Draft Biological and Conference Opinion on the Registration Review of Simazine Pursuant to the Federal Insecticide, Fungicide, and Rodenticide Act

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List of Abbreviations

A

Animal and Plant Health Inspection Service

(APHIS)

B

Biological Evaluation

(BE)

C

Census of Agriculture

(CoA)

Center for Biological Diversity

(CBD)

Comprehensive Environmental Response, Compensation and Liability Act

(CERCLA)

Crop Data Layer

(CDL)

D

E

ecotoxicology database

(ECOTOX)

Endangered Species Act

(ESA)

Environmental Protection Agency

(EPA)

F

Federal Insecticide, Fungicide, and Rodenticide Act

(FIFRA)

H

hazardous concentration 5th percentile

(HC05)

L

lethal concentration 50%

(LC50)

lethal dose 50%

(LD50)

Lowest Observed Adverse Effect Concentration

(LOAEC)

M

Master Record Identifier

(MRID)

may affect, not likely to adversely affect

(NLAA)

N

National Academies of Science

(NAS)

National Agricultural Statistics Service

(NASS)

National Marine Fisheries Service

(NMFS)

National Research Council

(NRC)

no effect

(NE)

No Observed Adverse Effect Concentration

(NOAEC) ....

P

R

S

(SLN).. 11

Species Sensitivity Distribution

(SSD)..

Surface Mining Control and Reclamation Act

(SMCRA) ....

U

U.S. Army Corps of Engineers

(USACE) ....

U.S. Department of Agriculture

(USDA) ....

U.S. Fish and Wildlife Service

INTRODUCTION

This document represents the U.S. Fish and Wildlife Service's (Service) Biological and Conference Opinion (Opinion) based on our review of the Environmental Protection Agency's (EPA) proposed national registration of simazine and its effects on endangered and threatened species and designated critical habitat (CH) in accordance with section 7(a)(2) of the Endangered Species Act (ESA) of 1973, as amended (16 U.S.C. 1531 et seq.). On November 12, 2021, EPA submitted the necessary information and a request to initiate formal section 7 consultation.

We based this Opinion on information in the final Biological Evaluation (BE) for simazine (USEPA 2021), many interagency meetings, meetings with stakeholders, conference calls, and other sources of information as described herein. The methods employed in EPA's BE follow the Revised Method for National Level Listed Species Biological Evaluations of Conventional Pesticides (referred to as the "Revised Method")[1]. In March 2020, EPA released the Revised Method for National Level Listed Species Biological Evaluations of Conventional Pesticides. EPA used the Revised Method to conduct the draft BE for simazine. The Revised Method incorporates recommendations from the National Research Council (NRC) of the National Academies of Science (NAS) for the process EPA developed with the Service and National Marine Fisheries Service (NMFS) for determining effects from the action to listed species and critical habitats. A preliminary approach developed in 2015 is referred to as the Interim Method, which was applied to the first three national-level pilot BEs (for chlorpyrifos, diazinon and malathion; discussed in more detail below in the Consultation Background section). EPA's "lessons learned" during the three pilot BEs provided the starting point for development of the Revised Method via public comments provided through stakeholder meetings, through the docket on the draft BEs for chlorpyrifos, diazinon and malathion, and through the docket on the proposed Revised Method; comments received during consultation with federally recognized tribes; and comments provided by the Service, NMFS, and the U.S. Department of Agriculture (USDA). On November 5, 2020, EPA released the draft BE for simazine for public comment. EPA received public comments on the simazine BE through January 5, 2021. Updates that were specific to simazine were incorporated into the final BE. A complete record of this consultation is on file at the Services' Headquarters office in Falls Church, Virginia.

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

[1] Available at: https://www.epa.gov/endangered-species/revised-method-national-level-listed-species-biological-evaluations-conventional

CONSULTATION BACKGROUND

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

Pesticide Litigation Summary

The pesticide lawsuits against the Service were preceded by lawsuits against EPA for failure to consult on pesticide registrations. The first of these suits, filed in 2002, alleged failure to consult on the effects of 66 pesticides on the California red-legged frog in *CBD v. Johnson*, No. 02-cv-1580-JSW (N.D. Cal.). The Center for Biological Diversity (CBD) and EPA settled this suit in 2006, and EPA agreed to make effect determinations on the 66 pesticides. Between October 2007 and October 2008, EPA requested initiation of formal consultation on the effects of more than 30 pesticides on the California red-legged frog. As mentioned above, the Agencies did not agree on the approach to assess the risk of pesticides on endangered and threatened species, and in a letter dated January 14, 2009, the Service informed EPA that we did not have the necessary information to initiate formal consultation.

The CBD filed a second lawsuit in 2007, *CBD v. EPA*, No. 3:07-cv-02794-JCS (N.D. Cal.), in which the plaintiff sought to compel EPA to initiate consultation on the effects of 75 pesticides on 11 federally endangered and threatened species in the San Francisco Bay area and to enjoin EPA from permitting the use of the pesticides in the area until consultation was completed. In May 2010, EPA and the CBD reached a settlement. EPA agreed it would complete effects determinations, under a set schedule, on the 75 pesticides and initiate consultation on pesticides for which "may affect" determinations were made. By July 2013, EPA had completed effects determinations for all but 16 of the 75 chemicals. In 2015, the parties amended their agreement to allow EPA to focus its effects determinations on four pesticides (atrazine, simazine, propazine, and glyphosate) for all endangered and threatened species and to complete BEs for the identified pesticides by June 30, 2020.

The Service became a part of the litigation in 2011 when the CBD filed a complaint against the Service and EPA, (*CBD v. FWS*, No. 3:11-CV-5108-JSW [N.D. Cal.]). The suit alleged failure to consult on the effects of 64 pesticides on the California red-legged frog. On November 4, 2013, the CBD, the Service, and EPA agreed to complete consultation on the effects of two pesticides on the California red-legged frog within a year of the court's approval of the agreement and on an additional five pesticides within 2 years. Following the NAS report and recommendations on the pesticide consultation process (described further below), the Agencies decided it would be more effective and efficient to conduct national consultations on the effects of individual pesticides on all protected resources pursuant to the ESA rather than consult on multiple pesticides considering only one or a few species at a time. On July 28, 2014, the CBD agreed to amend the 2013 settlement agreement so that EPA and the Service could conduct nationwide consultations on five pesticides (chlorpyrifos, diazinon, malathion, carbaryl, and methomyl) rather than focus on the effects of seven pesticides on the California red-legged frog.

On February 24, 2022, the Center for Biological Diversity (CBD) filed a complaint against FWS and the Secretary of the Department of the Interior, alleging violations of the Administrative Procedure Act ("APA") for unreasonably delaying completion of ESA section 7 consultation on EPA's FIFRA registration of the uses of pesticide products containing chlorpyrifos and diazinon. *Ctr. for Biological Diversity v. U.S. Fish & Wildlife Serv., et al.*, 772 F. Supp. 3d 1074 (D. Ariz. Mar. 12, 2025). On January 12, 2024, CBD filed a motion to amend the complaint to include additional unreasonable delay claims for four consultations involving other pesticide registrations (i.e., carbaryl, methomyl, atrazine, simazine). On March 12, 2025, the Court ordered FWS to issue biological opinions for these pesticide registration reviews on the following schedule: by March 31, 2025, for carbaryl; March 31, 2026, for atrazine and simazine; and by September 30, 2028, for chlorpyrifos and diazinon. At the time of the order, the Service had already issued a biological opinion for methomyl in December 2024.

NAS Report and Path Forward

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

(1) identifying best available scientific data and information;

(2) considering sublethal, indirect and cumulative effects;

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

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

(5) incorporating uncertainties into the evaluations effectively; and

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

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

1. In Step 1, EPA makes the no effect/may affect determination. If EPA determines that a pesticide's registration will have no effect on any endangered or threatened species or their designated critical habitats, it may move forward with a pesticide's registration without further consultation with the Service or NMFS. We review EPA's no effect determinations for species and designated critical habitats and adopt their determinations unless otherwise noted in the *Supporting Information for the Concurrence Section of the Consultation* (Appendix A).

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

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

CONSULTATION HISTORY

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

Early Coordination on EPA's Biological Evaluation:

November 05, 2020	EPA releases the draft BEs for atrazine, simazine, and propazine for public comment

June 8, 2021	EPA issues final cancellation order for propazine, which terminated all propazine products registered in the U.S.
November 12, 2021	EPA provides the Service with the final BEs for atrazine and simazine
February 9, 2022	The Service received the consent of EPA and the 3 technical registrants (who are "applicants" to the consultation), Syngenta, Drexel Chemical Company (Drexel), and Sipcam Agro, to extend the timeframe for completing the simazine consultation, pursuant to ESA Section 7(b).
January 29, 2025 – September 30, 2025	EPA, the Service, and simazine registrants meet regularly to discuss simazine where topics include: • Progress on Simazine Registration Review and Listed Species Assessments • Updates on simazine since the BE • Species/critical habitat analysis approach • Deliverables • Timeline • EPA NLAA/LAA/NE determinations and lists • Application of EPA's Herbicide Strategy to reduce exposure to listed species from agricultural use of simazine and the extent that effects remain after relevant mitigations are applied

CONCURRENCE

In their BE and subsequent correspondence for simazine, EPA provided determinations of "no effect (NE)" for 610 proposed and listed species and 433 proposed and designated critical habitats (see Appendix A, Table 1). Similarly, we concurred with EPA's "may affect, not likely to adversely affect (NLAA)" determinations for 346 listed and proposed species entities and 165 proposed and designated critical habitats under Service jurisdiction. Our discussion of these species and critical habitats is provided in Appendix A.

BIOLOGICAL OPINION

DESCRIPTION OF THE PROPOSED ACTION

The proposed federal action (hereafter, the proposed action) is the registration review of simazine under FIFRA. Pursuant to FIFRA, before a pesticide product may be sold or distributed in the U.S., it must be exempted or registered with a label identifying approved uses by EPA's Office of Pesticide Programs. Once registered, a pesticide may not legally be applied within the U.S. unless the use is consistent with directions on approved label(s) associated with products containing that pesticide. The EPA authorization of pesticide uses is categorized as FIFRA section 3 (new product registrations), section 18 (emergency use), or 24(c) Special Local Needs (SLN). FIFRA requires pesticides registered under section 3 and section 24(c) to have their registrations reviewed periodically. For simazine, the proposed action only includes active section 3 and 24(c) registrations (there are no registered or proposed Section 18 registrations for simazine at this time). As EPA has adopted a 15-year timeframe to review pesticides, the Service considers the duration of the proposed action to be 15 years.

For simazine, the proposed action includes registration review of the uses, as described by product labels, of all products making pesticidal claims and containing simazine as the active ingredient. There are five active registrants (or "applicants") with simazine products: Amvac Chemical Corporation, Drexel Chemical Company, Sipcam Oxon S.P.A, Syngenta Crop Protection LLC, and Winfield Solutions. Those registrants hold 21 registrations of products with simazine as an active ingredient. A complete listing of products and their registrant contact information is found in Table 2 of this Opinion. This section describes the proposed action, which is based upon the 21 registrations for simazine.

Chemical-Specific Uses

Simazine is registered for use as a pre- and post-emergence herbicide that is intended to control broadleaf and grassy weeds. Simazine is registered for Section 3 use throughout the conterminous U.S. (CONUS) for a variety of agricultural use patterns, including pome fruit, stone fruit, tree nuts, citrus, berries, grapes, corn, and sod farms. The SLN registrations for simazine allow use in Oregon and Washington on alfalfa (grown for seed) and cole crops (grown for seed). Simazine is also registered for Section 3 use on the following non-agricultural use patterns within the CONUS: turf (lawns and golf courses), nurseries and ornamental ponds. There are 21 registered simazine-containing products (Table 1); including: twelve section 3 end-use product (EP) labels, five SLN (24(c) EP labels, and four technical product labels[2]. The focus of EPA's BE is on the twelve section 3 and five SLN labels because technical products do not have direct outdoor uses[3]. EPA considered all section 3 and SLN labels in the BE as part of the action for simazine[4]. Simazine-containing products with agricultural uses include formulations that are emulsifiable concentrate, flowable concentrate, and water dispersible granules. Three of the twelve section 3 end use labels are co-formulations with other herbicide active ingredients. Two are co-formulated with atrazine, and a third with both imazaquin and prodiamine. Most of the labeled products allow tank mixing with those previously mentioned active ingredients, as well as other active ingredients and/or fertilizers.

Table 1. Summary of registered simazine uses that apply to individual Use Data Layers. Also included are annual usage data, including pounds (lbs) applied per year and acres treated (by UDL).

Use Data Layer (UDL)	Registered uses	Geographic restriction	Max annual application rate (lbs ai/acre)	Average annual lbs ai applied**	Average annual acres treated**
Agricultural uses					
Corn	Corn	CONUS	2.5	2,300,000	2,200,000
Other Orchards	Almonds Apples Avocados Cherries Hazelnut Macadamia nut Nectarines	CONUS	2-4	232,000	130,000

[2] An EP is a pesticide product whose labeling (1) includes directions for use of the product (as distributed or sold, or after combination by the user with other substances) for controlling pests or defoliating, desiccating or regulating growth of plants, and (2) does not state that the product may be used to manufacture or formulate other pesticide products.

[3] A manufacturing use product is any pesticide product other than an end use product. A product may consist of the technical grade of active ingredient only, or may contain inert ingredients such as stabilizers or solvents (40 CFR § 153(h)). MPs are intended and labeled for formulation and repackaging into other pesticide products.

[4] With one exception: A new SLN label was registered in 2022 that allowed simazine use on mixed green in Oregon. Since this use was previously allowed in Washington state, the modeling for Washington is considered representative for Oregon. Overlap data for the other crops UDL was already included for both Washington and Oregon.

Use Data Layer (UDL)	Registered uses	Geographic restriction	Max annual application rate (lbs ai/acre)	Average annual lbs ai applied**	Average annual acres treated**
	Olives Peaches Pears Pecans Plums Walnuts				
Citrus	Oranges Lemons Grapefruit	CONUS	4-8	228,000	103,000
Grapes	Grapes	CONUS	4	160,000	98,000
Vegetables and Ground Fruit	Blackberry Blueberry Boysenberry Sweet corn Cranberry Loganberry Raspberry Strawberry	CONUS	2-4	35,000	25,000
Other Crops	Sod farms	CONUS	6	30,000	40,000
Other Crops	Alfalfa* Broccoli* Brussels sprouts* Cabbage* Chinese cabbage*	OR and WA	1.6-2	Not surveyed at the national level	Not surveyed at the national level

Use Data Layer (UDL)	Registered uses	Geographic restriction	Max annual application rate (lbs ai/acre)	Average annual lbs ai applied**	Average annual acres treated**
	Cauliflower* Kale* Kohlrabi* Mustard* Radish* Rutabaga* Turnip greens*				
Christmas trees	Christmas trees	CONUS	4	Not available	Not available
Non-agricultural uses					
Developed	Lawns	Limited to parts of CONUS where warm season grasses grow	6	70,000[5]	30,000[5]
Open Space Developed	Golf courses	Limited to parts of CONUS where warm season grasses grow	6	200,000[5]	40,000[5]
Nurseries	Conifers, deciduous trees and	CONUS	4	Not available	Not available

[5] These data gathered by EPA are based on 2014 survey results from a market research firm and may no longer be applicable. The information we provide specific to lawn care applications of simazine with respect to species and critical habitat Integration and Synthesis analyses and in the Integration and Synthesis Section of this Opinion is based on information and discussions the Service and EPA have had with the Golf Course Superintendents Association of America, professional turf grass scientists, and lawn care professional industry experts in 2025.

Use Data Layer (UDL)	Registered uses	Geographic restriction	Max annual application rate (lbs ai/acre)	Average annual lbs ai applied**	Average annual acres treated**
	woody ornamental species				

*grown for seed

**see appendix 1-4 of BE

Environmental Fate

Once applied, the main routes of dissipation for simazine are microbial degradation under aerobic conditions, runoff, and leaching. Simazine's primary transport routes from treated areas to aquatic habitats include spray drift and runoff. Simazine is expected to move into surface and ground water given its persistence and mobility. Simazine has a low vapor pressure (6.1×10^{-9} at 20°C (Torr)) and low Henry's Law Constant (3.2×10^{-10} atm-m^3/mole; calculated at 25°C), suggesting low potential for volatilization. Simazine is moderately soluble in water with reported aqueous solubility values ranging from 3.5 to 11 mg/L at 20-25ºC. Bioaccumulation of simazine is expected to be low due to a low octanol water partitioning coefficient and low bioconcentration factors.

Environmental fate data indicate that deisopropylatrazine (DIA) and hydroxysimazine (HS) are the major transformation products of simazine (>10% applied a.i.) and that diadealkylatrazine (DDA) is a minor chlorotriazine degradation product (<10% applied a.i.). EPA determined that aquatic modeling of the parent compound alone for simazine is adequate for determining potential exposure concentrations to aquatic organisms, and we agree with that assessment. In the terrestrial environment, EPA considered formation of transformation products, through the consideration of toxicity data and foliar dissipation half-lives. More details on the environmental fate properties of simazine and how they relate to exposure are discussed in the *Exposure* section of this Opinion.

Simazine has demonstrated adverse effects on growth to aquatic and terrestrial plants, which is expected because simazine is an herbicide. For animals, simazine does not generally pose a concern for mortality from acute exposure. On a chronic exposure basis for animals, simazine may lead to adverse effects (depending on the exposure). In both terrestrial and aquatic animals, toxicity studies indicate that simazine may lead to growth, reproductive and survival effects at a range of chronic exposure concentrations. Available ecotoxicity data for transformation products shows similar or lower toxicity in comparison to simazine.

Geographic Scope

The twelve registered section 3 labels for simazine may be used throughout the CONUS; however, use is not permitted in Alaska, Hawaiʻi or the U.S. territories. The five SLN labels are registered for use in Washington and Oregon only.

Maps of the potential use sites and offsite locations binned by all agricultural and all non-agricultural areas are depicted in Figure 1 and Figure 2. These maps are developed using the Use data layers (UDLs) that are representative of the potential use sites for which simazine is registered. EPA did a cross-walk of the registered uses (both agricultural and non-agricultural) and available UDLs to represent potential use sites of simazine (see Appendix 1-6 of the BE and Table 2). The UDLs representing simazine registered agricultural and non-agricultural uses throughout the CONUS are included in Table 1.For the special local needs uses in Oregon and Washington, we considered the Other Crops UDL only within those two states because the five SLNs labels are for crops that are grown for seed, which is included in this UDL. It should be noted that the footprint for the Other Crops UDL overestimates the spatial footprint for crops grown for seed and treated with simazine because it includes crops that are not registered for simazine use.

The potential area of exposure includes the potential use site areas identified by the UDLs plus an off-site area representing the major transport routes (from use sites to non-target locations) of simazine spray drift and runoff. We considered the maximum offsite transport distance for spray drift to account for potential areas where simazine exposures may occur, as calculated by EPA using a maximum distance of 305 m for ground applications (aerial applications are prohibited). Therefore, Figure 1 and Figure 2 include those off-site areas that are within 305 m of the UDLs representing potential locations of agricultural and non-agricultural (respectively) uses of simazine.

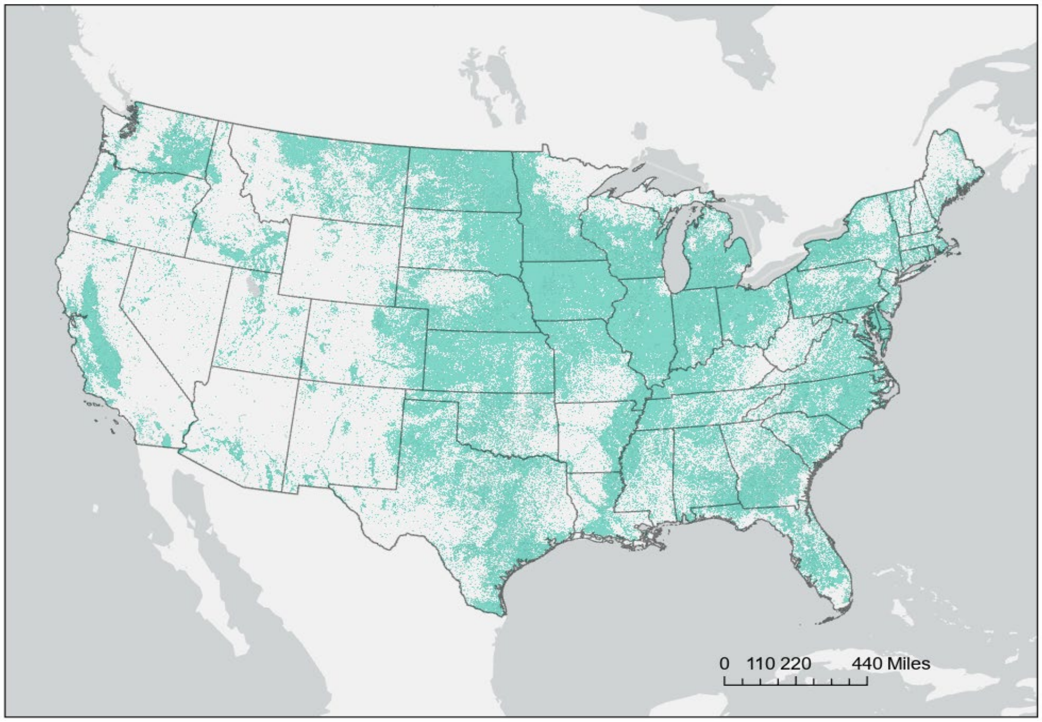

Figure 1. **Map depicting potential agricultural use sites of simazine and potential off-site transport area located 305 m beyond the use sites.**

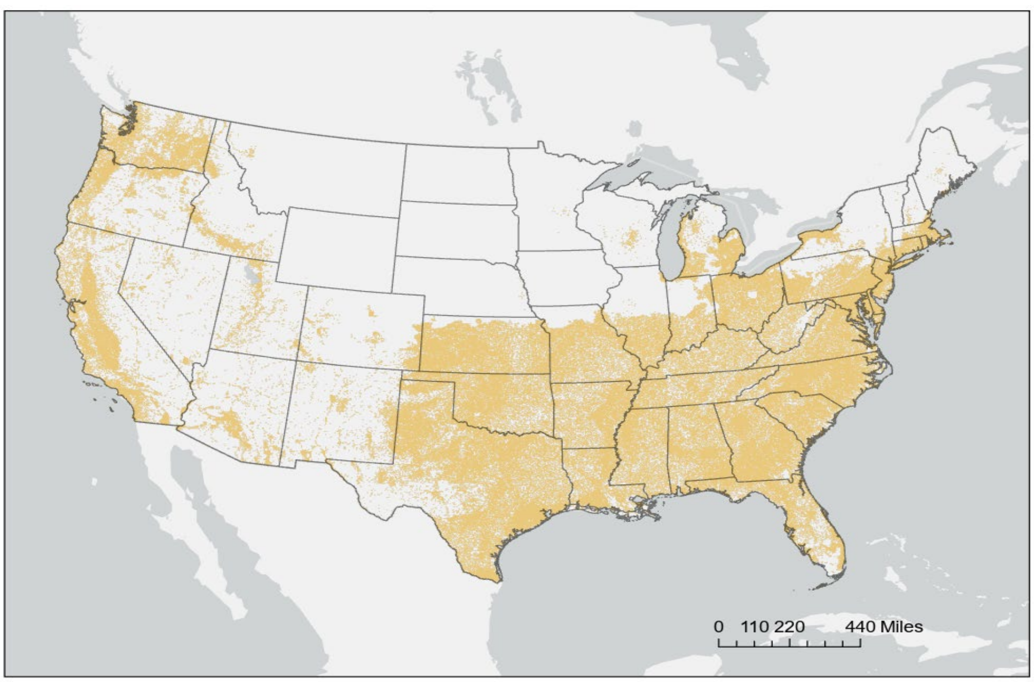

Figure 2. **Map depicting potential non-agricultural use sites of simazine and potential off-site transport area located 305 m beyond the use sites.**

The Service relies on EPA's current exposure models to account for potential on-site and off-site exposures to listed species, and available toxicity information from the scientific literature and unpublished studies submitted by registrants to support their FIFRA registrations, as applied in EPA's BE and implementation of the Herbicide Strategy during the consultation process (as described in further detail below). These processes identified potential effects to non-target plants as the primary concern for simazine. This means that impacts directly on listed plants (specifically growth decreases) and loss of habitat or food resources for listed animals is the predominant concern. We are working with EPA to apply their Herbicide Strategy[6] through this consultation to mitigate impacts on plants due to agricultural uses of simazine. Reductions in drift and runoff exposure that protect plants through these mitigations will also reduce exposure to other taxa, including terrestrial and aquatic animals that may have growth or reproductive effects from chronic exposures. Remaining effects not addressed by Herbicide Strategy drift and runoff conservation measures may be associated with direct effects to animals exposed to simazine on treatment sites and effects that are caused from applications to non-agricultural use sites.

Usage & Exposure Considerations

As discussed in previous sections, simazine is registered as an herbicide to control weeds in a variety of agricultural (e.g., corn, orchards, berries, crops grown for seed) and non-agricultural (e.g., turf and ornamentals) use sites. Use data summarized in Table 1 in the BE describe the use sites, associated UDLs, maximum application rates, method (e.g., ground spray), re-treatment intervals, number of applications that may occur according to registered product labels and conservation measures required on product labels. The crop with the highest labelled maximum application rates is citrus in Florida with 8 lbs a.i./acre, while most other fruit (e.g., berries and orchards) are 4 lbs a.i./acre. The maximum application rate for corn is 2.5 lbs a.i./acre.

We use recent data provided by EPA in the BE (Appendix 1-4) to represent potential usage of simazine. Table 2 in Appendix 1-4 of the BE includes the uses that correspond to each UDL and the relevant usage information, including pounds of simazine applied per year and acres treated per year with simazine. This information can be found above in Table 1 and from Appendix 1-4 in the BE and represents the usage across all registered of simazine within a UDL. When considering UDLs, the most acres treated per year were for corn (2.2 million acres), other orchards (130,000 acres), citrus (103,000 acres) and grapes (98,000 acres). Data from the BE indicates that applications to turf were approximately 40,000 acres of sod farms, 40,000 acres of golf courses, and 30,000 acres of treated lawns. However, the usage data for lawns and golf courses is from 2014, which we expect is out of date and is not representative of current simazine usage on these non-agricultural use sites. We provide more current information on simazine usage in lawns and golf courses in the *Usage Analysis* section of this Opinion. Appendix 1-4 in the BE also includes percent crop treated information for agricultural uses by state. Percent crop

[6] https://www.epa.gov/endangered-species/strategy-protect- endangered-species-herbicides

treated information was used by EPA to calculate overlap information for listed species and critical habitats.

Conservation Measures

Several conservation measures were developed for the registration of simazine that result in reduced exposure to listed species and critical habitat, either by limiting the geographic scope of the registration, or requiring practices to limit off-site transport from application sites. Below, we describe measures that were:

1) Voluntarily submitted by registrants prior to this consultation,
2) Required by the 2020 Simazine Interim Decision for Registration Review, and
3) Developed during this consultation using EPA's Herbicide Strategy and incorporated into the action following commitment by registrants to implement these measures through product labels and species-specific Bulletins.

Since simazine registrants have committed to implementing all conservation measures, whether developed prior to or during consultation, they are now part of the proposed action and considered in our assessment of exposure and effects to listed species and designated critical habitats. After describing these measures, we discuss generally how they were considered quantitatively and qualitatively in our analysis of effects.

1. *Conservation measures voluntarily submitted by simazine registrants prior to consultation:*

The simazine registrants agreed to substantial conservation measures that were incorporated into the BE. Appendix 1-2 of the BE includes the letters from registrants committing to implement these measures, which include limiting the geographic footprint of simazine use to the 48 conterminous states and substantial spray drift mitigations. Mitigations were also included for runoff. The registrants also agreed to add standard ESA language that directs users to obtain bulletins (when they are available). EPA has approved revised simazine labels to include these conservation measures.

Geographic restriction (for all uses):

- Prohibit all uses of simazine in Hawaiʻi, Alaska, and the United States territories (Puerto Rico, Guam, American Sāmoa, the United States Virgin Islands, and the Commonwealth of the Northern Mariana Islands), thereby restricting registered uses to the conterminous United States.

Spray drift conservation measures (for agricultural uses):

- Applications may only be made using ground equipment (no aerial uses are allowed).
- User must only apply with the release height recommended by the manufacturer, but no more than 4 feet above the ground or crop canopy.

- Applicators are required to use a coarse or coarser droplet size (ASABE S572)
- Do not apply when wind speeds exceed 10 miles per hour at the application site.
- Do not apply during temperature inversions.
- User must maintain a 15 foot (4.6 meter) in-field downwind buffer (in the direction in which the wind is blowing) from the edge of streams and rivers, as well as high-tide line for all estuarine/marine environments.

Runoff conservation measures (for agricultural uses):

- Product must not be mixed or loaded within 50 feet of intermittent streams and rivers, natural or impounded lakes and reservoirs.
- Product must not be applied within 66 feet of points where agricultural field (nurseries, Christmas tree plantings, and turf grasses for sod farms) surface water runoff enters perennial or intermittent streams and rivers or within 200 feet of natural or impounded lakes and reservoirs. If this product is applied to highly erodible land, the 66 foot buffer or setback from runoff entry points must be planted to crop, or seeded with grass or other suitable crop.
- Do not apply within 66 feet of standpipes in tile-outletted terraced fields.
- Apply this product to the entire tile-outletted terraced field under a no-till practice only when a high crop residue management practice is practiced. High crop residue management is described as a crop management practice where little or no crop residue is removed from the field during and after crop harvest.

Endangered Species Statement:

"It is a Federal offense to use any pesticide in a manner that results in an unauthorized 'take' (e.g., kill or otherwise harm) of an endangered species under the Endangered Species Act section 9. When using this product, you must follow the measures contained in the Endangered Species Protection Bulletin for the area in which you are applying the product. You must obtain a Bulletin no earlier than six months before using this product. To obtain Bulletins, consult http://www.epa.gov/espp/, call 1-844-447-3813, or email ESPP@epa.gov. You must use the Bulletin valid for the month in which you will apply the product"

2. *Conservation Measures required by the 2020 Simazine Interim Decision for Registration Review*:

- Mixer/loader/applicators for backpack application to grapefruit and oranges are required to wear coveralls over long-sleeve shirts and long pants clothing
- Applications made by mechanically pressurized handguns are restricted to spot treatment only for the following uses:
  - Citrus (Grapefruit, Oranges, Lemons)
  - Pome Fruits (Apples, Pears)
  - Stone Fruits (Cherries [sweet and tart], peaches, Plums, Nectarines)
  - Tree Nuts (Pecans, Walnuts, Filberts, Almonds, Macadamia Nuts

- Berry and Small Fruit (Blueberries, Blackberries, Loganberries, Raspberries, Grapes, Lowbush Blueberries, Cranberries)
- Tropical and Sub-tropical Fruits (Avocado, Olive)
- Nursery/Ornamentals
- Sweet Corn

- Applications made by mechanically pressurized handguns to strawberries are restricted to either spot treatment only or mixer/loader/applicators are required to wear coveralls over long sleeve shirts and long pants of clothing.
- For residential turf on lawns, institutional turf, parks or recreational fields, the maximum application rate may either be:
  - 1.6 lb ai/acre paired with the requirement of 0.5 inches of irrigation; or
  - 0.65 lb ai/acre without irrigation.
  - No change for golf course turf or sod farms
- Updated Language for gloves, respirators, and water-soluble package.

3. *Conservation measures developed during this consultation using EPA's Herbicide Strategy:*

After the simazine BE was finalized in 2021, EPA released its final Herbicide Strategy in 2024 that applies to agricultural uses within CONUS. As part of this ESA consultation with the Service on simazine, EPA is implementing the strategy to inform and identify any conservation measures necessary to follow the Herbicide Strategy. The Service provided input for these measures, which were then agreed to by the simazine registrants. The strategy measures identified below are based on potential population-level impacts to listed plants and indirect impacts to listed animals that depend on plants in terrestrial, wetland, and aquatic habitats in areas that may be affected by off-field simazine exposure. The conservation measures identified for simazine include:

Runoff conservation measures (for agricultural uses):

- Three mitigation points on the general label

For all agricultural uses, the registrants have committed to include three points on the general label for all simazine agricultural use sites. Applicators can achieve the required points using the mitigation measures identified on EPA's Mitigation Menu website[7]. The menu provides a suite of options, including relief points for certain field characteristics and likelihood for pesticide vulnerability.

[7] Mitigation Menu website: https://www.epa.gov/pesticides/mitigation-menu

- Six mitigation points applied to geographically specific areas (implemented using Bulletins Live! Two[8])

The Herbicide Strategy allows the flexibility to apply conservation measures to geographically specific areas (also referred to as Pesticide Use Limitation Areas [PULAs]). These areas generally correspond to locations of species that need higher levels of mitigation to reduce effects from the proposed action compared to other species. In the case of simazine, listed plants and some animals that occur in non-flowing wetlands need higher levels of mitigation (i.e., six points total) to avoid potential population-level impacts. This is because wetlands (specifically those that are non-flowing, e.g., bogs, vernal pools) have higher levels of effects from runoff exposure. There is also a subset of uses that need higher levels of mitigation for additional listed plants and listed animals in non-flowing wetlands, terrestrial, and aquatic flowing habitats. Applicators can achieve the required points using the mitigation measures identified on EPA's Mitigation Menu website[7]. In Table 4, we list all species in this consultation, including those that require geographically-based conservation measures or PULAs.

We continue to work with EPA and the registrants to develop PULAs[9] for simazine based on the locations of these species, which applicators will need to access using the Bulletins Live! Two (BLT) system to determine if their application site (and type of use) falls within the geographic area where six points are needed.

The Herbicide Strategy is designed to be adapted in the future to include additional mitigation options when available. If additional mitigation options become available in the future, after the final biological opinion is issued, EPA will provide documentation regarding the reductions in off-site transport provided by these measures, and, as applicable, the assignment of point values. If the Service agrees with EPA's findings such that options identified would result in equivalent reduction in offsite transport as those identified for this consultation, then end users of simazine will be able to meet mitigation requirements using EPA's updated mitigation menu. However, if EPA or the Service determines that incorporating these changes from any updated Herbicide Strategy into the proposed action would result in effects to listed species or critical habitat that were not previously considered in the Biological Opinion or written concurrence (i.e., the

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[8] Bulletins Live! Two is EPA's on-line tool for pesticide labels directing users to specific pesticide use limitations that are geographically specific: https://www.epa.gov/endangered-species/endangered-species-protection-bulletins

[9] If the EPA identifies a need for geographically specific mitigations to protect a federally listed endangered and threatened ("listed") species and/or designated critical habitat from the use of pesticides, EPA may communicate those mitigations and where they apply using a web-based system called Bulletins Live! Two (BLT). The locations where those mitigations apply are called Pesticide Use Limitations Areas (PULAs). Thus, the purpose of a PULA is to identify areas where pesticide mitigation measures must be implemented to conserve a listed species and its critical habitat (if designated). These areas are where pesticide exposures are likely to impact the continued existence of listed species, resulting in a reduction in survival or recovery of the species. PULAs focus mitigation to where they are most needed to protect populations and include one or more species that share the same mitigation. measures for a pesticide or group of pesticides. (Source: https://www.epa.gov/system/files/documents/2024-12/core_map_process.pdf)

measures do not provide equivalent conservation), then the agencies would reinitiate consultation to evaluate any effects of the action that were not previously considered.

Consideration of conservation measures in our analysis of exposure and effects

All the conservation measures in this section were considered in our analyses as described in this Opinion. We expect that conservation measures that reduce spray drift and runoff from treated fields will reduce the magnitude (e.g., lower simazine concentrations) and extent (e.g., less habitat exposed) from off-site exposure to listed species and critical habitat.

To address the magnitude of exposure, EPA provided estimated environmental concentrations of simazine in the BE, which we updated to reflect the incorporation of the general label measures and PULAs (when applicable to a species) and carried forward into our analysis in this Opinion.

To address the extent of exposure, we qualitatively considered the implementation of conservation measures and, in general, we expect less habitat will be exposed to simazine as spray drift and runoff measures are designed to limit the amount of simazine residue that leaves application sites. While we did not make quantitative adjustments to our overlap or usage analyses for off-field exposure, we factored this into our analyses, where appropriate, to reflect these measures and considered that reduction in our determinations.

For direct exposure to listed terrestrial and aquatic animals, or terrestrial plants on simazine use sites, which was not specifically reduced through Herbicide Strategy conservation measures, effects could remain at levels high enough to cause greater than low levels of adverse direct and/or indirect effects. However, further analysis is required to determine the extent of effects, if any, and the resultant risk to these listed species. We intend to continue coordinating with EPA and simazine registrants between the release of this draft Opinion and the transmission of the final Opinion to gain information regarding the exposure and effects of simazine registration, as proposed, to these species and critical habitats.

Table 2. Simazine Registrations and Summary of Action[10]

Simazine_Summary_of_Action_Table.xlsx

Consideration of Usage Data

Usage data describe how the pesticide has been applied to multiple use sites within a state, region, or the United States. In development of its BE, EPA reviewed usage data that documents

[10] To view the spreadsheet in MS Excel from the MS Word Opinion, double click on the Excel icon. To view the spreadsheet in MS Excel from the portable document format (pdf) Opinion, open the Opinion in the desktop version of Adobe Acrobat or Reader and open the embedded attachment corresponding to the numbered table.

the actual (field) applications of a pesticide, including information such as actual application rates and timing, and spatial distribution of applications across multiple sites (usually based on survey data). The difference between use and usage is that use refers to the authorized application under the label while usage refers to how it is actually applied on the landscape.

This Opinion considers the proposed action, specifically the registration review of simazine according to its labeled uses. We recognize that the geographic areas authorized under the labels are intentionally broad to cover a variety of current and future, less predictable pest pressures and user needs throughout the action area (defined below) over the course of the 15-year duration of the proposed action. We also recognize that it is not realistic to assume the chemical will be used in every location in the action area where labeled uses allow, nor do we expect that the highest application rates and frequencies authorized under the label will occur in all these locations each year. Based on how the labels are currently written, we acknowledge the full range of uses and use sites allowed under the proposed registration review. While we agree simazine will not be used everywhere, applied at the highest allowable frequency at each site, or applied at the highest application rates each time it is used (which would likely comprise more product than is currently manufactured or distributed), we also recognize that simazine can be used anywhere the label allows and at the highest rates and frequencies specified for a given use. Similarly, we also recognize that, while knowledge of past usage patterns and locations may be helpful in providing context for where some uses are likely to occur, the past does not necessarily predict future pest pressures, management, or pesticide uses.

Mindful of the limitations associated with usage data, we utilize usage data to inform our analysis, but it is not dispositive in determining "effects of the action." Because usage data represents historical patterns of how and where simazine was applied on the landscape, it is appropriately considered in determining "effects of the action," which, under ESA section 7 regulations and Administrative Procedure Act standards, respectively, must be "reasonably certain to occur" and rationally based. At the same time, particularly where there are informational gaps, we apply usage data in this Opinion using our best professional judgment to make assumptions that are not only reasonable but are appropriately conservative for the species and critical habitat to determine whether EPA's proposed action ensures against the likelihood of jeopardy of species or destruction and adverse modification of critical habitat. Although usage data is a portion of the best scientific and commercial data available, it is only one of many factors and points of data we consider in determining "effects of the action."

ACTION AREA

The action area is defined as all areas to be affected directly or indirectly by the federal action, and not merely the immediate area involved in the action (50 CFR 402.02). Consistent with the ESA section 7 implementing regulations, in delineating the action area for simazine, we evaluated the physical, chemical, and biotic effects of the proposed action on the environment that would not occur but for the proposed action and that are reasonably certain to occur. For the reasons mentioned below, the action area for this consultation is delineated by these effects to the environment and consists of the labeled uses only within the CONUS.

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

From EPA's BE, the action area was derived in ArcGIS Pro version 2.9.11 and by combining the data layers representative of simazine potential uses plus off-site transport to 305 meters based on the physical chemical properties of simazine potential for run-off (see the *Chemical Specific Uses and Environmental Fate* Section above). The currently registered uses (summarized above in the *Description of the Proposed Action* Section and Appendix 1-2 of the BE) include agricultural and non-agricultural uses (Figure 3).

