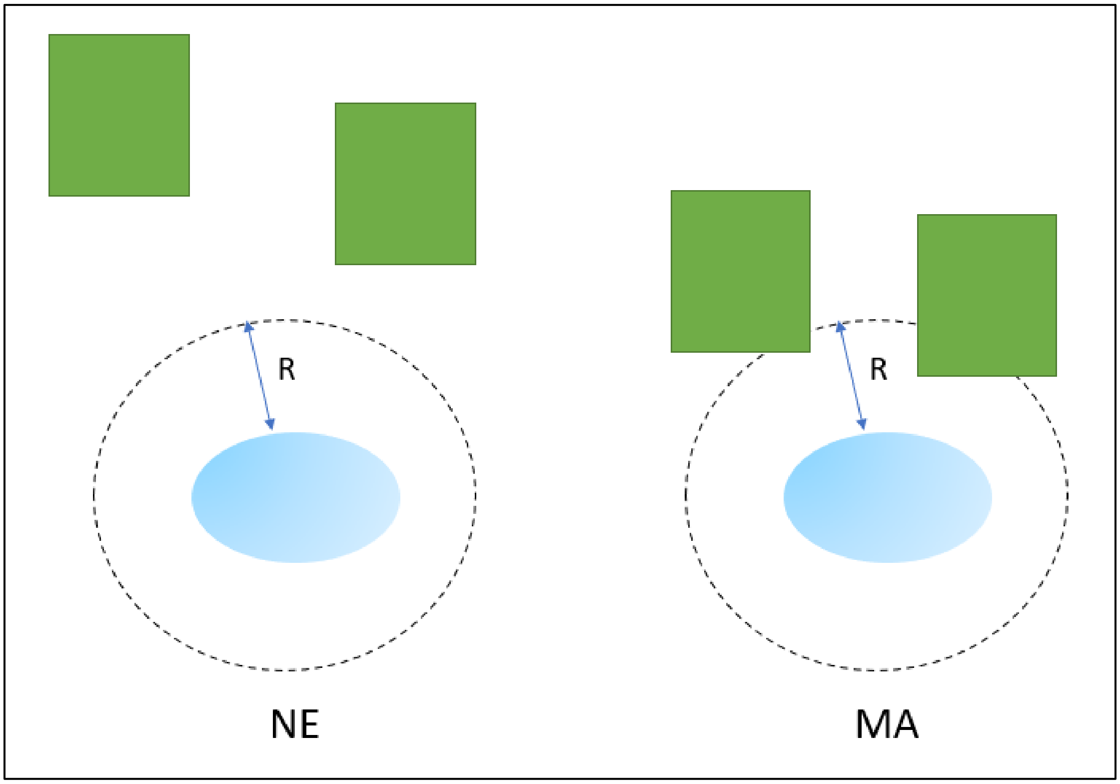
Background

One of the components of a biological evaluation is the action area, which represents the area where a pesticide exposure could potentially occur through direct application or off-site transport (e.g., through spray drift). When setting the initial extent of the action area, the off-field transport extent is based on the upper bound estimates for off-site transport. Previously, EPA only quantitatively included spray drift transport when establishing the action area. As noted in the risk assessment above, spray drift from applications of Enlist do not pose a risk; however, there is potential risk from runoff transport to terrestrial and wetland areas. Unlike spray drift, EPA did not have an approach to estimate distance for runoff or how far off site the pesticide concentrations in the runoff would continue to exceed the threshold of effect. The purpose of this appendix is to describe EFED's approach to calculate a conservative upper bound to represent the travel distance of runoff from a treated field to a wetland.

Conceptual Approach and Method

In this assessment, since runoff is identified as an exposure route of concern, the distance from the field where runoff could travel is used to establish the Enlist action area. The corn, cotton and soybean use footprint is expanded in all directions by this runoff area. Expanding the use areas in all directions to develop the action area is another conservatism in the method because runoff would only flow down slope, not in all directions around a field. **Figure M-1** depicts a hypothetical situation where a pesticide can be applied to use areas represented in green. Conceptually, a listed species occupies the wetland depicted in light blue. By considering the area found between the treated field and the species location, EPA can determine whether pesticide use may have an effect on listed species (May Affect) or if the species distribution is outside of the action area (No Effect).

Figure M-1. Conceptual model depicting an example of how the runoff distance (R) can be used to determine No Effect (NE) and May Affect (MA) in an Overlap Analysis. Green = treated field, Blue = wetland
Data



To estimate a conservative runoff distance, EPA used ArcGIS 10.8 and the National Hydrography Dataset (NHDplus Version 2[43]) to evaluate the catchment area and perimeter associated with each 1st order and 2nd order streams/river segments for the conterminous United States. The NHDplus is a feature-based database that interconnects and uniquely identifies the stream segments or reaches that make up the nation's surface water drainage system. The catchment features of the NHDplus are elevation-derived and define the extent of land area that contributes directly to the associated stream segment via overland flow rather than channelized stream flow from upstream reaches. Each catchment represents the watershed/drainage area for a single stream segment. NHDplus data was originally developed at 1:100,000-scale and exists at that scale for the whole country. While high-resolution NHDplus data, generally developed at 1:24,000/1:12,000 scale, is available, EPA used the medium resolution NHDplus data, as that data is currently being used by EPA for other GIS analysis. This resolution is sufficient for the purposes of this effects determination (**Section 4**).

Information from the NHDplus version 2 and tools found in ESRI ArcGIS 10.8 were used to estimates widths for each NHD catchment. The national seamless NHDplus database for the conterminous United States includes spatial layers representing each catchment[44] drainage and streams[45], in addition to

[43] https://www.epa.gov/waterdata/get-nhdplus-national-hydrography-dataset-plus-data
[44] National Seamless data NHD plus v21: NHDPlusV21_National_Seamless_Flattened_Lower48.gdb/NHDPlusCatchment/Catchment
[45] National Seamless data NHD plus v21: NHDPlusV21_National_Seamless_Flattened_Lower48.gdb/NHDPlusSnapshot/NHDFlowline_Network

descriptive tabular data for both. The available information from the NHD was supplemented using standard geometry tools found within ArcGIS 10.8 to calculate the perimeter for each catchment. Together this information was used to estimate the catchment width. While actual runoff distance will be less, the width estimate represents the maximum possible distance runoff can travel within a catchment outside of a channelized stream. The 90th percentile from the national distribution of estimated catchment widths is selected for use in evaluating potential exposure from runoff from a treated field to a listed species and critical habitat.

EPA selected 1st and 2nd order streams as the basis of this analysis based on proximity to pesticide-treated areas and susceptibility to agricultural runoff (e.g., sheet flow and shallow concentrated flow from a field). First-order streams are perennial streams, streams that carry water throughout the year but have no permanently flowing tributaries. A second order stream forms at the confluence of two first-order streams. According to the NRC[46], in many watersheds, the first-order streams encompass 60% to 80% of the total watershed area and that riparian wetlands (wetlands immediately adjacent to streams) can receive significant surface-water runoff. Use of 1st and 2nd order streams is also protective of the higher order streams because the catchments for the individual stream segments associated with the higher order streams are generally smaller based on available data for these catchments and would have smaller widths (NHDplus Version 2). Width estimates when only considering 1st and 2nd order streams resulted in a value of 1.6 km, this value remains the same considering all streams.
In addition, the connectivity of 1st and 2nd order streams to permanently flooded wetlands may occur at any point within 1st and 2nd order catchments (USEPA 2015[47]). For this analysis, EPA is not proposing to estimate a distance that the runoff will travel along the 1st and 2nd order streams to reach permanently flooded wetland, as this involves a different off-site transport mechanism (downstream transport). Other types of wetlands (e.g., depressional, seasonally dry, geographically isolated from streams) are also expected to be within the 1st and 2nd order stream catchments (USEPA 2015) and could receive runoff.

Due to the number of variables and impacting runoff concentrations and distance, EFED chose to develop a method to conservatively account for runoff as overland flow to wetland habitats as part of the action area. This distance allows for the initial screen of species found within the action area. This distance does not represent a distance to effect for a listed species and does not reflect how far runoff may travel as channelized stream flow (downstream transport). A more advanced methodology would be required for both analyses.

Results and Discussion

EPA used the NHDplus version 2 to identify the catchments for these 1st and 2nd order streams or river segments. Within each catchment, runoff (defined here as sheet flow and shallow concentrated flow outside of the stream channel, illustrated with red dashed arrows in **Figure M-2**) moves toward the stream found within the catchment (blue in **Figure M-2**). The example catchments highlighted in **Figure M-2** depict two scenarios, one where the stream is found roughly in the in the middle of the catchment

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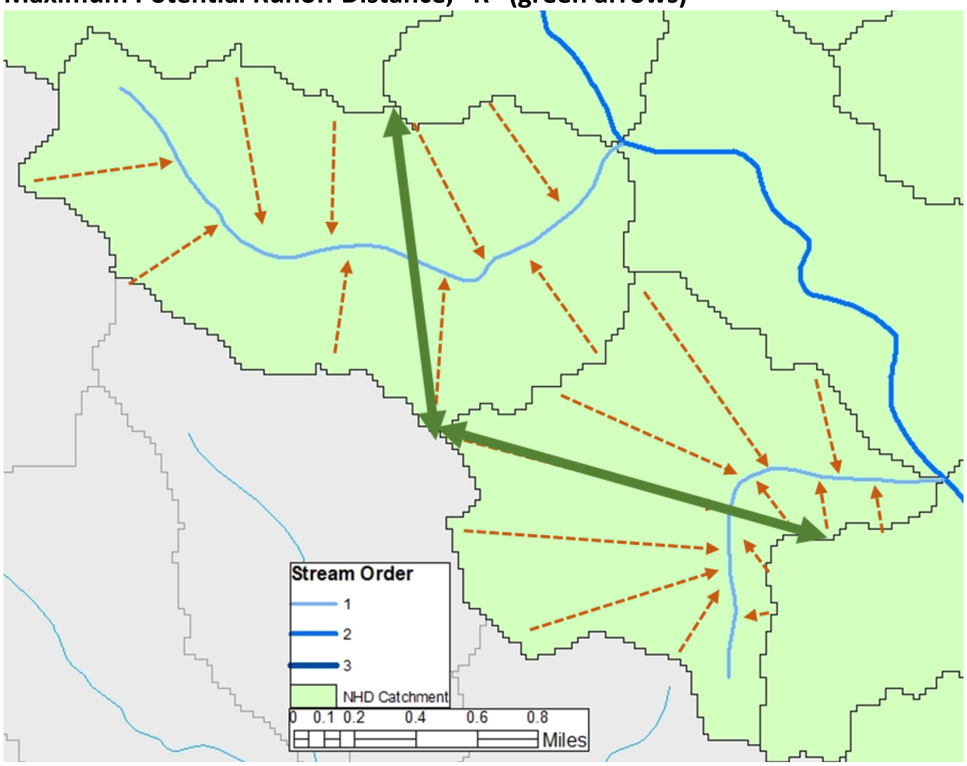
[46] National Research Council 2001. Compensating for Wetland Losses Under the Clean Water Act. Washington, DC: The National Academies Press. https://doi.org/10.17226/10134
[47] USEPA. 2015. Connectivity of Streams & Wetlands to Downstream Waters: A review & Synthesis of the Scientific Evidence. January 2015.USEPA Office of Research and Development, NCEA and NHEERL. https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=296414

(top of **Figure M-2**) and one where the stream is found close to one side of the catchment (bottom of **Figure M-2**). The furthest distance runoff could travel within a catchment is represented as the total width and it roughly represented by the second catchment. This approach uses the full width to represent the runoff distance for this reason. Under this scenario, runoff from a treated field starts on side of the catchment and travels to the far side. Not considering the location of the stream within the catchment makes the resulting distance a conservative estimate because it is assumed runoff always travels the full width of the catchment. However, actual runoff distances will vary, depending on the location of the streams within the catchment and on the locations of the treated areas from which runoff originates.

EPA used the area and perimeter of the catchment to calculate the width. After completing the width calculation for all catchments of interest, both nationally and by HUC-2, EPA used the 90th percentile value as the upper bound runoff distance. EPA considers the 90th percentile catchment width as a protective estimate to represent the runoff distance to a listed species and is consistent with other EPA metrics[48] derived from distributions.

Figure M-2. First order stream reaches with non-channelized runoff (red arrows) and estimated Maximum Potential Runoff Distance, "R" (green arrows)



The Thinness Ratio describes the relationship between a catchment's perimeter to its area based on the expected geometric parameters of a circle. By understanding this relationship between the perimeter

48 USEPA. 2020. Framework for Conducting Pesticide Drinking Water Assessments for Surface Water. September 2020.USEPA Office of Pesticide, Environmental Fate and Effect Division.

and area of the catchment the width can be estimated[49]. **Equations 1 to 3** provide standard equations for different geometric properties of a circle. It is possible to calculate the individual parameters used in these equations using geometry tools within ArcGIS 10.8.

Equation 1. Area of a circle $A = \pi r^2$
Equation 2. Perimeter of a circle $P = 2\pi r$
Equation 3. Diameter of a circle: $d = \frac{P}{\pi}$

If the catchment was a perfect circle the width could be calculated using standard tools found in ArcGIS. Due to the irregular shape of catchment width is estimated based on the deviation of the known parameters of the catchment from the expected parameters of a perfect circle. Solving for r using the perimeter equation results in a value for r that can be substituted into the area of a circle equation:

$$r = \frac{P}{2\pi}$$
$$A = \frac{P^2}{4\pi}$$

This relationship between the perimeter and area of a circle is the basis for the Thinness Ratio. For a circle, or a catchment that is a perfect circle, this Thinness Ratio would equal 1 (**Equation 4**).