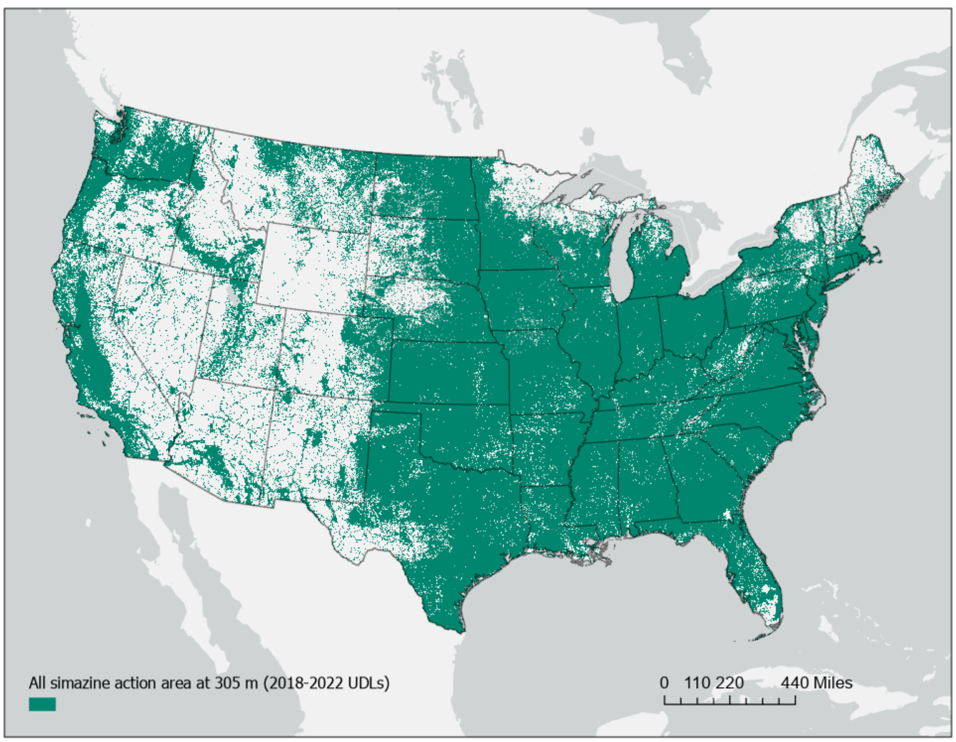

Figure 3. Map depicting the action area of simazine. This includes potential agricultural and non-agricultural use sites and potential off-site transport area located 305 m beyond the use sites. The action area does not include Alaska, Hawai'i or the United States territories.

All species' range or critical habitats that overlap with use sites and off-site transport areas, or species that are dependent upon a prey base which overlaps with use sites and off-site transport areas (Chapter 4 of the BE) are assessed. EPA's analysis used spatial data of species' ranges and critical habitat designations from the Service. In CONUS, agricultural potential use sites are represented using the USDA Crop Data Layer (CDL)[11] from 2013-2017 (Appendix 1-6 of the BE) that are grouped by similar uses into UDLs. Agricultural UDLs used for simazine include Corn, Vegetables and Ground Fruit, Other Crops, Citrus, Christmas Trees and Other Orchards. For simazine non-agricultural uses, use on conifers, deciduous trees and woody ornamental species, reliable data were not available to map the locations of the potential use sites. However, EPA determined that these uses were limited in geographic scope and adequately represented by uses within the Nurseries UDL. For lawn use and golf courses, the Developed and Open Spaced Developed UDLs were used, respectively. EPA assessed species whose ranges, resources (e.g., prey species, host fish), or critical habitats overlap use sites or off-site transport areas in the BE and in the implementation of the Herbicide Strategy to make species and critical habitat effects determinations.

To reduce uncertainty that can lead to over-estimations in overlap, EPA provided additional information using an alternative method of developing UDLs using Crop Sequence Boundaries (CSB) vector data[12]. Use of CSB data lessens the extent in the CDL of so-called "spurious pixels" (i.e., in raster data, individual pixel or clusters of pixels that misrepresent or deviate from the expected values due to errors or defects in the image or subsequent visual depiction of such). This was accomplished by assigning the approximate 119 CDL classes to the appropriate UDL and assessing five years of CSB classification and assigning a polygon to the UDL if the crop was classified in the UDL in any of the five years. A comparison of the UDL acreage to the Census of Agriculture acreage (by county) is then made and the CSB acreage is expanded to an equivalent acreage, if necessary, and again at the county level, utilizing an expansion algorithm. EPA and the Service evaluated this method in an on-going effort utilizing ground truthing tools (e.g., aerial photography) and find it to be a promising tool providing increased accuracy. In general, utilization of the CSB data results in more accurate representation of agricultural land uses and improved resolution of field-level remote sensing compared to the CDL data and greatly diminishes the "noise" of spurious pixels in the raster CDLs.

As explained in more detail in our analysis of species exposure and effects of the action, we identified some areas in which certain species are extremely unlikely to be exposed to generalized environmental effects arising from a specific registered simazine use (i.e., the effect is discountable to the species), or alternatively, exposure would occur, but in such low levels that the effects to species from exposure are likely to be insignificant. We discuss these species in Appendix A, *Supporting Information for the Concurrence Section of the Consultation*.

[11] USDA National Agricultural Statistics Service Cropland Data Layer. 2013-2017. Published crop-specific data layer [Online]. Available at https://www.nass.usda.gov/Research_and_Science/Cropland/SARS1a.php (accessed 3/2018; verified 02/2021). USDA-NASS, Washington, DC.

[12] The Crop Sequence Boundaries (CSB) developed with USDA's Economic Research Service, produces estimates of field boundaries, crop acreage, and crop rotations across the contiguous United States. Source: https://www.nass.usda.gov/Research_and_Science/Crop-Sequence-Boundaries/index.php.

During past agency and stakeholder workshops and communication, we were occasionally asked to consider whether the Agencies should eliminate certain federal lands from the action area based on past or recent consultations where another action agency had already consulted on the use of the subject pesticide in their management plans or other actions. Examples include actions occurring on lands under the jurisdiction of the Service, the National Park Service, Bureau of Land Management, and U.S. Forest Service. A specific review of previous simazine use on Service lands (e.g., National Wildlife Refuges (NWRs)) revealed some simazine usage (13,121 total lbs a.i. applied for all NWRs) for the 10-year period of 2013 to 2024 (PUP report 2024; Table 18). However, while informative, the queries of Service database information may not be definitive for other federal land management agencies (e.g., the Department of Defense). We are not aware of any agreements, plans, and/or other commitments by federal agencies related to the use and/or restriction of use of simazine within their jurisdictions. For this reason, and because the labels allow use on federal lands, we determined it would be inappropriate to remove federal lands from the action area. Previous consultations involving simazine use on federal lands are considered to be part of the environmental baseline.

Overlap with Species Ranges and Critical Habitats

In addition to species where no overlap was found with the action area, we assumed that the proposed action would not result in exposure or effects to the following categories of listed species[13], proposed species, or designated or proposed critical habitats that occur within the action area[14]:

(1) listed species presumed extinct within the CONUS and their designated or proposed critical habitats in CONUS, as applicable;

(2) listed species presumed extirpated within the CONUS with no expectation of recolonization or plans for reintroduction over the duration of the proposed action; or

(3) listed species that occur only in captivity with no plans for reintroduction in the CONUS over the duration of the proposed action.

Analytical Framework for Jeopardy and Destruction or Adverse Modification Determinations

Jeopardy Determination

Section 7(a)(2) of the ESA requires that federal agencies ensure that any action they authorize, fund, or carry out is not likely to jeopardize the continued existence of listed species. "Jeopardize

[13] This Opinion does not consider foreign listed species, due to the extent of the action area as described in EPA's BE.

[14] It is our understanding that EPA recognizes reinitiation of consultation may be necessary if individuals of species presumed extinct or extirpated are discovered within the timeframe of the proposed action.

the continued existence of" means to engage in an action that reasonably would be expected, directly or indirectly, to reduce appreciably the likelihood of both the survival and recovery of a listed species in the wild by reducing the reproduction, numbers, or distribution of that species (50 CFR § 402.02).

The jeopardy analysis in this Opinion considers whether the effects of the action, in the context of environmental baseline, status of the species, and cumulative effects, would be expected to appreciably reduce the survival and the recovery of the listed species. Thus, our analysis relies on four components: (1) the *Status of the Species*, which describes the condition of the species in its entirety, the factors responsible for that condition, and its survival and recovery needs; (2) the *Environmental Baseline*, which analyzes the condition of the listed species in the action area, without the consequences to the listed species caused by the proposed action; (3) the *Effects of the Action*, which includes all consequences to listed species that are reasonably certain to occur and would not occur but for the proposed action, including the consequences of other activities that are caused by the proposed action; and (4) the *Cumulative Effects*, which evaluates the effects of future, non-federal activities in the action area on the species.

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

Destruction or Adverse Modification Determination

Section 7(a)(2) of the ESA requires that federal agencies ensure that any action they authorize, fund, or carry out is not likely to destroy or to adversely modify designated critical habitat. A final rule revising the regulatory definition of "destruction or adverse modification" was published on August 27, 2019 (FR 44976). The final rule became effective on October 28, 2019 (84 FR 50333).

The destruction or adverse modification analysis in this Opinion relies on four components: (1) the *Status of Critical Habitat*, which describes the range-wide condition of the critical habitat as a whole in terms of the key components (i.e., essential habitat features, physical and biological features, or primary constituent elements) that provide for the conservation of the listed species, the factors responsible for that condition, and the intended value of the critical habitat overall for the conservation/recovery of the listed species; (2) the *Environmental Baseline*, which analyzes the condition of the designated critical habitat in the action area, without the consequences to the designated critical habitat caused by the proposed action; (3) the *Effects of the Action*, which includes all consequences to the critical habitat that are reasonably certain to occur and would not occur but for the proposed action, including the consequences of other activities that are caused by the proposed action; and (4) *Cumulative Effects*, which evaluate the effects of future non-federal activities that are reasonably certain to occur in the action area on designated critical habitat.

For purposes of making the destruction or adverse modification determination, the Service: (1) reviews all relevant information, (2) evaluates the current status of the critical habitat and environmental baseline, (3) evaluates the effects of the proposed action and cumulative effects, (4) add the effects of the action and cumulative effects to the environmental baseline and, in light of the status of the critical habitat, determines if the proposed action is likely to result in the destruction or adverse modification of critical habitat by appreciably diminishing the ability of critical habitat as a whole to provide for the conservation of the species.

STATUS OF THE SPECIES AND CRITICAL HABITAT

In their BE, EPA identified numerous listed and proposed species and proposed and designated critical habitats that may be affected by the proposed action. Species addressed in this Opinion are listed in Table 3. Species that were included in the BE but have been removed from this Opinion because the species are not currently listed are included in Appendix A of this Opinion. The detailed status of each listed and proposed species and their proposed or designated critical habitat is provided in Appendix B.

Table 3. Listed and proposed animal and plant species and proposed and designated critical habitats addressed in this Opinion.[15] [16]

Draft Table 3 animal and plant species in si

We included nonessential experimental populations (EXPN) in Table 3. The Service designated these experimental populations to support the recovery of listed species in taxa groups including birds, bivalves, fishes, insects, mammals, and snails. We do not provide separate analyses and jeopardy determinations for these populations. Rather, we treat all populations of the species (including populations designated as experimental) as a single listed entity when making jeopardy determinations or for other analyses in a section 7 consultation, such as our reviews of EPA's "may affect, not likely to adversely affect" determinations for our potential concurrence. An "essential experimental population" is a reintroduced population whose loss would be likely to appreciably reduce the likelihood of the survival of the species in the wild. However, there are no "essential experimental populations" in this consultation. A "nonessential experimental population" is a reintroduced population whose loss would not be likely to appreciably reduce the likelihood of survival of the species in the wild. By definition, a "nonessential experimental population" is not essential to the continued existence of the species. Therefore, no proposed

[15] For determinations and conclusions in Tables 4 and 5: LAA = "may affect, likely to adversely affect;" NLAA = "may affect, not likely to adversely affect;" NE = "no effect;" NA = Not Applicable (e.g., critical habitat has not been designated for a species).

[16] To view the spreadsheet in MS Excel from the MS Word Opinion, double click on the Excel icon. To view the spreadsheet in MS Excel from the portable document format (pdf) Opinion, open the Opinion in the desktop version of Adobe Acrobat or Reader and open the embedded attachment corresponding to the numbered table.

action impacting a population so designated could lead to a jeopardy determination for the entire species. In cases where our assessment of the listed entity (i.e., the nonexperimental population(s) of the species) leads to a "not likely to jeopardize" determination, we generally assume any added effects to the nonessential experimental population will not change these determinations. However, we consider the role of the experimental population in the survival and recovery of the species and consider this information in our jeopardy analyses as appropriate.

Environmental Baseline

The environmental baseline is defined as:

"The condition of the listed species or its designated critical habitat in the action area, without the consequences to the listed species or critical habitat caused by the action.

The environmental baseline includes the past and present impacts of all federal, State, or private actions and other human activities in the action area, the anticipated impacts of all proposed federal projects in the action area that have already undergone formal or early section 7 consultation, and the impact of State or private actions which are contemporaneous with the consultation in process. The impacts to listed species or designated critical habitat from federal agency activities or existing federal agency facilities that are not within the agency's discretion to modify are part of the environmental baseline" (50 CFR § 402.02, as revised May 6, 2024).

Because this consultation addresses a large geographic area and the distribution of species within the action area is widespread, this Opinion will consider the environmental baseline at a broad scale. Many of the ESA-listed species and their critical habitats are exposed to multiple stressors comprising the past and present impacts of actions and activities that are described below. The environmental baseline in this Opinion focuses primarily on the status and trends of the ecosystems in which these species and their critical habitats occur in the United States and the factors that contribute to the current status for ESA-listed species and their resources. We first explore factors that affected listing decisions over the last several decades, then describe factors that affect the environmental baseline for listed species and designated critical habitats, including pesticide use, land use change, invasive species, pollution, harvesting, water-related issues, climate change, and several others.

In Table 4, we present threats that contributed to listings 877 ESA-listed species identified through Federal Register documents up to August 1994 (Czech, Krausman and Devers 2000). We also present the factors associated with 143 ESA listing decisions (threatened or endangered) from February 2011 to October 2014 (Smith-Hicks and Morrison 2021). In both assessments, the most frequently referenced threats were: non-native species, urbanization/roads, agriculture, and loss of genetic viability/small population sizes. Before 1994, some species were listed due to threats that were not referenced in the 2011-2014 rules (e.g., aquifer depletion/wetland filling, native species competition, and vandalism). In the 2011-2014 rules, several new threats were presented (i.e., commercial fishing, climate change, and pesticides/herbicides). Some species may be affected by multiple stressors at the same time. Of particular interest is that several factors (e.g., pesticides, agriculture, fire suppression and related activities, urbanization, and water diversions) were influential to species' listings across both time periods (before 1994 and between 2011-2014).

Table 4. Threats identified for ESA-listed species from rules before 1994 (column 2) and between February 2011-October 2014 (column 3). Modified from (Czech, Krausman and Devers 2000) and (Smith-Hicks and Morrison 2021).

Threat	**Number (%) of Species Listed by Threat** (Czech, Krausman, & Devers, 2000)	**Number (%) of Species Listed by Threat** (Smith-Hicks & Morrison, 2021)
Non-native species	305 (35)	76 (53)
Urbanization	275 (31)	77 (54) (combined with Roads in "Land conversion")
Agriculture	224 (26)	55 (38)
Recreation	186 (21)	38 (27) (combined with Industry/Military in "Competing uses")
Ranching	182 (21)	49 (34) (combined with Fire suppression in "Modified disturbance regimes")
Reservoir and water diversions	161 (18)	52 (36)
Fire suppression	144 (16)	49 (34) (combined with Ranching in "Modified disturbance regimes")

Threat	**Number (%) of Species Listed by Threat** **(Czech, Krausman, & Devers, 2000)**	**Number (%) of Species Listed by Threat** **(Smith-Hicks & Morrison, 2021)**
Pollution	144 (16)	30 (21)
Mining/Oil & gas	140 (16)	47 (33) (combined with Logging in "Resource use")
Industry/military activities	131 (15)	38 (27) (combined with Recreation in "Competing uses")
Harvest	120 (14)	18 (13)
Logging	109 (12)	47 (33) (combined with Mining/Oil and gas in "Resource use")
Roads	94 (11)	77 (54) (combined with Urbanization in "Land conversion")
Loss of genetic viability	92 (10)	97 (68)
Aquifer depletion/wetland filling	77 (9)	N/A
Native species competition	77 (9)	N/A
Disease	19 (2)	31 (22)

Threat	Number (%) of Species Listed by Threat (Czech, Krausman, & Devers, 2000)	Number (%) of Species Listed by Threat (Smith-Hicks & Morrison, 2021)
Vandalism	12 (1)	N/A
Commercial fishing	N/A	3 (2)
Climate change	N/A	56 (39)
Pesticides/Herbicides	N/A	22 (15)
Unknown or Other	N/A	8 (6)

Treakle, Epanchin-Niell and Iacona assessed listing outcomes for 387 species between 1996-2018 in the CONUS, specifically if the species was ultimately listed or removed from consideration for listing due to preemptive conservation. They found that most species that were not listed occupied smaller ranges, intersected more states, and were found primarily on public lands. Many of the species not listed had higher baseline conservation and faced fewer threats due to preemptive conservation efforts than species that were listed. The authors suggest that greater sharing of information or resources may assist with preemptive conservation for species with broad or multi-state ranges (Treakle, Epanchin-Niell and Iacona 2023). Overall, species faced with a greater number of threats are more likely to be listed under the ESA (Smith-Hicks and Morrison 2021, Treakle, Epanchin-Niell and Iacona 2023).

Land Use and Land Cover Change

A primary factor negatively affecting imperiled species are changes to their habitat. Many habitat modifications have occurred in the United States throughout human history, the earliest of which likely included the use of fire to encourage or discourage the growth of certain plant communities. The types and extent of habitat changes have increased through time, with much of the land in the United States now being used for agriculture, forestry, urban and industrial development, and mining. Each of these land uses affect species and habitats differently. The land use categories that most affect species and habitat long-term are agriculture and urban/industrial development.

Over the last 300 years, forests in the eastern United States were reduced by at least half due to land use change for agriculture, urbanization, and infrastructure development. Intensive, large-scale land use changes began during European settlement and continued rapidly as settlers moved west, exploiting the land for tobacco and lumber for export (Keeney and Kemp 2002).

Between 1938 and 1992, urban areas expanded by 140%, wetlands decreased, and agricultural land uses (e.g., cropland and hay) decreased nationwide by 18% with higher decreases in the east. Forestland and grassland increased, primarily due to agricultural abandonment (Sohl, et al. 2016). Between the early 1900s and early 2000s, the area of forest cover in the United States was relatively stable (Masek, et al. 2011), though reforested areas may not provide the same quality of habitat as unharvested, old-growth forests do for ESA-listed species (Sutherland, Gergel and Bennett 2016). For example, marbled murrelets use old-growth forests that take 100-200 years to recover with necessary nesting habitat structures (USFWS 1997), and if these forests are removed, they may not recover into the same forest structure as was present before deforestation took place. In many cases, abandoned areas succeed into different communities from the ones that occurred before the land was converted to agriculture.

Agriculture

Agriculture is a principal industry in the United States, accounting for over 50% of the country's land uses (e.g., cropland, pasture and range, forested grazelands, animal operations). According to the USDA, there were about 1.8 million crop and livestock farms (a decrease of nearly 15,000 since 2023) across approximately 876,460,000 acres of land in the U.S. in 2024 (NASS 2025). The number of farms, particularly small farms, and the total acreage of farmland in the U.S. has decreased every year for decades (Lacy 2025). Most grasslands in the United States are plowed and planted for crops for human consumption, livestock grazing, and more recently, biofuel production (Mitchell, et al. 2010).

Despite an overall decreasing trend in farms and farmland across the country, between 2008-2016, croplands expanded by over 10 million acres across the CONUS, with over 1 million acres converted per year. Croplands expanded greatly in many midwestern states (e.g., North Dakota and Missouri) and were abandoned in others (e.g., Idaho and Maryland). Simultaneously, 3.52 million acres of cropland were converted to non-cropland uses, including abandonment (Figure 4). Rates of agricultural abandonment and conversion vary by year and by state, in many cases, dramatically (Lark, et al. 2020). Using USDA's Crop Data Layer from 2020, Xie et al. found that 53% of abandoned cropland was converted to grassland or pasture and 19.6% of abandoned croplands were enrolled in Conservation Reserve Programs with USDA's Farm Service Agency (Xie, et al. 2024). Land use change is non-linear and when one area is converted from a natural area to agriculture, another may be allowed to succeed into a novel natural area after abandonment.

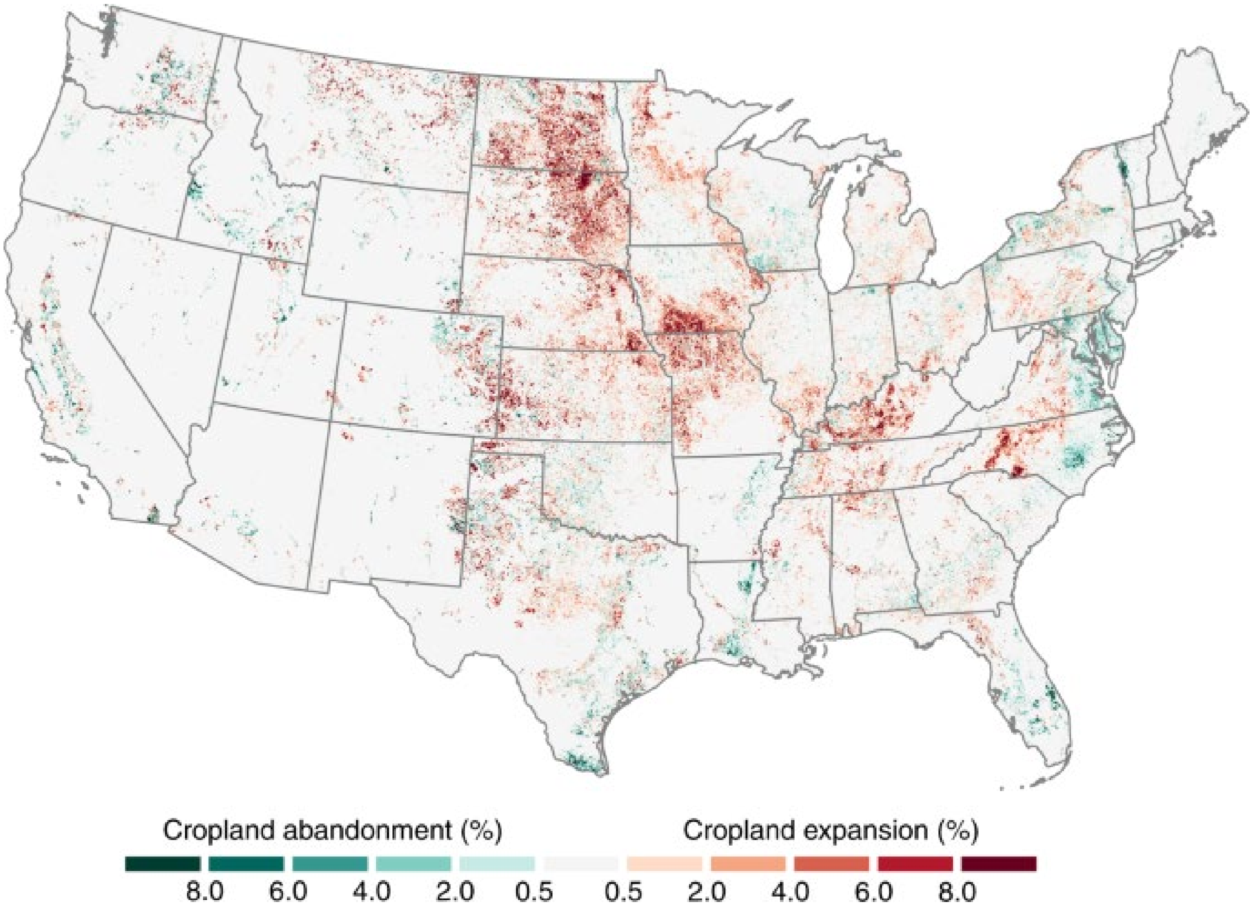

Figure 4. Cropland conversions between 2008-2016 in the continental United States, including abandonment and expansion (Figure 1 from (Lark, et al. 2020)).

Crop production can lead to pesticides leaching into groundwater and entering streams from surface water runoff (Spence, et al. 1996, Rao and Hornsby 2001). Several pesticides were detected in small streams and sloughs within agricultural and urban sites tested within Puget Sound (Bortleson and Davis 1997). In periodic reconnaissance studies of streams in nine Midwestern states, the U. S. Geological Survey documented that large quantities of herbicides and their degradate products were flushed into streams during post-application run-off (Scribner, et al. 2003). For more information about effects of pesticides, please see the *Use of Pesticides* section below.

Large animal husbandry operations are common in the Midwest and throughout the eastern United States. Many animal operations require grasslands for grazing and/or pasture farming (i.e., rangelands, pasturelands, and others). Agricultural grasslands, including rangelands and pasturelands, provide many ecosystem services to wildlife including food and shelter. Rangelands are managed as a natural ecosystem with mostly native grassy vegetation, while pasturelands are grazing lands used to permanently produce forage species, primarily for grazing animals (GLTI 1997). In the east, grasslands are mostly seeded pasturelands and, in the west, grasslands are mostly rangelands. Seeded pasturelands often receive more fertilizer and herbicides to control unwanted species than rangelands (Mitchell, et al. 2010). In some areas, intense grazing resulted in a general decline in range conditions, including increased fire risk and establishment of invasive, non-native vegetation (Oliver, Irwin and Knapp 1994). By the 1960s and 1970s, legislation allowed for monitoring, improvements, and better stewardship of rangeland (including those in National Forests). Despite these efforts, over 70% of federal land (e.g., Bureau of Land Management, U.S. Forest Service) was grazed by cattle and sheep in the western Unites States by 1970 (CAST 1974). Effects from livestock grazing can be considerable if management practices are not sufficient to protect habitat functions (Wissmar, et al. 1994, Belsky, Matzke and Uselman 1999). In overgrazed areas, native understory grasses are eliminated, tree seeds establish and are not consumed by grazers, and dense tree seedling areas further succeed in the absence of fire (Madany and West 1983, Franklin, et al. 2008), changing the vegetation composition of the habitat over time. Livestock trampling also damages fragile moss and lichen layers (i.e., biocrust) that provide nutrients to the soil, protect the soil against erosion, support native grasses, and limit colonization by non-native invasive vegetation (e.g., cheatgrass) (Finger-Higgins, et al. 2022).

Agriculture, which has contributed to the loss of native and riparian vegetation, degradation of water quality, and introduction of contaminants (Hamilton and Helsel 1995), is the leading cause of deforestation (Benayas and Bullock 2012); and is responsible for 10% of anthropic greenhouse gas emissions in the United States (USEPA 2021). In addition, agriculture is the leading cause of water quality concerns in the United States (Keeney and Kemp 2002), from changes in water temperature, increases in siltation and turbidity, and introduction of contaminants. In addition to effects on or adjacent to agricultural lands, effects to water quality may extend far downstream of agriculture activities through runoff. For example, livestock production often degrades water quality through the addition of excess nutrients from animal manures and agricultural fertilizer, which can contribute to excessive growth of aquatic plants, harmful algal blooms, reduced levels of dissolved oxygen, and adverse effects to fish (Embrey and Inkpen 1998, USEPA 2006) and other aquatic organisms.

Attempts have been made to mitigate past impacts on the ecosystems in the United States from agricultural operations. In 1970, the EPA took over implementation of FIFRA to regulate the registration and use of chemical pesticides, although some authors note challenges associated with its implementation. Additionally, state and federal programs were organized to aid landowners in voluntarily managing their properties to improve water and habitat quality (Edge 2001). The 2002 farm bill drastically increased funds for conservation and created the voluntary Conservation Security Program (Keeney and Kemp 2002), which provided funds to producers for conservation actions. Though revised a few times since its establishment, the Conservation Stewardship Program (formerly, Conservation Security Program) has been reauthorized with each new farm bill (Stubbs 2023).

Forestry Activities

At the beginning of European settlement in 1630, an estimated 423 million hectares (46%) of what would become the United States was forest lands. From 1850 to 1997, forest land remained relatively stable across the country and by 2012, forests comprised 309 million hectares (USDA

2014). Reserved forest land (e.g., state and federal parks and wilderness areas) doubled between 1953 and 2001. Significant additions to federal forest reserves occurred after the passage of the Wilderness Act in 1964 (USFS 2001). According to the U.S. Forest Service, the most acreage of forest lands occurs in the western United States, followed by large areas in the southern and northern parts of the country.

Intensive forest management generally results in adverse effects such as loss of older forest habitats and habitat structures, increased fragmentation of forest age classes, loss of large conterminous and interior forest habitats, decreased water quality, degradation of riparian and aquatic habitats, and increased displacement of individual species members. Intensive forest management on most private lands generally maintains these lands in an early seral stage (e.g., 40 to 50 years of age) with relatively few structures such as snags, down logs, large trees, variable vertical layers, and endemic levels of forest “pests” and “diseases,” when compared to what was present before intensive management.

Timber Harvest

Forested areas that were considered unsuitable for agriculture were often managed for timber. Pioneers used river systems to transport logs and other goods. Following World War II, truck road systems replaced railroads, but smaller streams continued to be used as transportation corridors. After 1930, timber was in demand and motorized trucks and chainsaws allowed for substantial increases in harvest. While timber harvest continues to occur across the United States, conversion of forest lands to other uses continued as the human population has grown.

Timber harvest changes the forest composition and can change forest ecosystem functions. Before timber harvest began, forest composition included many age classes, diverse species, and various canopy levels. Timber harvest initially focused on large-diameter trees, ultimately changing the forest composition and slowing recruitment (Sedell, Leone and Duval 1991, USFS 2003). Old-growth forests declined on federal and non-federal lands across the U.S.. Once old-growth forests are disturbed, they may not succeed or recover with the same characteristics that they had before the disturbance (i.e., they may have a different species composition), and old-growth trees take 150+ years to grow (Spies 2004). Many species rely on characteristics of old-growth forests that are rare or unavailable in other habitat types (e.g., tree snags), and many intact old-growth forests have intricate water cycles (Dawson 1998) and cross-species relationships that are severed when forest composition changes.

In addition to forest effects, timber harvest and associated activities, such as road construction and skidding, can increase sediment delivery to streams, clogging substrate interstices and decreasing stream channel stability and formation. Harvest in riparian areas decreases woody debris recruitment and negatively affects runoff patterns. Runoff timing and magnitude can change to deliver more water to streams in a shorter period, which causes increased stream energy and scouring and decreased base flows during summer months. Stream temperatures may rise with decreases in the forest canopy and riparian zone shading. Loss of large trees also increases erosion and simplifies stream channels (Quigley and Arbelbide 1997).

Improvements in forestry methodologies have reduced some effects from these practices. In some areas, harvest units have been restricted in size, and greater consideration has been given to the health and appearance of forest landscapes and the biotic communities that depend on them. In some cases, equipment is used and/or engineered in ways to minimize soil disturbance and other habitat impacts. In other cases, however, the methods used may result in increased soil disturbance and extreme fire hazards (e.g., machine piling and burning, accumulation of dead slash from thinning activities, etc.) (Oliver, Irwin and Knapp 1994).

Fire Suppression

Under historical fire regimes, natural disturbance from forest fires resulted in a mosaic of diverse habitats. In addition to facilitating germination of some species (e.g., jack pines) and making room for others to grow into the forest canopy (e.g., Douglas fir) (Cooper 1961), fires release nutrients back into the soil, maintain grassland and other early successional habitats that are otherwise overtaken by forests, and help diversify landscapes (Knapp, Estes and Skinner 2009). Before human interference, in some lowland areas, fires were frequent and not highly destructive, primarily burning off revegetation. At higher elevations and in cooler areas, fires were less frequent and highly destructive.

Starting in the late 1880s, fire suppression was used to protect human-dominated areas, and it became a priority of the U.S. Forest Service to suppress all fires in 1905. Historically, burned areas were maintained as early successional vegetation through grazing or were left to develop into dense stands with different compositions than was previously present. Many fire-dependent pine species were outcompeted by hardwoods (e.g., oaks, maples, yellow poplar) that do not need fire to reproduce and are otherwise restricted to wetter environments (Keane, et al. 2008). The environmental integrity of forests changed and denser forest stands may be more susceptible to disease and pests (Oliver, Irwin and Knapp 1994). Fire suppression led to a buildup of forest fuels, which increased the likelihood of large, intense forest fires in some areas. Large fires can cause longer-lasting damage than small fires because their heat effects run deeper into the soil and they can create larger burn areas (Keane, et al. 2008).

Although fire suppression was viewed as necessary to protect resources and private property, some advocated the use of prescribed fire to reduce fuels and protect stands against high-heat, damaging fires. In the 1960s, the National Park Service recognized that fire was an important natural process and began letting naturally ignited fires run their course under prescribed conditions. The U.S. Forest Service began allowing natural fires to burn in wilderness areas in 1974. Other land management agencies (e.g., U.S. Fish and Wildlife Service, Bureau of Land Management, Bureau of Indian Affairs) began implementing fire management, as opposed to fire control, in the 1990s and 2000s (van Wagtendonk 2007).The use of prescribed fire in certain environments was encouraged, with certain precautionary measures. Although scientists recognized the value of prescribed burning as one of many tools to help return landscapes to natural conditions, some managers have been slow to embrace prescribed burning partially due to liability. There are other constraints upon prescribed burning including short-term expenses and air-quality concerns.

Forest Diseases and Pests

Forest diseases and pests were present in forests before European settlement, including fungal pathogens and defoliating insects. Many diseases and pests were transported unintentionally to the United States as world travel became more common. By the mid-1900s, several defoliating insects were documented across the United States (e.g., tussock moths, pine butterflies, bark beetles, pine beetles) that kill trees, reduce their growth, and increase their susceptibility to other damage from insects or disease (Kulman 1971). Starting in the 1930s, surveys and control were used to combat pests, including selective harvesting or salvage harvest to remove infested trees, pesticide use (e.g., ethylene dibromide, dichlorodiphenyltrichloroethane (DDT), and other insecticides), and removal of host plants (e.g., currant [*Ribes* spp.], host of white pine blister rust). Between 1860-2006, about 2.5 new non-native forest insects were detected in the continental United States each year. By 2010, there were an estimated 450 non-native insects and 16 new pathogens in our forests and urban trees, and at least 14% of them caused notable tree damage (Aukema, et al. 2010). Invasive insects, plant and fungal pathogens, and parasitic plants can change forest composition and structure (Cobb and Metz 2017, Poland, et al. 2021). Forests that have been affected by defoliating insects and/or pathogens are more susceptible to other threats like drought, fire, and effects of climate change (Kliejunas, et al. 2008).

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

Urbanization

In general, urban land acreage quadrupled from 1945 to 2007 with an estimated 61 million acres in 2007 (Nickerson, et al. 2011). By 2012, USDA estimated that 70 million acres of the United States (3% of total land area) were urbanized. Urban land area increased at more than double the human population growth rate between 1945 and 2012, and between 1982 and 2012, the increase in developed land acreage was primarily driven by conversion of forest and cropland (Bigelow and Borchers 2017). Between 2001 and 2016, the most persistent and permanent land use change in the CONUS was development (5.6 of the total land area), most of which occurred between 2001-2006 (Homer, et al. 2020). Figure 5 depicts the 2020 human population density by county in the U.S. and serves as a coarse representation of urbanization. Between 2010-2020, the U.S. human population grew by over 22 million people, with a total population in the 2020 Census of 331,449,281 people (USCB 2021). Since 2020, U.S. population trends fluctuated due to changes in migration patterns, responses to the COVID pandemic, and other causes. Between 2022-2023, the northeast region of the U.S. saw a 0.1% decline, the Midwest saw a 0.2% increase, the south saw a 1.1% increase, and the west saw a 0.2% increase in population (USCB 2023).

In general, urbanization (including impervious land cover, manufacturing and waste, housing densities, and contributions to greenhouse gas emissions) concentrates effects of water, land, and mineral use; increases the pollutant load in water and on the land; increases the likelihood of noise and air pollution; contributes to degradation of ecosystems and habitat for fish, wildlife and plants; lessens biodiversity; and contributes to changes in climate at varying scales.

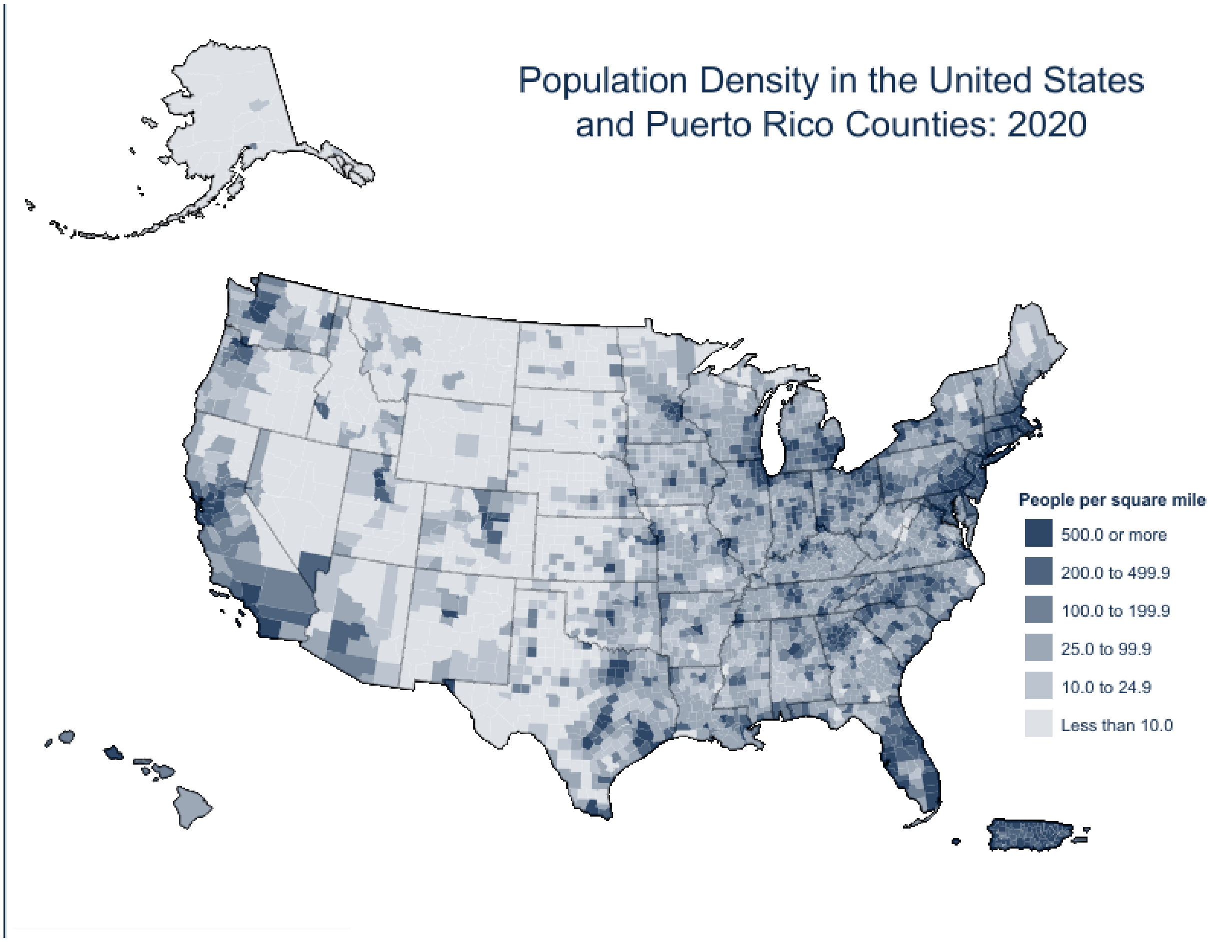

Figure 5. United States population density by county (USCB 2021).

Land uses in urban and suburban areas are cited as the primary cause of declining environmental conditions in the United States (Flather, Knowles and Kendall 1998) and other areas of the world (Houghton 1994). Urban and suburban development often includes construction of roads, railroads, associated rights-of-way (ROWs) and associated clearing of vegetation and other habitat features. These activities, as well as installation of below grade utility lines, pipelines, transmission lines and other infrastructure, can change terrestrial and riparian habitats and simplify and channelize streams, thereby reducing connectivity of surface water and groundwater. Historically, stream materials (e.g., sand, gravel and cobbles) were often used as fill, and excess excavation materials were pushed over the road bank, where they frequently entered streams. Riparian vegetation and stream banks were damaged using heavy equipment adjacent to and in streams. Side channels were often cutoff or eliminated, and stream channels were confined, resulting in increased bank erosion in certain areas. Lack of adequate drainage led to saturation of roadside soils. In many parts of the United States, road and ROW siting, construction, and maintenance practices have not changed significantly through time and thus continue to contribute to the decline of ecosystem function for fish, wildlife, and plants. Constriction of floodplains resulted in increased flooding (Palmisano, Ellis and Kaczynski 2003), which continues today in some areas. Construction, maintenance, and use of urban and suburban areas can also result in loss or degradation of riparian and wetland areas, degradation and fragmentation of terrestrial plant and animal habitats, sedimentation, erosion and slope hazards, reduction of species' passage, dispersal, or migration , and increased strike hazards to many classes of animals. Activities that involve land disturbance increase the risk of erosion and, therefore have the potential to affect the quantity of sediment that reaches waterways. Excessive sediment reduces stream depth, leads to increases in water temperatures and reductions in dissolved oxygen content (Ringler and Hall 1975, Henley, et al. 2000).

Most land areas covered by natural vegetation are highly porous and have limited sheet flow; precipitation falling on these landscapes infiltrates the soil, is transpired by the vegetative cover, or evaporates. The transformation of land into a mosaic of urban and suburban land uses has increased the area of impervious surfaces (e.g., roads, rooftops, parking lots, driveways, sidewalks). Precipitation that would normally infiltrate soils in forests, grasslands and wetlands falls on and flows over impervious surfaces and runs off the land. Runoff is channeled into storm sewers and released directly into surface waters (e.g., rivers and streams), which changes the magnitude and variability of water velocity and volume in those receiving waters. Runoff also can transport pollutants into waterways and across landscapes.