Equation 4. Thinness Ratio $1 = \frac{A}{(\frac{P^2}{4\pi})}$

The width of the catchment can be estimated using the ratio of the diameter of the circle with the same perimeter as the catchment and the area of the catchment over the area of the circle with the same perimeter as the catchment (**Equation 5)**.
If the catchment shape is a circle:

$$\mathrm{w}_{catchment} * \left(\frac{P^2}{4\pi}\right) = \left(\frac{P}{\pi}\right) * A_{\mathrm{catchment}}$$

Where:

Area of a circle calculated using the perimeter of the catchment = $\frac{P^2}{4\pi}$

Diameter of a circle calculated using the perimeter of the catchment = $\left(\frac{P}{\pi}\right)$

Equation 5. Width of a polygon $\mathrm{w}_{\mathrm{catchment}} = \frac{\left(\frac{P}{\pi}\right)*A_{\mathrm{catchment}}}{\left(\frac{P^2}{4\pi}\right)}$

That is the adjusted width of a catchment equals:

[49] Van Meter, K. J., & Basu, N. B. (2015). Signatures of human impact: size distributions and spatial organization of wetlands in the Prairie Pothole landscape. *Ecological Applications*, *25*(2), 451–465. http://www.jstor.org/stable/24432315

$$\frac{\textit{Diameter of a circle with the same perimeter as the } \text{catchmen} * \textit{Area of the } \text{catchmen}}{\textit{Area of circle with the same perimerter as the } \text{catchment}}$$

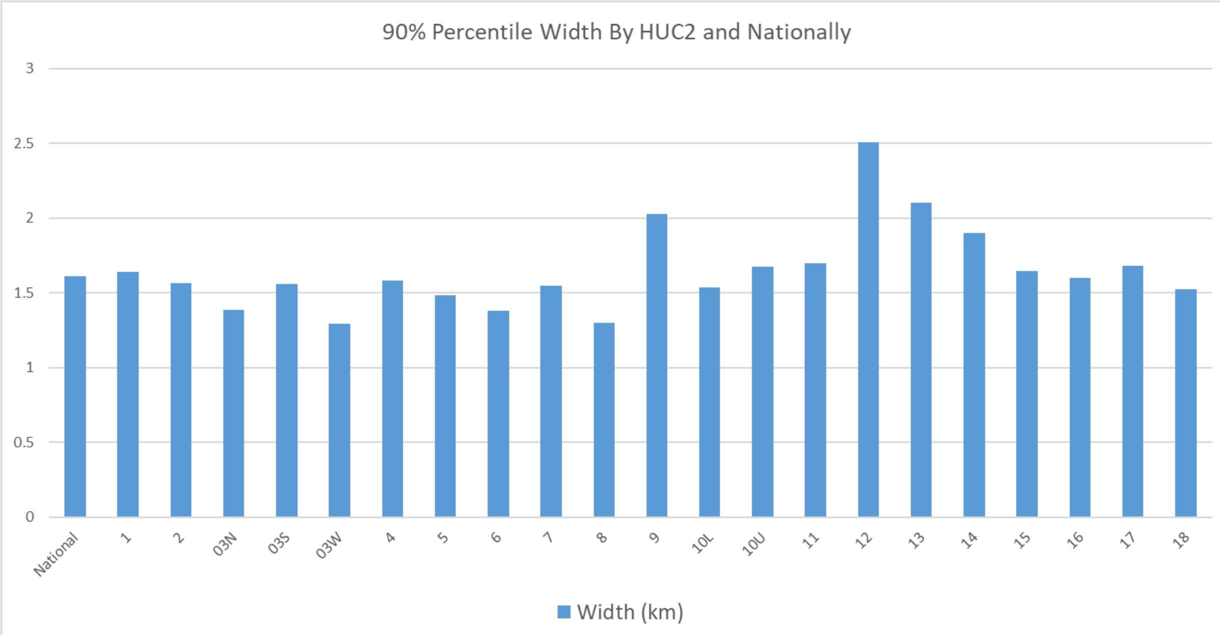
Specifically, the area and perimeter of the catchment can be calculated using standard geometry tools in ArcGIS 10.8 or extract from the tabular data from the NHDplus. Applying these values to **Equation 5** estimates the width of each catchment. The catchment attribute tables provided with the NHDplus include the area of each catchment in square kilometers, and the perimeter for each catchment was calculated in kilometers using ESRI's standard 'Calculate Geometry' tool. Prior to calculating the perimeter, the catchments data layer was projected into a coordinate system that preserves distance (North_America_Equidistant_Conic.prj: WKID: 102010).

Stream order for each stream segment is provided within the NHD flow line attribute table. Catchments associated with 1st and 2nd order stream/river segments are identified by joining the attributes for the streams and catchments using the 'FEATUREID' and 'COMID', respectively. A distribution of widths for all catchments associated with 1st and 2nd order stream or river segments were generated nationally and by Hydrologic Unit Code-2 (HUC-2) (**Table M-1**, **Figure M-3).** Given the minimal variation across HUC 2s, the 90th percentile national value of 1.6 kilometers was selected as the upper bound runoff distance. This estimated distance is used to set the action area extent. Python code used to calculate the catchment widths and filter catchments to 1st and 2nd order stream segments is available in **Attachment M1**.

Table M-1. Catchment Width in Kilometers Nationally and HUC 2.

HUC-2	90th Percentile Width (km)
National	1.6
1	1.6
2	1.6
03N	1.4
03S	1.6
03W	1.3
4	1.6
5	1.5
6	1.4
7	1.6
8	1.3
9	2.0
10L	1.5
10U	1.7
11	1.7
12	2.5
13	2.1
14	1.9
15	1.7
16	1.6
17	1.7
18	1.5

Figure M-3. 90% percentile catchment width in kilometers nationally and HUC 2.



EPA established the action area for the Enlist products using corn, cotton and soybean use data layers (UDLs) established from the USDA's Crop Data Layer. All UDLs are buffered in all directions to 1500, to account for offsite transport, in this assessment that is runoff transport or 1600 meters from the field. Prior to this assessment spray drift was the only offsite transport considered when setting the action area extent. When considering spray drift, EPA used buffered the UDLs to 1500 meter, or double the aerial spray drift limit. For this assessment the 1500 meters was used for the action area extent because the spatial analysis was completed and all species within the were identified at 90 meters or less. The spatial analysis was not updated to reflect the new 1600 meters because it would not impact the species identified within the action area for this assessment.

This upper bound estimate for runoff distance is intended to be conservative and is not expected to occur in the natural environment. This approach uses the spatially-defined NHD catchments to set the maximum potential distance runoff could move before reaching a stream channel and being transported into the next catchment. Actual distances are expected to be less, as they will be impacted by geographic factors, such as locations of treated areas, stream channels, and species or habitat locations within the catchment; physical factors, such as infiltration, surface roughness, micro-scale landscape changes that affect retention; and chemical degradation and sorption properties. This approach assumes the treated field is found on one side of the catchment and the runoff from the treated field travels to the far side of the catchment without impact by the previously stated geographic, environmental and chemical factors. EPA acknowledges that these conditions are extreme making the estimate intentionally conservative. Analyses of such conditions are more detailed, site-specific, and beyond the scope of this particular approach.