Impervious surfaces associated with residential and urban development create one of the most lasting impacts to stream systems. The amount of new impervious surfaces increased significantly in recent history, and this trend will likely continue in the future. There is a strong relationship between the amount of forest cover, level of impervious and compacted surfaces, and degradation of aquatic systems (Klein 1979, Booth, Hartley and Jackson 2002). Intensive development leads to losses of forest cover, increases in impervious surfaces, and changes to hydrology (e.g., increased peak flows, increased flow duration, reduced base flows, decreased evapotranspiration and groundwater infiltration) and these environmental changes can be detected when impervious surface in the watershed is as low as 5 to 10% (Booth, Hartley and Jackson 2002, May, et al. 1997). Some environmental changes, like increased peak flows and flow duration, often require engineering channels to address flooding, erosion, and sediment-transport concerns. Impervious surfaces also increase stormwater runoff, which causes many contaminant and pollution concerns (see the *Use of Pesticides* and *Pollution* sections below).

Additional water-quality concerns related to urban and suburban development include stormwater runoff, adequate sewage treatment and disposal, transport of contaminants to streams by storm runoff, and preservation of stream corridors. Human-dominated landscapes influence water availability, which has been and will continue to be a major, long-term issue in many areas. It is now widely recognized that ground-water withdrawals can deplete streamflows (Morgan and Jones 1999), and one of the increasing demands for surface water is the need to maintain instream flows for fish and other aquatic biota. For more information about impervious surfaces, water quantity, or pollutants, please see the *Impervious Surfaces*, *Water Quantity and Use*, and *Pollution* sections below.

To avoid or minimize negative environmental effects of impervious surfaces, developers and decision makers can implement actions to counter effects of impervious surfaces and stormwater runoff on natural resources. Narrower roads can be used in some cases to reduce the amount of impervious surface, and swales and rain gardens can be installed to reduce the amount of runoff. Land use planning, zoning, addition of parks, and natural area acquisitions are used in many communities to incorporate green infrastructure into developed landscapes that can help maintain functional floodplains, stream flows, water quality, fish and wildlife habitat, and other ecosystem functions and public benefits. Permeable pavement has been used to reduce stormwater runoff and pollution transport (Brattebo and Booth 2003, Drake, Bradford and Marsalek 2013), among other negative effects of impervious surfaces. Some states and localities have laws intended to control erosion and sedimentation (USEPA 2024, Fairfax 2024, Virginia.Gov 2024).

Mining and Mineral Extraction

The U.S. has a history of mining that dates to the early 17th century when iron, lead, silver, copper, and coal were discovered and mined by early colonial settlers of New England and the Mid-Atlantic states. Today, all states and Puerto Rico produce mined materials or extract minerals from below the Earth's surface. Mined materials include fuels (e.g., coal, oil, and gas) and building materials (e.g., sand, gravel, and clay). Extracted minerals include rare Earth minerals, aluminum, and copper. There are no readily available summary data to illustrate the extent of the various forms of mining; however, a 1975 U.S. Army Corps of Engineers (USACE) study on strip mining estimated 4.4 million acres and approximately 13,000 miles of rivers and tributaries were disturbed or adversely impacted by surface coal mining (USACE 1979).

In 1977, the U.S. passed the Surface Mining Control and Reclamation Act (SMCRA), "the primary federal law that regulates the environmental effects of coal mining in the United States" (OSMRE and SMCRA Programs 2024). SMCRA requires minimum standards for coal mining to be used nationwide with an aim to protect the environment and allows states to enact stricter regulations. Mined lands must be returned to pre-mining conditions as much as possible (i.e., reclamation), including successful revegetation and restoration of natural waterways. Acid-producing pyritic (FeS_2) materials need to be isolated below the final surface of the revegetated area. Post-SMCRA mine soils (i.e., 2002) had a higher pH than the finer-textured mine soils from mines sampled in 1980. In addition to the implementation of SMCRA, many technology improvements have occurred over the last several decades, and recent mining activities have bored deeper into unweathered rock as opposed to weathered rock closer to the surface (Daniels, Haering and Galbraith 2004).

Major coal producing areas in the U.S. include large swaths of the Appalachian Basin from Pennsylvania to Alabama; Colorado Plateau across New Mexico, Colorado, and Utah; Northern Rocky Mountains from North Dakota through Montana and Wyoming; Illinois Basin in Illinois, Indiana, and Kentucky; Western Interior from Iowa to Texas; and Gulf Coast from Texas through Louisiana, Arkansas, Mississippi, Alabama, and Tennessee. Comparatively small areas in Arizona, Washington, Oregon, and Idaho are also noted in national SMCRA biological opinions for coal mining activities (USFWS 2020).

Environmental effects from exploration, mineral extraction, and reclamation include habitat loss, reduction in surface and ground water quality, reduction in air quality, and pollution from mining waste disposal. Mining activities can alter downstream water chemistry, which may affect species, their habitat, and other resources on which they depend. Studies have shown that mining-impacted waterways often contain elevated levels of arsenic, selenium, iron, aluminum, manganese, and sulfate, and they typically have lower alkalinity concentrations and lower pH, while specific conductivity and total suspended solids are typically higher compared to streams unimpacted by mining (Skogerboe, et al. 1979, Wangsness, et al. 1981, Zuehls, Fitzgerald and Peters 1984, Herlihy, et al. 1990, Bryant, McPhilliamy and Childers 2002, Petty, et al. 2010, USEPA 2011, Presser 2013). Environmental impacts from mining have caused decreases in macroinvertebrate communities (Hartman, et al. 2005, Pond, et al. 2008) and fish (Hopkins and Roush 2013, Giam, Olden and Simberloff 2018, Sergeant, et al. 2022) downstream of mining activities. For some sites, even after years of reclamation and restoration efforts, the sites continued to show low levels of forest productivity compared to nearby native forests (Groninger, Fillmore and Rathfon 2006).

Water Quantity and Use

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

From the most recently published reports by the U.S. Geological Survey, freshwater withdrawals increased from 1950 until the 1980s, after which surface water use appeared to decrease even with population increases. Water use across the U.S. was estimated to be 322 billion gallons per day in 2015, which is the lowest overall withdrawal since 1970 and 9% less than the 2010 estimate. Freshwater withdrawals accounted for 87% of the total and saline-water withdrawals accounted for 13%. Overall, the largest water uses in 2015 were thermoelectric power and irrigation of agricultural lands (Dieter, et al. 2018). Overall water use and water use trends vary drastically across the U.S., and future assessments intend to focus on per capita water use instead of overall water use to reduce effects of variability (Alzraiee, et al. 2024).

Irrigation is used for agriculture and horticulture (e.g., forest nurseries, seed orchards, other crops), to maintain green spaces (e.g., golf courses, parks, turf farms, cemeteries, and other landscaping), and other water-related processes (e.g., frost protection, chemical application, weed control, harvesting, dust suppression, and leaching salts from the root zone). In 2015, most irrigation withdrawals (81%) were used in the western U.S. (i.e., North Dakota south to Texas and west to the Pacific Ocean). Groundwater was the primary source of irrigation water in California, Nebraska, Texas, Kansas, South Dakota, and Oklahoma, and surface water was the primary source elsewhere in the west (Dieter, et al. 2018).

Effects associated with water withdrawals include lower water volumes in rivers, streams, lakes, and aquifers; modification of natural flow regimes; water shortages downstream and during drought periods; reduced water quality; and degradation of wildlife habitat (Wissmar, et al. 1994, Saha and Quinn 2020). Irrigation also includes effects from water storage and drainage, increased water temperatures (which can become thermal barriers for salmonids and other aquatic species), introduction of pollutants (such as runoff containing pesticides and fertilizers), and increased sediment levels (Wissmar, et al. 1994, Krupka 2005).

There have been several attempts to reduce impacts from water withdrawal and water-diversion activities. Some efforts to minimize effects to anadromous fish were undertaken relatively early (Palmisano, Ellis and Kaczynski 2003), such as screening of irrigation diversions in the 1930s, although the screens did not protect all fish life stages, nor were they adequately maintained. More recently, the EPA published a handbook for developing watershed plans to restore and protect U.S. waters (USEPA 2008), in which they outline information needed for a watershed plan to meet water quality standards and protect water resources; many states have similar guides. Some projects were proposed specifically to address flow issues. For example, between 2000 and 2004, the Salmon Recovery Funding Board funded projects to alter river flows over 85 acres, slowing the stream flows to enhance salmon spawning and rearing habitats (SRFB 2005). Many similar projects exist across the country (NOAA 2023, WDOE 2023, YWA 2023).

Pollution, Contaminants, and Pesticides

Pollution is the introduction of harmful materials into the environment. Pollutants include a wide variety of materials such as excess nutrients, heavy metals (e.g., mercury, lead), persistent organic pollutants like polycyclic aromatic hydrocarbons (PAHs), poly-bromated diphenyl ethers (PBDEs), hazardous waste, and microplastics. The types and concentrations of pollutants in the environment vary depending on each pollutant's chemical characteristics and sources and can be influenced by environmental factors, habitat types, and regions. Altogether, pollutants represent a complex network of environmental stressors that contribute to habitat degradation, cause toxic effects in plants and animals, and impair ecosystems around the world. Given the wide variety of pollutants that currently contaminate the listed species' habitat, we are not able to fully address the breadth of impacts that pollutants have on the environmental baseline of listed species. Here, we provide a general survey of some common pollutants, a summary of their impacts on the environment, and description of how they contribute to the environmental baseline of listed species.

Pesticides and fertilizers are used on agricultural and developed lands and can enter the environment through stormwater runoff and spray drift (see *Use of Pesticides* section below for further discussion of the impact of pesticides on the environmental baseline). In their 2022 National Lakes Assessment, EPA reported that 47-50% of U.S. lakes were in poor condition with elevated phosphorus or nitrogen levels (i.e., excess nutrients), and lakes with excess nutrients experienced greater growth of harmful algae and cyanobacteria and hosted poor macroinvertebrate (e.g., insect larvae, snails, clams) communities. EPA did not find significant changes in nutrient levels, biological indicators, or microcystins (a type of cyanobacterial toxin) between their 2017 and 2022 survey results. They noted significant changes between 2017 and 2022: the number of lakes with good shallow water habitat (55% in 2022) and the number of lakes with good ratings for lakeshore disturbance (16% in 2022) each decreased by 9%, and detection of microcystins increased from 20% to 50% (USEPA 2022). Harmful algal blooms result in broad ecosystem effects like depleted dissolved oxygen resources for aquatic species, altered pH, reduced light availability, and increased turbidity (USEPA 2024). Some harmful algal blooms produce potent toxins and cause illnesses in wildlife and humans, such as paralytic shellfish poisoning. Harmful algal blooms commonly result in major environmental impacts, such as large-scale fish kills and hypoxic dead zones (Hallegraeff, Anderson and Cembella 1995).

Inorganic pollutants, including heavy metals like lead, mercury, and arsenic, occur naturally in the environment and can accumulate as pollutants as a result of human activities. Heavy metals are widely used in industrial, domestic, agricultural, medical, and technological applications, and can enter the environment through hazardous material spills, industrial emissions, vehicle emissions, stormwater runoff, discarded batteries, paints, and dyes. For example, in their 2022 Lakes Assessment, EPA found that 100% of fish sampled (n=413) contained mercury and 51% exceeded the recommended criterion of 300 ppb (USEPA 2022). Given their wide use, heavy metal contamination is a world-wide phenomenon. Heavy metals can be highly toxic, causing a wide range of effects like disruption of organ systems and metabolic function, developmental effects, neurological disorders, or other illnesses in wildlife and humans (Timothy and Williams 2019). Heavy metals do not degrade and thus can persist in the environment indefinitely without any remediation efforts.

Similarly, organic pollutants cover an incredibly wide array of chemical types. Many organic pollutants, such as PBDEs, per- and polyfluoroalkyl substances (PFAS), polychlorinated biphenyls (PCBs), and chlorinated compounds (including dioxins), are (or previously were) components of manufactured goods and their widespread use facilitates environmental contamination on a global scale. Some organic pollutants, such as PAHs, dioxins, and microplastics, are byproducts of industrial processes like combustion or leaching, or form during waste disposal and are unintentionally released into the environment. Regardless of their origin, organic pollutants are widespread and persistent in the environment. Their chemical characteristics (e.g., low water solubility, high volatility, slow degradation) make them long-lasting environmental contaminants as they permeate soils, are transported long distances by air, and accumulate in animal tissues, even long after removal of original sources.

Organic pollutants can have a variety of toxic effects, ranging from acute toxicity of various organ systems to long-term chronic effects like altered reproduction, endocrine disruption, and carcinogenesis. In the 2007 National Lakes Assessment, EPA found that several contaminants, including mercury, PCBs, dioxins, furans, and DDT (an insecticide), were widely distributed across surveyed lakes. Of particular concern is that some of these harmful pollutants (e.g., PCBs and DDT) remained detectable 30+ years after they were banned for use in the U.S. because of their effects on humans and the environment (USEPA 2007). In their 2022 Lakes Assessment, EPA found 100% of fish samples (n=413) contained PCBs and 93% contained at least one PFAS (USEPA 2022). Although the effects from many of these chemicals have been at least partially analyzed, multiple substances are present in the habitat and/or biota and little is known about their synergistic effects.

Other sources of toxic contaminants (including inorganic and organic pollutants mentioned previously) include solid waste and leaching from landfills, discharges of municipal and industrial wastewater, improper disposal of hazardous waste from different industries (e.g., printing, dry cleaning, auto repair shops), and channel dredging, which can result in resuspension of contaminated sediments. Discharges from wastewater treatment plants may be treated prior to discharge into receiving waters, but some persistent, bio-accumulative, endocrine-disrupting, or toxic compounds often remain in the water (Bennie 1999, CSTEE 1999, Daughton and Ternes 1999, Servos 1999). Stormwater runoff is another significant contributor of non-point source water pollution and can contain complex mixtures of multiple chemical and biological contaminants, which can have devastating effects on fish, like salmonids (KCDNR and WSCC 2000, Chow, et al. 2019), reefs, seagrass beds, and other aquatic life. The presence of roads and other impervious surfaces increase the distance pollutants can travel throughout runoff because they prevent water absorption into the ground, greatly exacerbating the environmental impact of many types of pollutants.

Even if contaminated areas are relatively small, their effects can be far-reaching and long lasting. Many pollutants, particularly those that have low solubility like organic pollutants, are taken up by living organisms through a variety of routes of exposure, such as inhalation, dermal contact, or ingestion. Many pollutants can biomagnify within an ecosystem, where body burdens disproportionately increase with increasing trophic levels. Consequently, predators can have very high contaminant levels, even if they have spent little or no time in contaminated areas.

Due primarily to risks to human health, much attention was given to hazardous dump sites and other areas of high pollution in the 1970s. In 1980, Congress established the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), which allows the EPA to clean up contaminated (i.e., Superfund) sites. CERCLA also forces responsible parties to either clean up their pollutants or reimburse the EPA for cleanup. CERCLA authorizes short-term removals and long-term remedial responses, depending on the nature of the contaminated site and the urgency of human and environmental health risks (USEPA 2024b). Many Superfund sites exist across the country, and success stories include Otis Air National Guard Base/Joint Base Cape Cod in Massachusetts, Brick Township landfill in New Jersey, Tobyhanna Army Dept in Pennsylvania, Kerr-McGee Chemical Corp in Mississippi, Celotex Corporation in Illinois, the USDOE Pantex Plant in Texas, Kansas City Structural Steel, Libby Asbestos in Montana, and Black Butte Mine in Oregon (USEPA 2024c).

Use of Pesticides

Pesticide use is common to kill or manage unwanted pests (e.g., plants, animals, fungi, microbes). Pesticides are often targeted for classes of pests: herbicides (i.e., plants), insecticides, rodenticides, fungicides, and others. In general, pesticides are beneficial to foresters and residential developers through control of unwanted or invasive non-native plants and aid in restoration of native habitat. They are beneficial to agriculture through control of pests that destroy crops, outcompete crops, degrade soils or water, and affect livestock. Pesticides can increase food production, increase profits for farmers, and prevent disease spread. Pesticides also benefit human health by killing pests mosquitos that that carry and transmit diseases (e.g., malaria, West Nile virus, and Zika).

When pesticides are applied, they can enter air, water, and soil across the environment. How long pesticides remain in the environment varies with the chemical itself (e.g.., how easily it degrades) and environmental conditions (e.g., soil water content) when its applied and after application (Arias-Estévez, et al. 2008). During a 10-year study by the U.S. Geological Survey (1992-2001), they detected pesticides in more than 90% of stream water samples, 80% of fish samples, and 50% of bed-sediment samples collected across the country (n=186). Pesticides were detected at concentrations above benchmarks for the protection of aquatic life in 50% of streams tested nationwide, 83% of streams in urbanized areas, and 94% of streambed sediments (Gilliom, et al. 2006). Pesticides were common throughout the year in streams of developed watersheds dominated by agriculture, urban, and mixed land uses. Fish and sediment in streams were contaminated with organochlorines like DDT, many of which have not been used for years due to discovered environmental and human health impacts. Other studies showed that pesticides were frequently detected in groundwater samples and while concentrations were often below human-health benchmarks, they did not assess wildlife or other environmental benchmarks (Toccalino, Lindsey and Rupert 2014, Bexfield, et al. 2021). From their 2022 report, EPA reported that 41% of samples lakes had detectable levels of atrazine, a significant 11% increase from their last report in 2017 (USEPA 2022).

Pesticide use as part of past federal and non-federal actions has resulted in impacts to listed species, their habitats, and other species on which listed species depend. Pesticides affect taxa groups differently. For example, insecticides are targeted for insect pests, so they typically have greater effects on listed insects and potentially predators of insects and plants pollinated by insects than on other taxa groups. In general, pesticides have been documented to cause many unintended effects to nontarget organisms such as changes to bird eggshell thickness, fish behavior and reproduction, and insect behavior and survival (D. Pimentel 1971, Köhler and Triebskorn 2013).

Some federal actions have undergone ESA section 7 consultations related to pesticide use. For example, the USDA Animal and Plant Health Inspection Service (APHIS) Pest Program uses pesticides to achieve its mission and has consulted with the Service on multiple occasions. APHIS's implementation of these activities is generally supported by a well-established program that includes environmental compliance, training, monitoring, and reporting, as well as incorporating species-specific conservation measures designed to avoid and minimize adverse effects (e.g., their use of pesticides for the Grasshopper and Mormon cricket suppression program includes conservation measures that led to Service concurrence on NLAA determinations for many species). Most APHIS activities occur on non-federal lands.

Aquatic Habitats

All waterbodies in the U.S. have been affected by anthropogenic stressors, which often lead to long-term environmental degradation, lower biodiversity, reduced primary and secondary production, and a lower capacity or resiliency of the ecosystem to recover to its original state in response to natural perturbations (Rapport and Whitford 1999). Freshwater habitats are among the most threatened ecosystems in the world (Leidy and Moyle 1998). Reviews of aquatic species' conservation statuses for the past three decades have documented the cumulative effect of anthropogenic and natural stressors on freshwater aquatic ecosystems, resulting in a significant decline in the biodiversity and condition of indigenous fish, mussel, and crayfish communities (Taylor, et al. 2007, Jelks, et al. 2008).

Wetlands

Wetlands include tidal salt marshes, mangroves, freshwater marshes, swamps, riparian forests, and peatlands (W. J. Mitsch, et al. 2009). Wetlands store atmospheric carbon, protect clean water, maintain cool water temperatures, retain sediments, store and desynchronize flood flows, maintain base water flows, mitigate storm damage to coastal areas, and provide food and cover for many species of fish, birds, aquatic organisms, and other wildlife (Mitsch and Gosselink 1993, Beechie, Beamer and Wasserman 1994, W. J. Mitsch, et al. 2009). Wetlands also improve water quality through nutrient and toxic-chemical removal and/or transformation (Hammer 1989, Mitsch and Gosselink 1993).

Riparian areas, the transitional zone between streams and uplands, protect the stream from excess sediments, sequester pollutants, contribute to the reduction in peak stream flows during floods, and act as holding areas for water that is released back into the stream during times of low flow. They create habitat features essential for wildlife, like pools, riffles, slack areas, and off-channel habitats. Agricultural development, urbanization, timber harvest, road construction, and other land-management activities are responsible for much of the loss of riparian buffers (70% of the original area of riparian ecosystems) in the United States (Swift 1984). Different riparian widths provide various ecological functions depending on the characteristics of a particular riparian zone. For many small stream systems, riparian areas are highly degraded or no longer exist, and their restoration is precluded by existing development. Although functional riparian areas have the capacity to mitigate for some of the adverse impacts of development (Morley and Karr 2002), they cannot effectively address significant impacts from changes to stream hydrology resulting from significant losses of forest cover (May, et al. 1997, Booth, Hartley and Jackson 2002).

Though efforts to create and restore wetlands and riparian buffers have dramatically reduced the rate of destruction or degradation, many wetland habitats continue to be lost. The U.S. originally contained almost 392 million acres of wetlands. Between the 1780s and the 1980s, 118 million acres of wetlands were lost after human interference. Wetlands were often excavated or filled to create upland for real estate development or converted to agriculture (Duke and Krucynski 1992). Arkansas, California, Connecticut, Illinois, Indiana, Iowa, Kentucky, Maryland, Missouri, and Ohio lost 70% or more of their original wetland acreage. California lost an estimated 91% and Florida lost 46% of its 1780s total (Dahl 1990). Between 2006 and 2009, approximately 13,800 acres of wetlands were lost per year (Dahl 2011). In 2019, wetlands occurred on approximately 116.4 million acres of the CONUS and most of them (95%) were freshwater (Lang, Ingebritsen and Griffin 2024). Most wetlands were vegetated, primarily freshwater emergent or scrub-shrub wetlands (92% of freshwater) and salt marsh (80% of saltwater). Net wetland loss between 2009-2019 increased by over 50% compared to 2004-2009, most of which was loss of vegetated wetlands (Figure 6). The authors believe some loss of saltwater wetland indicates a future loss of wetland to sea level rise and coastal storm impacts (Figure 7). Many remaining wetlands have been degraded and have reduced functionality compared to wetlands from the 1780s. Lang et al. (2024) also documented an increase in non-vegetated wetlands, a shift which reduces the prosperity, health, and safety of wetland and nearby communities compared to vegetated wetlands.

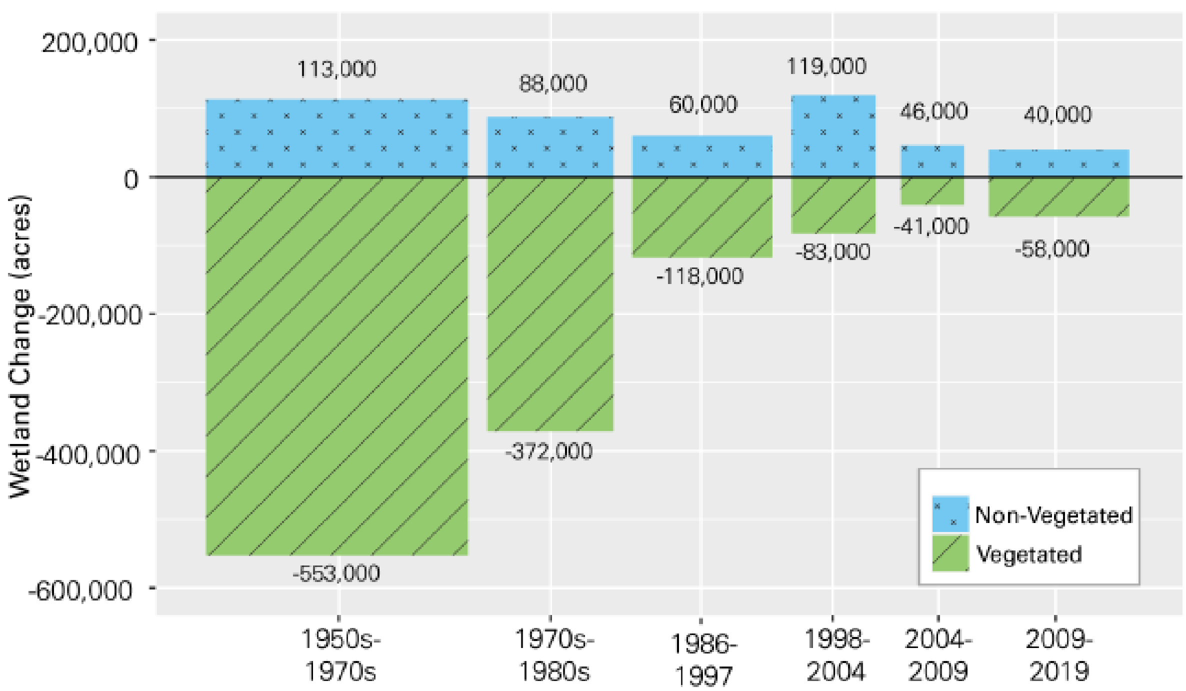

Figure 6. Average net annual non-vegetated and vegetated freshwater wetland acreage change estimates for the CONUS from the 1950s-2019. Figure on page 21 of (Lang, Ingebritsen and Griffin 2024).

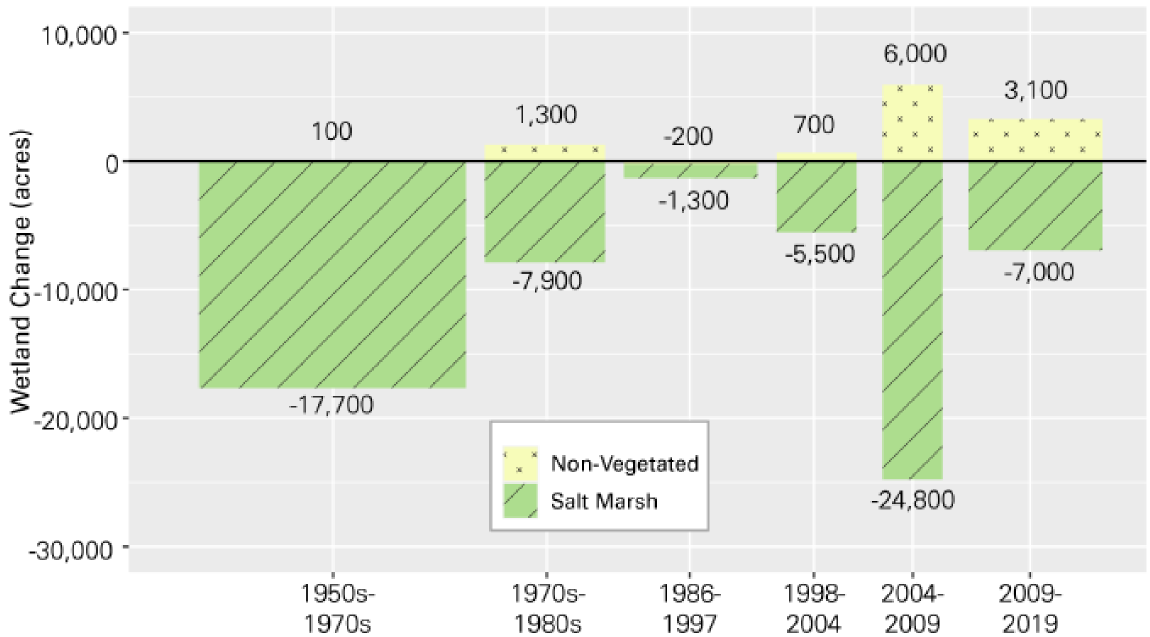

Figure 7. Average net annual salt marsh and non-vegetated saltwater wetland acreage change estimates for the CONUS from the 1950s-2019. Figure on page 21 of (Lang, Ingebritsen and Griffin 2024).

Rivers and Streams

Free-flowing rivers regularly flood and recede, collecting and depositing sediment laterally and downstream. Sediment is eventually deposited in deltas and estuaries where freshwater enters saltwater. Natural rivers typically are narrower and have more riparian and bank cover, habitat diversity, and pool volume than rivers managed for transportation or for other purposes. Past land use can leave legacy effects on streams and rivers, and restored riparian zones may not serve the same ecosystem function as the original habitat (Wohl and Merritts 2007).

Many streams have been channelized, diverted, and confined through the construction of dikes, levees, berms, revetments, embankments, and other structures. Channelization (and often its associated bank armoring) is used to reduce flood damage to property, exclude water, or store water for future use. While these changes may be favorable to property owners or project proponents, such actions often result in substantial changes to aquatic and terrestrial habitats and their use by wildlife. Channelization results in simplification of the stream and has resulted in changes in flow, velocity, temperature, and movement of water (Tarplee, Louder and Weber 1971, Bolton and Shellberg 2001). Channelization also degrades and fragments migratory corridors, eliminates historical foraging, migration, and overwintering habitats (Bolton and Shellberg 2001), and results in changes to songbird and small mammal communities (Possardt and Dodge 1978).

Barriers to Fish Movement

Water management structures (e.g., dams, dikes, levees) are often used for flood control, conversion of wetlands to agriculture, bank protection, water supply needs, power generation, recreation, or other development and can reduce connectivity among and within watersheds. By 2024, 600,000 miles of river in the United States (conservatively, 17%) have been modified by over 75,000 large dams (IWSRCC 2024). Dams serve as a barrier to fish passage (Limburg and Waldman 2009) and delay or block passage of anadromous fish to upstream reaches. The ability of anadromous fish to access areas above man-made barriers is important for the survival of individuals and populations of fish and for the integrity of the ecosystems they support (Cederholm, et al. 2000). Staging and spawning adult fish are prey for upstream aquatic and terrestrial predators. Rich marine-derived nutrients from anadromous fish are transported to the reach of stream in which they die, into the lower reaches of the stream and estuary through downstream drift, and across habitat or ecosystem boundaries by mobile mammals, birds, and fish (Doughty, et al. 2015, Mattocks, Hall and and Jordaan 2017). In addition, fish movement is essential for reproduction of many freshwater mussel species (Haag 2012). Barriers to fish passage also contribute to fragmented mussel populations.

Controlled flow from a dam often slows river movement and changes the natural cycle of river flows, resulting in areas that are either drier than normal (because the water is being held behind the reservoir) or flooded by much higher levels of water. Changing the depth and flow of rivers affects water quality, temperature, and material transport (e.g., sediments, nutrients, and large woody debris). Reservoirs fill with sediment and less sediment reaches downstream deltas and estuaries. For example, in a press release about the Iron Gate Dam drawdown, the Klamath River Renewal Corporation mentioned that 17-20 million cubic yards of sediment has been trapped behind three dams slated for removal (Brownell 2024).

Many projects aimed to mitigate or minimize effects of past or present dams or reservoirs on downstream habitats exist across the country (USFWS 2022, NRCS 2023, NOAA 2023). Fish ladders were added to some waterways to aid in fish passage, but some fish life stages still cannot get through, and they encourage congregation, which facilitates disease spread and resource competition. Over 1,200 dams have been removed across the U. S. according to Bellmore, et al. (2017). Few studies have assessed changes to habitat or ecosystem biodiversity after dam removal (Bellmore, et al. 2017), but when dams are removed, trapped sediment (often millions of cubic yards of sediment) runs downstream (Brownell 2024) and can change waterflow and cause turbidity. Some invasive and non-native species (e.g., Asian carp and lampreys) use fish ladders and benefit from dam removal. In some locations, dams are being used intentionally to limit movement of an unwanted or invasive fish species from affecting target species, like trout, chubs, and salmon (McLaughlin, et al. 2013).

Improperly installed, incorrectly sized, and failed culverts serve as barriers to fish movement and migration. Several groups have made efforts to inventory and remove fish barriers under their jurisdictions, often either removing barrier culverts or replacing them with a more-suitable structure. Removal of a barrier culvert is often undertaken when a crossing is no longer needed (Peck 2005). If a crossing is necessary, other options include bridges or other specific methodologies: stream simulation, roughened-channel design, no-slope methodology, or hydraulic design.

Estuaries

Estuaries, including salt marshes, are some of the most productive ecosystems in the world (Correll 1978), and they include salt marshes, mangrove forests, mud flats, tidal streams, rocky intertidal shores, reefs, and barrier beaches. Estuaries are home to thousands of species of birds, mammals, fish, and other wildlife in the U.S. Salt marshes filter pollutants that flow through it and trap nutrients, which explains why salt marshes serve as nursery and breeding grounds for many wildlife species. Estuaries and associated wetlands also stabilize shorelines and protect nearby coastal and inland areas from flooding and other storm damage (NOAA 2024). Many animals, including most commercially important fish (e.g., salmon, sturgeon), sea turtles, and waterbirds, depend on estuaries for nursery, rearing, foraging, and/or migration habitat.

Diking and filling reduces the tidal prism, reduces freshwater inflows, changes sediment flows, and eliminates emergent and forested wetlands and floodplain habitats. Dikes may have marked effects on tidal channel biota, specifically on the seaward side of the structure, and their construction may result in decreased sinuosity and complexity, preventing energy dissipation during flood events in some places (Hood 2004). Similarly, dredging activities in shallow coastal estuaries can increase the tidal prism, increase salinities, increase turbidity, release contaminants, lower dissolved oxygen, and reduce nutrient outflow from marshes, resulting in a host of negative consequences to these ecosystems. Diking, filling, and dredging has: reduced fishery productivity; contributed to land losses (e.g., Louisiana, Florida); contributed to fish kills; reduced avian habitats and use; and reduced the resiliency of estuarine areas to stochastic events (e.g., hurricanes) (Johnston 1981, Nightingale and Simenstad 2001). For example, changes in habitat and food-web dynamics have altered their capacity to support juvenile salmon (Bottom, et al. 2005, Fresh, et al. 2005, Allen, Pondella and Horn 2006, LCFRB 2010).

Mitigation of losses of estuarine marsh in the mid-Atlantic and Gulf areas may roughly keep pace with the losses of the last two decades, but they have not reversed the large losses of the mid-twentieth century (Dahl 2011). The Estuary Restoration Act of 2000 was developed to address wetland loss and damage from human activities, and the USACE received funding for project implementation across the country.

In Florida, the Kissimmee River Restoration Program was authorized by Congress and initiated in 1992. In July 2021, the South Florida Water Management District and USACE Jacksonville District completed the project's construction. Overall, they restored >40 mi^2 of the river floodplain, 20,000 ac of wetlands, and 44 mi of historic river channels (SFWMD 2021). Also, the Comprehensive Everglades Restoration Plan was authorized by Congress in 2000 to "restore, preserve, and protect the south Florida ecosystem while providing for other water-related needs of the region, including water supply and flood protection" (SFNRC 2016). The greater Everglades ecosystem historically encompassed 18,000 sq. miles from central Florida to the Florida Keys. Water flowed south into Lake Okeechobee and then spilled over its banks into the sawgrass plains, open water sloughs, rocky glades, and marl prairies and finally into the Gulf and

Florida and Biscayne Bays. The USACE installed a massive network of canals, levees, and water conservation areas that blocked sheet flow to urban areas and provided water for dry season use. The Comprehensive Everglades Restoration Plan is ongoing as of 2022 (NPS 2022).

In Washington, restoration efforts focused on the benefits of restoring ecosystem functions affected by water diversion structures. In 2002, the Nisqually Tribe removed a portion of a dike in Red Salmon Slough, reconnecting 31 acres of former pastureland to the Nisqually River Estuary (SPSSEG 2002). This action was undertaken to benefit juvenile salmonids, other fish, and migratory birds. At Spencer Island in Snohomish County, two 250-foot-long breaches were made in an estuary dike to reconnect approximately 250 acres of estuarine marsh (Carlson 2005). Other similar restoration work has occurred across the country (USACE 2013).

Shorelines

Significant shoreline development and urbanization has occurred throughout the U.S. Habitats at risk from shoreline alteration include riparian buffers, freshwater habitats (e.g., streams, lakes), and shallow subtidal, intertidal, and shoreline habitats known collectively as the “marine nearshore.” Submerged aquatic vegetation (i.e., seagrass beds) on the Pacific and Atlantic coasts grow in the intertidal zone and in mud and sand in the shallow sub-tidal zone. Turtle grass, shoal grass, manatee grass, and wigeon grass occupy similar ecological niches in the estuaries in northern areas of the Gulf. Many of these areas house migratory shorebirds and waterbirds, spawning and rearing salmonids, shellfish reefs, and other wildlife (Duke and Krucynski 1992).

Portions of nearshore and shoreline habitats have been altered with vertical or steeply sloping bulkheads and revetments to protect various developments and structures (e.g., railroads, piers) from wave-induced erosion, stabilize banks and bluffs, retain fill, and create moorage (e.g., docks, harbors) for vessels. Depending on shoreline characteristics and structure placement, shoreline armoring can interrupt natural sand inputs from landward bluffs and result in sediment deficits (Prosser, et al. 2017). Docks, bulkheads, and other shoreline structures likely contribute to reductions in submerged aquatic vegetation and spawning and rearing areas for fish. For example, in some areas, 20-100% of highly productive submerged aquatic vegetation habitats were lost in estuaries of the northern Gulf (Handley, Altsman and Demay 2007). Often, submerged aquatic vegetation serves as an indicator of lake or shoreline health and die offs result from decreases in water quality or contamination (Moorman, et al. 2017) from development on or near the shore.

Clean Water Act

Several laws and regulations help improve the state of our aquatic resources, the principal one being the Clean Water Act (CWA). The original 1948 statute was re-written in 1972 and defined its current purpose: “to restore and maintain the chemical, physical, and biological integrity of the Nation's waters” (Federal Water Pollution Control Act, Public Law 92–500). Congress made substantial amendments to the CWA in the Water Quality Act of 1987 (P.L. 100-4) in response to significant and persistent water quality problems.

The CWA generally prohibits all point source discharges into waters of the U.S. (as defined in 40 CFR 120.2[17]), unless otherwise authorized under the CWA. One of the main ways that point source discharges are regulated is through permits issued under the National Pollutant Discharge Elimination System (NPDES) for bacteria, oxygen-consuming materials, and toxic pollutants like heavy metals, pesticides, and other organic chemicals. EPA has also promulgated regulations setting effluent limitation guidelines and standards under CWA sections 301, 304, and 306 for more than 50 industries [40 CFR Parts 405 through 471] that are based on pollutants of concern. These effluent limitations have been credited for helping reduce the amount of pollutants like toxic metals entering the aquatic environment (Smail, et al. 2012). While provisions of the CWA have helped significantly improve the quality of aquatic ecosystems, nonpoint sources of water pollution, which are believed to be responsible for most of modern water quality problems in the U.S., are not subject to CWA permits or regulatory requirements. Instead, nonpoint sources of pollution are regulated by programs overseen by the states.

Invasive Species

Invasive species are species capable of causing great economic or ecological impacts in areas where they become established; they are often, but not exclusively, non-native to the ecosystem. Ecological impacts from biological invasion include predation, disease transmission, competition (for food, light, space), and hybridization. The rate of species invasion increased over the past several decades due to human population growth, alterations of the environment, and technological advances that allow for the rapid movement of people and products (Pimentel, Zuniga and Morrison 2005). Invasive species are considered a contributing factor in the decline of half of the imperiled species in the U.S. (Wilcove, et al. 1998). On islands where species are also usually threatened by small populations, small ranges, and high rates of endemism, the impact of invasive species is even greater.

An estimated 50,000 or more non-native terrestrial and aquatic species are believed to have been introduced into the U.S. across its history. Non-native mammals include species of dogs, cats, horses, sheep, pigs, goats, deer, and rodents. About half of these species are plants, 5,000 of which were introduced to the U.S. as food or ornamental plants and have since escaped and established on their own. In some cases, non-native plants are capable of dominating new habitats, forming dense monocultures, and completely excluding other native plants (Pimentel, Zuniga and Morrison 2005). Invasive plants can accelerate carbon cycling, alter hydrologic cycles, change nutrient cycles, and reduce sunlight penetration in aquatic habitats (Poland, et al. 2021). Approximately 97 non-native birds exist in the U.S. with self-sustaining populations, 56% of which are considered pest species. Many non-native birds compete with or displace native birds, and they are vectors for avian diseases. As of 2005, 138 non-native fish were introduced into the U.S., and at least 44 native fish species are threatened or endangered because of invasive fish. Approximately 53 species of reptiles and amphibians have been introduced to the U.S., and they often prey upon native species. More than 4,600 non-native invertebrate species are found

[17] See https://www.ecfr.gov/current/title-40/chapter-I/subchapter-D/part-120/section-120.2.

in the U.S., some of which are well known for vast ecological impacts (e.g., balsam woolly adelgid [*Adelges piceae*], red imported fire ant [*Solenopsis invicta*], and European green crab [*Carcinus maenas*]), including the decline or extirpation of native species (Pimentel, Zuniga and Morrison 2005).

Once an invasive species is established, management strategies available include prevention of further spread, early detection, eradication, control, and adaptation. Prevention includes actions like ship inspections and eradication at entry ports before it is brought into the location. If a species is missed during prevention efforts, it can be detected early and potentially eradicated, particularly if there are only a few individuals or a small population. Control includes efforts to limit the growth and spread of an established species or population (e.g., physical barriers). Adaptation can include use of pesticides on the invasive species or harvest of the species. The optimal choice for managing invasive species varies with the species of concern, environment affected, and policy and fiscal considerations (Marbuah, Gren and McKie 2014, Espanchin-Niell 2017). The Lacey Act of 1900 is a tool used to limit transportation of "injurious" wildlife. In 1996, the U.S. amended the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 to include the National Invasive Species Act of 1996, which aims to prevent introductions and spread of invasive aquatic species in the Great Lakes through ballast water.

Collection and Harvesting

Some ESA-listed species, such as salmonids and freshwater mussels, are economically important species harvested as food. Harvesting and exploitation is identified as a contributing factor to 18% of the imperiled freshwater mussels of the United States through the pearl industry (Strayer, et al. 2004). After species are listed as threatened or endangered under the ESA, they receive protection from overharvesting because harvest requires a permit issued by the Service, and permits are generally limited to certain categories of activities that would benefit the conservation and recovery of the species. Although harvest is a historical threat to many ESA-listed species and illegal harvest still likely occurs to some degree, it rarely affects species substantially now, and it is not expected to greatly affect currently listed species in the action area in the future.

Climate Change

All species discussed in this Opinion are or may be threatened by the effects of global climate change. The Intergovernmental Panel on Climate Change (IPCC) reported that global surface temperatures were 1.09°C higher between 2011-2020 than 1850-1900; greater increases were recorded over land (1.59°C) than the ocean (0.88°C) and temperatures increased faster between 1970-2020 than any other 50-year period in the last 2,000 years. Human-induced causes like greenhouse gas emissions are strongly believed to be responsible for about 1.07°C of this change (Lee and Romero 2023).

Increasing atmospheric temperatures have contributed to changes in the quality of freshwater, coastal, and marine ecosystems and the decline of populations of endangered and threatened species (Mantua, et al. 1997, Karl, Melillo and Peterson 2009, Littell, et al. 2009, Staudinger, et al. 2012). Climate change is anticipated to impact the timing and intensity of seasonal stream flows (Staudinger, et al. 2012), reduce snow accumulation, increase winter stream flows, cause spring snowmelt to occur earlier in the year, and reduce summer stream flows in rivers that depend on snow melt (Littell, et al. 2009). Warmer water temperatures were identified as a factor in the decline and disappearance of mussel and barnacle beds in the northwest U.S. (Harley 2011), complete loss of some macroinvertebrate species in Arizona (Sponseller, et al. 2010), wetland desiccation and declines in four amphibian species in Yellowstone National Park (McMenamin, Hadly and Wright 2008). Changes in stream flow from changes in seasonal run-off patterns may alter predator-prey interactions and change species assemblages in aquatic habitats.

Warmer global air temperatures are rapidly melting sea ice and rising global sea levels. Between 1880 and the 2010s, global mean sea level increased between 21-24 cm, the fastest rate of sea level rise over the last 2,800 years. Higher sea levels worsen effects of coastal storms, storm surge, tidal flooding, and waves. Climate change is also anticipated to increase storm frequency and intensity, which would exacerbate these concerns. Wave action, beach inundation, marsh flooding, and general sea level rise affect coastal habitats and wildlife, including geomorphology and sediment cycling, and modify the future flood risk profile of communities and ecosystems (Sweet, et al. 2017).

Warmer temperatures are expected to increase water use for agriculture, both for existing fields and the establishment of new ones in once unprofitable areas (ISAB 2007). If agriculture requires more water, streams, rivers, and lakes will experience additional water withdrawals and potentially higher contaminant loads from returning effluent.

Other effects of climate change include changes in sea surface temperatures, alterations in precipitation patterns, and increased success of non-native, invasive, and pathogenic species. Biota may be forced to respond to climate-induced changes in their environment like altered reproductive seasons/locations, shifts in migration patterns, reduced distribution and abundance of prey, and changes in the abundance of competitors and/or predators. Climate change is most likely to have its most pronounced effects on species whose populations are already in tenuous positions (Isaac 2009, McElwee, et al. 2023). Warmer water stimulates biological processes that can lead to environmental hypoxia. Oxygen depletion in aquatic ecosystems can result in anaerobic metabolism increasing, thus leading to an increase in metals and other pollutants being released into the water column (Staudinger, et al. 2012). Effects of aquatic nuisance species invasions are also likely to increase as ecosystems become less resilient to disturbances (USEPA 2008). Invasive species that are better adapted to warmer water temperatures could outcompete native species that are physiologically adapted to lower water temperatures; such a situation already occurs along central and northern California (Lockwood and Somero 2011).

The EPA has several programs and standards in place to help combat greenhouse gas emissions, and thereby combat climate change. In 2005, EPA created the Renewable Fuel Standard, which requires all fuels sold in the U.S. to contain a certain amount of renewable fuels to offset petroleum-based fossil fuels and reduce greenhouse gas emissions (USEPA 2023). EPA implements a carbon dioxide emission standard for commercial and large business aviation and a greenhouse gas emissions standard for passenger cars and light trucks for model years 2023-2026. The passenger standards are estimated to save over 3 billion tons of greenhouse gases up to 2050 (USEPA 2024).

Change of Ecosystem Function and Biodiversity Loss

The environmental and habitat changes discussed in the previous sections affect ecosystem function and biodiversity. Biodiversity, the variety of life in a community often measured in number of species and equity of those species (i.e., richness and evenness, respectively), has been declining globally and in the United States for decades. Many aspects of biodiversity and its effects on ecosystem function that are unknown, but evidence supports that communities with higher biodiversity in terrestrial, aquatic, and marine ecosystems are more productive than monocultures in the same environments. Productivity comes from optimal use of limited resources, lower incidence of disease and herbivory, higher nutrient stores, and more nutrient-cycling feedback loops. Communities with higher biodiversity are more resistant to non-native species invasions because few resources are unconsumed and available for invaders. Highly diverse communities have a greater bacteria diversity, which makes them more resistant to some pathogens (Tilman, Isbell and Cowles 2014). Climate change and drivers of climate change exacerbate biodiversity loss across taxa and regions (McElwee, et al. 2023). Specifically, insect and invertebrate declines across regions and habitats are well-documented and pose great threats to ecosystem resilience and repercussions for human systems.

Insect Pollinator Decline

Of particular concern to national pesticide consultations is the documented insect pollinator decline that has occurred over the last several decades. Insects have been experiencing a worldwide decline in biomass, abundance, and diversity with potentially negative implications for plant pollination. Long term surveys in North America and Europe show terrestrial insects declined in abundance by an average of 9% per decade (0.92% per year), whereas freshwater insects increased by 11% per decade (1.08% per year). The most compelling evidence for declines in terrestrial insect assemblages was found in North America, but strong evidence exists for directional trends in temperate zone, Mediterranean, and desert climates. The declines appear to be associated with changes in land use. Moderate evidence exists for a negative relationship between terrestrial insect abundance trends and landscape urbanization that may be explained by habitat loss and light and/or chemical pollution (Van Klink 2020).

Consequences of insect declines could include reduction in ecosystem services like pollination and seed dispersal (Dornelas and Daskalova 2020). By 2010, there were 54 studies covering 89 plant species that showed the most frequent proximate cause of reproductive impairment of wild plant populations in fragmented habitats was pollination limitation (Potts, et al. 2010). Over the last 10-30 years, many pollinators are at risk of extinction, and they have shifted or contracted their ranges due to several factors, including habitat loss, environmental changes, competition with invasive or non-native species, and potentially other reasons (McElwee, et al. 2023). The scope of global and national pollinator decline has been evaluated in numerous studies, and we summarized a few below.

In Illinois, Burkle (2013) used historical data sets to determine the degree of change over 120 years in a temperate forest understory community. Results showed that 50% of bee species in the study area were extirpated and 46% of the original forb-bee interactions were lost (246 of 532), even though all 26 forbs remained present. More specialist pollinators were lost then generalists, even though specialist host plants were still present. Specialist bees, parasites, cavity-nesters, and those that participated in weak historical interactions were more likely to be extirpated. Bee species richness visiting the forb *C. virginica* did not change between 1891 and 1971, but it declined by over half in the following 40 years, likely due to changes in forested habitat during that time. Also in Illinois, Marlin & LaBerge (2001) found 140 bee species between 1970 and 1972, implying a 32% reduction in biodiversity compared to historical records from the same location 75 years earlier. Only 59 of the 73 prairie-inhabiting bees and 15 of the 27 forest-dwelling bees were found. Another study evaluated changes in the distribution of six bumble bee species by comparing historical records with intensive surveys across 382 locations in the U.S. Half of the species declined in abundance by as much as 96% of their initial populations in the last 30 years, and their geographical range was reduced between 23 and 87% (Lozier, et al. 2011). In Oklahoma, only five of the ten species of bumble bees that were present in 1949 were found in 2013 after extensive surveys across 21 counties. Additionally, the species *B. variabilis* was presumed extinct (Figueroa and Bergey 2015).

In southern Ontario, bumble bee community composition was compared between 2004 and 2006 and 1971 and 1973 at the same sites. These areas formerly had diverse bumble bee communities and underwent declines in bumble bee species richness, diversity, and relative abundance between these two time periods. Between 1971 and 1973, 14 bumble bee species were found, and between 2004 and 2006, eleven species were found. Fourteen species found between 1971 and 1973 were either absent or decreasing in relative abundance between 2004 and 2006. For example, the rusty patched bumblebee (*B. affinis*) was previously widespread and common but underwent drastic decline and has likely been extirpated throughout much of its range. It was not found during the 2004-2006 surveys. No new species were identified (Colla and Packer 2008).

GENERAL EFFECTS

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

For this Opinion, our analysis of the effects of the proposed registration review of simazine on listed resources under the Service's purview is presented first by discussing the effects of simazine to different taxa groups in the *General Effects* section. The *General Effects* section of this Opinion is divided into several sections and subsections. First, we briefly summarize the anticipated toxicological effects related to the proposed action, including the anticipated general pathways of exposure to listed species taxa groups and their designated critical habitat. Next, in the *Exposure* and *Usage Analysis* sections, we describe specific aspects of simazine (e.g., chemical properties, applications rates, routes of exposure), its use and usage on the landscape, and how it will impact species and critical habitats based on these properties. We describe those factors that influence exposure and effects and how we chose to incorporate them into our analysis. Theses sections are broadly broken into sections for Terrestrial Animals, Aquatic Animals, and Plants due to fundamental differences in how these groups of species may be exposed, and in turn, respond to simazine use. We included taxa-specific, meaningful information to the analysis wherever possible.

Toxicological Effects

As described in the BE, simazine is a pre-emergent herbicide that is intended to control broadleaved and grassy weeds. Simazine belongs to a class of herbicides known as chlorotriazines. Chlorotriazines differ only in the number or position of the methyl groups on moieties branched in identical positions off of the triazine ring (Figure 8) and thus are nearly structurally identical and therefore have similar herbicidal effects on terrestrial and aquatic plants (USEPA 2016).

Simazine acts by binding with a protein complex of the photosystem II mechanism in chloroplast photosynthetic membranes (Schulz, Wengenmayer and Goodman 1990). The result is an inhibition in the transfer of electrons through the light reactions of photosynthesis that in turn inhibits the release of oxygen, production of adenosine triphosphate (the main energy molecule in all living cells), and the fixation of carbon dioxide into sugars. As a result, the plant will experience a marked decline in growth (PŁoszynski and Zurawski 1971).

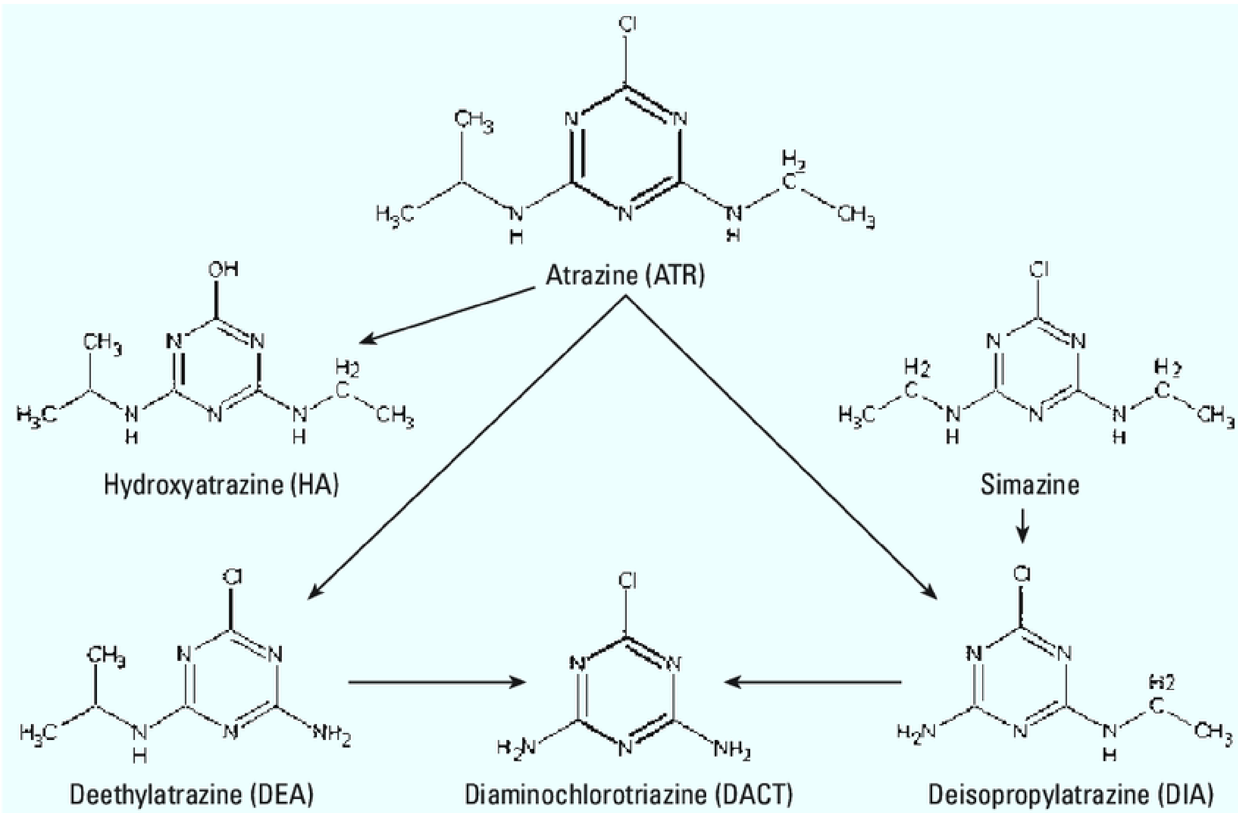

Figure 8. Structure of the environmental metabolites of two chlorotriazine herbicides, atrazine and simazine. Atrazine can be metabolized to HA, DIA, or DEA. The DEA and DIA forms are both quickly converted to DACT (Enoch, et al. 2007).

Evaluating the effects of endocrine disrupting compounds

Simazine has been identified by EPA's Endocrine Disruptor Screening Program as a chemical with evidence for potential interaction with the estrogen and androgen signaling pathway. Chemicals that interact or bind to endogenous hormone receptors are referred to as endocrine disrupting compounds (EDCs) and can interfere with hormone signaling in vertebrates, eliciting changes in hormone production and feedback loops of sex steroids, thyroid hormones and neurotransmitters. Such disruptions can adversely affect outcomes such as reproduction, growth, development, immune function, nervous system signaling, and behavior (USEPA 2015, Marlatt, et al. 2022). Endocrine disruption can occur in vertebrates following both acute and chronic exposures to certain contaminants and result in lasting effects. Early life stages are particularly sensitive to EDC exposure, especially in organisms with developmental plasticity (i.e., the influence of environmental factors such as temperature, population sex ratios, and other abiotic, external factors on growth and reproductive maturation in some taxa), such as fish, amphibians, and reptiles (Bergeron, Crews and McLachlan 1994, Coe, et al. 2010, Hoskins and Boone 2018). One such example is the impact of endocrine disruptors on sexual development in organisms exposed during embryonic or larval stages, which can result in demographic changes and skewed sex ratios in the subsequent adult population (Coe, et al. 2010).

The adverse outcome pathway (AOP) established for endocrine disrupting compounds, described by (Ankley, et al. 2010), illustrates a sequential series of events through which molecular responses induced by exposures to endocrine disruptors can lead to organismal and population level responses. Briefly, molecular initiating events, such as gene expression, induce a cascade of higher order biological effects at the cellular, tissue, organismal, and population level (Figure 9; (Ankley, et al. 2010). Using this framework, studies measuring these molecular initiating events and cellular responses, can be used to qualitatively assess potential risks to the organism or population. While scaling up effects throughout the levels of organization described in the AOP can be challenging and unique to species, it is a useful framework for interpreting studies measuring altered gene expression.

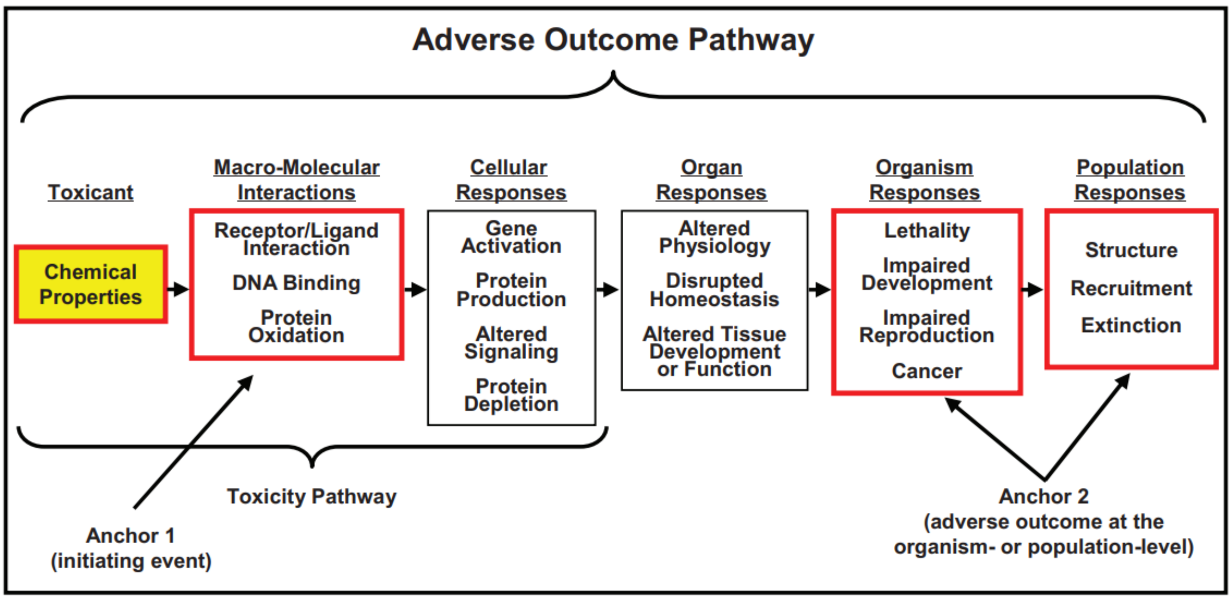

Figure 9. Conceptual diagram of the adverse outcome pathway established for endocrine disruptors (Ankley et al., 2010).

However, even when molecular-level effects (e.g., alterations in hormone signaling) are detected in response to chemical exposure in laboratory studies, we often do not have adequate information to determine if effects of EDCs will alter the fitness of an individual in the wild (i.e., result in effects to survival or reproduction) or result in a population-level response. For instance, individuals may compensate for molecular-level effects at the cellular, organismal, or behavioral level with ultimately no detectable effects to fitness. Similarly, population level effects of endocrine disruptors can be highly nuanced and influenced by environmental factors and species life history. As such, the AOP provides, at times, an oversimplified framework to infer the possible impacts (Lagadic, Wheeler and Weltje 2020).

Scaling up endocrine effects to infer outcomes at the population level has been conducted using empirical data from model fish species (Miller and Ankley 2004, White, et al. 2017). However, populations responses can be nuanced between different species and are dependent on population demographics, life history, and environment (White, et al. 2017). Furthermore, there is evidence that exposure to low levels of EDCs can impact populations for generations through maternal offloading to offspring, exposure to developing oocytes, or from offspring inheriting genetic or molecular (i.e., epigenetic) modifications induced by initial exposure from previous generations (Major, et al. 2020). These long-term, multigenerational or transgenerational effects suggest that EDC exposure can have lasting impacts on population of exposed organisms; however, it is unknown if organisms will be able to adapt to the effects of EDC exposure over time or if effects will become additive over generations.

In evaluating listed species response to endocrine disruptors, we consider effects described throughout the AOP, such as molecular signaling and cellular or organ responses, if available for each taxon. However, given the uncertainty of many of these types of effects on individual and population level outcomes, we rely on studies evaluating effects to reproduction, development, and survival in toxicity testing for pesticides to determine the effects of the action to listed species and critical habitats. We rely on these studies because we believe they integrate any effects within the AOP, including those at the molecular, cellular, and organ-level, as a measure of their effect on individual fitness and overall threats to the survival of species.

Below we describe the toxicological data available for each taxonomic group in our assessment, including effects to growth and reproduction, and where available, effects throughout the AOP. Ultimately, we expect that the analysis of simazine effects to growth and reproduction adequately represents and includes the potential effects of EDCs as they measure the resultant effect of responses from any point in the AOP as manifested through an outcome that we can confidently use to predict effects to an individual's fitness. We can then, in turn, use this information to analyze the effects of the action to listed species, using these studies to represent multiple levels along the AOP, and establish thresholds at which we expect adverse effects to occur (e.g., Lowest Observed Adverse Effect Concentration; LOAEC and No Observed Adverse Effect Concentration; NOAEC) for each taxa group. Using this framework allows for a comprehensive approach to evaluating the effects of endocrine active pesticides based on a wide body of scientific literature and the ability to translate these endocrine disrupting effects to individual- and population-level effects.

Effects by Taxa

The effects of simazine have been studied extensively in many taxa, particularly in plants, amphibians, fish, birds, and aquatic invertebrates. Studies include acute and chronic laboratory and field studies from both registrant-submitted studies and the open literature, with either technical or formulated simazine. A technical pesticide is the pure form of a pesticide as it is manufactured prior to being formulated into an end-use product (e.g., wettable powders, granules, emulsifiable concentrates).

An analysis of the metabolites of concern for simazine is provided in Appendix 1-8 of the BE and a comparison of the toxicity data for the parent triazines and major degradates is presented in BE Appendix 1-8 Table 2, Table 3, and Table 4 for fish, aquatic invertebrates, and aquatic plants, respectively. The toxicity data show that degradates tend to be less toxic than the parent, but may, based on some studies for sublethal or chronic effects, have a similar toxicity to the parent.

Aquatic monitoring data also supports this conclusion for degradates in the aquatic environment, as detected values are generally observed at concentrations an order of magnitude lower than values of the parent compounds. Considering these factors overall, we consider aquatic modeling of the parent compound alone for simazine is adequate for determining potential exposure concentrations to aquatic organisms.

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

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

Toxicity data in this Opinion were divided into ten taxonomic groups (i.e., mammals, birds, fish, reptiles, amphibians, aquatic insects, crustaceans, mollusks, terrestrial insects, and plants), which are somewhat similar to those groups assessed in the BE. Depending on availability, we identified dose-response curves, quantitative endpoints, or other qualitative information to assess the expected biological response for multiple endpoints (i.e., direct and indirect effects[18], including mortality, growth, and reproduction) at predicted exposures. Where these analyses have already been performed in the BE, they have been directly carried over.

For each taxonomic group, we selected endpoints for mortality and their accompanying slopes to ensure we captured the sensitivity of the species being assessed. Mortality endpoints include the median lethal dose (LD_{50}) (lethal dose that causes 50% mortality of test subjects), median lethal concentration (LC_{50}) (lethal concentration that causes 50% mortality of test subjects), and hazardous concentration (HC) values (hazardous concentration extrapolated from Species Sensitivity Distribution (SSD) curves). For LD_{50} and LC_{50} data, the most sensitive endpoint was generally chosen. For taxa with SSDs, hazardous concentration 5^{th} percentile (HC_{05}) values (representing the LD_{50} or the LC_{50} of the 5th percentile most sensitive species of the SSD) are generally chosen. Slopes for dose-response curves were derived from information in the BE and were either contained in the studies that generated the toxicity endpoint, contained in one of studies near the HC_{05} in the case of SSDs, or using EPA's default slope of 4.5. Data were also examined to determine if species-specific data were available or if sufficient information existed to group into finer taxonomic categories (e.g., Order or Family level) that may be more or less sensitive to toxicological effects, and therefore more or less susceptible to the impacts of the herbicide. Within the finer taxonomic groups, factors we considered included the number of species, how representative they may be of listed species within the taxa, and the variability of response. The data were also examined for information related to specific life-stages and it was noted if no data were found.

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

We conducted a similar process for each sublethal response endpoint (i.e., growth, behavior, reproduction). For these lines of evidence toxicity data are generally derived from hypotheses-based testing (i.e., effects observed at a limited number of doses). For this reason, rather than constructing dose-response curves, information about the magnitude of response was generally

[18] While our Opinion considers all consequences of the proposed action (per the definition of effects of the action at 50 CFR Part 402.02), the terms "direct" and "indirect" effects were used in EPA's BE, and are used in environmental risk assessment terminology in general, and do not have the same meaning as used in the prior ESA regulations. As used in the effects analysis section, direct effects to species are those caused by the pesticide itself through dietary, dermal, or inhalation routes of exposure. Indirect effects occur when the pesticide acts on elements of the ecosystem that are required by the species, such as alterations to prey or shelter. Thus, in the effects analysis section, we may sometimes continue to use these terms to link back to the analysis in EPA's BE.

gathered from effects described at different pesticide exposure concentrations. For some taxonomic groups, a large number of studies were available for one or more response endpoints, and the entire data array presented in the BE was used to determine the ultimate response endpoint used for that taxa group for the Opinion. For other taxonomic groups, few studies were available to describe effects for one or more response endpoint, and the magnitude of response was wholly based on those data. In other cases, no data were available to describe a response endpoint line of evidence. In these cases, effects were either extrapolated from data from another taxonomic group, or that response was not carried forward in the analysis, as applicable.

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

General Effects to Terrestrial Animal Species

Terrestrial species may be exposed to herbicides such as simazine through one or more routes of exposure, including contact, ingestion, dermal absorption, or inhalation. We extrapolate results of laboratory studies to predict the likely effects of each type of exposure to listed species. However, the difficulty in recreating natural settings and exposure routes in the laboratory limits the relevance of these studies when assessing effects to species in their natural environment. Some of these limitations, especially for terrestrial vertebrates, are discussed below, followed by a description of the available data for each taxonomic group.

<u>Effects to Terrestrial Vertebrates</u>

<u>Mortality</u>

For terrestrial vertebrates, most laboratory studies measure effects of toxicity from the ingestion route of exposure. Researchers provide test subjects with contaminated food (concentration based, for derivation of LC_{50} values) or administer a single dose through oral gavage or injection (dose-based, for derivation of LD_{50} values). Generally, only orally administered routes are considered to be environmentally relevant and directly comparable to estimated environmental concentrations, as the route of transport in the body is equivalent to how individuals would be exposed to these concentrations in the wild. However, the intraperitoneal exposure route has been demonstrated to have an absorption route with a similar circulatory pathway (initial absorption into portal system) as ingested substances for organic compounds and may be the type of exposure route selected for toxicity testing (for derivation of LD_{50} values) to avoid potential regurgitation of the administered dose in certain cases (Lukas, Brindle and Greengard 1971). Both dietary endpoints (LC_{50} values) and dose-based endpoints (e.g., LD_{50} values) produced from these tests are derived in a manner that is reflective of certain aspects of how species are likely to be exposed in the wild. Both assess the sensitivity of species to potentially toxic food sources only, but not other routes of exposure (i.e., dermal or inhalation) nor other methods of ingestion such as drinking water. The LC_{50} studies provide an estimate of toxicity based on constant exposure to a set concentration of pesticide in food over a series of days, while the LD_{50} studies provide an estimate of toxicity based on a single potentially lethal exposure. Both of these methods capture a subset of conditions in which terrestrial species may be exposed to pesticides. Species in some feeding guilds such as granivores or insectivores are likely to feed and ingest pesticide throughout the day if confined to a contaminated area, while predatory or scavenging species may be exposed to a dose of a pesticide from an exposed carcass and not feed again for one or more days. However, listed species may undertake a large variety of feeding styles beyond those emulated in toxicity testing. Highly mobile species may receive intermittent doses of pesticides from feeding at different locations with varying levels of contamination. Secondary predators may get a large dose of pesticide that has not been fully digested nor on the surface of prey, but remains in the gastrointestinal tract in its parent form (i.e., unmetabolized) (Hill and Mendenhall 1980). Frequency or types of dietary items vary throughout the year, depending on availability, needs for migration, or reproduction. Long-distance migrators such as the red knot may gorge feed at stopover locations, then travel long distances on food stores from these events.

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

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

Sublethal endpoints

For sublethal endpoints, while all data are considered, analyses often rely on concentration-based studies. Most studies that are designed to examine sublethal effects such as growth, behavior, and reproduction are chronic dietary studies. Many endpoints carried over into our analysis are derived from registrant-submitted studies that examine these endpoints as part of long-term reproduction studies (e.g., 20 weeks for birds). Since these studies incorporate many aspects of the reproductive cycle (e.g., litter size, copulation, egg formation, parental care, growth of young), one or more responses to pesticide exposure may be incorporated into ultimate effects to reproduction. In this way, many parts of the reproductive cycle are examined, but it is often difficult to tease out specific effects or which aspect of the reproductive process was compromised. As mentioned previously in the *Evaluating the effects of endocrine disrupting chemicals* section of this Opinion, given the uncertainty of molecular-level effects such as gene expression or the cascade of signals during endocrine initiating events on individual and population level outcomes, we likewise rely on these studies of growth and reproductive outcomes to integrate effects associated with endocrine disrupting compounds as a measure of their impact to individual fitness. We rely on these studies because we know that molecular signaling events are part of the systems and processes that dictate observations made at the organismal level on growth or reproduction.

For these types of studies, we consider the nature and magnitude of effects at test concentrations as well as in the No Observed Adverse Effect Concentration (NOAEC). In some cases, effects may be observed at the concentration identified as the NOAEC, but they are not statistically different from controls due to test design and sensitivity. While we cannot assign these effects to the test substance in these cases, we can consider these observations in the larger context of the study. In all cases, it is important to consider effects that could occur in the span of concentrations between the NOAEC and the Lowest Observed Adverse Effect Concentration (LOAEC), especially when there are high effects at the LOAEC.

Effects to Birds

There are open literature and registrant-submitted studies involving birds, including acute oral, sub-acute dietary and chronic reproduction with technical grade or formulated simazine. Studies were excluded from EPA's BE (and thus, excluded from this Opinion) if they were considered invalid or not associated with an environmentally relevant exposure route. Thresholds are based on the most sensitive lethal and sublethal effects identified among registrant-submitted studies and open literature in the ECOTOX database.

Mortality: Dose-based oral exposure

Simazine is classified by EPA as practically non-toxic to birds on an acute exposure basis (Table 5). The acute oral toxicity of simazine is based on a 14-day study to 14-day old mallard ducks (*Anas platyrhynchos*) (MRID 00072798). No mortality was observed during the study and the

LD_{50} exceeded the highest dose tested (>4640 mg a.i./kg bw). This value is conservatively used to derive the acute mortality threshold for birds. While mortality was not observed, reduced reaction to external stimuli (sound and movement), wing droop, and depression were observed at the 1,000, 2,150, and 4,640 mg a.i./kg doses one hour after dosing, as compared to the control group. As a result, the observational NOAEC from this study is 464 mg ai/kg bw. An additional study observed 40% mortality of young chickens (*Gallus sp.*) at 5000 mg a.i./kg and no adverse effects or symptoms in pigeon (*Columba livida*) at any tested concentrations (up to 5000 mg a.i./kg).

Table 5. Available Dose-Based Toxicity Data (oral) for Birds Exposed to Simazine.

Scientific Name	Common Name	LD_{50} or other endpoint (mg/kg-bw)	Duration (days)	MRID/ECOTOX ref #
Anas platyrhynchos	Mallard duck	>4640	14	00072798 Fink, 1976
Gallus spp.	Chicken	>5000		00037750, Ciba-Geigy Corp., 1958a
Columba libida	Pigeon	>5000		00037750, Ciba-Geigy Corp., 1958a

Mortality: Dietary-based oral exposure

Subacute avian dietary toxicity values for the technical grade and 80% formulation indicate that simazine is practically non-toxic (Table 6). (Hill, et al. 1975) reported no mortality in four species of birds at the highest concentrations of technical simazine tested (MRID 00022923). Corresponding LC_{50} values for the mallard duck, bobwhite quail, and ring-necked pheasant (*Phasianus colchicus*) are > 5000 mg a.i./kg; the LC_{50} value for the Japanese quail (*Coturnix coturnix japonica*) is >3720 mg a.i./kg. No mortality was observed during the study and the LC_{50} exceeded the highest dose for each species tested. The LC_{50} value of 5000 mg/kg-diet based on the mallard duck and bobwhite quail is conservatively used to derive the acute mortality threshold for birds.

Table 6. Available Dietary-Based Mortality Data for Birds Exposed to Simazine.

Scientific Name	Common Name	LC_{50} (mg/kg-diet)	MRID/ECOTOX ref #
Coturnix coturnix japonica	Japanese quail	>3720	(Hill, et al. 1975) 00022923
Anas platyrhynchos	Mallard duck	>5000	(Hill, et al. 1975) 00022923
Colinus virginianus	Bobwhite quail	>5000	(Hill, et al. 1975) 00022923

Scientific Name	Common Name	LC_{50} (mg/kg-diet)	MRID/ECOTOX ref #
Phasianus colchicus	Ring-necked pheasant	>5000	(Hill, et al. 1975) 00022923
Colinus virginianus	Bobwhite quail	>20000	(Gough and Shellenberger 1983) 00139393
Colinus virginianus	Bobwhite quail	8800	Woodard Research Corp., 1965 00023318
Anas platyrhynchos	Mallard duck	>25,600	Woodard Research Corp., 1965 00023318

Growth and Reproduction

The available avian reproductive studies determined NOAECs of 100 mg a.i./kg-diet (MRID 00163134) and 150 mg a.i./kg-diet (MRID 43576901), based on effects to growth and reproduction. In MRID 00163134, a one-generation reproduction study with the bobwhite quail, effects noted at 500 mg a.i./kg-diet included reduction in number of eggs laid (20% reduction), viable embryos (28% reduction), 3-week embryos (33% reduction), hatchling survival (33% reduction), and 14-day old chick survivors (32%). Results were statistically significant for 3-week embryos, hatchling survival, and 14-day old chick survivors.

No additional studies on growth and reproduction effects due to oral simazine exposure in birds were identified in the ECOTOX database, as such we based on our analysis on this study. Due to the difference between the NOAEC and LOAEC in this study and the magnitude of effects at the LOAEC, we used the MATC (i.e., maximal acceptable toxicant concentration; geometric mean of the NOAEC and the LOAEC) of 223 mg a.i./kg-diet as a reasonable and conservative threshold concentration for adverse effects. No additional studies on other sublethal effects due to oral dietary simazine exposure in birds were identified in registrant studies or the ECOTOX database.

Drinking Water, Inhalation, and Dermal

No studies involving avian exposure via drinking water, inhalation, or dermal pathways were identified in registrant studies or the ECOTOX database. We further describe our assessment of these routes of exposure below in the *Exposure* section of this Opinion.

Incident Reports

As part of BE development, EPA reports any incidents related to the pesticide under consultation from their Ecological Incident Information System or aggregate 6(a)(2) incident reports. While incident reports can inform our analysis, we expect that only a very small percentage of organisms that die from any pesticide exposure will be located and reported (N. Vyas 1999) As such, the existence of verified incidents can increase our understanding of the risk associated with pesticide exposure, but the absence of verified incidents does not further contribute to the weight of evidence.

As reported in EPA's BE, only three simazine incidents have been reported involving terrestrial animals, two with an "unlikely" certainty index (i.e., evidence exists that a stressor other than exposure to this pesticide caused the incident, but that evidence is not conclusive) and one "probable" (circumstances of the incident and properties of the pesticide indicate that this pesticide was the cause, but confirming evidence is lacking). In the incident with a certainty index of "probable," which occurred on June 26, 1998, five Canada geese were found dead in a corn field in Rockingham County, Virginia, following spray application of Princep 4L (#I008168-001), which contains simazine as its only active ingredient. Soil and vegetative samples were collected along the bank near the creek in which the dead geese were found. Concentrations of simazine and atrazine found in the samples ranged from 0.16 to 2.3 ppm in soil and 8.5 to 20.5 ppm in foliage. These concentrations are well below concentrations studied in laboratory toxicity tests that resulted in no mortality in birds. As such, and given there are no other known incidents for birds associated with simazine applications, we do not have enough information to conclude that this mortality is attributable to simazine. Therefore, this incident did not influence our analysis of the effects of simazine to birds at estimated environmental concentrations modeled for the Opinion.

Effects to Reptiles

No toxicity data are available for reptiles exposed to simazine. The available toxicity data and thresholds for birds are used as a surrogate for reptiles. There is notable uncertainty in using birds as surrogates for reptiles as it is assumed that they will have similar responses to simazine.

Effects to Terrestrial Amphibians

No toxicity data are available for terrestrial-phase amphibians exposed to simazine. The available toxicity data and thresholds for birds are used as a surrogate for terrestrial-phase amphibians. There is notable uncertainty in using birds as surrogates for terrestrial-phase amphibians as it is assumed that they will have similar responses to simazine. Toxicity data for aquatic-phase amphibians are discussed in the *General Effects to Aquatic Species* section below.

Effects to Mammals

The effects of simazine on mammals have been studied extensively. EPA excluded studies from the simazine BE if they were considered invalid or not associated with an environmentally relevant exposure route. Thresholds are based on the most sensitive lethal and sublethal effects identified among the available registrant-submitted studies and open literature in the ECOTOX database.

Mortality: Dose-based oral exposure

The most sensitive acute toxicity endpoint was an acute LD_{50} study on the gray-tailed vole (*Microtonus canicaudus*), LD_{50} values with TGAI were 2,014 and 2,363 mg a.i./kg-bw for males and females, respectively. Additional signs noted in the study included hind limb extension, lethargy, muscle spasm, lacrimation and depression (E70756, (Cholakis, Wong and Lee 1978). The corresponding LD_{50} value for the TGAI in rats is >5,000 mg a.i./kg-bw (MRID 00148897). Although a definitive LD_{50} was not established in the study with rats, mortality was noted.

Acute mammalian oral toxicity data are also available for one degradate of simazine, DIA (MRID 43012301). In this study both the female and male LD_{50} values for the degradate showed that the degradate appears to be more toxic to laboratory rats than technical grade values for the parent simazine with respective values of 810 mg a.i./kg for the female and 2,290 mg a.i./kg for the male. The combined LD_{50} value for males and females is 1,240 mg a.i./kg.

Based on the available acute mammalian toxicity data, the endpoint used to derive the acute oral toxicity threshold, based on mortality observed in the male gray-tailed vole, is 2,014 mg a.i./kg-bw. Although there is degradate data with a lower LD_{50} value in females, there is a wide range in the degradate toxicity between males and females and the range of values overlaps with those of the toxicity threshold being used. Additionally, there is uncertainty around the degree to which this degradate will form in the terrestrial environment.

Growth and Reproduction

Reproductive and developmental mammalian toxicity studies provide adequate toxicity data on chronic developmental and reproductive effects of simazine. Chronic studies using laboratory rats show consistent reductions in adult body weight gain and adult body weight at simazine concentrations of 100 mg a.i./kg-diet (Table 7). The corresponding NOAEL value for these studies is 10 mg a.i./kg-diet (0.56 and 0.7 mg a.i./kg/day for males and females respectively; MRIDs 41803601 and 40614405). Body weight gains for the 100 mg a.i./kg-diet P males were decreased during Days 0-70 (≈↓13%) and during Days 7-14 (≈↓11%) for the 100 mg a.i./kg-diet F_1 males at first mating, but were increased from rest period to term for F_1 males at second mating. In the 100 mg a.i./kg-diet group, decreased body weights were observed during the premating phase in P and F_1 first mating females (approximately 6%).In addition, reproductive effects including increased abortions, reduced fetal weight, and increased skeletal variations were observed in New Zealand white rabbits at a concentration of 200 mg a.i./kg/day, with a corresponding NOAEL value of 75 mg a.i./kg/day (MRID 00161407).

Based on the available data on growth and reproduction, the sublethal toxicity threshold based on decreased body weight (6%) and decreased body weight gain (13%) is a NOAEC value of 0.56 mg a.i./kg-bw (LOAEC = 5.61 mg a.i./kg-bw, MATC = 1.77 mg a.i./kg-bw).

Table 7. Selected data on sublethal effects to growth from simazine in mammals.