Attachment M1: Python code use to calculate the catchment width, filter dataset to 1st or 2nd stream and river segments and calculate percentiles, using Python 2.7.

```
import pandas as pd
import arcpy
from math import pi

# Extract attribute data from NHD files

fields_line = [ u'COMID', u'RESOLUTION', u'GNIS_NAME', u'LENGTHKM', u'REACHCODE', u'FTYPE', u'StreamLeve',
u'StreamOrde', u'StreamCalc', u'Pathlength', u'AreaSqKM', u'Tidal', u'MAXELEVRAW', u'MINELEVRAW',
u'MAXELEVSMO', u'MINELEVSMO', u'SLOPE', u'SLOPELENKM','VPUID']

# perimeter 'perm_calc' distance calculated in the attribute table for the catchments prior to extracting the
attribute information
fields_catchment = [ u'GRIDCODE', u'FEATUREID', u'SOURCEFC', u'AreaSqKM', u'perm_calc'])

line_array = arcpy.da.TableToNumPyArray(r"path\NHDPlusV21_National_Seamless_Flattened_Lower48.gdb\
NHDSnapshot\NHDFlowline_Network", fields_line)

cat_array = arcpy.da.TableToNumPyArray(r"path\NHDPlusV21_National_Seamless_Flattened_Lower48.gdb\
NHDPlusCatchment \Catchment", fields_catchment)

# Convert attribute table data to pandas data frames
cat_df = pd.DataFrame(data=cat_array)
line_df = pd.DataFrame(data=line_array)

# join the attributes of the catchments and flow lines
merged = pd.merge(cat_df, line_df, how='left', left_on ='FEATUREID', right_on ='COMID')

# Calculate estimated width for catchment using Equation 5 width of a polygon based on thinness ration
merged['Width'] = ((merged['perm_calc']/pi)*merged['AreaSqKM_x'])/((merged['perm_calc']**2)/(4*pi))

# Filter to 1st and 2nd order streams segments then filter stream segments to include streams and rivers only
filter = merged.loc[(merged['StreamOrde']==1)|(merged['StreamOrde']==2)].copy()
filter = filter.loc[(filter['FTYPE']=='StreamRiver')].copy()

# sorted based on with
sorted =filter.sort_values(by='Width',ascending=False)

# extract columns need to calculate percentiles, and calculate percentiles nationally and by HUC2
quantile_df = sorted[['FEATUREID','VPUID', 'AreaSqKM_x','Width']].copy()
national = quantile_df.quantile([0.99,0.95,0.9,0.8,0.7,0.6,0.5,0.4,0.3,0.2,0.1]).reset_index()
national['VPUID'] ='National'
huc2 = quantile_df.groupby('VPUID').quantile([0.99,0.95,0.9,0.8,0.7,0.6,0.5,0.4,0.3,0.2,0.1]).reset_index()
huc2.rename(columns={'level_1':'index'}, inplace=True)
common_col = [c for c in national.columns.values.tolist() if c in huc2.columns.values.tolist()]
out_quantiles = pd.merge(national,huc2,how='outer',on = common_col)
```

Appendix N. Listed Species and Critical Habitat Overlap

See **Section 4.1.2.** for methodology on determining the action area and overlap analysis.

The overlap analysis results are in the attached file "Appendix N_11-3-21.xlsx"

APPENDIX O. Generation of the ESA Agricultural Use Data Layers (UDLs) from the Cropland Data Layer (CDL)

Use Data Layers (UDLs) spatially represent application sites for agricultural and non-agricultural label uses in EPA's Endangered Species Biological Evaluations (BEs). They leverage several different landcover and land use datasets acquired from remote sensing[50] technology to create a spatial footprint for a given label use. EPA uses USDA's Cropland Data Layer[51] (CDL) for the agricultural use sites found in the conterminous United States. Updated annually, this publicly available dataset includes a robust accuracy assessment which is used by EPA to ensure the UDLs used in the BEs are of sufficient accuracy for decision making. This document provides a brief history of how this remotely sensed data is assessed for accuracy, introduces key topics related to assessing remotely sensed data, and outlines the criteria used by EPA when generating the agricultural UDLs and finally outlines the UDLs used in the Enlist One and Enlist Duo effects determination.

Introduction to Accuracy Assessments

When selecting data sources to use to create its UDLs, EPA prefers to use publicly available national level datasets; however, it may use proprietary data if it cannot identify appropriate publicly available data. By using existing datasets, EPA leverages the expertise of other agencies and organizations, rather than becoming a 'data maker'. Generally, the selected datasets follow national standards for the creation of spatial data and, in the case of remotely sensed data, include accuracy assessments. Accuracy assessments provide a measure of correctness for the data layer. Without this measure of understanding in the spatial layers, decisions based on the dataset may lead to unexpected and possibly unacceptable results (Congalton, 2019). The goal of a quantitative accuracy assessment is to identify and measure map errors so that the map can be as useful as possible to the persons making decisions.

Two distinct types of quantitative accuracy assessments exist for spatial data: positional and thematic. Positional accuracy deals with the locational correctness of a map feature by measuring how far a spatial feature on a map is from its true or reference location on the ground (Bolstad, 2005). The Federal Geographic Data Committee (FGDC) produced the U.S. National Cartographic Standards for Spatial Accuracy (NCSSA) (FGDC, 1998) to create positional accuracy standards for medium- and small-scale maps/data. When possible, EPA leverages datasets adhering to these standards. Thematic accuracy deals with the labels or attributes of the features in the resulting GIS product and will be the focus of the discussion in this document. The thematic labels or attributes are the specific cover classes assigned in the landcover dataset. Each landcover dataset targets specific types of landscape features. In the case of the UDLs, and the underlying CDL, the primary goal of the datasets is to identify cover classes that represent agricultural crops. Other remotely sensed products may target but are not limited to non-agricultural features, non-agricultural plant cover, or water features. Each of the remotely sensed

[50] Remote sensing is defined as the collection and interpretation of information about an object from a distant vantage point. Remote sensing systems involve the measurement of electromagnetic energy reflected or emitted from an object and include instruments on balloons, aircraft, satellites, and unmanned aerial systems (UAS) (Congalton, 2019).

[51] Available at USDA's National Agricultural Statistic Survey website: https://www.nass.usda.gov/Research_and_Science/Cropland/SARS1a.php

products may use the same satellite imagery, but due to the different goal of each project, the end results can differ. Thematic accuracy assessment provides measures of how different the mapped cover classes are from what occurs on the ground at specific reference locations. This is completed by comparing reference data, known/true classification of samples sites, and classified data for the same sample sites.

History of Map Making

Before the invention of aircraft, maps were created from human observations using survey equipment. Today, most map/data makers use remote sensing data rather than collecting data using field observations. To create the spatial data from remotely sensed data, decision trees algorithms use the imagery and information from known sites, referred to as training data, to generate the cover class classifications. These algorithms look for spectral signatures across multiple wavelengths to identify unique cover classes – in the CDL these are crop cover classes. Spectral signatures of various vegetation components include things such as canopy architecture, stem characteristics, leaf orientation, light angle, and shadowing of vegetation (Shah, 2019). Even though advances in technology have provided access to remotely sensed information, field observations are still important and provide information at specific sample locations, used as known data for the decision tree, or as a reference site for the accuracy assessment; rather than providing a complete survey of the project area's map extent.

Map/data making has moved to using remotely sensed data to make maps because it:

- is less expensive and more efficient than creating maps from human observations;
- offers a bird eye perspective, improving the understanding of spatial relationships and the context of our observations; and
- captures information in electromagnetic wavelengths that humans cannot see, such as the infrared portions of the electromagnetic spectrum, allowing for characterization of the landscape a human could not achieve.

However, no remotely sensed dataset is perfect. It is not possible to reach a complete one-to-one correlation between variation in remotely sensed data and the true variation found on the landscape. This means no resulting dataset will be error free. Several factors influence errors occurring in remotely sensed data, including but not limited to aircraft movement, topography, lens distortions, and other environmental factors (e.g., shadows, clouds, forest cover, snow morphology). These influences can reduce the strength of the relationships between the remotely sensed data and the landscape. However, errors are not limited to remotely sense datasets. The historical method of field observation also included errors due to factors such as observer bias, equipment malfunctions, inaccuracies from sampling errors, or goals of the projects.

Regardless of the collection method, no dataset will be error free. The accuracy assessment allows for an understanding of those errors and provides the user the necessary information to decide if the accuracy level meets their decision-making needs. As discussed above, remotely sensed data typically includes two types of accuracy assessment: positional and thematic. The use of remotely sensed data requires an understanding of both.

Positional accuracy is assessed by comparing the coordinates of sample/reference points on a map against the coordinates of the same points derived from a survey or some other independent source. The Federal Geographic Data Committee (FGDC) produced the U.S. National Cartographic Standards for

Spatial Accuracy (NCSSA) (FGDC, 1998) to create positional accuracy standards for medium- and small-scale maps/data. When possible, EPA leverages datasets adhering to these standards.

Unlike positional accuracy, there is no government or professional society standard for assessing thematic accuracy. This omission is partially due to the inherent complexity of thematic accuracy but primarily because historically, thematic accuracy was generally assumed to be at acceptable levels (Congalton, 2019). The following sections explores the history of thematic accuracy and the accuracy goals set by EPA for the UDLs in absence of the government or professional society standard.

History of Thematic Accuracy

The history of assessing thematic accuracy of maps derived from remotely sensed data is relatively brief, beginning around 1975 and was divided into four parts or epochs by Congalton in '*Assessing the Accuracy of Remotely Sensed Data'* (2019). Initially, no real accuracy assessment was performed on maps; rather, a "it looks good" mentality prevailed. This approach is typical of a new, emerging technology in which everything is changing so quickly that there is no time to assess how well you are doing. Despite the maturing of the technology over the last half century or so, some remote sensing analysts and map users still lean heavily on this mentality.

The second epoch is called the age of non-site-specific assessment. During this period, total acreages for each cover class were compared between reference estimates and measured without regard for location. It did not matter whether you knew where it was; only the how similar the total amounts were when compared. While total acreage is useful, it is equally if not more important to know where a specific landcover exists. Therefore, this second epoch was relatively short-lived and quickly led to the age of site-specific assessments.

In a site-specific assessment, reference locations for cover classes are compared with the classified cover class at the same location, and result in a measure of overall accuracy across all cover classes in the form of a 'percent correct'. This method far exceeded the non-site-specific assessment but lacked information on individual landcover categories. Site-specific assessment techniques were the dominant method until the late 1980s.

The fourth and current age of accuracy assessment is called the 'age of the error matrix'. An error matrix compares cover class information for a number of reference sites to the remotely sensed cover class results for the same location, across each cover classes in the data layer. The error matrix is a square array of numbers set out in rows and columns, accounting for each of the cover classes. Generally, the reference data cover classes are represented as the columns and the remotely sense/classified cover classes are represented by the rows. The number in each cell represent the sample sites in the corresponding cover classes from the reference data and the classified data. The major diagonal of this matrix identifies the sites where the reference and classified cover classes match, meaning the classified data correctly identified the cover class. (**Figure O-1)**.

Some key terminology when considering these matrices:

- Reference data cover classes: the class label of the accuracy assessment site derived from field or human collected data, assumed to be correct

- Classified data cover classes: the class label of the accuracy assessment site derived from the remotely sensed data.

Figure O-1. Example Error Matrix and Accuracy Values (Congalton 2019). Numbers within the bolded section of the matrix are the total number of sample sites that were identified for each cover class. In this example there are a total of 434 sample sites. The number in each cell represents the total number of sample sites found with the corresponded reference and classified cover class. For example, the 65 in the top left corner indicates that 65 samples site were identified as "D" for deciduous in both the reference and classified data. However, 65 does not account for all "D" sample sites in either classified or reference data. Moving over once cell to right, there are 4 sample sites identified as "C", conifer, in the reference data but "D" in the classified data. The classified data misidentified the cover class by including it in the incorrect category – this is an error of commission. Moving down to the cell directly below 65, there 6 sites known to be "D" from the reference data but "C" in the classified data -- here the misidentified cover class results in the exclusion from a category or an error of omission. The diagonal of the error matrix represents the number of sample sites matching in the reference and classified data. The column total provides the number of sample sites found each cover classes based on the reference data, and the row total provided the number of sample sites found in each cover class based on the classified data.

		Reference Data				
		D	C	AG	SB	row total
Classified Data	D	65	4	22	24	115
	C	6	81	5	8	100
	AG	0	11	85	19	115
	SB	4	7	3	90	104
	column total	75	103	115	141	434

Land Cover Categories

D = deciduous

C = conifer

AG = agriculture

SB = shrub

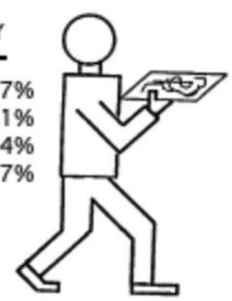
OVERALL ACCURACY = (65+81+85+90)/434 = 321/434 = 74%

PRODUCER'S ACCURACY

D = 65/75 = 87%
C = 81/103 = 79%
AG = 85/115 = 74%
SB = 90/141 = 64%

USER'S ACCURACY

D = 65/115 = 57%
C = 81/100 = 81%
AG = 85/115 = 74%
SB = 90/104 = 87%



With each annual release of the CDL, USDA provides error matrices for their thematic classification of cultivated land at both the national and state level.

The next sections provide additional details on the types of reported accuracy metrics provided with the error matrices, how the matrices are collapsed, and accuracy metrics are recalculated to represent the agricultural UDLs. Along with these descriptions is an example of the use of these metrics as outlined in **Figure O-1**.