Test organism	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Observed Effect	Duration (days)	Reference (MRID or ECOTOX #)
Rat	Not identified	14.25	decreased body weight gain, decreased food consumption and hematological changes.	90	00143265
Dog	6.9 – 8.2	64.3 – 65.2	decreased body weight/body weight gain, decreased food consumption, organ weight changes, decreased serum glutamate oxaloacetate (SGOT) and reduced alkaline phosphatase activities (females).	13	00146655
Rat (maternal)	30	300	Decreased body weight/body weight gain and decreased food utilization		40614403
Rat (developmental)	30	300	skeletal variations		40614403
Rat (maternal)	5	75	decreased body weight gain, decreased food consumption, increased tremors, and stool alterations.		00161407
Rat (developmental)	75	200	based decreased fetal weight and increased skeletal variations.		00161407
Rat (adult)	0.56 – 0.7	5.61 – 7.04	Decreased body weight/body weight gain		41803601
Rat (offspring)	31.93	Not identified	NA		41803601
Dog	0.76-3.41	3.64-42.9	Males: decreased body weight gains, increased platelet counts, and increased adrenal/brain weight ratio Females: decreased body weight gain, hematological effects		40614402

Test organism	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Observed Effect	Duration (days)	Reference (MRID or ECOTOX #)
			(decreased levels of red blood cell counts, hemoglobin and hematocrit) and increased adrenal weight, adrenal/brain weight ratio, and adrenal/body weight ratio.		
Rat	100	300	Organ weight effects and vaginal cytology		43598614
Rat	5	40	Body weight effects, effects to pre-peak, peak, and post-peak luteinizing hormone (LH) concentrations, adjusted peak LH response	28	45471002

<u>Drinking water, Dermal, and Inhalation</u>

No studies involving mammalian exposure via drinking water were identified in the ECOTOX database or in review of registrant submitted studies. Three rat dermal exposure studies in mammals are available for simazine (Table 8). In general, we anticipate dermal absorption of simazine is low, as one study calculated less than 1% of doses applied were absorbed after 24-hours. However, 11-41% of doses remained on the skin and is potentially absorbable. A systemic study was not able to identify a LOAEL but identified a NOAEL of 1000 mg/kg/day. Another study calculated an acute dermal LD_{50} of > 5050 mg/kg/day. One inhalation study in mammals for simazine is available. The study identified an acute inhalation LC50 > 1.22 mg/L for rats.

Table 8. Mammalian Dermal and Inhalation Studies for Simazine.

Exposure Scenario	Dose	Endpoint	Reference (MRID or ECOTOX #)
Acute Dermal	5050 mg/kg/day	Mortality (LD_{50})	43474102
Dermal (rat)	1000 mg/kg/day	NOAEL	00005767
Dermal Absorption (rat)	0.1, 0.5 mg/cm^2	Dermal absorption was less than 1% at both doses and all time points. However, 11-20% of the low dose and 31-	40614409

Exposure Scenario	Dose	Endpoint	Reference (MRID or ECOTOX #)
		41% of the high dose remained on skin, and potentially absorbable.	
Acute inhalation (rat)	1.22 mg a.i./L	Mortality (LC_{50})	43474103

Incident Reports

As part of BE development, EPA reports any incidents related to the pesticide under consultation from their Ecological Incident Information System or aggregate 6(a)(2) incident reports. While incident reports can inform our analysis, we expect that only a very small percentage of organisms that die from any pesticide exposure will be located and reported (N. Vyas 1999) As such, the existence of verified incidents can increase our understanding of the risk associated with pesticide exposure, but the absence of verified incidents does not further contribute to the weight of evidence.

As reported in EPA's BE, only three simazine incidents have been reported involving terrestrial animals, two with an "unlikely" certainty index and one "probable." There were no incidents with a "probable" certainty index that involved mammals. As such, incident reporting did not influence our analysis of the effects of simazine to mammals at estimated environmental concentrations modeled for the Opinion.

Effects to Terrestrial Invertebrates

The terrestrial invertebrates taxonomic group was designated in the BE and is described as all invertebrates with a terrestrial lifecycle. The Service further divides the terrestrial invertebrates as a taxonomic group to be addressed in this Opinion based on available toxicity data into terrestrial insects and arachnids, a group which includes: all insects with a terrestrial or partial terrestrial lifecycle, spiders and their relatives. The other groups is the terrestrial snails. We more narrowly apply the terrestrial invertebrate data from the BE based on insect toxicity data to terrestrial insects and discuss toxicity data to terrestrial snails below using data specifically for terrestrial snails.

Few studies on the effects of simazine on terrestrial invertebrates are available. Thresholds are based on the most sensitive lethal and sublethal effects identified among the available registrant-submitted studies and open literature in the ECOTOX database.

Mortality – Terrestrial insects

Simazine is categorized as practically non-toxic to terrestrial invertebrates on an acute contact basis. An acute contact toxicity study of simazine in adult honey bees (*Apis mellifera*) reported 6.5% mortality at the highest test concentration (96.7 µg a.i./bee). As such, we presume the LD_{50} value for acute contact mortality is > 96.7 µg a.i./bee. Two studies in earthworms showed no mortality or adverse effects to growth after 96-hours of exposure to 10 µg a.i./cm^2 and 7 days of exposure at 100 mg/kg-soil (the highest test concentration used in the study). Similarly, a study in the rove beetle (*Aleochara bileneata*) using formulated product (Gesatop 50-WP, 50% simazine) found no mortality or adverse effects to reproduction (i.e., egg production) after 5 days of treatment at an application rate of 600 L/ha.

Based on the available terrestrial invertebrate toxicity data, the endpoint used to establish the mortality toxicity threshold for terrestrial insects is 96.7 µg a.i./bee (equal to 756 mg a.i./kg-bw, using assumptions for body weight from the BeeRex model). This value is also used as the sublethal threshold based on observed mortality (6.5%) at this concentration. No data was available for terrestrial invertebrates for exposure units of mg a.i./kg-diet.

<u>Mortality - Terrestrial Snails</u>

For the toxicological analysis for terrestrial snails, we find the open literature data available on atrazine exposure in both aquatic and terrestrial more appropriately address the effects of simazine on terrestrial snails than the studies described above for bees, beetles, or earthworms. The exposure routes (dietary or contact) described in the atrazine studies are also a more appropriate means by which snails could be exposed to simazine.

There are few open literature studies for atrazine that assess exposure to terrestrial snails and aquatic snails that are not part of a microcosm or mesocosm study (Schuytema, Nebeker and Griffis 1994, Gustafson, Belden and Bolek 2015, Roses, Poquet and Munoz 1999, Omran and Salama 2013).

Both groups of snails were relatively tolerant of atrazine exposure in terms of mortality. While there are few studies to use for the terrestrial snail toxicity endpoint, we posit it is more appropriate to compare aquatic snail data to terrestrial snail data than using simazine data from bees, beetles, or earthworms to assess effects of simazine on terrestrial snails. We believe the terrestrial snail endpoint is appropriate as it verifies that snails in general are not sensitive to simazine exposure and the endpoints for both terestial and aquatic snails are within a similar order of magnitude. We use the most sensitive 14-d LC_{50} value of 4,706 ppm from a study by (Schuytema, Nebeker and Griffis 1994) using the terrestrial brown garden snail species *Helix aspersa* exposed to an atrazine amended diet.

<u>Sublethal - Terrestrial Snails</u>

There are no available chronic studies on the sublethal impacts of simazine on mollusks. Instead, we reviewed other available triazine data for mollusks/snails to address toxicity for simazine. We reviewed data for atrazine and determined these data are an acceptable substitute for simazine data as atrazine shares a similar structure to simazine. They are both moderately soluble in water, are persistent in aquatic systems, and exert the same mechanism of action (photosynthesis inhibition), which while does not target mollusks, we observe a similar toxicological response in terms of mortality for aquatic mollusks thus we anticipate sublethal effects will also be within the same order of magnitude. Data are shown in Table 10. We use the LOAEC value of 330 µg a.i./L as the threshold for assessing sublethal adverse effects to listed mollusk species based on the study by (Barky, et al. 2012) from atrazine exposure to the aquatic snail, *Biomphalaria alexandrina* resulting in a significant decrease in number of eggs, and percent hatchability after two weeks of exposure.

Incident Reports

As part of BE development, EPA reports any incidents related to the pesticide under consultation from their Ecological Incident Information System or aggregate 6(a)(2) incident reports. While incident reports can inform our analysis, we expect that only a very small percentage of organisms that die from any pesticide exposure will be located and reported (N. Vyas 1999). As such, the existence of verified incidents can increase our understanding of the risk associated with pesticide exposure, but the absence of verified incidents does not further contribute to the weight of evidence.

As reported in EPA's BE, only three simazine incidents have been reported involving terrestrial animals, two with an "unlikely" certainty index and one "probable." There were no incidents with a "probable" certainty index that involved terrestrial invertebrates. As such, incident reporting did not influence our analysis of the effects of simazine to terrestrial invertebrates at estimated environmental concentrations modeled for the Opinion.

General Effects to Aquatic Animal Species

The breadth of toxicity data, in terms of species and taxa representation, available for our effects assessment for listed species (from the BE) was based on studies generated by registrants as well as open literature studies and government reports retrieved through ECOTOX. As a result, there tends to be an abundance of data for taxa that are more commonly tested or studied for regulatory purposes (i.e., fish, aquatic insects, and aquatic crustaceans), compared to less well-studied taxa, such as mollusks (including mussels and aquatic snails) and amphibians. Similarly, within taxa, there may be numerous studies for common aquatic test species, such as rainbow trout (*Oncorhynchus mykiss*), fathead minnow (*Pimephales promelas*), bluegill (*Lepomis macrochirus*), sheepshead minnow (*Cyprinodon variegatus variegatus*), water flea (*Daphnia* spp.), or the amphipod *Hyalella azteca*, but fewer studies for species representing other genera, families, or orders. As a result, the taxa for which toxicity data are available may or may not be strong surrogates for listed species. Considering the high variability in toxicity values between species for some taxa groups (e.g., five orders of magnitude difference between the highest and lowest fish acute mortality data or LC_{50} values), it is important that we take this uncertainty into account when assessing risks to listed species.

Listed aquatic species that may be affected by simazine in aquatic habitats include fish, amphibians (aquatic phases), and various taxa of aquatic invertebrates (i.e., aquatic insects, crustaceans, and mollusks). For those species that are exclusively aquatic, all life stages may be affected by exposure to simazine in water. Some species of aquatic insects (e.g., dragonflies, damselflies, and stoneflies) and amphibians (e.g., frogs, toads, and some salamanders) have both aquatic and terrestrial life stages and may therefore be affected by exposures in either aquatic or terrestrial habitats, or both. Certain species also have obligate relationships with other species. For example, early life stages of freshwater mussels (glochidia) are parasitic and require a host fish to complete their development. Consequently, we also assess the potential effects of simazine on host fish in the effects analyses for mussels. Similarly, effects to a listed species from impacts to their food items (such as aquatic invertebrates or prey fish) were included in our analyses. Our approach to applying the acute mortality data (LC_{50} values) for assessing lethal effects to listed species relies on the SSDs developed in the BE (Appendix 2-5 of the BE), when available. The HC_{05} (from the SSD) and its corresponding slope is generally used to assess mortality for each taxonomic group. When an SSD was not available, we used the lowest (most sensitive) LC_{50}. Unlike the acute mortality data, sublethal effects endpoints were largely reported as NOAECs and LOAECs for a variety of measurement endpoints and species within each effect category (i.e., growth, reproduction). In our analysis of effects to listed aquatic species, we consider all data related to sublethal responses including those affecting the endocrine system. As mentioned previously in the *Evaluating the effects of endocrine disrupting chemicals* section of this Opinion, given the uncertainty of molecular-level effects such as gene expression or the cascade of signals during endocrine initiating events on individual and population level outcomes, we likewise rely on these studies of growth and reproductive outcomes to integrate effects associated with endocrine disrupting compounds as a measure of their impact to individual fitness. We rely on these studies because we know that molecular signaling events are part of the systems and processes that dictate observations made at the organismal level on growth or reproduction.

EPA organized sublethal effects data as effects arrays in the BE. Depending on the taxonomic group, we used these arrays to assess the likelihood or risk of species experiencing sublethal effects as a result of exposure to simazine.

Effects to Fish and Aquatic-Phase Amphibians

We rely on toxicity data carried forward from the BE for our effects analysis to fish and aquatic phase amphibians. The most sensitive reliable endpoint for mortality and sublethal effects are used to represent thresholds to adverse effects for fish and aquatic-phase amphibians.

We generally use the fish toxicity endpoints as surrogates for aquatic and aquatic-phase amphibians where there are few data for amphibians and discuss both taxa groups together in this section. The toxicity data used to assess the effects of simazine are provided below and in Table 9. Incident reports are discussed at the end of this section. All data referenced in the following sections are from the Effects Characterization (Chapter 2) of the BE.

Mortality

Mortality data for fish and aquatic-phase amphibians are available for simazine. The mortality acute toxicity estimates (ranging from 24-hour to 96-hour LC_{50}) for simazine for aquatic vertebrates varied by five orders of magnitude, ranging from 90 µg/L to >910,000 µg/L. There were not sufficient data available to generate a species sensitivity distribution for fish and aquatic-phase amphibians, and so we use the most sensitive reliable endpoints to represent mortality thresholds for aquatic vertebrates.

In Appendix 2-1, 2-2, and 2-3 of their BE, EPA provides a list of studies that they evaluated when selecting the most sensitive endpoints for their ESA risk assessment for fish and aquatic-phase amphibians (Table 9). Perciformes and Cypriniformes, in general, appear to be more sensitive to simazine than other fish groups, such as Salmoniformes. Acute toxicity estimates (96-hour LC_{50}) for simazine span four orders of magnitude, indicating a wide range of sensitivity to simazine among fish. The lowest LC_{50} in fish reported for simazine is 4.2 mg a.i./L seabream larvae exposed to formulated product (Arufe, et al. 2004), which is nearly identical to the lowest LC_{50} in fish exposed to technical grade active ingredient (TGAI) of 4.3 mg a.i./L for sheepshead minnow (MRID 42503702). Available ECOTOX report data in aquatic-phase amphibians indicate the lowest LC_{50} value for amphibians is 7.55 mg a.i./L (Saka, Tada and Kamata 2018). Given the difference in LC_{50} values between fish and aquatic-phase amphibians, we expect using the fish mortality endpoint will be protective of aquatic-phase amphibians. As such, we use the lowest LC_{50} value of 4.2 mg a.i./L as our mortality threshold for this consultation.

Sublethal effects

The most sensitive chronic exposure endpoint for fish was observed in an early life-stage study in common carp (*Cyprinus carpio*; (Velisek, et al. 2012), which reported statistically significant reductions in body weight and total length (up to 29% decrease) at exposures of 600 µg a.i./L (NOAEC = 60 µg a.i./L). The study also reported altered tubular systems of the caudal kidney. Another study in carp also found similar histopathological changes in the kidneys at test concentrations of 45 µg a.i./L, although no changes in weight or length were associated with these changes (Oropesa, Cambero and Soler 2009).

A study in African clawed-toed frog (*Xenopus laevis*) reported decreased growth rate in tadpoles and found a significant reduction in the number of tadpoles completing metamorphosis (up to 22% fewer individuals) and a significant increase in the number of days required to complete metamorphosis (up to 6.3% more days) at treatments as low as 11.1 µg a.i./L (Sai, Qu, et al. 2016). A different study in *Xenopus* using the same treatment levels observed up to 57% reduction in gonad weight and gonadosomatic index, in addition to histopathological changes in testicular tissue, in treatments as low as 11.1 µg a.i./L (Sai, Liu, et al. 2015)

While these reproductive and growth effects studies from Sai et al. represent the most sensitive sublethal endpoints in aquatic vertebrates, we do not view these data as reliable because there are several uncertainties that limit our ability to evaluate the effects of simazine exposure to the aquatic amphibian tested for several reasons:

For example, these studies were not conducted according to standard OCSPP[19] or OECD[20] guideline methods to be able to assess the reliability of these studies. While this alone does not preclude the consideration of these studies for use in our Opinion, there were several other aspects of the methodology and results we provide examples of below that led the Service and EPA to question the ability of the study to show how simazine exposure impacted amphibian development such as 1) the use of dimethyl sulfoxide (DMSO) as a solvent (simazine is fairly soluble in water and a vehicle is not needed to get simazine into solution for aquatic exposure testing). A vehicle such as DMSO can also affect the absorption, distribution, metabolism, and excretion of a compound and is not recommended for *in vivo* studies, 2) the environmental conditions were poorly described, 3) larvae were fed an unspecified amount of diet per day, 4) the loading rate exceeded the American Society for Testing and Materials (ASTM) rate of 1 larva/L, 5) there was a weak treatment response, 6) there was a significant amount of mortality in the control group (>10%), 7) time to metamorphosis varied from 60 to ~90 days in some control animals and treatment groups (tadpoles should normally complete metamorphosis in 6 to 8 weeks), thus leading to 8) high variability limiting the ability to differentiate treatment effects. The Sai et al. studies contain multiple confounding effects that could obscure the ability of the study to evaluate treatment effects. See Appendix F for more details.

The endpoint for freshwater fish based on the early life stage toxicity study with Common Carp from (Velisek, et al. 2012) with a NOAEC, MATC, and LOAEC values of 0.060, 0.190 and 0.600 mg/L, respectively, showed a 29% reduction in body weight at the LOAEC which was more sensitive than the LOAEC identified for simazine in the Amphibian Metamorphosis Assay using *X. laevis* at 1.9 mg/L under standardized test conditions by (Schneider, Kendall and Krueger 2012) as part of the Endocrine Disrupting Screening Program Tier I Study.[21]

As such, we consider the growth NOAEC observed in fish (60 µg/L) from the (Velisek, et al. 2012) study to be the most sensitive sublethal endpoint for both fish and aquatic-phase amphibians.

Table 9. Toxicity values for simazine for Fish and Aquatic-phase Amphibians (Table 2-8 from the BE).

Taxa	Threshold Type	Effect (endpoint)	Value(µg a.i./L)	Duration of exposure/Species	Reference (or MRID or ECOTOX #)
Freshwater Fish	Mortality	LC_{50}	6400	4 days	00033309

[19] EPA Office of Chemical Safety and Pollution Prevention
[20] Organization for Economic Cooperation and Development
[21] https://www.regulations.gov/document/EPA-HQ-OPP-2013-0251-0033

Taxa	Threshold Type	Effect (endpoint)	Value(µg a.i./L)	Duration of exposure/Species	Reference (or MRID or ECOTOX #)
Freshwater Fish	Growth	NOAEC (LOAEC)	60 (600)	90 days	(Velisek, et al. 2012)
Estuarine/Marine Fish	Mortality	LC_{50}	4300	4 days	42503702
Aquatic-phase Amphibians	Mortality	LC_{50}	7550	4 days	(Saka, Tada and Kamata 2018)

Incident Reports

As part of BE development, EPA reports any incidents related to the pesticide under consultation from their Ecological Incident Information System or aggregate 6(a)(2) incident reports. While incident reports can inform our analysis, we expect that only a very small percentage of organisms that die from any pesticide exposure will be located and reported (N. Vyas 1999). As such, the existence of verified incidents can increase our understanding of the risk associated with pesticide exposure, but the absence of verified incidents does not further contribute to the weight of evidence.

As reported in EPA's BE, a review of EPA's Incident Data System in July, 2020 identified ten reported incidents of fish kills reported for simazine between 1976 and 2004, with seven of these incidents with a certainty index of "highly probable" or "probable" and three incidents with certainty index of "possible" or "unlikely". One incident resulted from runoff into a lake due to a valve failure in a chemical application hose that was dispensing glyphosate and simazine to golf course turf. The incident resulted in the death of 6 unknown species of fish. Six incidents resulted from treatment of a lake, pond, or lagoon; two incidents were associated with simazine use on corn and from simazine use along railroad tracks; one was due to a spill from golf course use, and the treatment site for the other incident was not reported. In a number of the incidents involving direct application of simazine to lakes, ponds, and lagoons, the legality of use was listed as "misuse" or "undetermined." For those incidents where the legality of use is reported as "registered use," the volume of the water bodies is not provided; therefore, it is unclear whether simazine was applied in accordance with its intended use. Of the ten reported incidents, three were reported in California, two were reported in Nebraska, two were reported in South Carolina, and one was reported in Michigan, Oklahoma, and in Tennessee. Fish species listed in these kills include smelt, bullheads, stickleback, striped bass, bluegills, channel catfish, croaker, menhaden, mullet, northern pike, pinfish, yellow perch, sea trout, black bullhead, and fathead minnows.

The six incidents involving direct application of simazine to water all occurred prior to 1996, when label language was clarified to restrict direct applications to ornamental ponds and aquaria. It is important to note that in a number of the incidents involving direct application of simazine to water, low dissolved oxygen, caused by decaying aquatic vegetation, is attributed as an indirect effect related to the fish kills. The certainty index associated with the remaining three incidents (those resulting from use on corn, railroad tracks, and an unspecified treatment site) was reported as "unlikely."

We are unable to relate the circumstances of these reports to expected exposure of fish from currently registered uses of simazine. As such, incident reporting did not influence our analysis of the effects of simazine to fish at estimated environmental concentrations modeled for the Opinion.

Effects to Aquatic Invertebrates

We rely on toxicity data carried forward from the BE for our effects analysis to aquatic invertebrates. The most sensitive reliable endpoint for mortality and sublethal effects are used to represent thresholds to adverse effects for aquatic invertebrates. The toxicity data used to assess the effects of simazine are provided below and in Table 10. Incident reports are discussed at the end of this section. All data referenced in the following sections are from the Effects Characterization (Chapter 2) of the BE.

<u>Mortality</u>

Aquatic Insects and Crustaceans:

Mortality data were available (submitted by registrants or available in ECOTOX database) for several different orders of aquatic invertebrates. Acute mortality data (48- and 96-hour EC/LC_{50}'s) are available for 14 species of aquatic non-mollusk invertebrates. These types of studies are generally conducted using juvenile stages of invertebrates. Toxic effects to non-mollusk aquatic invertebrates can vary widely. For instance, across nine studies of simazine exposure to water fleas (*Daphnia spp.*), EC_{50} values range from >3,500 to 60,000 µg a.i./L with both formulated product and TGAI. While the lowest reported EC_{50} is 1,000 µg a.i./L (observed for *Daphnia* and a calanoid copepod) (E10460), EPA did not use this as the endpoint in the BE given a number of issues regarding the reliability of these studies. EPA identified an EC_{50} of 1,900 µg a.i./L in stonefly (*Petronarcys californica*) as the lowest reliable mortality endpoint for non-mollusk aquatic invertebrates (MRID 40098001/E6797). As such, we use this value as the threshold for assessing mortality to non-mollusk aquatic invertebrates in this Opinion.

Mollusks (mussels and aquatic snails):

One acute toxicity study is available for mollusks. A study in the Eastern Oyster (*Crassostrea virginica*) tested with TGAI reported an EC_{50} value >3,700 µg a.i./L (MRID 42503703). There was a 6.8% reduction in shell growth reported at the highest test concentration in the study. Although non-definitive, this represents the most sensitive acute endpoint for mollusks and will be conservatively used as the mortality threshold for mollusks.

Sub-lethal

Aquatic Insects and Crustaceans:

Few studies on the sublethal effects of simazine to aquatic invertebrates are available. One study in *Daphnia magna* exposed to formulated product observed a significant stimulation of offspring produced at 800 µg a.i./L treatment over 21 days, but observed no adverse effects, even at the highest test concentration of 2,000 µg a.i./L (MRID 00043676). A registrant-submitted study in mysid shrimp (*Americamysis bahia*) exposed to TGAI observed a statistically significant reduction in the number of offspring produced per day and a reduction in total adult male and female length at 608 µg a.i./L. Additionally, the study found a significant reduction (15%) in F_0 survival at 151 µg a.i./L (NOAEC = 63 µg a.i./L). We use the NOAEC value of 63 µg a.i./L and LOAEC value of 151 µg a.i./L as the thresholds for assessing sublethal impacts to non-mollusk aquatic invertebrate species in this Opinion.

Mollusks (mussels and aquatic snails):

There are no available chronic studies on the sublethal impacts of simazine on mollusks. There are few open literature studies for atrazine that assess exposure to terrestrial snails and aquatic snails that are not part of a microcosm or mesocosm study (Schuytema, Nebeker and Griffis 1994, Gustafson, Belden and Bolek 2015, Roses, Poquet and Munoz 1999, Omran and Salama 2013).

While there are few studies to use for the aquatic snail sublethal endpoint, we posit it is more appropriate to use atrazine data for effects to aquatic mollusks. We reviewed data for atrazine and determined these data are an acceptable substitute for simazine data as atrazine shares a similar structure to simazine. They are both moderately soluble in water, are persistent in aquatic systems, and exert the same mechanism of action (photosynthesis inhibition), which while does not target mollusks, we observe a similar toxicological response in terms of mortality for aquatic mollusks thus we anticipate sublethal effects will also be within the same order of magnitude. Data are shown below in Table 10. We use the LC_{10} value of 330 µg a.i./L as the threshold for assessing sublethal adverse effects to listed mollusk species based on the study by (Barky, et al. 2012) from atrazine exposure to the aquatic snail, *Biomphalaria alexandrina* resulting in a significant decrease in number of eggs, and percent hatchability after 2 weeks exposure.

Table 10. Effects endpoints used to derive mortality and sublethal thresholds for determining effects to listed aquatic invertebrates exposed to simazine.

Taxa	Threshold Type	Effect (endpoint)	Value (µg a.i./L)	Reference (or MRID or ECOTOX #)
Non-Mollusk Aquatic Invertebrates	Mortality	EC_{50}	1,900	MRID 40098001/E6797, (Mayer and Ellersieck 1986)
Mollusks	Mortality	EC_{50}	3,700	MRID 42503703
Non-Mollusk Aquatic Invertebrates	Sublethal	NOAEC (LOAEC)	63 (151)	MRID 48680006
Mollusks	Sublethal (reproduction)	LC_{10}	330	(Barky, et al. 2012)

Incident Reports for Aquatic Invertebrates

As part of BE development, EPA reports any incidents related to the pesticide under consultation from their Ecological Incident Information System or aggregate 6(a)(2) incident reports. While incident reports can inform our analysis, we expect that only a very small percentage of organisms that die from any pesticide exposure will be located and reported (N. Vyas 1999). As such, the existence of verified incidents can increase our understanding of the risk associated with pesticide exposure, but the absence of verified incidents does not further contribute to the weight of evidence.

As reported in EPA's BE, as of July 2020, there were no reported incidents of simazine specifically impacting aquatic invertebrates. As noted in the discussion of incident reports for fish and aquatic-phase amphibians, a number of the incidents involving simazine and aquatic vertebrates are attributed as an indirect effect related to low dissolved oxygen caused by decaying aquatic vegetation. As such, we can assume that aquatic invertebrates were similarly impacted in those incidents as well. However, since the incidents are not related to toxicity of simazine, these reports did not influence our analysis of the direct effects of simazine to aquatic invertebrates at estimated environmental concentrations modeled for the Opinion.

General Effects to Plants

Plant toxicity data from both registrant-submitted studies and studies in the scientific literature have been reviewed for this assessment. Registrant-submitted studies are conducted under conditions and with species defined in EPA's test guidelines. Endpoints such as plant growth, dry weight, and biomass are evaluated for both monocots and dicots, and effects are evaluated at both seedling emergence and vegetative life stages. Studies were excluded if they were considered invalid or not associated with an environmentally relevant exposure route.

Discussion of endpoints are provided for effects on terrestrial plants and terrestrial plant communities. These serve as a surrogate for effects on an individual of a listed species and the effects on the pollination, prey, habitat, or dispersal of a listed species, respectively. Based on the results of the submitted and available open literature terrestrial plant toxicity tests, it appears that the seedling emergence stage of plant development is more sensitive to simazine than the vegetative vigor stage of development. However, all tested plants, with the exception of corn in the seedling emergence and vegetative vigor tests, exhibited adverse effects following exposure to simazine. The registrant submitted data represents the most sensitive endpoints for effects to listed species

Effects to Aquatic Plants

Most of the available toxicity studies with aquatic plants have focused on growth, reproduction, physiological effects, and population effects. Threshold values and effects data arrays in this assessment are based on endpoints expressed in, or readily converted to, environmentally relevant concentrations in terms of the amount of the simazine (i.e., µg a.i./L). Single-species aquatic plant toxicity studies are used as one of the measures of effect to evaluate whether simazine may affect primary production and diversity in aquatic ecosystems. Numerous aquatic vascular plant toxicity studies have been submitted to the EPA and/or published in the open literature.

Physiological endpoints include measures of various sub-organismal effects, including tissue permeability, photosynthesis, carbon fixation, water uptake, and photosystem II inhibition. While several of these measures are clearly relevant to apical endpoints, especially growth, these endpoints are naturally variable with potential for rapid recovery and the study designs generally do not allow for connection to apical endpoints or are for short durations that would not capture the potential for recovery. This was particularly true for the most sensitive endpoints from these groupings. For these reasons, the following discussions of the single species aquatic toxicity data will focus on growth effects. These growth effects are sometimes captured under population level effects due to the manner in which aquatic plants grow as a community of organisms.

Effects to Non-Vascular Aquatic Plants

Numerous aquatic non-vascular plant toxicity studies have been submitted to EPA and/or published in the open literature, representing a broad diversity of unicellular and multicellular organisms collectively referred to as "non-vascular aquatic plants." These include Eubacteria (*e.g*., blue-green algae), Archaeoplastida (*e.g*., red algae, glaucophytes, green algae, and aquatic bryophytes), Chromalveolates (*e.g*., aveolates, cryptomonads, dinoflagellates, diatoms, water molds, and brown algae), Excavates (*e.g*., euglena), and a few lineages of the Unikonts (*e.g*., fungi, and collared-flagellates). These single-species toxicity studies serve as the foundation for evaluating whether atrazine may affect primary production and diversity in the aquatic ecosystem.

Effects to non-vascular plants were observed on various measures of physiology and growth at the individual and population level. The most sensitive endpoints were generally related to effects on growth and measures of photosynthesis. The most sensitive quantitative endpoint comes from the registrant submitted toxicity test with *Anabaena flos aqua* (MRID 42662401). For this study, a 28% reduction in cell density was observed at the lowest test concentration of 20 µg a.i./L so the threshold is based on the IC_{05} value of 5.4 µg a.i./L.

Effects to Vascular Aquatic Plants

Studies have reported a number of adverse effects to vascular aquatic plants, including various measures of physiology, reproduction, and growth at the individual and population level. The most sensitive endpoints were generally related to effects on growth. The threshold for aquatic plants comes from the registrant submitted toxicity test with *Lemna gibba* (MRID 42503704) with a NOAEC and LOAEC of 50 and 110 µg/L, respectively, based on a 39% decrease in frond number.

Effects to Aquatic Plant Communities

EPA used the median effect concentration (EC_{50}) values for aquatic plants to derive the threshold for effects to the PPHD of an individual of a listed species. Studies with effects on measures of growth (e.g., biomass, cell counts, number of fronds); were conducted with technical grade simazine; and had 4-day, 7-day, or 14-day exposure durations were used to derive a Species Sensitivity Distribution (SSD). These parameters were selected to maximize comparability of results.

Toxicity estimates for simazine range from 8 – 28,000 µg a.i./L and span three orders of magnitude, indicating a wide range of sensitivity to simazine among aquatic plants. The most sensitive non-vascular aquatic plant endpoint is from (Bednarz 1981), with an IC_{50} value of 8.0 µg a.i./L for the green alga, *Chlorococcum sp*., based on reductions in growth rate. The most sensitive species from the registrant submitted 850.4500 guideline was for the blue-green algae *Anabaena flos-aqaue* with an IC_{50} value of 36 µg a.i./L (MRID 42662401). Vascular plants have a similar sensitivity to simazine as non-vascular plants, with the most sensitive vascular plant EC_{50} having a value of 67 µg a.i./L, based on fresh weight reduction (biomass reduction) in *Vallisneria americana* (Wilson and Wilson 2010). In comparison, the registrant submitted 850.4550 guideline study using *Lemna gibba* (MRID 42503704) reported an IC_{50} of 140 µg a.i./L based on a reduction in frond number.

For the SSD, five distributions were tested, and a variety of methods were used. The logistic distribution and maximum likelihood (ML) method were ultimately chosen to represent HC_{05} through HC_{95} values for vascular and nonvascular aquatic plants. Table 6-1 and Figure 6.5 in EPA's BE provide a summary of the results. The threshold for species that rely upon aquatic plants for their PPHD is based on the HC_{05} from the species sensitivity distribution (SSD).

Table 11. Summary statistics for aquatic plant SSD fit (adapted from table 12-3 from EPA's BE).

Statistic	All Aquatic Plants (µg a.i./L)
HC_{05}	12.19
HC_{10}	25.31
HC_{50}	217
HC_{90}	1856
HC_{95}	3854

In addition to reviewing the toxicity data for individual species and deriving SSDs, the toxicity of simazine to aquatic plant communities is evaluated by considering microcosm and mesocosm (cosm) data available in the open literature. Cosm studies conducted with simazine provide measurements of primary productivity that incorporate the aggregate responses of multiple species in aquatic plant communities. Because plant species vary widely in their sensitivity to simazine, the overall response of the plant community may be different from the responses of the individual species measured in laboratory toxicity tests. Cosm studies allow observation of population and community recovery from simazine effects and of indirect effects on higher trophic levels. In addition, cosm studies, especially those conducted in outdoor systems, incorporate partitioning, degradation, and dissipation, factors that are not usually accounted for in laboratory toxicity studies, but that may influence the magnitude of ecological effects. From these studies, effects on the aquatic community, including reductions in survival and biomass, have been observed at concentrations of 50 µg a.i./L and greater. This exposure value approximates the HC_{25} species of the SSD for all aquatic plants.

Table 12. Summary of simazine field and cosm studies (adapted from EPA's BE table 6-2).

Study type/test material	Study Design	Test Organism	Effects	Reference (ECOTOX # or MRID)
Field study (84 days)	0.05, 0.5, and 5 mg/L simazine applied to outdoor pond microcosm systems for 84 days. Biological and water quality components measured 14, 28, 56, and 84 days.	Submerged rooted vegetation (*Myriophyllum spicatum* and *Elodea canadensis*) Emergent rooted vegetation (*Persicaria amphibia* and *Glyceria maxima*) Floating aquatic vegetation (*Lemna minor*) Phytoplankton	Application rate of 0.05 mg/L caused significant reduction in growth (length, number of shoots, biomass, number of cells) yield, and floral development after 28 days. Decreased DO and pH also observed after application of simazine.	(Vervliet-Scheebaum, et al. 2010) (150229)
Field study (210 days)	1.0 mg/L simazine applied to <0.4 ha pond (control pond also tested). Biological and water quality components measured 3, 5, 7, 10, and 18 days after application, and biweekly and monthly (210 d).	Phytoplankton Macrophytes Zooplankton Macroinvertebrates	Application rate of 1.0 mg/L cause significant reduction in growth and survival of fish (likely from low DO and reduced food resources). Die-off of macrophytes, resulting in decreased DO, and increase in CO_2, TSS, total carbon, and specific conductivity. DO decrease concurrent with phytoplankton and macrophyte mortality. Zooplankton biomass decreased.	(Gordon, et al. 1982) (15428)

Study type/test material	Study Design	Test Organism	Effects	Reference (ECOTOX # or MRID)
		Largemouth bass (*Micropterus salmoides*) Bluegill (*Lepomis macrochirus*)	No significant differences in total abundance or biomass of macroinvertebrates (observed increase in Ostracoda species). Significantly fewer young of year bluegill sunfish survived, increased mean weight of survivors. Reduced juvenile and adult largemouth bass growth.	
Field study (20 weeks)	- 9 x 0.01-A rectangular concrete pools w/MS river water (3 ft deep). - Bottom covered w/3” layer loam soil. - 4 lbs *Elodea canadensis* in each pool. - 3 pools treated once at 1, 2.5, and 5 ppm; 3 pools treated w/1, 2.5 and 5 ppm every 4 wks for total of 5 treatments/each; and 3 controls (untreated)	Algae *Elodea candensis* Zooplankton Macroinvertebrates Goldfish Bluegill (*Lepomis macrochirus*)	*Elodea candensis* and algae eliminated from all treated pools for duration of study. Fish survival highly variable. Reduced goldfish survival at 2.5 and 5.0 ppm, higher survival in monthly-treated pools and pools treated once. Reduced bluegill survival in pools treated only once, no dose-response in pools treated monthly. Bluegill survival reduced in pools treated with 5 ppm and in pools treated with 2.5 ppm monthly. The mean number of zooplankton and benthic fauna showed no dose-response effects in zooplankton or benthic fauna abundance.	(Gilderhaus 1969) (MRID# 00025433)
Microcosm (126 days)	1.0 mg/L simazine applied to 4 circular fiberglass pools (4.12 m2, depth = 45 cm) at 0, 24, 56, 87, and 106 days after pools were stocked with 100 *Tilapia nilotica* swim-up fry each. Four untreated pools were used as controls.	Nile tilapia (*Tilapia nilotica*) (swim-up fry, <12mm in length)	Decreased tilapia fingerling yield after 126 days (52% reduction) when treated periodically with 1 mg/L simazine as compared to untreated ponds. Reduced survival a combination of direct impacts, loss of food resources, and poor water quality.	(McGinty 1984) (10969)

Study type/test material	Study Design	Test Organism	Effects	Reference (ECOTOX # or MRID)
	Pools were not cleaned and only water lost by evaporation was replaced.		No significant differences in survival and treatments (91% and 87% survival, respectively). Significant decrease in fish weight after 42 days.	
Field study (60 days)	1.3 mg/L simazine applied to channel catfish ponds infested with *Chara vulgaris*. Catfish were stocked into 0.06 ha earthen ponds at 12,350 fish /ha. Four heavily infested ponds were treated w/simazine and 4 ponds containing little or no *Chara* were monitored for changes in water quality before and after simazine treatment. The ponds were equipped with emergency aeration that was initiated when DO fell below 2.5 mg/L.	Channel catfish (*Ictalurus punctatus*) (average weight = 55 g)	Reduced fish production by 20%. Fish stopped feeding immediately after application. Reduced feeding persisted even after water quality variables in treated ponds returned to control pond levels. Possible direct effect of simazine on feeding response. The magnitude of effects was greatest in 2 weeks following treatment. Temporal changes in DO, CO_2, pH, TA-N in treated pools are related to the response of the plant community to simazine.	(Tucker, Busch and Lloyd, Effects of Simazine Treatment on Channel Catfish Production and Water Quality in Ponds. 1983) (10669)
Field study (120 days)	14 earthen ponds (0.04 - 0.06 ha) used; 6 ponds stocked w/7400 channel catfish fingerlings, and 8 ponds stocked with 5000 bluegill. Prior to filling ponds w/water, 3 of the 6 catfish ponds treated with Aquazine at rate of 13.4 kg/ha (~12 lb/acre). Aquazine was applied as a suspension in water and applied evenly over the entire pond bottom. Four of the 8 bluegill ponds were treated w/ 1.5 mg/L (1.88 mg/L Aquazine 80W). A slurry of the chemical was dispersed over the pond surface.	Channel catfish (*Ictalurus punctatus*) Bluegill (*Lepomis macrochirus*)	Application in catfish ponds prior to flooding results in persistent levels of simazine (200 ppb for more than 4 months). Persistence results in reduced chlorophyll α and percentage of pond bottoms covered by macrophyte growth. Simazine resulted in extended period of reduced DO. Reduced catfish yield by 19% and feed conversion efficiency (likely resulting from indirect effects to food and water quality). Bluegill ponds treated with triazine had (non-significant) reduction in average yield of bluegills (11% less than controls). Single application of	(Tucker and Boyd 1978) (71314)

Study type/test material	Study Design	Test Organism	Effects	Reference (ECOTOX # or MRID)
			simazine did not result in season-long control of macrophytes.	
In situ enclosures (27 days)	Littoral enclosures (240 x 120 cm sheets of 1.5-mm PVC plastic on long axis w/ends cemented together) placed in water ~ 60-cm depth and embedded into sediment to depth of 45 cm. Artificial substrata placed upright in sediments. Concentrations of 0.1, 1.0, and 5.0 mg/L simazine dispensed in 300-l enclosure volume. Sampled substrata 9 days following simazine application and at weekly intervals for 5 wks. Colonization of substrata by periphyton was monitored by measuring chlorophyll *a* and carbon assimilation rate.	Periphyton	Periphyton chlorophyll synthesis EC_{50} between 0.1 and 1.0 mg/L simazine. No change in chlorophyll α accumulation and carbon assimilation rate observed at 0.1 mg/L simazine. Algal biomass increased over time in all treatments w/the most notable increases in treated enclosures following flooding. No detrimental long-term effect on productivity of periphyton may be predicted.	(Goldsborough and Robinson, The Effect of Two Triazine Herbicides on the Productivity of Freshwater Marsh Periphyton 1983) (11289)
In situ enclosures (42 days)	Littoral enclosures (diameter = 78 cm; volume ~300 l) situated in a marsh. Rods used as substrata for periphyton growth were positioned vertically. Simazine added to enclosures at concentrations of 0.1, 1.0, and 5.0 mg/L (plus one control). Substrata collected 9 days after simazine application and at weekly intervals for 6 weeks. Measurements included carbon assimilation, chlorophyll a concentration, densities of algal taxa, and total algal biovolume. Flooding during the experiment provided opportunity to monitor extent and rate of recovery of the community.	Periphyton	No reduction in total biovolume observed at 0.1 mg/L simazine treatment and increased inhibition (94-98% reduction) at pre-flooded concentrations of 1.0 and 5.0 mg/L. Community LC_{50} between 0.1 and 1.0 mg/L simazine. Observed increased biovolume in all but highest treatment levels following flooding and removal of herbicide, with rates of colonization similar to control. After flooding ,substratum colonization dominated by *Cocconeis placentula.* No evidence that a clearly herbicide resistant colony had developed prior to flooding, suggesting that filamentous algae selectively inhibited to a greater extent than the others.	(Goldsborough and Robinson 1986) (12264)

Study type/test material	**Study Design**	**Test Organism**	**Effects**	**Reference (ECOTOX # or MRID)**
			High abundance of periphytic blue-green alga suggests that this taxon possesses some means of herbicide tolerance.	
In situ enclosures (18 days)	Cylindrical enclosures placed in marsh water ~ 60-cm depth and embedded into sediment to depth of 45 cm. Artificial substrata placed vertically in enclosures. Simazine dispensed to give ~1.0 mg/L in 300-l enclosure volume. Treatment consisted of 7-day exposure before flooding, and an 11-day exposure following re-addition of simazine 9 days after the flood. Sampled substrata @ 1-, 3-, and 5-week intervals. Substrata segments received 0.1, 0.5, 1.0, 2.5, or 5.0 mg/L (3 reps/ treatment plus 3 controls). Colonization of substrata by periphyton was monitored by measuring chlorophyll *a* and carbon assimilation rate.	Periphyton	No significant differences in photosynthetic yields, indicating that herbicide resistance can develop in lentic periphyton after short (7 days) exposure, but only at simazine concentrations greater than or equal 0.8 mg/L (i.e., increased EC_{50} observed at treatments greater than 0.8 mg/L simazine).	(Goldsborough and Robinson 1988) (3136)
Mesocosm (6 months)	25 lb dose of granular simazine applied to alternate halves of a 1/5 acre (3-ft deep) pond on 2 consecutive weekends. Changes in aquatic plant communities over time were observed.	Aquatic plants	Specific endpoints or effect values were not reported. A 4-yr old farm pond containing *Najas flexilis* and *Potamogeton foliosus* was treated in the spring. After decay of the higher plants, phytoplankton did not dominate, but instead herbicide resistant seeds and subsurface structures of *Potamogeton foliosus* developed. Benthic algae covered and stabilized the bottom. Following stabilization, the water cleared and *Chlara vulgaris* became established in a portion of the pond where the substrate was firm.	Crawford, 1981 (MRID 45088203)

Study type/test material	Study Design	Test Organism	Effects	Reference (ECOTOX # or MRID)
			Treatment of the pond with simazine resulted in death of the majority of macrophytes. However recovery of the macrophytes was noted within two to three months post application. Seeds and tubers of *P. foliosus* maybe resistant to simazine.	
Mesocosm	Microcosms consisted of 12 x 3 liter Erlenmeyer flask plugged w/cotton. Algal cultures were obtained from a chicken processing oxidation pond allowed to grow to stationary phase. Nominal concentrations of 50, 150, 400 ppb simazine were used. Photosynthesis, respiration, dry weights, diversity, species dominance, and chlorophyll a were measured.	Aquatic plants	Simazine caused a shift in time of highest productivity peaks by about 2 weeks at 150 and 400 ppb. A lag in net productivity, but larger peaks of productivity, were seen in the higher doses. Pigments and dry weights were relatively unaffected. Although it was stated that successional sequence was affected, there were very few organisms on which to base this observation. Algal species exposed to the highest concentration had delayed net and gross productivity and respiration, which was followed by rate increases in both that exceeded the same rates for algal species exposed to lower concentrations. Gross productivity was greatest for the high exposure group at the end of the bioassay. Algal biomass in control and lower treatment groups were not different. The type of successional sequence of species was affected by treatment level with Chlorella dominating at the higher levels. Uncertainty exists as to whether this community shift remains in the absence of simazine.	(Bryfogle and McDiffett 1979) (MRID 45088205)

Effects to Terrestrial Plants

Single-species terrestrial plant toxicity studies are used as one of the measures of effect to evaluate whether simazine may affect primary production and diversity in terrestrial ecosystems. Numerous terrestrial plant toxicity studies have been submitted to the EPA and/or published in the open literature.

The registrant submitted data represents the most sensitive endpoints for effects to listed species. Seedling emergence and vegetative vigor were studied on ten non-target crops (including soybean, lettuce, radish, tomato, cucumber, cabbage, oat, ryegrass, corn, and onion) following application of Princep 4L herbicide (simazine) at 4 lb a.i./acre (MRIDs 42634603 and 42634604).

For seedling emergence, the most sensitive dicot is lettuce with NOAEC, LOAEC, and MATC values of 0.0018, 0.0054, and 0.0031 lb a.i./acre, respectively. The most sensitive monocot is onion, with NOAEC, LOAEC, and MATC values of 0.014, 0.049, and 0.028 lb a.i/acre, respectively.

Table 13. Summary of non-target terrestrial plant seedling emergence toxicity data for simazine (Table 12-2 in the BE).

Species	**Taxa**	**Endpoint**	**NOAEC (lbs a.i./acre)**	**LOAEC (lbs a.i./acre)**	**MATC (lbs a.i./acre)**
Corn (*Zea mays*)	Monocot	No Effect	4	>4	4
Oat (*Avena sativa*)	Monocot	Biomass	0.016	0.049	0.028
Onion (*Allium cepa)*	Monocot	Biomass	0.014	0.049	0.028
Ryegrass (*Lolium perenne*)	Monocot	Biomass	0.016	0.049	0.028
Radish (*Raphanus sativus*)	Dicot	Biomass	0.049	0.15	0.086

Species	**Taxa**	**Endpoint**	**NOAEC (lbs a.i./acre)**	**LOAEC (lbs a.i./acre)**	**MATC (lbs a.i./acre)**
Soybean (*Glycine max*)	Dicot	Biomass	0.016	0.049	0.028
Lettuce (*Lactuca sativa*)	Dicot	Biomass	0.0018	0.0054	0.0031
Cabbage (*Brassica oleracea alba*)	Dicot	Biomass	0.016	0.049	0.028
Tomato (*Solanum lycopersicum*)	Dicot	Biomass	0.016	0.049	0.028
Cucumber (*Cucumis sativus*)	Dicot	Biomass	0.016	0.049	0.028

For vegetative vigor, the most sensitive dicot is lettuce with NOAEC, LOAEC, and MATC values of 0.016, 0.049, and 0.028 lb a.i/acre. Similarly, the most sensitive monocot for vegetative vigor is oat with NOAEC, LOAEC, and MATC values of 0.016, 0.049, and 0.028 lb a.i/acre.

Table 14. Summary of non-target terrestrial plant vegetative vigor toxicity data for simazine (Table 12-2 in the BE).

Species	**Taxa**	**Endpoint**	**NOAEC (lbs a.i./acre)**	**LOAEC (lbs a.i./acre)**	**MATC (lbs a.i./acre)**
Corn (*Zea mays*)	Monocot	No Effect	4	>4	4

Species	**Taxa**	**Endpoint**	**NOAEC (lbs a.i./acre)**	**LOAEC (lbs a.i./acre)**	**MATC (lbs a.i./acre)**
Oat (*Avena sativa*)	Monocot	Biomass	0.016	0.049	0.028
Onion (*Allium cepa)*	Monocot	Biomass	0.016	0.049	0.049
Ryegrass (*Lolium perenne*)	Monocot	Biomass	0.016	0.049	0.049
Radish (*Raphanus sativus*)	Dicot	Biomass	0.049	0.15	0.086
Soybean (*Glycine max*)	Dicot	Biomass	0.049	0.15	0.086
Lettuce (*Lactuca sativa*)	Dicot	Biomass	0.016	0.049	0.028
Cabbage (*Brassica oleracea alba*)	Dicot	Biomass	0.016	0.049	0.028
Tomato (*Solanum lycopersicum*)	Dicot	Biomass	0.049	0.15	0.15
Cucumber (*Cucumis sativus*)	Dicot	Biomass	0.016	0.049	0.028

Effects to Terrestrial Plant Communities

Twenty five percent inhibition concentration (IC_{25}) values for terrestrial plants are used to derive the threshold for effects to the PPHD of an individual of a listed species. Studies with effects on measures of growth (e.g., height, weight, and biomass) for both monocots and dicots were conducted with technical grade simazine and had 14- and 21-d exposure durations used to derive Species Sensitivity Distributions (SSD). These parameters were selected to maximize comparability of results. Studies used to derive the SSDs are compiled in Appendix 2-6 of EPA's BE. SSDs were developed for both seedling emergence and vegetative life stages.

Toxicity estimates for simazine range from 0.009 to greater than 4 lb a.i./acre and span three orders of magnitude, indicating a range of sensitivity to simazine herbicides among terrestrial plants. Based on the results of the submitted terrestrial plant toxicity tests, it appears that the seedling emergence stage of plant development is more sensitive to simazine than the vegetative vigor stage of development.

For seedling emergence, the most sensitive dicot is lettuce and the most sensitive monocot is onion. IC_{25} values, on an equivalent application rate basis, for lettuce and onion, which are based on a reduction in dry weight, are 0.009 and 0.02 lb a.i./acre, respectively. For vegetative vigor studies, the most sensitive dicot is lettuce with an IC_{25} and the most sensitive monocot with a definitive IC_{25} was oat (IC_{25} = 0.033 lb a.i./acre).

For the SSD, five distributions were tested, and a variety of methods were used. The gumbel distribution and linearization (GR) method were selected to represent HC_{05} through HC_{95} values for vegetative vigor endpoints and the logistic distribution and maximum likelihood (ML) method were selected to represent the HC_{05} through HC_{95} values for seedling emergence endpoints. The threshold for species that rely upon terrestrial plants for their PPHD is 0.0129 lb a.i./acre based on the HC_{05} from the SSD for seedling emergence.

Table 15. Terrestrial plant SSD quantiles for vegetative vigor and seedling emergence (adapted from table 12-3 from EPA's BE).

Statistic	Vegetative Vigor (lbs a.i./A)	Seedling Emergence (lbs a.i./acre)
HC_{05}	0.0256	0.0129
HC_{10}	0.0283	0.0164
HC_{50}	0.0445	0.0335

Statistic	Vegetative Vigor (lbs a.i./A)	Seedling Emergence (lbs a.i./acre)
HC_{90}	0.09702	0.0683
HC_{95}	0.0119	0.0871

A summary of available data evaluating the phytotoxicity of simazine to woody plants was submitted to the EPA in 2007 (Wall, 2007). A total of 79 species were tested in 110 separate trials at application rates of 0.5 to 12 lbs a.i./acre. Signs of phytotoxicity were summarized and reported. Fifty-four species exhibited either no or negligible (<10%) phytotoxicity. Further examination of data for the remaining 25 woody species showing phytotoxicity values > 10% indicates that the species were exposed to simazine concentrations greater than those expected to be present at environmentally relevant concentrations.

The data indicate that simazine is not likely to have an adverse effect on woody plants when used at labeled application rates (or even at higher rates, which is often tested in field phytotoxicity trials). The species were exposed to simazine in a direct application, which represents a worst-case exposure scenario. It is expected that woody plant species adjacent to treated areas would not be exposed to simazine at the tested rates. Furthermore, simazine is labeled for use around numerous wood species including citrus, tree nuts, grapes. Based on the available data and expected lower predicted concentrations away from the treated field, it is unlikely that simazine will cause adverse effects to non-target woody plant species.

Incident Reports

As part of BE development, EPA reports any incidents related to the pesticide under consultation from their Ecological Incident Information System or aggregate 6(a)(2) incident reports. While incident reports can inform our analysis, we expect that only a very small percentage of organisms that die from any pesticide exposure will be located and reported (N. Vyas 1999). As such, the existence of verified incidents can increase our understanding of the risk associated with pesticide exposure, but the absence of verified incidents does not further contribute to the weight of evidence.

As reported in EPA's BE, four simazine incidents have been reported for terrestrial plants, with two rated as being "unlikely" a result of simazine. In one incident, water from a simazine-treated swimming pool affected a section of lawn grass. The certainty index for the lawn incident (# I003567-001) is "highly probable." Another incident report from New York, was a Syngenta 6(a)2 submission regarding an allegation that the spraying of three herbicide formulations (a.i.s included were atrazine, simazine and primisulfuron-methyl) caused loss of milk production and loss of corn and hay production. The treatment site nor the magnitude of the incident were given, neither was the magnitude or any analysis (IO16790-007, September 20, 2005). The certainty index for the incident involving simazine is listed as "probable." We are unable to relate the circumstances of these reports to expected exposure of plants from currently registered uses of simazine. As such, incident reporting did not influence our analysis of the effects of simazine to plants at estimated environmental concentrations modeled for the Opinion.

Exposure

Once applied, the main routes of dissipation for simazine are microbial degradation under aerobic conditions, runoff, and leaching. Simazine's primary transport routes from treated areas to aquatic habitats include spray drift and runoff. Simazine is expected to move into surface and ground water given its persistence and mobility. Simazine has a low vapor pressure (6.1×10^{-9} at 20°C (Torr)) and low Henry's Law Constant (3.2×10^{-10} atm-m^3/mole; calculated at 25°C), suggesting low potential for volatilization. Simazine is moderately soluble in water with reported aqueous solubility values ranging from 3.5 to 11 mg/L at 20-25ºC. Bioaccumulation of simazine is expected to be low due to a low octanol water partitioning coefficient (K_{ow} = 122) and low bioconcentration factors ($BCF_{steady\ state}$ = 4.92, $BCF_{kinetic}$ = 7.30). Bioconcentration factors represent the ability of a substance to accumulate via aqueous exposure and can be determined under steady-state conditions as the ratio of the substance concentration in a fish compared to the water concentration or kinetic conditions where the rate at which the organism absorbs the substance compared to how fast the organism can metabolize the substance and remove it (USEPA 2016)). Soil sorption coefficients for simazine (K_F) range from 0.5 to 4.3 ml/g (1/n=0.79-1.40). Information on leaching and adsorption/desorption indicate that simazine is considered moderately mobile according to the Food and Agricultural Organization (FAO) mobility classification system.

Simazine is resistant to direct photodegradation in water ($t_{1/2}$ > 386 d; MRID 42503708) and to photodegradation on soil ($t_{1/2}$=132 d; MRID 42739101) which means it does not break down in water when exposed to light.

Although the submitted guideline studies for triazines would indicate they do not degrade by abiotic hydrolysis at pH 5, 7, and 9 (MRID 00027856), open-literature studies show variable acid-catalyzed hydrolysis half-lives for triazines in different matrices including soils, clay suspensions, organic matter, and groundwater (Mabey and Mill 1978, Hance 1967, Armstrong, Chesters and Harris 1967, Burkard and Guth 1981, Khan 1978, Widmer, Olsen and Koskinen 1993, Gamble, Khan and Tee 1983, Plust, et al. 1981) and (Navarro, et al. 2004). The hydrolysis half-life for simazine specifically is observed to be 310 d in non-sterile groundwater at 20 °C and pH of 6.66 (Navarro, et al. 2004). The hydrolysis of triazines leads to formation of hydroxytriazine compounds. Thus simazine is persistent and does not appear to break down quickly in groundwater.

Simazine is moderately persistent to persistent in aerobic loam soils ($t_{1/2}$ ranges from 80 to 650 days) based on the (Goring, et al. 1975) classification. Simazine has a half-life of 74 d in aerobic aquatic environments (MRID 43004501, 46561301, 43004502). Simazine is also persistent in anaerobic aquatic environments ($t_{1/2}$ > 1,766 d) and potentially persistent in anaerobic soil environments as no degradation occurred within a 51-day study duration (MRID 40614411, 00027857).

Field dissipation studies show that simazine dissipation is driven by microbially mediated degradation and by leaching. The half-lives of simazine in several terrestrial field studies indicate it is not susceptible to dissipation and is detected in up to 480 days (MRID 40634202, MRID

00027863). The half-life of simazine in four aquatic field dissipation studies in multiple states ranged from 10 d to stable (MRIDs 00027829, 40614420, 40614421, 40614422). Microbially mediated degradation is therefore an important route of dissipation.

Environmental fate data indicate that deisopropylatrazine (DIA) and hydroxysimazine (HS) are the major transformation products of simazine (>10% applied a.i.) and that diadealkylatrazine (DDA) is a minor chlorotriazine degradation product (<10% applied a.i.). The degradate diadealkylatrazine is often commonly referred to as DACT. DIA and DDA are formed through dealkylation of the amino groups on the simazine molecule, while HS is formed through substitution of a chlorine by a hydroxy group. Dealkylation is a microbial-mediated process, while hydroxylation is both abiotic and microbial-mediated process. In the terrestrial environment, EPA gave consideration to formation of transformation products, through the consideration of toxicity data and foliar dissipation half-lives. Available ecotoxicity data for transformation products shows similar or lower toxicity in comparison to simazine across taxa groups. Based on EPA's analyses, we assume that modeling of the parent compound alone for simazine is adequate for determining potential exposure and effects concentrations to organisms in the aquatic and terrestrial environments.

A foliar dissipation half-life on plant surfaces of 35 days was used for estimating terrestrial exposure in EPA's BE. EPA uses a default non-chemical specific foliar dissipation half-life of 35 days for terrestrial modeling based on data reported by (Willis and McDowell 1987) unless chemical specific data are available. It is an upper-bound value (second highest reported among the 447 half-lives) from the empirical data presented in a meta-data analysis by (Willis and McDowell 1987).

This potentially has the most implications for animals species that preferentially visit certain areas where simazine has been sprayed and/or consume large amounts of a plant surface that has been treated, or those listed plant species that may be in close proximity where applications are made.

Rate, Frequency, and Number of Applications

Estimated environmental concentrations (EECs) are influenced, in part, by the allowable manner of pesticide use as described by the label, including the application rate, frequency of application, and the maximum number of applications per season or year. Generally, EPA modeled EECs using the highest allowable application rate and minimum re-entry interval for each labeled use. We recognize that simazine will not always be used in a manner that produces maximum concentrations in the environment. Where we found these concentrations result in effects to listed species, we looked to usage data to determine whether it is reasonable to assume that simazine is used in a manner to produce such concentrations.

In selecting application dates for aquatic modeling, EPA considers a number of factors including label directions, timing of pest pressure, meteorological conditions, and pre-harvest restriction intervals. Agronomic information was consulted to determine the timing of crop emergence, pest pressure and seasons for different crops. General sources of information include crop profiles, agricultural extension bulletins, and/or available state-specific use information.

Simazine may be applied during different seasons, and the directions for use indicate the timing of application, such as, dormant season (pre-emergent) or foliar (e.g. when foliage is on the plant; post-emergent). For simazine uses, the Pesticide in Water Calculator (PWC) model inputs for the application dates were chosen based on these timings, the crop emergence and harvest timings specified in the PWC scenario, and precipitation data for the associated meteorological station. Application dates were selected to represent conservative and reasonable estimates. If applicable, dormant seasons were assumed to occur between November and February, the predominant period throughout the country when crops are dormant. Pre-harvest intervals (the minimum time between an application and harvest) were also considered. Applications would not occur closer to harvest than allowed by the pre-harvest interval.

Determining Percent of the Population That Could Be Exposed to Simazine

Overlap with species range: We derive the estimate of exposure for each species, in part, by determining the extent that the range of a species overlaps with use site categories for which the pesticide is registered, combined with anticipated off-site transport. The process for establishing the use site footprint is generally described in Attachment 1-3 of EPA's BE. Briefly, simazine use sites were binned (i.e., categorized) by the general land cover class that best represents the use pattern (e.g., peaches are categorized with other orchards while cole crops – e.g., cabbage, broccoli, Brussels sprouts, and kale – are binned with vegetables and ground fruit; see Table 1). EPA lists information on crop or use, application timing, application rates, method, and any geographic restriction in the Master Use Summary Table (Appendix 1-3 of the BE and Table 2 of this Opinion). To map use sites on the landscape, EPA used the 2017 National Agricultural Statistics Service (NASS) Census of Agriculture (CoA) crop acreage reports and the 2017 NASS CoA crop harvested data to confirm the presence or absence of individual use sites or crops within a county. Unless the label limits a use pattern to a particular geographic area, all regions are modeled where there are crop acres or harvested data. For those crops/use sites where NASS harvested data are unavailable, the crop or use site was assumed to occur within that county based on the information provided by the crop data layer (CDL) representing the landcover groups.

The process by which EPA obtained species or critical habitat ranges and analyzed the overlap of the simazine UDLs with those ranges as well as determined the usage applicable to those overlaps is described in the BE in Appendix 1-5, Appendix 1-6, and Attachment 1-1. Briefly, EPA developed an analytical process using tools developed in Arc GIS and using the programming language Python, by which ranges and critical habitats from the Service's ECOS[22] on-line system are downloaded, sorted, and overlap and usage data for all UDLS for the action

[22] Environmental Conservation On-Line System: https://ecos.fws.gov/ecp/

area are applied to determine the percentages of overlap with the uses or usage of the herbicide that occurs within a species range or critical habitat.

The "percent overlap" for each use site is generally divided between on-field overlap, off-field overlap, and total overlap. On-field overlap refers solely to the footprint of the use site itself. Off-field overlap is comprised of the 305-m offsite transport area outside of use sites. This is the distance at which EPA determined there is an attenuation of the spray drift and the maximum distance at which they expect impacts to non-flowing wetland plants (i.e., the most sensitive species group). The total overlap combines these two metrics. When mapping use sites, EPA found redundancies among various use sites. That is, mapped use sites are not mutually exclusive of one another. For instance, there may be landcover that is considered to be part of both the Other Orchards category and the Citrus category. For this reason, combining the percent overlap for use sites may overestimate the total amount of a species' range that is overlapping with use sites. To compensate for this redundancy, for our overlap analysis, we use the highest value of those crops where redundancies are shared to assess the totals for those given crops. Additionally, we cap our calculated total overlap metrics at 100% as totals greater than 100% are artifacts of redundant acreage between different UDLs. We discuss this as well in the *Agricultural Overlap* portion of the *Integration and Synthesis* section of this Opinion.

Distribution of individuals within the range:

We determined the exposure of species to pesticides at a population level by considering the overlap of pesticide use sites and associated off-site transport with individuals within the landscape, as determined by the range of the species and the anticipated distribution of individuals within the range. We estimate the distribution of individuals by several types of factors, including: habitat preference, life history traits, behaviors such as colonial nesting or flocking, type of water body (flowing or static), size of water body (for aquatic or semi-aquatic species), and known areas of high or low density of individuals of the species. Distribution can also include areas where species may congregate to breed or roost on a short-term basis, such as leks or spawning sites. Areas of high densities of individuals can increase the vulnerability of a species if they overlap with pesticide use sites. Conversely, vulnerability may increase for species with few individuals that overlap use sites and are not widely distributed outside of these sites. Our approach considered this scenario as well. However, the availability of specific information regarding the distribution of species varies. Where information is readily available for individual species or taxonomic groups, it is incorporated into the analysis in a qualitative manner. For species where no information is available, we will assume that species are uniformly distributed throughout the range. However, we may consider that species may be more or less likely to be in use areas based on the suitability of habitat and availability of resources. The assumption of a uniform distribution can either increase potential exposure by artificially expanding the area of exposure to the whole range or decrease the potential exposure by failing to identify high density areas that overlap with pesticide use sites.

<u>Seasonal exposure:</u>

Species may be precluded from exposure to a pesticide due to life history factors such as migration, estivation, or hibernation. Where species may avoid exposure to a pesticide for a particular life stage or life event, it was considered in the analysis. For example, whooping cranes in the Aransas-Wood Buffalo National Park population do not breed in the United States (they only winter and migrate within the United States) and, therefore, effects to breeding were not anticipated to occur from the action under consideration. When species may not be present during pesticide applications, consideration was made as to whether residues were likely to remain in the environment when the species returns to the site. As our analysis generally evaluated the effect of a single exposure per year, we did not modify the anticipated risk based on the percent of the time spent in the action area, as each species could be exposed at least once per year regardless of that factor.

Volatilization and Atmospheric Drift

Based on a relatively low Henry's Law Constant and vapor pressure as previously stated in the *Exposure* section of this Opinion, and low soil/water partitioning, simazine has low volatilization potential.

Terrestrial-specific Exposure Factors

Terrestrial organisms can be exposed to pesticides in the environment through diet, direct spray, preening, drinking water, and inhalation at different life stages. Various factors influence the likelihood and extent of this exposure at both the individual and population level including both properties of the pesticide (e.g., number of applications, persistence) and life history factors of the species (e.g., dietary preference, feeding habits, species distribution, and local and long-distance movement).

Routes of Exposure

<u>Ingestion - dietary exposure</u>

A primary route of exposure to pesticides in general for terrestrial organisms is from ingestion, either by feeding on food items that have been contaminated after a pesticide application or through direct consumption of the pesticide (e.g., in the water-dispersible granular form). For contaminated food items, exposure may be to residues that have either been biologically incorporated into plant or animals or deposited on the surface or the plant or animal. Secondary predators may also be exposed to pesticide within prey that has not yet been biologically incorporated but resides within the gastrointestinal tract of prey (Hill and Mendenhall 1980).

The frequency of food ingestion can vary by species. Some species may hunt or graze on dietary items daily, either at certain times (e.g., dawn and dusk), or throughout the day. Other species, such as predators and scavengers (e.g., California condor, snakes) may ingest a prey item or carcass and not feed again for one or more days. Life stage may also affect the frequency of feeding, as young of altricial species may be reliant on parents to bring food back to the nest site one or more times per day. Long-distance migrators such as the red knot may gorge feed at stopover locations, then travel long distances on food stores from these events. Terrestrial invertebrates such as many Coleoptera or Lepidoptera may also spend a significant portion of their larval and/or other juvenile stages consuming large amounts of food or prey to progress into the next life cycle stage.

For terrestrial species, EPA's BE provides EECs based on output from the T-REX model on and in food items of terrestrial vertebrates as both dietary-based and dose-based values (as described in the 2016 Preliminary Ecological Risk Assessment (PRA) for Simazine, Tables 32- 34) for exposure on use sites and via spray drift. Pesticide concentrations vary by dietary item and use (i.e., incorporating use-specific application rates and frequency). Therefore, individual species may be exposed to a range of EECs based on the number of food items consumed and the number of use sites that the species overlaps with.

For our analysis, listed terrestrial species have been documented to consume from 1 to 11 dietary items. For many species, dietary preferences are unknown or the information is not readily available. For these species, we assume that individuals are equally likely to consume any of the dietary items identified. Some species may have known dietary preferences. In these cases, we have increased confidence in the likelihood of exposure to the pesticide concentration associated with preferred dietary items. However, even if a dietary item is less preferred, it should be considered whether it may be consumed at a high enough rate to cause effects even once over the course of the entire year. In some cases, prey exposed to pesticides could be taken preferentially, as such exposure may make it more susceptible to predation (e.g., (Hunt, et al. 1992)).

The breadth of EECs that are likely to be encountered by individuals may also be influenced by the degree of mobility of the species. The EECs derived from the T-REX model are based on empirical values of dietary items collected from fields following herbicide applications that vary both across and within application sites. As such, a range of potential EECs is generated based on these values and the designated application rate. The PRA for simazine provides two EECs from this range, the mean and upper bound.

For each application of simazine, T-REX produces a time series of concentrations on each dietary item, starting immediately after application and progressing on a daily basis. For our assessment, we have chosen to look at the peak EECs from this time series; as explained below, use of peak EECs is a rational approach for analyzing species response. Accordingly, for some dietary items, such as plants, peaks will occur immediately after an application and decrease through time. For other dietary items, such as small mammals and birds, peaks may not occur until days after an application as the prey item itself continues to be exposed to residues prior to it being preyed upon by the listed species under consideration. Peak values can also be influenced by multiple applications and the length of time between those applications. For mobile species, we acknowledge that looking at peak values may overestimate exposure, as individuals may not be present or may be foraging in a different location when peak values occur. However, mobile individuals may also have more opportunities for exposure to peak values if their foraging areas pass through multiple areas of pesticide use. For instance, wood storks typically forage 5 to 12 miles from nesting sites but have been documented foraging as far as 80 miles. Species such as this may be exposed to simazine as a consequence of multiple application events (i.e., from different fields or use sites, or from multiple applications on the same field), or from feeding multiple days on the same use site where concentrations may remain high enough to result in adverse effects. Our analysis does not capture the risk to species that may be exposed repeatedly or on multiple occasions throughout the year; we assess the risk of effects to individuals following a single exposure event. However, the above approach is a rational and balanced one for several reasons. As previously explained, our analysis evaluates effects from a single exposure event, but it is also possible that simazine may not be applied in areas of overlap, or if applied and the species is exposed, it may be the case that exposure is at concentrations that would not illicit an adverse response from the subject species. By using peak EECs, the Service is evaluating the full breadth of species response. For example, for species with little to no movement, individuals on or near use sites have a high likelihood of seeing peak EECs following an application, as well as subsequent EECs from the same application that may result in adverse effects. However, they may be unlikely to experience exposure from spray events from other use sites, and therefore, are likely to have less chance of exposure from multiple applications in different sites.

Peak EECs are used to assess mortality and sublethal effects from both acute and chronic exposure. As described above (Effects to Terrestrial Species), most toxicity studies that are designed to examine sublethal effects such as growth, behavior, and reproduction are chronic studies in which test subjects may be exposed to pesticides for long periods of time (e.g., 20-week reproduction studies for birds). Endpoints measured in these studies aggregate the combined effects of that exposure that may be a result of one or more responses (e.g., parental behavior of adults versus developmental effects to young that combined result in reducing hatching). It is not generally possible to ascertain the specific response, or timing of that response, that caused the ultimate effects. For reproduction in birds, for example, it is possible that short exposures at some point during the 20-week exposure cycle were ultimately responsible for effects. Without information to suggest that effects are only likely to result from longer exposures, we assess the potential for simazine to affect individuals based on a single peak EEC value.

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

Terrestrial species may be exposed to pesticides through direct contact with a pesticide followed by dermal absorption. Exposure may occur from pesticides directly deposited on an individual during a spray or individuals contacting contaminated media after a spray, such as walking on a treated field or brushing against treated foliage. For example, studies involving cholinesterase-inhibiting pesticides, particularly organophosphates, have shown this can be a significant route of pesticide exposure for terrestrial vertebrates, especially for birds (Hudson, Haegele and Tucker 1979, Schafer, et al. 1973, Henderson, et al. 1994, Vyas, et al. 2006). For simazine, while data are lacking for contact toxicity of simazine in other terrestrial vertebrates, we anticipate dermal absorption of simazine is low in mammals, as one study in rats calculated less than 1% of doses applied were absorbed after 24-hours. A systemic study was not able to identify a LOAEL but identified a NOAEL of 1000 mg/kg/day, significantly greater than the threshold identified for assessing effects by dietary exposure. As such, we base our analysis on dietary toxicity as the primary route of exposure and effects to terrestrial vertebrates. While we acknowledge dermal contact can be an additional route of exposure that may increase the total amount of simazine that terrestrial vertebrates may be exposed to, we do not anticipate this type of exposure will result in additional measurable impacts to individuals that are not already accounted for given the conservative nature of the dietary assessment (i.e., diets consisting of only forage/prey items exposed at maximum concentrations) and the comparative data between the two routes of exposure.

For terrestrial invertebrates, though individuals can be exposed through diet, contact exposure (i.e., individuals exposed by direct spray or contact with contaminated vegetation or other media) is typically the most sensitive route of exposure. Thus we assess effects to terrestrial invertebrates based on exposure and effects via this route. To estimate effects of contact exposure, we use the output from the BEE-REX model, as provided by EPA, describing the concentration of pesticide on the surface of the terrestrial invertebrate, and compare that value to adverse effects thresholds for terrestrial invertebrates, as described above.

Ingestion from preening or grooming

Birds and mammals exposed to pesticides on their feathers or fur through direct spray or contact with contaminated media can ingest that pesticide through preening. For example, in one study, dermal exposure, including preening, was found to be a greater contributor to toxicological response from 8 to 48 hours post-spray than oral exposure in northern bobwhite exposed to simulated aerial crop applications of the cholinesterase-inhibiting pesticide methyl parathion (Driver, et al. 1991).

EPA did not assess exposure of birds and mammals through preening or grooming in the BE. We considered data regarding dermal toxicity and found this route to be a less sensitive endpoint than dietary exposure. For triazine herbicides, we generally expect dietary exposures through the consumption of prey that have accumulated triazine residues will result in higher body burdens that preening or grooming, particularly given EPA's conservative dietary exposure modeling assumptions (e.g., an individual exclusively feeds on an application site, an individual feeds on a single dietary item that results in the highest exposure concentration, etc.). As such, we anticipate our assessment of dietary exposure will account for any exposures resulting from preening or grooming.

Inhalation

Exposure may occur via inhalation of spray droplets (at the time of application) or volatilization that occurs after the application. Inhalation of spray droplets occurs at the time of or within minutes of an application, which is considered an acute exposure. We expect a low likelihood that listed terrestrial vertebrates will be exposed during this short period. In addition, simazine has low oral acute toxicity to birds and mammals, and in available studies with the rat, no mortality was observed at the highest inhalation dose tested (Chapter 2 of the BE).

Inhalation exposure via volatilization is not considered likely because simazine is considered non-volatile[23] (vapor pressure of simazine is 3.0×10^{-7} and 6.1×10^{-9} Torr).

Therefore, given that we expect a low likelihood of exposure and low toxicity for this route of exposure, inhalation is not of concern for simazine.

Ingestion - drinking water

Terrestrial animals obtain the water they need from their food and likely ingest drinking water from various sources. In general, mammals that consume foods with high water content (e.g., in general, leaves, fruit and arthropods are approximately 80% water) obtain their water needs largely from their diet. Birds, reptiles and amphibians that consume food items with high water content, may ingest some drinking water; however, exposures are at least an order of magnitude lower through drinking water compared to diet due to no or limited drinking water consumption. Based on this information, mammals and birds that primarily consume seeds, which have lower water content (approximately 10% water) need to drink water to fulfill their daily water requirements. Sources of drinking water may include dew (in the morning) and puddles on treated fields or adjacent areas, as well as ponds and flowing water bodies (streams, rivers). In general, drinking water sources on or near the field with smaller water volumes (i.e., dew and puddles) result in higher pesticide concentrations compared to larger water bodies (with larger volumes that dilute concentrations). Relative to direct consumption of simazine from dietary items on or near the field, drinking water is expected to be a much lower route of exposure. Animals likely consume water from a variety of sources, resulting in lower exposures than if animals consumed water from on-field. To have a risk from drinking water, granivore animals would need to drink only water from puddles or dew on treated fields. Chronic exposures are unlikely because residue contaminated puddles and dew on the treatment field are generally ephemeral. Although individuals may be exposed, it is unlikely that enough granivore individuals will consume drinking water on a chronic exposure basis from only treated fields so that there is a population-level effect. As such, we consider this route of exposure unlikely to lead to adverse effects in listed species.

Estimated Environmental Concentrations (EECs) on Use Sites and from Offsite transport

For the overlap with species range, the BE considers the aggregate of the six years (2013-2017) of available Cropland Data Layers data for pesticide use categories to ensure the full footprint is captured for each use. For the Opinion, we bring forward the same analysis as is used in the BE. Terrestrial exposure concentrations are uniquely calculated for each species depending on relevant use overlap with the species range, application rates associated with these relevant uses and the dietary items, habitat and obligate relationships for that species. To provide a bounding of potential terrestrial EECs used in the effects determinations, EECs were calculated for the range of application rates for simazine (a minimum application rate of 0.8 lb a.i./acre with 1 application per year and a maximum single application rate of 8 lb a.i./acre) and are provided in

[23] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/guidance-reporting-environmental-fate-and-transport#I_B

the Simazine Summary of Action Table (Table 2). The BE summarizes the mean and upper bound dietary-based EECs and the associated base model that is used. However, EECs could be slightly higher with mid-range application rates applied multiple times.

Terrestrial EECs and overlap values for exposure via spray drift at two distances: 0 meters (representing concentrations directly on use sites) and at 305 meters from the application site, as this the distance at which EPA determined there is an attenuation of the spray drift and the maximum distance at which they expect impacts to plants (i.e., the most sensitive taxa) based on available toxicity data. These estimates assume drift extends these distances off fields, and typically represents open areas with flat topography. Pesticides may drift farther in some instances. In other instances, drift may be minimized by application methods, timing, or landscapes that impede its movement (e.g., forest).

For all species, we assume spray drift will increase the area of overlap with the species range, with this assumption particularly important for species that are not anticipated to enter use sites, as it may represent the only exposure to simazine that is likely to occur. However, it is important to note that spray drift areas from different uses can overlap with one another, or even overlap with use sites, depending on their proximity on the landscape. We account for this overlap in use site treatment footprint by combining all use site and spray drift overlaps in our exposure analyses, which we discuss in more detail in the *Exposure* sub-section in the *Integration and Synthesis* section of this Opinion.

Mixtures

Pesticide mixtures can be divided into three categories: formulated products, tank mixes, and environmental mixtures. Formulated products are produced and sold as one product containing multiple active ingredients. We have the most confidence in species being exposed to these types of mixtures, as application of these products ensures that both active ingredients enter the environment at the same time. Formulated products containing simazine have been identified as part of this action and are shown in Table 2. Tank mixes refer to a situation where the pesticide applicator applies multiple pesticides simultaneously at the use site. Unless explicitly prohibited on the pesticide labels, any two active ingredients may be combined in a tank mix. Though we have less certainty in these types of mixtures occurring, specific tank mixes are often described on product labels and their use may be encouraged to increase pesticide efficacy. Environmental mixtures result from unrelated pesticide use over the landscape and are typically detected in ambient water quality monitoring efforts. From monitoring efforts, we have high confidence that these types of mixtures occur. Monitoring data from state and federal agencies described in the BE and elsewhere have indicated that multiple pesticides often co-occur in aquatic habitats located throughout the United States. Studies conducted by the U.S. Geological Survey, under the National Water Quality Assessment program, have routinely detected the presence of multiple chemicals in surface water and groundwater samples.

While simazine may be tank mixed with other pesticides, there is no information available on the use of tank mixtures (e.g., what the composition of each tank mixture is, where tank mixtures are being used, how often they are used). Given the lack of available use and usage data on tank mixtures as well as the lack of tank mixture toxicity data, we limit our analysis to consider only the effects of simazine alone. However, available data on pesticide mixtures indicate that effects from exposure to one or more pesticide will result in an additive effect; that is, we do not expect that any toxic interactions will occur. In light of this, the National Academy of Sciences recommended in its 2013 *Report Assessing Risks to Endangered and Threatened and Species to Pesticides* that in the absence of data showing a synergistic (i.e., more than additive) response between a pesticide active ingredient and another mixture component, the analysis of effects should proceed on the assumption of additivity. Therefore, we consider that mixtures of pesticides with simazine, including tank mixes, will exert independent effects on species. Thus, given the lack of information to predict tank mixtures and our expectation that other pesticides will not enhance the effects of simazine, we do not further consider these mixtures in our analysis.

Factors to Determine Percent of the Population Exposed – Terrestrial Species

Utilization of pesticide use site

Concentrations of pesticides on food items and contaminated media such as plants are generally higher on pesticide use sites than on adjacent areas contaminated only by off-site transport from spray drift. Individuals that are predicted to experience effects from pesticide exposure on use sites may have reduced effects, or in some cases no effects, from exposure to pesticide as a result of spray drift because concentrations of pesticides are known to attenuate further from the point of application on a use site. For this reason, the tendency of individuals to enter or forage within a use site, when known, can affect the likelihood of exposure and effects. Species experts within Service field offices were asked to comment on whether species will enter, forage, roost, breed, pass through, or otherwise utilize pesticide use sites that overlap with the range of the species. Where this information was obtained from species experts or otherwise available from Service-produced documents or the open literature, we incorporated it into the analysis to verify or limit potential exposure as appropriate. For example, if a species may breed or forage on a use site, exposure was considered both on the use site and as a result of spray drift off site. If a species is only likely to travel through a use site and not forage within use sites, we primarily focused our analysis on dietary exposure from spray drift or other off-site transport. If a species was deemed unlikely to enter a use site, we did not consider effects from on-field exposure. Where data were lacking on whether use sites would be avoided, we assumed that a species could enter, forage, roost, breed, pass through, or otherwise utilize sites of pesticide use based upon their location within the species range. More specific information regarding a species' behavior on or near use sites results in better exposure assessments and reduced need for conservatism. We continue to expand our knowledge of the relationship between listed species and occurrence on pesticide use sites, as more specific information regarding a species' behavior will result in better exposure assessments and reduced need for conservatism.

Mobility of individuals

The percent of a population exposed to a pesticide may be influenced by the distance an individual travels to forage. As a default, we assume the proportion exposed is roughly equivalent to the percent of overlap between pesticide use sites and the species range. We may have more confidence in this assumption for species that have limited mobility compared to those with high mobility. For species that travel large distances to forage, this overlap is likely to be less predictive of pesticide exposure, depending on the manner in which use sites are distributed throughout the range. For instance, wood storks can travel large distances to forage, and use sites occurs throughout their range such that any individual could access that landcover type. In these cases, we would have less confidence that the percent overlap equates to the proportion exposed, as individuals from outside of the overlap area are likely to enter the area to forage. However, we would still consider and acknowledge that these use sites only represent a certain fraction of their range.

Determining Percent of the Population Exposed – Aquatic Species

Aquatic-Specific Exposure Factors

Aquatic species are likely to be exposed to pesticides that are deposited in surface waters through runoff and drift transport pathways. Our analysis focuses on exposure from contact with contaminated surface water. While dietary exposure may also be a relevant route of exposure, response data to the dietary exposure route is generally not available for these species or related surrogates. In addition, concentrations of simazine are not expected to accumulate in prey or other food resources, and as such, we do not expect significant exposure through this route. As such, contact with surface water is expected to be the primary route of exposure for aquatic species and is likely to capture any effects that may occur from the dietary route. Consequently, exposure was only evaluated using surface water concentrations estimates derived by EPA in the BE.

Aquatic Habitats

Aquatic species depend upon a variety of aquatic habitats which vary in size, volume, flow, etc. To better estimate pesticide exposure in these different types of surface waters, EPA modeled different aquatic habitats. Aquatic exposures are quantitatively estimated for different generic habitat types (Table 16), using different EPA models to assess a combination of run-off and drift into different water bodies with different volumes and flows. Two of these water bodies are aquatic, and one of which is a semi-aquatic habitat (or aquatic-associated terrestrial habitat).