Error Matrices, Overall, Producer's, and User's Accuracies, Kappa Statistic

Error matrices are effective representations of map accuracy, because the individual accuracies of each map cover class are plainly described on the major diagonal (i.e. classified data that matches the reference data), along with both the errors of inclusion (also referred to as "commission errors") and the errors of exclusion (also referred to as "omission errors") when the classified and reference data cover classes do not match. An omission error occurs when a sample site is left out, or omitted, from the correct classes in the classified dataset. This is considered a false positive of the classified data or Type 1 error. A commission error occurs when a sample site is included in an incorrect class in the classified dataset. This is considered a false negative/false match of the classified data or Type 2 error.

In addition to clearly showing errors of omission and commission, the error matrix can be used to compute overall accuracy, producer's accuracy, and user's accuracy, which were introduced to the remote sensing community by Story and Congalton (1986). Overall accuracy is simply the sum of the major diagonal divided by the total number of sample units, providing a 'percent correct' across all cover classes. In the example error matrix found in **Figure O-1,** the overall accuracy is the sum of the values on the major diagonal, where the classified and reference data match, divided by the total number of sample sites or 321/435; resulting in an overall accuracy of 74%. This value is the most commonly reported accuracy assessment statistic. In addition to the overall accuracy, the reporting of producer's and user's accuracies allow for additional considerations, specifically of individual cover classes.

Computed to determine individual cover class accuracies, producer's and user's accuracies provide important information related to error within the individual cover class from different perspectives. The producer of the map may want to know how well a class matched the reference data, referred to the producer's accuracy. This value is computed by dividing the value from the major diagonal (the agreement between the reference and classified data) for the class of interest, by the total number of reference data points for the class. Looking at **Figure O-1,** the map producer identified 65 sites as deciduous, while the reference data indicate there were a total of 75 deciduous sites. So, 65 of 75 samples were correctly identified, resulting in a producer's accuracy of 87%, which is quite good. However, this is only half of the story. If you now view the map from the user's perspective, a user wants to know how many classified data points matched the reference data. In **Figure O-1,** you see once again that 65 sites were classified as deciduous on the map that were actually deciduous, but the map shows a total of 115 site classified as deciduous, resulting in a user accuracy of 57%. In evaluating the accuracy of an individual map class, it is important to consider both the producer's and the user's accuracies.

The kappa statistic or coefficient is used as another measure of agreement for the resulting remotely sensed data (Cohen, 1960). This measure of agreement is based on the difference between the actual agreement in the error matrix (i.e., the agreement between the remotely sensed classification and the reference data as indicated by the major diagonal) and the chance agreement, which is indicated by the row and column totals (i.e., marginals). The kappa reflects agreement between the classified cover classes and the reference cover classes, and ranges from 0 to 1. If the kappa equals 0 than there is no agreement between the classified and references label. The closer to 1 the kappa, the closer the agreement is, and if it reaches 1 then the classified and reference data match perfectly. Ultimately, a Kappa of 0.85 means there is an 85% or better agreement than chance alone.

$$\widehat{K} = \frac{observed\ accuracy - chance\ agreement}{1 - chance\ agreement}$$

The power of kappa is in its ability to test whether one error matrix is statistically significantly different from another and not in simply reporting this value as another measure of accuracy.

Use of Accuracy Values in Understanding Thematic Errors

In the past, an overall accuracy level of 85% was often adopted as representing the cutoff between acceptable and unacceptable data. This standard was first proposed in Anderson et al. (1976) despite the lack of any research being performed to establish this standard. Accuracy depends on many factors, including the amount of effort, level of landscape or classification detail, and variability of the classes. In some instances, an overall accuracy of 85% is more than sufficient; in others it would not be accurate enough; and in others, such an accuracy would be way too expensive to ever achieve (Congalton, 2019). In the example described above and presented in **Figure O-1,** the error matrix has an overall map accuracy of 74%. This value tells about how accurate the map is, in general or across all classes, but provides no information within individual classes. For additional information on the deciduous cover class, the producer's and user's accuracies can be considered. The producer's accuracy for this class of 87% is quite good and even higher the overall accuracy of the dataset. However, if we stopped there, one might conclude that although the dataset appears to be average overall (i.e., 74%), it is more than adequate for the deciduous class. Making such a conclusion could be a serious mistake because the user's accuracy of 57% tells a different story. In other words, although 87% of the deciduous areas have been correctly identified as deciduous, only 57% of the areas called deciduous on the map are actually deciduous based on the reference data. This lower user accuracy tells us that there are errors of commission in the map related to the deciduous classes, meaning there are sample sites that were classified as deciduous that based on the reference belong to a different class. The result of this is more area in the map classified as deciduous than actually occurs on the ground.

A more careful look at the error matrix reveals significant confusion in discriminating deciduous from barren and shrub. Therefore, although the producer of this map can claim that 87% of the time an area that was deciduous on the ground was identified as such on the map, a user of this map will find that only 57% of the time that the map says an area is deciduous will it actually be deciduous on the ground, and may often be barren/scrub.

The intended use of the data/map can drive the need to address some of the error. For example, the lower user accuracy in the example above often resulted from the confusion between discriminating deciduous from barren/shrub. Collapsing these two classes together into a deciduous/barren/shrub class increase the user's accuracy to 83% but lowers the producer's accuracy to 85% (**Figure O-2)**. The higher user's accuracy means when the map identifies this grouped cover class it matches what is found on the ground more often than the two individual classes. Under certain situations it may be worth the slightly lower producer accuracy and sacrificing one of the cover classes, meaning the map will no longer distinguish between deciduous and shrub/barren.

Figure O-2. Example Collapsing Cover Class to Address Error of Commission, Building off the Error Matrix in Figure 1. Here, the Deciduous and Barren/Shrub are Combined and Accuracy Metric Recalculated.

	D/SB	C	AG	Row total
D/SB	183 (65+4 +114)	11	25	219
C	14 (6+8)	81	5	100
AG	19 (0+19)	11	85	115
Column total	216	103	115	434

Producer's accuracy = $\frac{183}{216} = 85\%$ User's accuracy = $\frac{183}{219} = 83\%$

For the purposes of the UDL, EPA's targets at least 85% in both the producer's and user's accuracy and at least 90% for an overall accuracy when combining individual crops from the CDL into the UDL cover classes.

EPA's Accuracy Value Goals for Use Data Layers Used in BEs

The native CDL landcover dataset includes over 100 cultivated cover classes in its thematic classification. The error matrices released with the CDL data provide overall, producer and user measures of accuracy at both the state and national level as well as the associated Kappas. In recent years, the overall accuracy of the CDL dataset has been in the low to mid-80% with Kappa just over 0.80. The producer's and user's accuracy for the individual cultivated classes range from less than 5% to 98%, and less than 15%-97%, respectively (Boryan, 2011). When considering the individual cultivated classes of the CDL, the user's accuracy is slightly better than producer's accuracy, resulting in a lower commission error, or false negative/Type 2 error. However, when considering these BEs, reducing the false positive/Type 1 error is equally or more important. Improving all accuracy metrics as well as leveling out the producer and user accuracies is an overall goal when grouping crops into the UDLs cover classes.