Aquatic exposures (surface water and benthic sediment pore water) were quantitatively estimated for representative simazine uses included in the master use summary document by HUC 2 Regions (Figure 10) and by aquatic habitat using the Pesticide Root Zone Model (PRZM5) coupled to the Variable Volume Water Model (VVWM)[24] in the Pesticides in Water Calculator (PWC). The master use summary document for simazine includes over 1,000 use/formulation/application-type combinations. In order to limit simulations to a manageable number, grouping of uses into general categories was performed where possible (see BE

[24] The exposure models can be found at: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment

Appendix 1-3). Within these use groups, as well as within non-grouped uses (*e.g.*, golf courses), formulations and application methods were selected that were expected to generate maximum Estimated Environmental Concentrations (EECs). Thus, use/formulation/application methods that had the highest application rates and/or lowest retreatment intervals were generally chosen. The maximum resulting EECs for each use/bin/HUC combination were then selected (only ground applications were modeled since simazine is only applied by ground) and are assumed to represent exposures for all uses in the category for the relevant aquatic habitat/HUC combination. Ornamental direct applications to trees and spot treatments were not simulated.

For simazine, when using PWC, the EPA has relied on two standard waterbodies which they have traditionally used to estimate EECs for the various aquatic habitats. The standard farm pond was used to develop EECs for the medium and large static waterbodies and for the medium and large flowing waterbodies using the PWC tool. For the smallest flowing and static waterbodies, EPA derived estimates from the Wetland Plant Exposure model (WPEZ) from the Plant Assessment tool (PAT) tool and AgDrift® models.

The Service identified the representative aquatic habitats utilized by each listed species. A single species may occur in a range of habitats represented by multiple aquatic habitats. Aquatic habitat characteristics were defined by the Service to facilitate the estimation of pesticides in surface water for comparison to relevant toxicity endpoints for listed species assigned to the appropriate habitat, based on habitat requirements. Table 16 summarizes the characteristics of each habitat type that were modeled by EPA. It should be noted that the same waterbody used in PWC may be used as a surrogate to represent multiple habitat types defined by the Service (e.g., the WPEZ model to represent both low flow and low volume waterbodies such as vernal pools or first order streams).

Table 16. EPA's standard models currently used to assess exposure to herbicides (adapted from Table 2 of EPA's Final Herbicide Strategy).

Environment	Exposure/Transport Pathway (Relevant Habitat)	Models or Assumptions
Terrestrial	Off-field spray-drift exposure	AgDrift®[25]
Terrestrial	Run-off and drift to terrestrial areas adjacent to treated areas	PAT[26] (TPEZ)

[25] AgDRIFT® version 2.1.1 available online at: https://www.epa.gov/pesticide-science-and-assessing-pesticiderisks/models-pesticide-risk-assessment#AgDrift

[26] PAT = Plant Assessment Tool version 2.8 available online at: https://www.epa.gov/endangeredspecies/provisional-models-and-tools-used-epas-pesticide-endangered-species-biological#pat

Environment	Exposure/Transport Pathway (Relevant Habitat)	Models or Assumptions
Wetland	Off-field spray-drift exposure	AgDrift®
Wetland	Run-off and drift to wetlands (includes vernal pools, non-riparian wetlands, and similar systems	PAT (WPEZ)
Aquatic	Run-off and drift to EPA Farm Pond or larger water body (includes riparian wetlands, medium/fast flowing waters, ponds, lakes, reservoirs	PWC[27]

Aquatic Exposure Modeling

EPA currently uses the PWC to calculate runoff/erosion herbicide concentrations. EPA uses standard PWC agricultural crop scenarios with weather information to assess runoff/erosion potential from vulnerable agricultural use sites. The PWC model generates high-end EECs associated with a particular pesticide, aquatic habitat, and use pattern within a specific geographic region. Each scenario is specific to an area where the use occurs (*i.e.*, where a crop is commonly grown). The EECs generated represent maximum annual concentrations that occur once every 10 years and consider the runoff/erosion and spray drift pathways of exposure. EPA considered the habitat requirements of currently listed plants, as well as any obligates, and identified which of EPA's standard model scenarios is most representative of the expected exposures for that species. In some cases, the standard model is a reasonably good fit for the habitat of the species in other cases, EPA expects that the model will overestimate exposures to the species' habitat (*e.g.,* the standard pond will likely have much higher exposures than rivers with larger volumes, dilution, and flow).

The U.S. Geological Survey (USGS) delineated watersheds in the United States based on surface hydrologic features classified by hydrologic unit. Each hydrologic unit is identified by a unique hydrologic unit code (HUC) consisting of two to twelve digits based on the level of classification in the hydrologic unit system (these levels range from region to subwatershed). The HUC-02 is the first level of classification and represents specific hydrologic regions distributed across 21 HUC-02 regions of the United States, eighteen of which are within the conterminous 48 states (CONUS; Figure 10). The EPA conducted surface water aquatic modeling with the HUC-2 regional Pesticide in Water Calculator (PWC) scenarios matching the registered uses. The PWC

[27] PWC = Pesticide in Water Calculator, available online at: https://www.epa.gov/pesticide-science-and-assessingpesticide-risks/models-pesticide-risk-assessment#PWC

uses soil, hydrology, land cover/land use, weather, and waterbody properties to simulate environmental conditions. This is described in more detail in the Exposure Section of the BE.

The EPA grouped non-commodity (*i.e.*, crops other than Corn and Citrus) crops based on agronomic practices to reduce the level of uncertainty in the spatial footprint for individual minor crops. The EPA selected a single 90th percentile scenario for each crop/group of crops within each hydroregion or subregion where the crop is present, based on CDL data, for a total of up to 21 scenarios to represent each group of crops on a national scale. Since pesticides with different soil organic carbon normalized sorption coefficient (K_{OC}) values will behave differently in the different scenarios and what is vulnerable for one set of chemicals may be different for another, EPA selected separate sets of 90th percentile scenarios to represent chemicals based on three ranges of K_{OC} values. The scenarios were developed, analyzed, and ranked using an automated methodology to identify the 90th percentile vulnerability scenario within each National Hydrography Dataset Hydroregion (NHDPlus HR)[28]

Beyond 305 meters for simazine, EPA assumes the runoff becomes concentrated (channelized) into rivulets, gullies, etc., which are represented by the wetland plant exposure zone (WPEZ). The WPEZ is intended to represent a non-target wetland or aquatic plant community that is exposed to pesticide via overland flow and spray drift is modeled using AgDrift®. The wetland can be immediately adjacent to the treated field or some unspecified distance away. The WPEZ is intended to represent any plant community that can exist in a saturated to flooded environment (e.g., a depression or shallow wetland that would collect and hold runoff from an upland area) and would receive all the runoff from an adjacent treated field. For modeling exposures to listed species in wetlands, the scenarios were modified to account for a 15 cm benthic depth to represent the typical active root zone for wetland species of forbs and woody plants.

[28] The NHDPlus HR is a national, geospatial model of the flow of water across the landscape and through the stream network. The NHDPlus HR is built using the National Hydrography Dataset High Resolution data at 1:24,000 scale or better, the 1/3 arc-second (10 meter ground spacing) 3D Elevation Program data, and the nationally complete Watershed Boundary Dataset (https://www.usgs.gov/core-science-systems/ngp/national-hydrography/nhdplus-high-resolution#WhatIsIt).

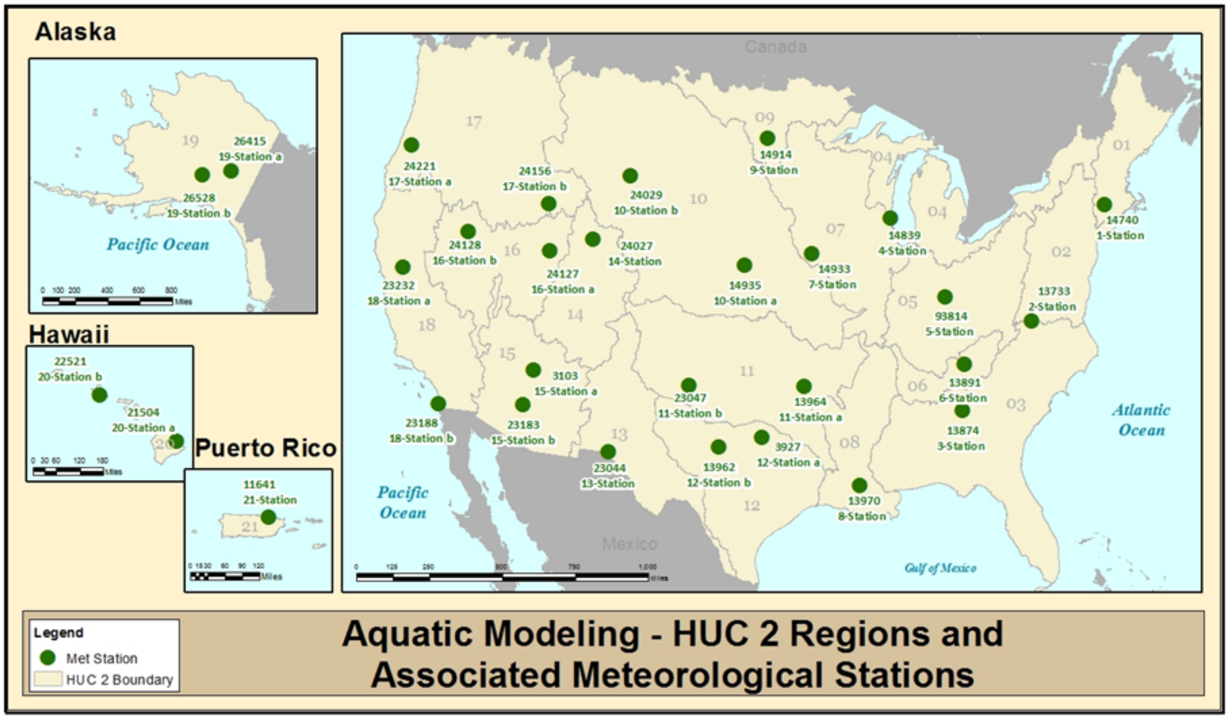

Figure 10. Hydrologic Unit Code (HUC) 2-digit Regions and Associated Metrological Data.

Estimated Environmental Concentrations (EECs) for Aquatic Habitats

We based exposure of aquatic species to simazine on the overlap of simazine use sites with the HUC12(s) that comprised their ranges. For the static-water habitats and the smallest flowing-water habitats within HUC12s, EECs are calculated for each overlapping UDL (e.g., Corn, Vegetables and Ground Fruit, Other Crops). EPA modeled each use as if the water body was immediately adjacent to the site (i.e., near field). The medium and large streams/rivers were modeled at the subwatershed/HUC12 scale (USEPA 2017). The EECs derived from the PWC modeling based on maximum labeled rates included in the master use summary document are summarized for the various aquatic habitats in Appendix 3-1 in the BE. The complete set of modeling inputs and results are available in Appendix 3-2 of the BE.

Proximity to Pesticide Use Sites

The likelihood that individuals will be exposed to simazine will be influenced by many factors including the proximity of populations to pesticide use sites. For our analysis, we consider that exposures may occur if pesticide use sites overlap with HUC12(s) that comprise the species range. For some species, there may be specific information regarding the location of populations within their range (i.e., occurrence in specific waterbodies or waterbody segments), and we considered this information qualitatively. Where this information was not available, we assumed the species would occur throughout its range (i.e., in all HUC12s), and individuals to be uniformly distributed within and between HUC12s. For species that occur in waterbodies of low flow and volume or large volume, under the uniform distribution assumption, we approximate the percentage of individuals in the population that are likely to be exposed by the percent overlap of pesticide use sites within the range. For species that occur in medium and large rivers we assume 100% of individuals in populations within HUC12s (where there is overlap with pesticide use sites) are assumed to be exposed because the exposures in these aquatic habitats were modeled at the subwatershed scale.

Mobility of Individuals

Some aquatic species, including many aquatic invertebrates and narrow endemic fish species, do not (or cannot) move large distances and are more likely to be exposed as a result of localized pesticide use. However, highly mobile or migratory species, such as anadromous fish (e.g., Atlantic salmon and Atlantic (Gulf) sturgeon), travel great distances and individuals could be exposed to pesticides from multiple use sites along the migratory corridor. Alternately, these species may be absent from any particular area at the time of pesticide use. For these reasons, the percentage of the population exposed may be lesser or greater than would be predicted based solely on overlap of use sites in individual HUC12s within the range depending on the presence of the species.

Aquatic Exposure Assessment

As mentioned above, we carried forward EECs generated for the BE into the Opinion. The method we use captures the variability in EECs derived by incorporating geographically specific estimates that are accounted for from two sources: (1) the occurrence of pesticide use sites within the species range (six-year data set), and (2) daily precipitation (30-year data set). In brief, this analysis was based on the 30-year annual maximum EECs from the 30-year annual time series (1-day time step) generated for each pesticide use/scenario/HUC2/aquatic habitat combination. The 1-in-10-year exposure concentrations are estimated using the daily time series of estimated concentrations from 30-year PRZM5/VVWM simulations. In this manner, the generation of the EECs is based on probabilistic methods. We incorporate a maximum from these scenario values to establish an upper bound for EECs within the aquatic habitat a species may be found in within its range.

Exposure Pathways for Cave Species

Listed cave-dwelling organisms consist of terrestrial invertebrate species (cave arachnids and beetles), crustaceans (cave amphipods), and fish. These species may be exposed to pesticides in water from over land flow or leaching from soil from agricultural practices over or near sinkholes, karst systems, or other porous features near the surface of cave habitats. The environmental fate, transport, and physicochemical properties of simazine are such that it is quite mobile in soil matrices and water, and persistent enough in the environment that it would be able to remain at levels toxic enough from run-off of fields after application and to impact cave species or contaminate their dietary items outside of caves. While the time scale of recharge of karst cave systems, or the process of aboveground water reaching the groundwater supply will often take several days to weeks to months, simazine is not known to degrade in that time frame (see *Exposure* section in this Opinion) and would be present in the water that enters the cave. Thus we discuss these dynamics below.

Simazine may enter the environment via spray drift as well as run-off onto soil, foliage, and/or water. Simazine is moderately soluble in water with a reported solubility range of 3.5 to 11 mg/L at 20-25ºC. Simazine is resistant to direct photodegradation in water ($t_{1/2}$ > 386 d; MRID 42503708) and to photodegradation on soil ($t_{1/2}$=132 d; MRID 42739101) which means it does not break down in water when exposed to light and thus will not degrade in the absence of light such as that it may be associated with cave systems. The hydrolysis half-life for simazine is 310 d in non-sterile groundwater at 20 °C and pH of 6.66 (Navarro, et al. 2004). The hydrolysis of triazines leads to formation of hydroxytriazine compounds. Thus simazine is persistent and does not appear to break down quickly in groundwater.

Simazine is moderately persistent to persistent in aerobic loam soils ($t_{1/2}$ ranges from 80 to 650 days) based on the (Goring, et al. 1975) classification. As previously discussed in the *Exposure* Section of this Opinion, soil sorption coefficient values for simazine correspond to soil organic carbon content being the primary determinant of simazine's mobility in soil. The average KFOC of 123.25 mL/gOC (n = 4; MRID 41442903) indicates a UN Food and Agriculture Organization (FAO) mobility classification of moderately mobile in soil (FAO, 2000).

Simazine has a half-life of 74 d in aerobic aquatic environments (MRID 43004501, 46561301, 43004502). Simazine is also persistent in anaerobic aquatic environments ($t_{1/2}$ > 1,766 d) and potentially persistent in anaerobic soil environments as no degradation occurred within a 51-day study duration (MRID 40614411, 00027857).

Based on monitoring data from the Water Quality Data Portal[29], we know that simazine has been detected in measured residues of both surface and ground water from data recorded from 1975-2020 (see Table 3-9 in the BE).While these data cannot provide a specific source of the simazine applied, they do provide evidence that simazine can be detected in both ground and surface waters at levels that would significantly impact listed species and their critical habitats in cave environments.

Karst systems are known to have enhanced porosity and permeability and are therefore susceptible to pesticide contamination that could be present in run-off water (Vesper, Loop and White 2000). Run-off water is likely to contain simazine from field applications, it is likely to reach karst systems from the surface waters and enter the subterranean habitats where many listed cave species reside (cave arachnids, cave crustaceans, and cave fish) because simazine is likely to persist (as mentioned above) after it has traveled from the surface and into karst cave reaches. Some karst system watersheds in specific areas of the country may provide enough overland flow to dilute simazine concentrations before they enter cave sinkholes or other porous features.

[29] http://www.waterqualitydata.us/

Simazine is predominantly present in the water column and bound to sediments thus exposure to sediment-dwelling organisms is likely to occur but to a lesser extent as compared to organisms in the water column. The low octanol/water partition coefficient (K_{ow} = 122) and low bioconcentration factors ($BCF_{steady\ state}$ = 4.92, $BCF_{kinetic}$ = 7.30) indicate a low potential to bioconcentrate or bioaccumulate in aquatic and terrestrial organisms. Thus, we do not anticipate simazine will accumulate in terrestrial organisms that may be external food sources for cave species.

Cave dwelling organisms may also feed on dietary items near the cave entrance. Many of the listed cave dwelling species rely on surface-derived nutrients that include leaf litter fallen or washed in, animal droppings, and animal carcasses. Several studies cite that nutrients in cave ecosystems are derived from exterior sources (Poulson and White 1969, Howarth 1983, Culver 1986, Howarth 1983) particularly from organic material washed in or brought in by animals. Bats are usually the major source of these nutrients, as well as the major source of contaminants (Kunz 1982). Pesticides can be introduced into caves by bats from their exposed carcasses that decay in caves or from bats defecating in caves (McFarland 1998, Sandel 1999, Land 2001, Eidels, Whitaker and Sparks 2007). Bats within a population/colony may consume pesticide-exposed insects while foraging in or near use areas and guano accumulated from multiple bats within the cave will reflect that exposure. However, we do not anticipate that cave-dwelling organisms that forage on guano will be exposed to simazine as it does not bioconcentrate or bioaccumulate

However, we feel the Herbicide Strategy mitigations for most agricultural uses of simazine are sufficient such that most aquatic and all terrestrial cave species (cave fish, cave crustaceans, cave beetles and cave arachnids will not be impacted by run-off, spray drift, or leaching through porous substrate, such as karst. In addition, estimated environmental concentrations are not likely to reach levels where we would observe mortality or sub-lethal effects to aquatic or terrestrial cave species from exposure from non-agricultural uses of simazine. However, some agricultural uses of simazine may still impact aquatic cave species. We reviewed the data and the proximity of these species habitats to these use sites on a species by species basis in the Integration and Synthesis summary Appendices for the relevant taxonomic groups.

In summary, we do not anticipate that direct application from run-off, spray drift, or leaching through porous karst systems from most agricultural uses of simazine would be likely pathways for terrestrial cave species when they are in subterranean habitats based on the Herbicide Strategy mitigations and label measures. Nor do we anticipate terrestrial or aquatic cave species would be exposed to simazine from contaminated food sources entering the cave. However, there is a possibility that aquatic cave species could be exposed to simazine run-off from some agricultural uses and we review this route of exposure for these species on a case by case basis in the Integration and Synthesis Summaries for these taxonomic groups.

Usage Analysis

The overlap information above describes the footprint of the simazine use based on the product label and any off-site transport. We apply usage data to describe how the pesticide has been applied in the past to use sites based on available data sources. The key difference between use and usage is that use data extends to all simazine uses authorized by EPA, whereas usage refers to how simazine has been actually applied on the landscape. To determine effects to listed species, we employ usage data to refine the scope of analysis from any area where simazine is authorized to be applied, to those areas where simazine applications are reasonably certain to occur. While we recognize that past usage data may not fully predict future usage, we believe this information better informs where we would expect usage to occur in the future and provides more context for our assumptions related to uncertainty.

As part of its BE and supplemental submissions, EPA provided the following usage information:

- National and State Use and Usage Summary for Simazine
- USDA Census of Agriculture (CoA) data for CONUS species
- California Department of Pesticide Regulation Pesticide Use Reporting data

We briefly describe each data source and how it was applied in our analysis below.

EPA's Simazine National and State Summary Use and Usage Matrix (SUUM; Appendix 1-4 in the BE)

Data provided in EPA's Use and Usage Summary for agricultural crops are obtained by EPA from USDA, the state of California, and a commercial source (Kynetec), as described in more detail in the BE. Analysis of this data by EPA indicate that simazine usage peaked in the early 2000's in terms of both pounds applied and total acres treated but has shown a strong trend toward decreased usage over the past decade. During the most recent five years of available survey data (2013-2017), an annual average of 3,000,000 pounds of simazine were applied to an average of 2,600,000 acres of agricultural crops (see Table 1 in Appendix 1-4 in the BE). Most of the data provided for states outside of California describing past agricultural usage of simazine are from the proprietary source Kynetec. According to materials provided by the company, Kynetec data is "designed to address market questions asked most often by senior executives, and those involved in product development, sales, and marketing." Surveys are designed to reach a particular percentage of the total crop grown at a national level, though statistics are reported at the state and Crop Reporting District (CRD) level when sample size is adequate. The data provided to the Service is lacking the statistical foundation to understand the robustness at the state level or any geographic specificity at the sub-state level. Neither EPA nor Kynetec was able to provide us with this information (e.g., how many applicators responded to the survey, how many acres are represented by the survey at the state level), nor any standards used to determine an adequate sample size at these levels, nor the minimum threshold required for reporting these values. Our understanding is that this varied on a case-by-case basis, according to the surveyor, crop, and state. The Kynetec data are provided at the state level and indicate how many acres of a crop has been treated with simazine over a 5-year period (2013 – 2017). Acres that are reported as "treated" are compared to the total number of acres grown for each crop at the state level, to produce a "percent crop treated (PCT)" value. EPA provided the Service with PCT values at the national and state level (mean, minimum, and maximum) over a 5-year period. The data are not comprehensive of all crops for which simazine is registered, and do not address every state in which surveyed crops are grown. In addition, with no indication of the robustness of the agricultural data provided by EPA at the state level, there is particularly high uncertainty associated with this dataset and we are unable to evaluate how representative these data are of past usage in these states. However, in a previous analysis of usage data, we did not find other data sources that would broadly inform our understanding of agricultural usage of pesticides on a nationwide scale (USFWS 2022). As such, we consider these data as our primary source of agricultural usage data for all CONUS states except for California, as described further below. We employed the conclusions from our 2022 analysis to inform our application of these data to our analysis of simazine usage in these states. In short, our analysis of various data usage sources led us to adopt a conservative approach when applying this survey data to better ensure that we capture the extent of usage occurring within states. Specifically, we consider the percent of a species' range treated with simazine using EPA's "upper maximum" scenario, which compares the total number of acres treated within a state to the total number of acres in the range of the species (see BE Appendix 1-7). In addition to using the maximum yearly usage across 5 years, this method assumes a 2.5% PCT for crops that were surveyed and no usage was reported to buffer against the uncertainty associated with these surveys and low usage estimates.

USDA's Census of Agriculture

USDA's Census of Agriculture (CoA) data is a complete count of United States farms and ranches that includes any plot of land, whether rural or urban, if $1,000 or more of agricultural products were produced and sold, or normally would have been sold, during the census year. The Census of Agriculture is conducted once every five years, looks at land use and ownership, producer characteristics, production practices, income, and expenditures. Response to Census of Agriculture is required by federal law and is therefore considering mandatory reporting data. As part of the data requested from operators, respondents report the number of acres treated with pesticides that year. In summarizing the data collected, USDA analyzes and reports results at the national, state, and county level. In its analysis of CONUS species, EPA used the 2017 CoA data to estimate the number of acres treated with herbicides within counties that overlapped with the ranges of listed species, and then compared that with the total number of acres in the species' range. EPA did not provide estimates of the percent of the range treated for every CONUS species, rather they reported when the number of total acres treated within the range of the species was <5% of its range. As this percentage reflects usage of all herbicides, and not just simazine, we consider this as an additional line of evidence, when appropriate, as an upper bound for simazine usage.

California Department of Pesticide Regulation Pesticide Use Reporting (CalPUR)

In California, annual reporting of pesticide usage is required for all agricultural and certain non-agricultural uses. California Department of Pesticide Regulation maintains a highly robust dataset of Pesticide Use Reporting (CalPUR). For the purposes of reporting, agriculture is broadly defined, and includes usage on parks, golf courses, cemeteries, rangeland, pastures, and along roadside and railroad rights-of-way. Unlicensed, non-professional, residential pesticide applications around a home or garden are not required to be reported, though licensed professional pesticide applications in or around the immediate environment of a household are reported as non-agricultural use (usually “structural pest control” or “landscape maintenance”). Agriculture pesticide usage is reported at the section level (640 acres or per square mile) and non-agricultural usage is reported at the county level. Information is publicly available and can be downloaded from their website[30]. Because of the robust nature of this data set, we exclusively apply CalPUR data to estimate agricultural usage for species wholly within California, based on information provided by EPA (U.S. EPA 2025, U.S. EPA 2025a). For these species, EPA used a three-tiered approach to characterize potential exposure of Service listed species to simazine, calculating the extent that each species’ range overlapped with any pesticide usage, any insecticide usage, or simazine only usage for the years 2013 - 2022. We used the maximum yearly value to estimate future usage for these species, as described further in (U.S. EPA 2025, U.S. EPA 2025a). In general, we considered the simazine-only overlap in our species-specific analyses. This value represents high maximum single year overlap of treated areas with the species’ range over the 10-year period. In general, these values represented a large sample size (i.e., many pesticide reporters within the range of the species being assessed), and are expected to be robust against small changes in pesticide use patterns. However, in instances where the sample size is very small (i.e., few pesticide reporters within the range), this value will have greater uncertainty and we may consider one of the higher tier values reported by EPA, such as usage of all herbicides with the range. This value is considered a more protective estimate as it is likely to account for the possibility that users may switch their herbicide choice to simazine within the time frame of the registration review. The overlap metric that considers any pesticide usage within the range is the most conservative value provided by EPA and will likely overestimate simazine usage as it includes usage from other pesticide classes such as insecticides and fungicides.

Non-agricultural usage

Simazine is registered for non-agricultural use on lawns, golf courses, and woody ornamental trees and plants. EPA’s BE provides usage data indicating a large number of acres of lawns and golf courses have been treated with simazine in the past. However, this data is from 2014[5] and more current information on triazine usage in these non-agricultural areas indicate that the lawns and golf course usage data from the BE is out of date and not reflective of current simazine usage in these areas. More current information provided by EPA and private stakeholders on simazine usage on turf grass species allowed us to significantly refine the area in which we expect listed species to be exposed. Thus limiting those areas to geographic zones in which warm-season grasses occur, and then further refining the extent and manner of usage within these areas.

[30] http://www.cdpr.ca.gov/docs/pur/purmain.htm

Depending on region, cool-season, warm-season, or a combination of turf grass species are managed on golf courses and lawns. Cool-season grasses grow best in cooler conditions, and warm-season grasses thrive in hot, dry weather (USDA, 2004); there is a transition zone across the U.S. where either category of turf grasses may be planted based on microclimate conditions. Exposure to simazine will kill cool-season grasses, and as such, simazine usage for weeds in turf dominated by cool-season grasses is not expected. However, warm-season grasses can tolerate exposure to simazine. As such, EPA estimated where in the United States only cool-season grasses are exclusively used in turf based on the U.S. Department of Agriculture's plant hardiness zone map as simazine use is not expected in these areas (USDA, 2023). Because hardiness zones will change over time with environmental conditions, EPA created a static map based on the hardiness zones where they expect warm- and cool-season grasses are grown based on the most recent data mapped (i.e., 1991-2020). EPA determined zones 1a-6a represent cool-season grasses (i.e., white areas) and zones 6b-13b may include warm-season grasses (i.e., black areas; Figure 11). We expect the cool- and warm-season grass assessment to apply to all turf, including residential, commercial, and golf course turf. We refer to EPA's cool-season map in species assessments where relevant, particularly if a species occurs exclusively in the cool-season zone where we expect simazine will not be used on turf and no exposure will occur from this use.

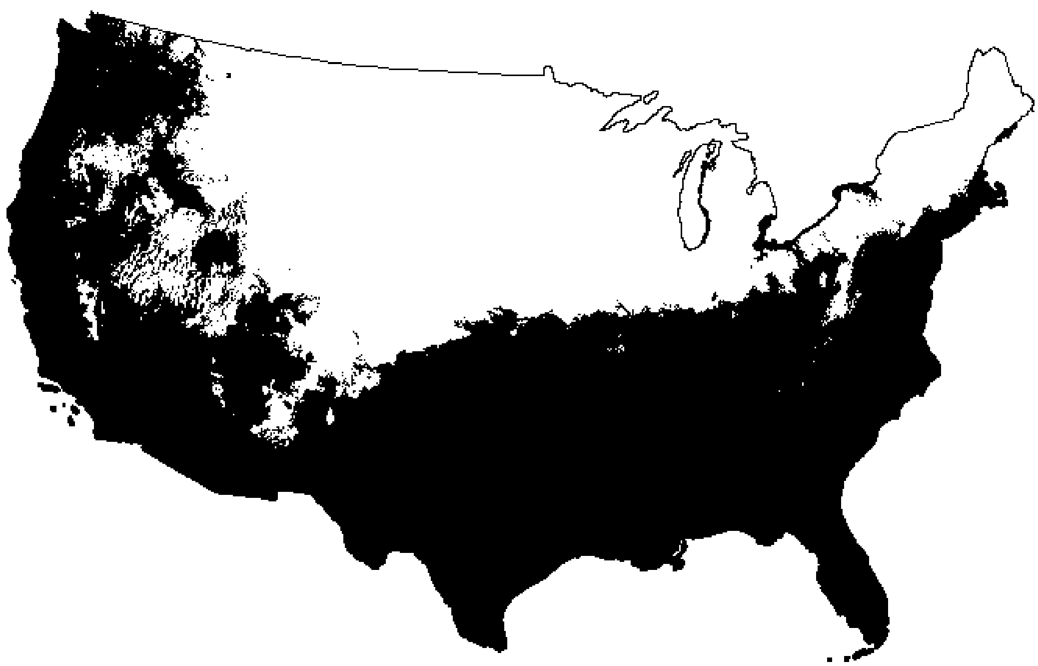

Figure 11. Map showing where cool-season grasses (white areas) and warm-season grasses (black areas) are used on turf across the continental United States..

Particularly for residential and commercial turf uses, qualitative usage information obtained by EPA from the National Association of Landscape Professionals (NALP) indicate that simazine is no longer commonly used on residential or commercial turf as potential consequences to turf areas related to timing of application has led to preferential use of other herbicides that can be applied more broadly. If simazine were used on residential or commercial turf, it would be applied during the fall and spring as a pre-emergent. In addition, commercial and residential applicators typically apply herbicides with hand-held equipment that release course droplets, limiting the potential for spray drift such that exposure to species off treated turf would be unlikely.

Particularly for golf course turf uses, we obtained qualitative usage information directly from the Golf Course Superintendents Association of America (GCSAA) and an academic turf scientist that indicate that simazine is used to control winter annual broadleaf and annual bluegrass weeds on golf courses. They are applied as a pre-emergent in early fall and early winter to fairways and roughs, which make up approximately 30% of a golf course's acreage. Simazine is not applied to tee boxes or greens, which make up an additional 6% of golf course acreage. Most applications are made at rates lower than what is on the label (i.e., 1-1.5 lbs a.i./acre). These applications are made only once or twice a year, 45-60 days apart. In general, golf courses typically apply herbicides using dedicated ground equipment with a low boom height (as per the label), and golf course superintendents make use of several tools to monitor soil moisture before any applications are made to help ensure turf and soil conditions do not lead to off-target movement of herbicides. In addition, riparian buffer zones are often used on golf courses between all water features to reduce off target movement (Golf Course Superintendents Association of America [GCSAA], pers. comm., 2025). The no-till methodology and continuous cover of a turf grass area inherent in managing golf course turf are equivalent to additional runoff mitigations (i.e., equivalent to six points on EPA's mitigation menu), and we considered them in our assessment.

Federal Lands

Federal lands cover about 640 million acres, which equates to 28% of land in the United States. Of these federal lands, 65% are managed by DOI agencies, 30% by the U.S. Forest Service, 2% by the Department of Defense, and 3% by other federal agencies (Congressional Research Service 2020). DOI land management agencies (the Service, National Park Service, and Bureau of Land Management) and the U.S. Forest Service each employ designated pesticide coordinators, provide policy and direction on pesticide use, have a process in place to review and approve pesticide use proposals, and maintain reports on usage. Similarly, the Armed Forces Pest Management Board (AFPMB) recommends policy, provides guidance, and coordinates the exchange of information on all matters related to pest management throughout the Department of Defense (AFPMB 2020).

We expect pesticide use on federal lands for a variety of reasons, including invasives control and the protection of human health. Simazine is registered for use on both agricultural crops and non-agricultural uses. While we recognize that some federally managed lands may contain agriculture, we expect these areas to account for a small percentage of these areas and that simazine usage will be limited. For instance, cooperative agriculture is a long-standing practice on National Wildlife Refuges (NWRs) in which the Service partners with farmers to meet wildlife management objectives. A search of the Service's Pesticide Use Proposal System

(PUPS) database indicated that simazine usage or subsequent applications of simazine have occurred between the years 2013-2024 (Table 17). Table 17 below indicates the year of usage of simazine, the specific simazine product and the number of acres treated. Several years had several NWRs where simazine was applied thus totals for that year are indicated. The different NWR locations where applications were made are also included, showing that simazine usage was limited to just four NWRs during this time period.

Table 17. Pesticide Use Proposal System Query for Simazine Use on National Wildlife Refuges from 2013 – 2024.

Field Station	Year	Trade Name	Trade Name lbs Applied (total for the year)	Acres Treated (total for the year)
PEE DEE NATIONAL WILDLIFE REFUGE THEODORE ROOSEVELT NATIONAL WILDLIFE REFUGE	2013	Princep 4L	340.3	600
HOLLA BEND NATIONAL WILDLIFE REFUGE PEE DEE NATIONAL WILDLIFE REFUGE	2014	Princep 4L	180	363
PEE DEE NATIONAL WILDLIFE REFUGE THEODORE ROOSEVELT NATIONAL WILDLIFE REFUGE COMPLEX	2015	Princep 4L	1,632	1469
PEE DEE NATIONAL WILDLIFE REFUGE THEODORE ROOSEVELT NATIONAL WILDLIFE REFUGE COMPLEX	2016	Princep 4L	1512	1296
HOLLA BEND NATIONAL WILDLIFE REFUGE PEE DEE NATIONAL WILDLIFE REFUGE THEODORE ROOSEVELT NATIONAL WILDLIFE REFUGE COMPLEX	2017	Princep 4L	1000	2392
HOLLA BEND NATIONAL WILDLIFE REFUGE PEE DEE NATIONAL WILDLIFE REFUGE THEODORE ROOSEVELT NATIONAL WILDLIFE REFUGE COMPLEX	2018	Princep4L	2582	1010
HOLLA BEND NATIONAL WILDLIFE REFUGE PEE DEE NATIONAL WILDLIFE REFUGE THEODORE ROOSEVELT NATIONAL WILDLIFE REFUGE COMPLEX	2019	Princep 4L	932	645
HOLLA BEND NATIONAL WILDLIFE REFUGE PEE DEE NATIONAL WILDLIFE REFUGE THEODORE ROOSEVELT NATIONAL WILDLIFE REFUGE COMPLEX	2020	Princep 4L	1759	535
PEE DEE NATIONAL WILDLIFE REFUGE	2021	Princep 4L	205	280
CRAB ORCHARD NATIONAL WILDLIFE REFUGE PEE DEE NATIONAL WILDLIFE REFUGE THEODORE ROOSEVELT NATIONAL WILDLIFE REFUGE COMPLEX	2022	Princep 4L	1490	1371.3
CRAB ORCHARD NATIONAL WILDLIFE REFUGE THEODORE ROOSEVELT NATIONAL WILDLIFE REFUGE COMPLEX	2023	Princep 4L	1488	1384
CRAB ORCHARD NATIONAL WILDLIFE REFUGE THEODORE ROOSEVELT NATIONAL WILDLIFE REFUGE COMPLEX	2024	Princep 4L, Simazine 4L Flowable	1403.4	574

We anticipate simazine usage on NWRs and other federal lands will continue to be low, as we do not have any information suggesting that future usage is expected to increase. Where information suggests that agriculture or managed turf, such as golf courses, may be present in federally managed lands within a species range, we consider this on a case-by-case basis. Where no information indicates that these simazine use sites represent a significant influence in these areas, we assume that use of simazine on federal lands will be low over the duration of the proposed action, and only occur in very localized areas as needed.

CUMULATIVE EFFECTS

Cumulative effects are defined in ESA section 7 implementing regulations as "those effects of future State or private activities, not involving federal activities, which are reasonably certain to occur within the action area of the federal action subject to consultation." (50 CFR 402.02). Cumulative effects are considered broadly in this Opinion, due to the national scope of the action. More refined species-specific information on cumulative effects is also found in the species accounts of the Integration and Synthesis summaries in Appendix K of this Opinion. Future federal actions that are unrelated to the proposed action are not considered in this section because they require separate consultation pursuant to section 7 of the ESA.

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

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

All manner of development and competing use projects and activities (as above) are likely to continue in many areas, resulting in clearing, addition of impervious surfaces, and introductions of non-native species. Similarly, the incremental effects of climate change from such activities are anticipated to continue and intensify over the course of the proposed action. Some examples of such effects include, but are not limited to, more extensive and severe droughts that reduce the extent or quality of aquatic habitats, more extensive and severe wildfires that impact habitat more intensely, alterations of local temperature regimes that alter vegetation and water availability and composition. These activities are expected to result in various impacts to water quality (degradation, as with increased pollutants), habitat quality (loss or degradation), and other negative effects to listed species and their critical habitats. In some cases, increased pesticide use, including those in addition to simazine, may occur to address new or emerging pest pressure (e.g., weeds and other invasive plants) in agricultural and non-agricultural settings. We anticipate some use of pesticides, including those in addition to simazine, may be used directly or indirectly to benefit listed species or their critical habitats. For example, future pesticide use is anticipated to eliminate or reduce competing or invasive species within a species' habitat. While we are not aware of any such proposed projects at this time that would use simazine to specifically benefit listed species, we anticipate that simazine or other pesticides may be used in the action area for this purpose over the life of the proposed action. Where implemented with appropriate avoidance and minimization measures to reduce the potential for lethal, sub-lethal, and indirect effects to listed species and their critical habitats, such projects could improve habitat conditions, thereby benefitting the species. However, in the absence of specific information for such activities, or for sufficient avoidance and minimization measures for other pesticides, we anticipate listed species will continue to be impacted as described previously in the *Environmental Baseline* section of this Opinion.

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

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

INTEGRATION AND SYNTHESIS

In this section of the Opinion, we consider whether the proposed action is likely to jeopardize any of the proposed or listed species considered in this consultation. We also consider whether the proposed action is likely to destroy or adversely modify designated and proposed critical habitat. In the *Integration and Synthesis* section, we consider the effects of the proposed action in the context of the status of the species and critical habitats (as appropriate), the environmental baseline, and cumulative effects. The first section below is a review of the overall considerations for the Opinion. The next section provides a brief summary of the *Environmental Baseline*, *Status of the Species and Critical Habitat*, *Cumulative Effects* (together "Background Information"), and *Effects of the Action* sections. The final sections provide an overview of our approach to the integration and synthesis along with determinations and rationales for our Opinion for each plant and animal species and critical habitat, presented by taxa group and habitat group, and further discussed in Appendix C (for each species) and Appendix D (for each critical habitat designation), as applicable.

Overall Considerations for the Opinion

The proposed action is the registration review of simazine, which authorizes all the uses of the pesticide per the products labels. None of the registered formulated products are restricted use products, meaning these products may be purchased and used by anyone; no training or licensing is required. As the proposed action is the approval of labels containing the active ingredient simazine, once approved, these labels become the law and are legally enforceable. The proposed registration review of the pesticide authorizes use of the pesticide on any of the crops or land categories described previously, with labels specifying one or more uses, associated restrictions, and guidance for that use. Proposed registration review labels have guidance that generally use terminology that is considered advisory or recommended, and these proposed registration review labels do not serve as enforceable restrictions until the technical registrants have committed to implementing them. Some labels may also include recommendations for tank mixtures. Tank mix recommendations may specify other ingredients that can be added to increase efficacy, such as surfactants, emulsifiers, oil, or salts, or may include another product with a different active ingredient. Listed species (as well as other species and habitats on which they depend) and their critical habitats exposed to pesticide mixtures may be at greater risk of adverse effects than when exposed to single pesticides, as described in the *Effects of the Action* section of this Opinion.

Agricultural Uses

We and EPA are aware that there are often general trends and patterns related to agriculture, throughout the action area. We understand the most recent available land use data is a reasonably good indicator of present land use or land uses over the next few years or decades. While this information may suggest where pesticides such as simazine may be applied in the future, we also recognize that land uses and pesticide usage may change over time due to a variety of often unforeseeable factors, such as future market forces, pest pressures, individual grower preferences and decisions, development and other land use changes, as well as changes in environmental conditions such as drought, floods, and maximum/minimum seasonal temperatures (e.g., unanticipated heat waves or freeze/frost events). We have incorporated these considerations by using a refined overlap analysis that considers use sites (by land use type) with labeled uses specific to simazine, and by calculating estimates of anticipated simazine usage, as described previously in the General Effects section of this Opinion. We find pesticide usage datasets are collected for very different purposes than addressing the limits of overlap of simazine usage and listed species and their critical habitats in the action area. However, we were able to use this information, with its inherent uncertainties and our assumptions, to better identify simazine use sites and gauge anticipated usage that is reasonably certain to occur for all use categories throughout the action area over the 15-year duration of the proposed registration of simazine. We anticipate this information is also likely to have some value in determining appropriate avoidance and minimization measures in localized areas where adverse effects to listed species would be anticipated.