To improve the overall, user and producer accuracies for the UDLs, the 100+ thematic cultivated classes found in CDL are reclassified into 13 crop groupings. Consolidating CDL into aggregated categories is a documented way to significantly improve the accuracy of assessments by eliminating misclassification errors within the combined classes (Johnson, 2013a; Johnson, 2013b; Wright, 2013; and Lark, 2017). Each of the 100+ thematic cultivated classes from the original CDL, are found in at least one state but not every state will include all 100+ classes. For this reason, while the focus is on the accuracy at the national level, there are instances when the state accuracy for a UDL would be higher than observed at the national level.

When deciding how to group crops from the CDL, EPA refers to the grouping used by the U.S. Geological Survey (Baker and Capel, 2011) and the Generic Endangered Species Task Force (Amos *et al.*, 2010). This information considers environmental factors that influence the location of crops and the error matrices provided by USDA with the original CDL data. By considering these agronomic factors in addition to the error matrices it is possible to improve the accuracy for these UDLs while retaining agronomic similarities. There is an infinite number of ways to group the crop cover classes found in the CDL, and each structured grouping can be reviewed in terms of recalculated accuracy compared to the native dataset.

When collapsing the available error matrices provided with the CDL into the 13 UDL groups, the sample site values for each of the CDL crops found in a UDL are summed across both rows and columns in the error matrix. Currently the 13 UDL groups bring the overall accuracy to 90%, increased from 80% for the CDL, with a Kappa of 0.88 (**Table O-1**). As described above, it is important to consider the producer's and user's accuracy of the individual thematic classes in addition to the overall accuracy.

When considering the user's and producer's accuracy, EPA targets at least 85% for each UDL, while retaining at least a 90% overall accuracy. Following the thematic grouping into the 13 UDLs and the recalculation of the user and producer accuracies, by year of the CDL, to help address errors of commission, additional steps to lower the omission errors, are implemented. These include the temporal aggregation of multiple CDL years into the UDL and expanding the crop area found in the UDL layer to meet or exceed the area for the same suite of crops as reported in the Census of Agriculture. The goal of each of these steps is to improve the accuracy of the UDLs by minimizing the rate of omission error. However, these steps are not directly related to the existing error matrices provided with the CDL, and therefore new accuracy values are not calculated following the temporal aggregation, and area expansion. By reducing the omission errors, these steps result in a more protective landcover classification for each UDL.

If an individual crop class in the CDL has both the producer and user accuracies that are over 85%, the corresponding UDLs is that same as the CDL crop cover class, for example cotton from the CDL is found in the cotton UDL. These UDLs include corn, cotton, grapes/other vineyards, rice, soybeans and wheat. Five of these UDLs have user and producer accuracies in the low to mid 90%, with Kappas ranging from ~0.89 to 0.97. The user's and producer's accuracy for the remaining cotton UDL falling above 85% with Kappas of ~ 0.85. Due to the geographically limited occurrence of cotton, this crop is only grown in the south, lower national accuracy is expected compared to other crops with a broader geographic range. This is due to the fact that cotton growing states may classify cotton well, however, there is a lower accuracy in identifying cotton in states where cotton doesn't grow, and this brings down the national accuracy.

When an individual crop cover class in the CDL is below 85%, grouping multiple crops together and ultimately reducing the number of total thematic crop groups, improves the accuracy of the resulting UDL. When deciding which crops to group, error of omission and commission of the remotely sensed data are considered, in addition to environmental and agronomic practices. EPA targets an accuracy of at least 85%; however, it is not always possible to reach the target without compromising the environmental/agronomic practices. For this reason, some of the UDLs that contain multiple crop classes have slightly lower than 85% accuracy.

The UDLs containing a number of crops include alfalfa/other agricultural grasses, citrus, other crops, other grains, other orchards, other row crops, and vegetables and ground fruit. Two of these UDLs, other crops and other grains, did not meet an 85% accuracy for user's and producer's accuracy. Two additional UDLs, other row crops and vegetables and ground fruit, did not reach 85% for just the producer's accuracy. See **Table O-1** for a complete list of accuracy values across all 13 UDLs. Of the 13 UDLs 3 were used to map the agricultural label uses for the Enlist One and Enlist Duo effects determination.

A list of the pertinent UDLs can be found in **Figure O-3.** As mentioned above, the focus of the discussion is on the national accuracies. But due to the variety and regional nature of some crops found in the

UDLs, state based accuracy assessments often reach 85% even though the national level assessment for the same UDL does not.

Additional challenges when identifying some crops include higher frequency of change in agricultural practices (*e.g*., crop rotation), and/or lower total area on the landscape for minor crops. These two challenges are related to errors of omission, rather than errors of commission addressed by grouping crops into the UDL categories a common practice implemented to increase accuracy of remotely sensed data (Johnson, 2013a; Johnson, 2013b; Wright, 2013; and Lark, 2017). Two additional steps address some of the uncertainty related to these errors of omission, specifically, the known downward estimates of acres for remotely senses data and changes in crop patterns over time. These steps are implemented on all UDLs, but have the most impact in addressing uncertainty around error of omission for the UDLs containing multiple crops with lower accuracy values. First, a temporal aggregation of multiple years of the CDL into the UDLs is performed to account for changing agricultural practices, for example crop rotation, from year to year. Second, the total area of the temporally aggregated UDL is compared to the reported area found in the Census of Agriculture, accounting for some of the error/difficulty in identifying minor crops. If the area of the UDL is less than the reported area in the Census of Agriculture, the UDL is grown out to meet or exceed the Census of Agriculture. Referred to as region growing, expanding the UDL area to meet or exceed the area reported in the Census of Agriculture is a conservative measure take to minimize the error of omission. However, the Census of Agriculture generated once every 5 years, represents a single year in time. The CDL generated every year may capture agricultural practices, such as rotations, not captured in the Census Agriculture. For this reason, there is uncertainty around the crop area found in the Census of Agriculture being representative across all years of the CDL.

At the end of the whole process, the resulting UDLs provide a more protective landcover estimate for the purposes of the Endangered Species Biological Evaluations, making them the best available spatial agricultural data to use in the ESA BEs.

Figure O-3 provide a summary of the UDLs used to map the agricultural label uses for the Enlist One and Enlist Duo effects determination with a complete crosswalk of the original CDL crops to the UDL class provided in **Table O-2.**

Table O-1. Collapsed National Error Matrix from the 2018 CDL, Example of the 3 National UDLs with Associated Measures of Accuracy

	Corn	Cotton	Soybeans	**User's Accuracy**	**Commission**
Corn	4222089	6598	124498	97%	6%
Cotton	9800	974234	36809	95%	13%
Soybeans	139339	54449	4754850	96%	7%
Producer's Accuracy	97%	94%	97%		
Omission	7%	11%	6%		
		Overall Accuracy	96%		

Figure O-3. Summary of Use Data Layer Classes for the Enlist One and Enlist Duo effects determination.

Summary of Use Data Layers (UDL) Classes

Reclass Value	UDL General Classes
10	Corn
14	Corn/soybeans
15	Corn/wheat
18	Corn/grains
20	Cotton
25	Cotton/wheat
26	Cotton/vegetables
40	Soybeans
42	Soybeans/cotton
45	Soybeans/wheat
48	Soybeans/grains

These classes are not mutually exclusive to one another and are further reclassified into 13 national agricultural UDL classes. 3 UDLs are used to map Enlist One and Enlist Duo labelled agricultural uses. The complete crosswalk for all 13 UDL classes can be found in ***Table 2.***

Corn: 10, 14, 15, 18

Cotton: 20, 25, 26, 42

Soybeans: 40, 42, 45, 48, 14

Table O-2. Cross-walk Between CDL Class and UDL Agricultural Classes

CDL Value	CDL Class Name	Reclass Category for UDLs	Double Crop (Y)	Reclass Code
1	Corn	Corn		10
2	Cotton	Cotton		20
3	Rice	Rice		30
4	Sorghum	Other grains		80
5	Soybeans	Soybeans		40
6	Sunflower	Other row crops		90
10	Peanuts	Other row crops		90
11	Tobacco	Other row crops		90
12	Sweet Corn	Vegetables and ground fruit		60
13	Popcorn Corn	Vegetables and ground fruit		60
14	Mint	Vegetables and ground fruit		60
21	Barley	Other grains		80
22	Durum Wheat	Wheat		50
23	Spring Wheat	Wheat		50
24	Winter Wheat	Wheat		50
25	Other Small Grains	Other grains		80
26	Double Crop Winter Wheat/Soybeans	Soybeans/Wheat	Y	45
27	Rye	Other grains		80
28	Oats	Other grains		80
29	Millet	Other grains		80
30	Speltz	Other grains		80
31	Canola	Other grains		80
32	Flaxseed	Other grains		80
33	Safflower	Other grains		80
34	Rape Seed	Other grains		80
35	Mustard	Vegetables and ground fruit		60
36	Alfalfa	Alfalfa/agricultural grasses		110
38	Camelina	Other grains		80
39	Buckwheat	Other grains		80
41	Sugarbeets	Other row crops		90
42	Dry Beans	Vegetables and ground fruit		60
43	Potatoes	Vegetables and ground fruit		60
44	Other Crops	Other crops		100
45	Sugarcane	Other grains		80
46	Sweet Potatoes	Vegetables and ground fruit		60
47	Misc Vegs & Fruits	Vegetables and ground fruit		60
48	Watermelons	Vegetables and ground fruit		60
49	Onions	Vegetables and ground fruit		60

CDL Value	CDL Class Name	Reclass Category for UDLs	Double Crop (Y)	Reclass Code
50	Cucumbers	Vegetables and ground fruit		60
51	Chick Peas	Vegetables and ground fruit		60
52	Lentils	Vegetables and ground fruit		60
53	Peas	Vegetables and ground fruit		60
54	Tomatoes	Vegetables and ground fruit		60
55	Caneberries	Vegetables and ground fruit		61
56	Hops	Other row crops		90
57	Herbs	Vegetables and ground fruit		60
58	Clover/Wildflowers	Other crops		100
59	Sod/Grass Seed	Other crops		100
60	Switchgrass	Alfalfa/agricultural grasses		110
61	Fallow/Idle Cropland	Other crops		100
66	Cherries	Other orchards		70
67	Peaches	Other orchards		70
68	Apples	Other orchards		70
69	Vineyards	Vineyards		71
70	Christmas Trees	Other trees		75
71	Other Tree Crops	Other orchards		70
72	Citrus	Citrus		72
74	Pecans	Other orchards		70
75	Almonds	Other orchards		70
76	Walnuts	Other orchards		70
77	Pears	Other orchards		70
92	Aquaculture	Other crops		100
141	Deciduous Forest	Forest		140
142	Evergreen Forest	Forest		140
143	Mixed Forest	Forest		140
152	Shrubland	Shrubland		160
204	Pistachios	Other orchards		70
205	Triticale	Other grains		80
206	Carrots	Vegetables and ground fruit		60
207	Asparagus	Vegetables and ground fruit		60
208	Garlic	Vegetables and ground fruit		60
209	Cantaloupes	Vegetables and ground fruit		60
210	Prunes	Other orchards		70
211	Olives	Other orchards		70
212	Oranges	Citrus		72
213	Honeydew Melons	Vegetables and ground fruit		60
214	Broccoli	Vegetables and ground fruit		60
216	Peppers	Vegetables and ground fruit		60

CDL Value	CDL Class Name	Reclass Category for UDLs	Double Crop (Y)	Reclass Code
217	Pomegranates	Other orchards		70
218	Nectarines	Other orchards		70
219	Greens	Vegetables and ground fruit		60
220	Plums	Other orchards		70
221	Strawberries	Vegetables and ground fruit		61
222	Squash	Vegetables and ground fruit		60
223	Apricots	Other orchards		70
224	Vetch	Alfalfa/agricultural grasses		110
225	Double Crop Winter Wheat/Corn	Corn/Wheat	Y	15
226	Double Crop Oats/Corn	Corn/Grains	Y	18
227	Lettuce	Vegetables and ground fruit		60
229	Pumpkins	Vegetables and ground fruit		60
230	Double Crop Lettuce/Durum Wheat	Wheat/Vegetables	Y	56
231	Double Crop Lettuce/Cantaloupe	Vegetables and ground fruit		60
232	Double Crop Lettuce/Cotton	Cotton/Vegetables	Y	26
233	Double Crop Lettuce/Barley	Vegetables/Grains	Y	68
234	Double Crop Durum Wheat/Sorghum	Wheat/Grains	Y	58
235	Double Crop Barley/Sorghum	Other grains		80
236	Double Crop Winter Wheat/Sorghum	Wheat/Grains	Y	58
237	Double Crop Barley/Corn	Corn/Grains	Y	18
238	Double Crop Winter Wheat/Cotton	Cotton/Wheat	Y	25
239	Double Crop Soybeans/Cotton	Soybeans/Cotton	Y	42
240	Double Crop Soybeans/Oats	Soybeans/Grains	Y	48
241	Double Crop Corn/Soybeans	Corn/Soybeans	Y	14
242	Blueberries	Vegetables and ground fruit		61
243	Cabbage	Vegetables and ground fruit		60
244	Cauliflower	Vegetables and ground fruit		60
245	Celery	Vegetables and ground fruit		60
246	Radishes	Vegetables and ground fruit		60
247	Turnips	Vegetables and ground fruit		60
248	Eggplants	Vegetables and ground fruit		60
249	Gourds	Vegetables and ground fruit		60
250	Cranberries	Vegetables and ground fruit		61
254	Double Crop Barley/Soybeans	Soybeans/Grains	Y	48

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APPENDIX P. Enlist One and Enlist Duo May 2021 Product Labels

The risk assessment and effects determination relied upon the attached product labels dated 5/14/2021.

Enlist One label in attached file "Enlist One-695 MSTR 14May21d_revised.pdf"

Enlist Duo label in attached file "Enlist Duo-649 14May21d_revised.pdf"