We recognize that growers will ultimately choose when and where crops and other commodities will be grown, and that growers, various local jurisdictions, and other property owners will likely determine where pesticide applications are needed. The label language, as currently written, is thus likely considered an asset for stakeholders to allow for greatest flexibility of use. However, we do not anticipate that simazine will be used in all the areas it is authorized to be applied under the label over the duration of the proposed action. As we must also consider what effects are reasonably certain to occur, we considered the best available scientific and commercial data for usage to better predict the consequences from the proposed action.

For some uses, overlap of pesticide use sites with species ranges is extremely low (i.e., <1%). When considered in context, however, we emphasize that even where the overlap is extremely low, the very small degree of overlap may nonetheless lead to adverse effects to the species, and if usage occurs in an area that is an important site for the species it may even have a disproportionate adverse effect on the species. For example, certain areas may support important foraging, migrating, overwintering, or breeding habitat for a species. Where such habitat may be limited or of lower quality elsewhere within the range, pesticide applications in this area where the species is congregating or is otherwise dependent on could lead to species-level effects. Alternatively, the area of overlap may be an area that is rarely used by the species in its range, either at all or during the time in which applications would occur. Thus, where overlap with species ranges and critical habitat appeared extremely low, we would still consider the value of that area to the species or critical habitat using geospatial data and species information. It was only when we had information that indicated there was no true overlap that these areas were not considered further in our analyses, based on a closer look at the geospatial data and species information. However, for many species, our analysis included an assessment of small areas of overlap with simazine use when we could not refine and/or exclude these areas based on additional information. These small overlaps were still part of the analysis because no additional information was available to exclude them, and exposure in these areas is still a concern for a species. Such an approach is appropriate when even extremely low levels of overlap may still be of concern for species.

Non-Agricultural Uses

Simazine has several registered non-agricultural uses, including use sites within developed, open space developed, and nurseries UDLs, such as residential buildings, lawns, golf courses, turf, and woody ornamental trees and plants. Given the expansive presence of human development across the national landscape, we anticipate most listed species' ranges contain at least some developed or open space developed use sites. However, UDLs for non-agricultural uses tend to be less defined than those for agricultural UDLs and may not accurately represent the actual footprint of these use sites on the landscape. As such, we assess exposure of species to non-agricultural uses of simazine in a qualitative manner by considering the life history of species, methods of application, available past usage data, and any existing conservation measures to reduce drift and runoff or otherwise limit exposure to species. When available, we incorporate additional past usage data for specific use patterns to further contextualize the level of usage we expect is reasonably certain to occur over the duration of the proposed action. For most species in this Opinion, we anticipate that non-agricultural uses will not meaningfully add to the overall level of anticipated exposure considered in our analysis of agricultural uses. Due to runoff and spray drift considerations described above, off-site exposure is not expected to result in effects to most species in this Opinion. In addition, we expect most listed species' habitat requirements precludes them from occupying non-agricultural use sites where simazine may be used. For species whose habitat is known or presumed to occur in non-agricultural use sites of simazine, we consider, individually and qualitatively, the extent and manner of non-agricultural simazine usage within the species' range to generally determine whether a small, moderate, or large number of individuals are likely to be exposed and the expected level of adverse effects from non-agricultural exposure of simazine.

We assess each listed species' life history traits, known locations, habitat preferences, and known behaviors to determine whether a species is likely to occur in or near developed, open space developed, or nursery use sites. In cases where a listed species is likely to occur in these use sites, we qualitatively assess the level of anticipated exposure based on available information on the species and possible usage trends in the areas relevant to the species.

Overview of Integration and Synthesis Analyses

We considered the consequences to proposed and listed species from the proposed action in the context of the species background information (i.e., *Status of the Species*, *Environmental Baseline*, *Cumulative Effects*, and when applicable, *Designated Critical Habitat*). Species were evaluated individually and are presented as individual Integration or Synthesis rationale narratives or by groupings of species with very similar rationales. While we recognize the species in this Opinion have variable life histories, distributions, recovery needs, and responses to the proposed action, as we reviewed the background information about the species and the anticipated consequences of the proposed action, we observed patterns in both species considerations (e.g., life history traits, habitat preferences, feeding behaviors, etc.) and pesticide exposure that helped us sub-group terrestrial and aquatic species for the initial stages of our analysis. Additionally, where relevant taxonomic groupings (e.g., terrestrial vs. aquatic snails, families of mussels, sea turtles or marine mammals) or habitat groups (e.g., cave systems) exist, we considered them simultaneously in the integration and synthesis analysis to streamline our discussion by avoiding repeating our findings when species are affected similarly.

As part of EPA's Herbicide Strategy, species were grouped based on whether general label measures (including a 15-foot spray drift buffer plus three runoff points) or additional conservation measures were required to reduce off-site transport. For spray drift, we found the 15-foot buffer on the general label to adequately reduce off-site concentrations to a level where we expect only low levels of effects to all listed species. For runoff, additional mitigation points were required for species occurring in non-flowing wetlands, or for those in proximity to certain simazine uses.

Species-specific information (e.g., environmental baseline, cumulative effects, status of the species, exposure, and toxicity) was considered for all species, including those species in the grouped analyses, and are presented in full in Appendices B and E. We discuss this approach in greater detail in the *Species Grouping* subsection further below in this Opinion.

The rationale for our conference opinion[31] for proposed species and proposed critical habitat designations are included in this section and its appendices. We applied the same approach used for listed species and designated critical habitats for proposed species and proposed critical habitats. Similarly, proposed critical habitat designations were considered in the same manner as designated critical habitat. We integrate and summarize our analysis and conference opinion together with listed species in the following subsections.

Summary of Status of the Species and Critical Habitat, Environmental Baseline, Cumulative Effects, and Effects of the Action

In the *Status of the Species* and *Critical Habitat*, *Environmental Baseline*, and *Cumulative Effects* sections of the Opinion, we established the effects of past and ongoing activities in the overall action area would maintain the existing degraded habitat conditions that are prevalent, although restoration activities and other conservation efforts may address some of the habitat conditions for some of the species, at least in part. We considered the status of the species and critical habitat through species- and critical habitat-specific accounts (i.e., detailed in Appendix C). The *Environmental Baseline* and *Cumulative Effects* sections in the body of this Opinion were broadly summarized in the generalized overview of the effects of previous and present and future ongoing activities in the action area for the proposed action. Species-specific environmental baseline and cumulative effects considerations are included for species and

[31] Our assessments and conference opinions for all species and critical habitat included as proposed in EPA's BE are included in this Opinion or in the concurrence section and Appendix A of the Opinion, except species that were ultimately not listed, species that were delisted, and proposed critical habitats that were not designated (see Appendix D). For species that have been listed or critical habitat that has been designated since the final BE was submitted, the listing status has been updated in this Opinion.

habitat groups in their respective integration and syntheses summaries for each taxa group (Appendix C) and in the *Status of the Species* and *Critical Habitat* (Appendix B).

Numerous activities across the landscape have impacted the habitats and ecological communities on which listed species depend. A variety of land uses associated with human activities, such as agriculture and grazing, residential and commercial development, and forestry, have altered habitat over the long-term. Changes in land use such as development, land clearing, diking, and other activities have affected terrestrial and aquatic habitats. Water diversions and storage, replacement of pervious soils and surface with impervious materials, impacts to riparian buffers, loss of wetlands, stream channelization, and other activities have affected the water quality and quantity for many aquatic habitats. Discharges and runoff from many land uses also result in the degradation of water quality due to contaminants, such as excess nutrients, fertilizers, pesticides, and other chemicals. Numerous pesticides have been detected in various waterbodies throughout the country. In many habitats, pesticides and other pollutants are present in the environment at detectable levels, although these levels cannot generally be tied to specific application events or all of the sources that may be contributing to accumulative concentrations. Additionally, as noted in the *Effects of the Action* section, monitoring data from state and federal agencies described in the BE and other sources have indicated that multiple pesticides often co-occur in aquatic habitats located throughout the action area.

It is reasonable to assume that as some ecological communities are affected by extreme stresses or changing conditions over the short- or long-term future, pest pressures may increase. As discussed earlier with forests, activities such as timber harvest, grazing, fire suppression, road construction, and management practices, together with other influences (e.g., introduction of invasive species, climatic conditions) have resulted in increases in disease and pests. Although pests and disease have always been present in habitats, an increase in both native species viewed as pests, as well as introduced non-native pest species, may be of increasing concern in the future. Some pest species may impact various agricultural and non-agricultural actions related to the use categories, resulting in the use of various pesticides in the future that are not considered part of the action. We also recognize pesticides may, in some cases, also be used to benefit listed species or their critical habitats by reducing or eliminating competing, predatory, or otherwise harmful species as part of a suite of activities to enhance or restore species habitat and support survival and recovery of the species.

Stressors that have influenced the environmental baseline and/or continue into the future as cumulative effects may often combine to result in an increased threat to sensitive species, where a single threat may have been less of a concern to a given species, its food base, habitat or other species (such as pollinators or hosts) on which it relies. The introduction of invasive species, together with other stressors, such as habitat impacts, pollution, harvest, and many other threats, is a major factor associated with species endangerment and loss of biodiversity across the action area. Combined with more frequent extreme weather events and other stressors on the landscape, including but not limited to increased frequency of drought or precipitation events, damaging storms, more or less frequent fire regimes, these stressors often exacerbate conditions that threaten a species' ability to persist. In coastal areas, sea level rise and ocean acidification are also expected to impact persistence of sensitive species that live in littoral, estuarine, or marine habitats.

In summary, we expect that numerous activities and resultant effects have occurred over the years and will continue into the future, and in many cases, will further degrade habitat conditions. We anticipate that, in some areas, restoration and recovery actions have and will continue to be undertaken to benefit listed resources to reduce adverse impacts from these activities but are not necessarily anticipated to completely mitigate these impacts.

Recovery Considerations

We also generally considered threats and factors associated with the needs of listed species in order to support their potential for recovery in addition to their continued survival in our analysis. Recovery is achieved when the status of a listed species is improved to the point at which protection of the ESA is no longer needed based on the criteria in section 4(a)(1) of the ESA. When determining whether an action will likely jeopardize the continued existence of a listed species, we evaluate whether the action is reasonably expected, directly or indirectly, to reduce appreciably the likelihood of both the survival and recovery of a listed species in the wild by reducing the reproduction, numbers, or distribution of that species, in accordance with the ESA section 7 implementing regulations at 50 C.F.R. 402.02.

We reviewed the available recovery plans, 5-Year Reviews, and other Service information for each species to gather information about the status of the species, habitat areas and environmental elements essential for species' survival and recovery, as well as threats to the species and actions needed for recovery. The recovery goals, objectives, and reclassification and delisting criteria identified in recovery plans were reviewed to help us understand and assess threats to each species and also to understand the effects of the proposed action on the recovery potential for the species. Reclassification and delisting actions result from successful recovery efforts. Achieving recovery so that species can be delisted is the ultimate goal of the ESA. Information related to the species' recovery is included in the Status of the Species and Critical Habitat (Appendix B).

Approach to the Effects Analysis

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

1. Mortality to portions of the population(s) of a listed species from direct, acute exposure from the use of simazine according to registered labels;

2. Altered growth among portions of the population(s) (potential for decreased survival and/or reproduction) from the use of simazine according to registered labels;

3. Reduced or impaired reproduction among portions of the population(s) from the use of simazine according to registered labels, and

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

To assess each response, we determined what percentage of the individuals were anticipated to be exposed to simazine at concentrations that may cause adverse effects, and when possible, the expected magnitude of those effects. To determine the proportion of individuals exposed, we considered the overlap of the species or critical habitat range with pesticide use sites, incorporating life history information when available and relevant. To determine the magnitude of effect, we used the most applicable dose-response relationship for each species to assess mortality or sublethal effects and assessed indirect effects such as prey, host species, or reliance on vegetative habitat. For sublethal effects, we used the magnitude of effect, when available, associated with endpoints derived from hypothesis-based toxicity (e.g., NOAEC, LOAEC).

The process by which EPA obtained species or critical habitat ranges and analyzed the overlap of the simazine UDLs with those ranges as well as determined the usage applicable to those overlaps is described in the BE in Appendix 1-5, Appendix 1-6, and Attachment 1-4. Briefly, EPA developed an analytical process using tools developed in Arc GIS and using the programming language Python, by which ranges and critical habitats from the Service's ECOS[32] on-line system are downloaded, sorted, and overlap and usage data for all UDLs for the action area are applied to determine the percentages of overlap with the uses or usage of the herbicide that occurs within a species range or critical habitat.

As part of our assessment, we use qualitative rankings of high, medium, or low for a listed species' vulnerability, exposure, and toxicity. Each of these factors considers several pieces of information to inform the assignment of organizational descriptions for each species. We used these descriptions of high, medium, or low to organize our review of certain species, based on common, specific factors (e.g., species that may have high levels of exposure and have high toxicity, species that have high levels of exposure and are highly vulnerable). The rankings, however, are not necessarily indicative that a species is likely to be jeopardized. For example, we may find that a species with high exposure, high toxicity ratings, and high vulnerability rankings is not likely to be jeopardized because, for instance, the species' life history indicates that it would spend very little time in use sites or other areas where we expect high concentrations of simazine.

[32] Environmental Conservation On-Line System: https://ecos.fws.gov/ecp/

Vulnerability

We considered several factors to summarize the current status and vulnerability of a listed species to additional stressors. This effort allows us to consider whether a species' current condition is moving toward recovery or further decline. In general, we expect the species' vulnerability to additional stressors to be higher if they are moving toward further decline than if their condition is improving. We also identify which species are most (and least) susceptible to additional stressors in general based on information that could be surmised from species listing and recovery documents, or other sources as cited and considered in the *Status* section of this Opinion.

Our assessment of vulnerability focuses on six factors: (1) the species listing status and recent 5-Year Review recommendation (if available), (2) distribution, (3) number of populations, (4) species population trends, (5) if pesticides have been noted as a threat, and (6) impacts from activities associated with environmental baseline and cumulative effects. We obtained the information to create the vulnerability summary from the *Status of the Species* accounts (Appendix B), the overarching *Environmental Baseline* section of this Opinion, 5-Year Reviews, species recovery plans, species status assessments, and other sources containing the best available scientific information for the species.

Vulnerability factors related to distribution, number of populations, and species population trends are described further below.

Distribution

We considered the distribution of a species as a vulnerability factor with the general view that the smaller or more confined the range, the more susceptible the species may be to a disturbance or stochastic event. If a species was a narrow endemic, or otherwise limited to small, isolated, or fragmented habitats or habitat patches, we assigned a "high vulnerability" ranking to this factor. Where species were wide-ranging and/or able to easily recolonize new or existing habitats, we assigned a low vulnerability ranking to this factor. A "medium vulnerability" ranking was assigned to species that did not clearly fall into either the constrained or widespread categories.

Species that migrate are generally considered inherently wide-ranging based on the extent of their ranges, especially for those that are long-distance migrants. However, parts of a species range that the species relies on seasonally, such as for breeding or overwintering, may be fragmented and constrained. The assignment of vulnerability rankings takes into consideration how vulnerable the species may be across its range as well as in seasonally used portions of its range within the United States. In some cases, even though a "low vulnerability" ranking generally applies to wide-ranging species, a "high vulnerability" or "medium vulnerability" ranking for this factor may be assigned to migratory species in instances where information on the species' seasonal habitat requirements indicates increased vulnerability.

Numbers of Populations

For numbers of populations, we considered whether a species was limited to a single population, few populations, or many populations. The use of “few” versus “many” was necessarily subjective, as it is related to the species’ distribution, redundancy, and resiliency to the effects of stochastic events that could result in extirpations of populations or subpopulations. Generally speaking, we consider “few” to be fewer than 10 populations, though for some species, we may consider “few” to be only two populations (or sub-populations, depending on the available species information). We assigned vulnerability ranking factors of: “high vulnerability” to species with a single population (or in some cases a single, small metapopulation, as appropriate); “medium vulnerability” to species with “few” populations, which allow for at least a limited level of redundancy to protect against stochastic events or localized extirpations; and “low vulnerability” to species with numerous populations, which may provide a greater level of redundancy.

Species Population Trends

For species population trends, we considered whether populations are declining, stable or increasing, based on the best available scientific information, including the most recent information from listing rules, recovery plans, 5-Year Reviews and other Service sources for the species (e.g., Service species experts). We assigned vulnerability factors of “high vulnerability” to species with one or more declining populations; “medium vulnerability” to species with all stable populations where none are known to be increasing or decreasing, or unknown population trends, and “low vulnerability” for species with increasing population(s) trends. This factor indicates whether the species is moving towards extinction or recovery as part of the species status and baseline.

We acknowledge that for species population trend information, various life history considerations or the species status can complicate an observation of its trend. For example, a species that appears “stable” according to this ranking factor (i.e., neither increasing nor decreasing) may actually have a very small population size(s), which in some cases may not be sufficiently robust to maintain the population over the long term even though numbers may appear stable. While we recognize this is a potential shortcoming in this ranking factor, by evaluating this factor in combination with species distribution, population size, and the other considerations described above, we are less likely to assign the factor undue weight in determining the vulnerability of the species in such a scenario.

Pesticides Listed as a Threat

As we reviewed species information in listing rules and recovery documents to generate the vulnerability factors, we also noted when pesticides were identified as a threat to the species in these documents and included this as an indicator in our overall assessment of species’ vulnerability. Where available information indicated the threat was due to a particular class of pesticides (e.g., rodenticides, insecticides, herbicides), we described that information. However, pesticide threats were not always mentioned or consistently evaluated for a species in listing rules or recovery documents, and such an omission does not necessarily mean the species would not be vulnerable to that factor. As such, where pesticides were not noted as a threat in the listing or recovery documents, we treated this consideration as a neutral factor in our overall vulnerability ranking.

Vulnerability Assessment

We scored each of the six vulnerability components with high, medium, or low scores. A medium score for an individual component is considered neutral and not weighed. We assigned high vulnerability to a species if vulnerability components were all high, a mixture of medium and high scores, or if the species was recommended for uplisting. We assigned medium vulnerability if a species' scores were all medium, a mix of high, medium, and low, or a mix of high and low (unless the species has been recommended for uplisting or delisting). We assigned low vulnerability to species with low scores across all components, a mixture of low and medium or if the species was recommended for delisting. Considerations regarding specific aspects of the species' vulnerability beyond what was included in the vulnerability were applicable for some species depending on unique aspects of their life history. This information is reflected in the rationales for the jeopardy determination for each listed or proposed species and for the destruction or adverse modification determination for each designated or proposed critical habitat.

Exposure

As described previously, we expect simazine applications to occur on a site-specific basis for the duration of the proposed action. Where appropriate, our analyses include a quantification of areas where the pesticide can be applied according to the labels as currently written. We characterize the expected level of exposure using the extent of overlap between simazine use sites and the species' range, past simazine usage data, and any species-specific considerations such as life history information (e.g., habitat preferences, dispersal behavior), conservation measures, and existing protections or other conservation actions.

Agricultural Overlap

Overlap data refers to the extent that simazine use sites (i.e., on-field areas) and adjacent areas likely to be exposed through off-site transport (i.e., off-field areas) occur within a listed species' range and is reported by the EPA as a percentage for each relevant use type. Given that agricultural uses of simazine are limited to ground application methods, we extend our off-field analysis to 305 meters from the edge of application sites as EPA determined this was the maximum distance at which effects are likely to occur to listed species. Our default approach is to assume that individuals of listed species are uniformly distributed throughout their range (see *Assumptions and Uncertainties for all species, Species Range Maps* section of this Opinion). We use this percent overlap to represent the proportion of individuals that may be exposed throughout the duration of the proposed action. We address species where available information indicate that a uniform distribution assumption is not appropriate on a case-by-case basis (see the *Additional Exposure Considerations* section below for more details).

We determine the total overlap between the species' range and the action area by summing the on- and off-field area overlaps with the species' range for each relevant use type (except for listed aquatic species, which we address below). We aggregate the overlaps across all non-highly redundant crop groups. Non-highly redundant crop groups refer to those crops that are not likely to be grown using the same fields (for example, crops in UDLs that are not rotated out or replaced within the same field location such as Other Orchards or Citrus). The Other Orchards UDL consists of berries and fruit trees which need to be established over several years or growing seasons before they can bear fruit and thus are not rotated out after only a few growing seasons. In contrast, row crops (such as those in the Vegetables and Ground Fruits use category) may be rotated out over growing seasons for various reasons such as replenishing soil nutrients or availability or demand for certain crops. Redundancy in relation to use type refers to the fact that mapped use sites are not exclusive of one crop type over time. As such, for highly redundant UDLs such as Corn and Soybean, or Citrus and Other Orchards, we use the higher overlap between the two redundant UDLs in our total overlap calculations.

Based on the value of a listed species' total overlap, each species' overlap is given a score. Species with greater than 10% overlap are assigned a high overlap score, species with 5-10% overlap are assigned a medium overlap score, and species with less than 5% total overlap are assigned a low overlap score. This assignment of high, medium, or low rankings to these specific percentages of overlap are used for purposes of organizing our analyses along assumptions that generally apply with all the species evaluated in this Opinion. For example, the Service has generally found that the greater the extent of overlap between species range and use and spray drift sites can be indicative of increased exposure of individuals to simazine. However, available information may indicate that general assumption does not apply to certain species. For example, even if the range of a species has a high overlap with simazine use and spray drift sites, the Service may have information indicating that individuals would not be present in certain areas of overlap.

Our approach for characterizing overlap for aquatic listed species is modified from the above overview. We go into the specific overlap characterization process for these species below.

<u>Aquatic species overlaps</u>

We anticipate listed aquatic species will primarily be exposed to simazine through contact with contaminated water in their habitats. We do not expect these species will occur on-field, and thus expect exposure will only result from off-field transport via spray drift or runoff into their aquatic habitats. Given that the ranges for listed aquatic species are generally delineated using the relevant U.S. Geologic Survey (USGS) Hydrologic Unit Code 12 (HUC 12) watersheds, we anticipate that all residues that leave use sites will be collected in the waterbodies within the species range where individuals occur regardless of how residues leave treated sites or where in the range they are deposited. As such, on-field overlap represents the total extent of agricultural activity within the species' ranges, and we do not extend overlap metrics off-field as this would not functionally change the expected exposures that listed aquatic species are likely to experience. Simazine does not degrade quickly (i.e., persists for several months) in aerobic aquatic habitats and as such is likely to persist in water bodies for long periods of time, be transported long distances in surface waters, and occur in groundwater sources.

Agricultural Usage

Usage data refers to the maximum annual percent of a crop that has been treated with simazine in the past. EPA uses past usage data, as summarized by the State Summary and Usage Matrix (SUUM) document in Appendix 1-4 in the BE, to calculate the percent of a species' range or critical habitat that is likely to be treated annually. Briefly, EPA calculates a percent crop treated at a state level, which they use to calculate the number of acres of a crop within a state that is treated within a year. Since the data do not indicate where within the state past usage has occurred, we conservatively assume that all treated acres of a crop occur within a listed species' range to determine the percent range treated annually. Similar to overlap, we assume that individuals of a listed species are uniformly distributed throughout their range, and that the percent range treated represents the likely proportion of individuals that will be exposed annually.

Similar to overlap, we determine the maximum percent of each species' range likely to be treated annually with simazine by aggregating the percent range treated of all non-highly redundant crop groups. For most species, we do not expect all areas of a specific crop use site will be treated with simazine each year. As such, total usage is typically smaller than overlap.

We score total usage based on the total percent area that is likely to be treated with simazine annually. Species that data indicate will have a large portion of their range (>10%) treated with simazine each year are assigned a high usage score. Species that will have a medium portion of their range (5-10%) treated with simazine each year are assigned a medium usage score, and species for which data indicate will have a low portion of their range (<5%) treated with simazine each year are assigned a low usage score. In the sections below, we outline cases where available data results in a slightly different approach to assessing usage, including for species occurring entirely in the state of California.

<u>California Pesticide Use Report</u>

The California Department of Pesticide Regulations' California Pesticide Use Report (CalPUR) provides spatially specific information regarding pesticide usage in the state of California. The state of California mandates pesticide usage reporting for all agricultural applicators and a subset of nonagricultural applicators. The EPA summarizes these data in terms of the percent of a species' range that has been reported to be treated with any pesticide, treated with any herbicide, and treated with simazine over a 10-year period (2013-2022). The EPA also provides estimates of the average number of growers/applicators that report pesticide usage within the species' range in that same 10-year period, which we use as a surrogate metric for the potential variability in pesticide usage over time (e.g., a large number of growers reporting pesticide usage within a species' range indicates less variability in the total area treated each year as changes in pesticide usage of a few growers is not likely to affect the proportion of the range treated). Given that this state level data is spatially specific to the species' ranges and is this reporting is mandated by the state, we have a high confidence that these data more accurately represent likely exposure than other sources of usage data. As such, we replace the usage data provided in EPA's BE SUUM Appendix 1-4 with CalPUR data for species and critical habitats that occur entirely or largely within the state of California.

Additional Exposure Considerations

When information on a specific species indicates that exposure assumptions are not likely true (e.g., species are known to avoid agricultural areas, species that are only found in protected areas with no agricultural pesticide use), we qualitatively incorporate that information into our exposure rankings. Some examples of relevant information include knowledge of species' distribution on protected lands that are less likely to be treated with simazine (e.g., national parks and some national wildlife refuges), life history information that indicates a low likelihood of exposure (e.g., avoidance of agricultural areas, fossorial life history strategy), or additional sources of usage data, such as USDA's Census of Agriculture.

<u>Life History Traits</u>

Listed species often exhibit different and unique characteristics and behaviors that enable them to survive in their environments. For instance, species that occupy habitats that naturally accumulate lower levels of pesticides (e.g., aquatic habitats with high flow rates, terrestrial habitats located in remote areas far from agriculture) are not likely to experience high levels of exposure compared to species that live in areas surrounded by cultivated land or habitats that are likely to accumulate high levels of pesticides. Behavioral traits such as how and where individuals forage, and their tendency to use particular habitats can also be highly influential in their susceptibility to pesticide exposure. We qualitatively incorporate relevant life history traits that are expected to modify the level of expected exposure relative to our baseline assumptions where relevant species information is available.

<u>U.S. Department of Agriculture's Census of Agriculture Data</u>

The USDA's Census of Agriculture is reported at a county level and includes information on pesticide usage summarized by pesticide class (i.e., all insecticide usage). The EPA provides information in cases where there are low levels of general herbicide usage within the counties that a listed species' range occurs in. Given that these data are more spatially specific than simazine-specific usage data available (with the exception of California, where data are available at a sub-county level) and covers all herbicides used (not just simazine), we consider instances where the CoA reports low levels of usage for all herbicides within a species' range as strong evidence that simazine usage is unlikely to exceed low levels of usage throughout the course of the action.

Non-Agricultural Exposure

As discussed above in the *Overall Considerations for the Opinion* section, we differentiate our evaluations of potential exposure to agricultural and non-agricultural uses of simazine. In contrast to agricultural UDLs, UDLs for non-agricultural uses 1) tend to be less defined and use footprints only generally approximate use site locations, 2) likely incorporate large areas that will not be treated with simazine, and 3) are not likely accurate representations of the actual footprint of non-agricultural use sites on the landscape. As such, we assess exposure of species to non-agricultural uses of simazine in a qualitative manner by considering the life history of species, methods of application, available past usage data, and any existing conservation measures to reduce drift and runoff or otherwise limit exposure to listed species.

Exposure Assessment

We determine the overall exposure ranking by qualitatively considering both the total overlap and total usage (when available), as well as any additional exposure considerations that might modify the level of exposure likely to occur. When overlap and usage scores are the same, we assign the overall exposure ranking the same score (e.g., if both overlap and usage is high, the overall exposure ranking is high). In cases where overlap is high and usage is medium or when overlap is medium and usage is low, we use the overlap score as the overall exposure ranking to maintain conservative exposure assumptions, as usage is a subset of overlap and so the overlap score will always be greater than the usage score. In cases where overlap is high but usage is low, we anticipate a moderate portion of the range may be treated over the duration of the proposed action even if only a small portion of the range is treated in any given year (particularly if the areas treated occur in different locations each year). Thus, species with high overlap but low usage have an overall exposure ranking of medium. In cases where no usage data is available, in the absence of any additional exposure considerations for these species, our ranking is based on total overlap of simazine use sites for species that occur in these areas. For all species, where there are additional exposure considerations, we adjust the overall exposure ranking to reflect this additional information, as appropriate.

Toxicity

We characterize the expected toxic effect to species based on the anticipated level of direct and indirect adverse effects to individuals. Our analysis of toxicity assumes individuals are exposed to simazine at levels estimated by EPA's environmental exposure modeling and is focused on determining the level of adverse effect expected to occur once exposure has taken place. Direct effects are based on the anticipated level of mortality and sublethal effects (e.g., reduced growth) likely to occur in exposed individuals. Indirect effects are based on the impact a listed species is likely to experience when the organisms they rely on, such as those that act as food or habitat resources, are exposed to simazine and those resources experience adverse effects.

Direct adverse effects refer to adverse physiological impacts resulting from exposure to simazine (e.g., through contact or ingestion). We use available toxicity data in surrogate species as reference points to estimate the level of mortality or sublethal effects (e.g., growth or reproduction) to listed species. We also use available toxicity data in surrogate species for taxa groups where toxicity data is not available for a given taxonomic group (e.g., avian data to address endpoints for reptiles). We determine the overall toxicity ranking for species by qualitatively assessing both the expected levels of direct adverse effects (e.g., sublethal effects to growth and reproduction) and indirect adverse effects (e.g., prey and vegetation loss).

Indirect adverse effects refer to adverse impacts resulting from simazine exposure to other organisms that the subject species relies on (e.g., prey species that are exposed to simazine). These impacts may result even if an individual is not exposed to any simazine itself (e.g., loss of host plants or certain vegetation upon which the species depends). We qualitatively score the expected level of indirect adverse effects a listed species will experience based on the dietary items the species relies on or the effects to another species with which the listed species shares an obligate/symbiotic relationship with (e.g., host fish for mussels, ant species for myrmecophilous butterflies). Species that are particularly reliant on species that are sensitive to simazine at estimated environmental concentrations (e.g., plants) may be assigned a high indirect effect score while species that use a variety of food items with a range of sensitivities to simazine and species that use food resources that are not affected by simazine are assigned a medium or low indirect effect score, respectively.

To characterize the toxic effect of simazine to listed species, we first select an appropriate reference point from the available toxicity data (e.g., lowest reported LD_{50} or LC_{50}, the HC_{05} from a species sensitivity distribution, lowest reported LOAEC for sublethal effects). We then compare estimated environmental concentrations that EPA provides for each species to the appropriate toxicity reference point to determine the general magnitude of adverse effect likely to occur. The reference data used to characterize the magnitude of direct and indirect adverse effects will vary by taxa and is dependent on the breadth and depth of information available. We summarize the different toxicity considerations taken for the different taxa groups in the sections below.

Toxicity Assessment

We determine the overall toxicity ranking for listed species by considering the expected levels of direct adverse effects (i.e., mortality and sublethal effects) and indirect effects (i.e., prey or habitat loss). Given the immediate impact of mortality on the continued existence of a species, we generally assign mortality the greatest weight in our toxicity assessments, followed by sublethal direct effects and then indirect adverse effects. While we generally weigh sublethal and indirect effects less than mortality, depending on the level of sublethal impact (e.g., do we anticipate impacts to growth only? What is the anticipated impact to growth or reproduction?) and the importance of food or habitat resources to a particular species (e.g., does the species have an obligate relationship with a particular plant species? is the species known to be food limited?), we may still determine that a species with no anticipated mortality may still rank as high risk in our toxicity assessment based on potential sublethal or indirect effects.

Invertebrates

We expect contact exposure is the primary route of exposure for listed invertebrate species. We separate our invertebrate analyses into arthropods and mollusks/snails as available toxicity data indicate that insects and crustaceans are more sensitive to simazine exposure while mollusks are likely to experience adverse effects from simazine at higher environmentally relevant exposure levels. We compare estimated environmental concentrations resulting from simazine to the lowest terrestrial arthropod reference LD_{50} to determine the level of mortality listed terrestrial arthropod species are likely to experience. We compare estimated environmental concentrations in water to the aquatic invertebrate LC_{50} to determine the level of mortality listed aquatic arthropod species are likely to experience. We compare estimated environmental concentrations in water to the lowest mollusk LC_{50} or NOAEC to determine the level of mortality or sublethal effects to listed snails and bivalves, respectively.

For simazine sublethal effects may occur in listed mollusk species as available toxicity data indicate adverse effects to reproduction are likely to occur at some environmentally relevant exposures but are more likely to occur in lower flow or lower volume aquatic habitats.

For listed invertebrate species that rely on other invertebrates (e.g., predatory insects, butterflies with symbiotic relationships with ants), we use the lowest insect LD_{50} or the aquatic invertebrate LC_{50} to estimate the loss of prey or symbionts in terrestrial and aquatic environments, respectively. For species that rely on vertebrates (e.g., listed bivalves that use fish host species for reproduction), we estimate the level of vertebrate effects expected to occur at estimated environmental concentrations predicted to occur within the species' range. We do so by using the lowest fish NOAEC value. For listed invertebrate species that consume or otherwise rely on vegetation, we assumed that plants exposed on-field were likely to die, but plants exposed within the 305-m offsite transport zone would experience no more than low levels of adverse effects to the overall plant community. For invertebrates that were obligate to a particular plant species or group of species, we considered on a case-by-case basis the extent to which the plants that the listed species relied upon could be affected after the incorporation of conservation measures.

Terrestrial Vertebrates

We expect dietary exposure is the primary route of exposure for terrestrial vertebrates. The EPA provided dietary dosage estimates for listed terrestrial vertebrate species for a variety of potential dietary items. based on body weight, diet, metabolic rate, assimilation efficiency, mass of food consumed per day, and simazine concentration on food for each dietary item a species consumes on-field and off-field. For mammals, where the most sensitive endpoint was dose-based, we converted dietary-based exposure estimates to dose-based estimates following equations set out in EPA's KABAM model[33], which incorporates basic assumptions of species' body weights, commonly consumed dietary items, and ingestion rate. We compared species-specific dose-based

[33] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/kabam-version-10-users-guide-and-technical-2#G3

exposure estimates with the most sensitive observed endpoints (including LD_{50}, NOAEL, and LOAEL) to determine the level of direct adverse effects to listed mammal species. For birds, reptiles, and terrestrial amphibians, the most sensitive endpoint was based on a dietary study that measured concentrations of simazine in diet. As such, we compared estimated dietary dosages to the MATC derived from the lowest NOAEC or LOAEC available for terrestrial vertebrates, as appropriate, to determine whether sublethal effects are likely to occur. While pesticide exposure can result in a broad scope of sublethal effects, our analysis is confined to the data submitted by registrants or available in the open literature, which for simazine, was limited to growth and reproduction. Given that there is not sufficient toxicity data for amphibians or reptiles to create a separate analysis for these taxa, we used available bird toxicity data as a surrogate for terrestrial-phase amphibians and reptiles. We qualitatively adjusted the level of direct adverse effect based on available knowledge of whether a listed species is likely to exclusively consume one dietary item, whether individuals are likely to forage on-field or forage on prey that have recently foraged on-field, whether foraging is likely to occur soon after simazine application, and other relevant life history features (e.g., foraging distance, home range size, specificity of diet).

We expect terrestrial vertebrates that consume other animals may experience the loss of individual prey items but are unlikely to experience an overall reduction in food resources. For terrestrial vertebrate species that consume vegetation, we assumed that plants exposed on-field were likely to die, but plants exposed within the 305-m offsite transport zone would experience no more than low levels of adverse effects to the overall plant community. For terrestrial vertebrates that were obligate to a particular plant species or group of species, we considered on a case-by-case basis the extent to which the plants that the listed species relied upon could be affected after the incorporation of conservation measures. We qualitatively adjust the anticipated level of indirect effects based on any relevant life history traits, including information regarding prey preferences, ability to use multiple food resources, relevant foraging behavior, changes in diet across life stages, etc.

Aquatic Vertebrates

We expect contact with contaminated water is the primary route of exposure for aquatic vertebrates. The EPA provided estimated environmental concentrations (EECs) of simazine for different types of aquatic habitats (e.g., low flow/shallow habitats, high flow/large volume habitats) within the each USGS hydrologic unit code level 2 (HUC2) watershed. We compare maximum EECs corresponding to the UDLs with the greatest overlaps with the species' range to the lowest LC_{50} reported in EPA's BE to determine the general level of mortality likely to occur. We consider the LC_{50} a conservative threshold for qualitatively estimating anticipated mortality to listed fish. If maximum EECs are below the LC_{50} (i.e., below the level where we anticipate 50% of fish species will not experience high levels of mortality), then we have high confidence that mortality is likely low for a listed aquatic vertebrate species. We compare EECs to the lowest reported NOAEC or LOAEC, as appropriate, to determine whether sublethal adverse effects are likely to occur. We qualitatively modified the expected level of direct and indirect effect based on any available information on general preference for specific types of habitats, if species use certain habitats at certain life stages or time of year, etc. Given that there is not sufficient data on amphibians to create a separate analysis for this taxon, we use these lethal and sublethal endpoints for fish as a surrogate for aquatic-phase amphibians.

We use the aquatic invertebrate LC_{50} to estimate the level of invertebrate prey loss that is likely to occur at estimated environmental concentrations of simazine. We use the fish LC_{50} to estimate the level of fish prey loss that is likely to occur at environmental concentrations of simazine to listed piscivorous species. We qualitatively adjust the likely level of prey loss based on available information on life history traits (such as known prey preference, ability to use multiple food resources, habitat use, changes in dietary requirements across life stages, etc.).

Plants

We assessed plants with respect to the EPA's Herbicide Strategy PULA groupings as well as grouped them by the percent of exposure in their environment. We discuss this approach in more detail below in the section *Species Groupings*. The mode of action of triazine herbicides, such as simazine, involves inhibiting the photosystem II mechanism in plants. Thus, it blocks the plant from photosynthesizing, the process by which plants convert light energy to food energy and therefore impedes the plant's ability to grow which ultimately can lead to mortality. As such, the focus of our analysis on listed plants is based on direct effects to the plant itself.

In EPA's BE, the most sensitive value for monocot plants was an MATC, representing the geometric mean between the NOAEC and LOAEC, of 0.028 lb a.i./acre. EPA developed a species sensitivity distribution (SSD) to capture the breadth of effects to both monocot and dicot plants for the Herbicide Strategy to distinguish between species that EPA determined were likely to be adversely affected versus not likely to be adversely affected. In implementing its Herbicide Strategy, EPA used the 5th percentile of the plant species SSD (HC_{05} = 0.013 lb a.i./acre, 95% CI = 0.0091-0.017) to establish their population level impacts. Because the most sensitive monocot plant threshold value (MATC = 0.028 lb a.i./ace) is above the HC_{05}, EPA felt using the HC_{05} value (0.013 lb ai/acre) was more protective for all monocot species to determine effects to these plants.

The majority of listed plants are flowering dicot plants, and for dicot plants, EPA used, and we bring forth in our analyses, the MATC (which is based on a 25% reduction in seedling emergence weight for lettuce; see Chapter 2 of BE) endpoint of 0.0031 lb a.i/acre for listed dicot plants. Therefore Herbicide Strategy mitigations (based on the HC_{05} =0.013 lb a.i./acre) do not necessarily reduce exposures below the individual level endpoint used to identify significant effects for dicots because but within an order of magnitude of the threshold for effects. As such, we expect these mitigations will reduce the extent and magnitude of effects such where exposure occurs, we expect no more than low level adverse effects to growth.

For non-flowering plants, EPA used the dicot endpoint to represent effects to non-flowering listed plants (e.g., lichens, ferns) and we bring that analysis forward in this Opinion for non-flowering plants.

Rationales and Conclusions

Once the overall categories for each factor are determined for each species using the Integration and Synthesis Worksheet (Appendix E), we continue the jeopardy analysis by considering the combination of the overall vulnerability, exposure, and toxicity rankings described above. We also include and consider any additional information relevant to the consequences of the proposed action that may reduce or enhance the species reproduction, numbers, and distribution, and therefore modify the overall risk of exposure of the species to simazine.

Species Groupings

To streamline our discussion in this Opinion, we group species that have the same or very similar rationales for their conclusions to increase efficiency and avoid repetition. We considered relevant information and data unique to each individual species when assigning species to groups, which we incorporated into the rationales as appropriate. Species-specific information (e.g., environmental baseline, cumulative effects, status of the species, exposure, and toxicity) was considered for all species, including those species in the grouped analyses, and are presented in full in Appendices B and E. In cases where a combination of rankings and additional considerations provides a clear narrative for a determination that the proposed action is not likely to jeopardize the continued existence of a listed species, we provide a group rationale that outlines how the combination of vulnerability, exposure, toxicity, and additional considerations results in this conclusion for all relevant species. Within these grouped rationales, we add additional information, when relevant, to support our conclusions. We review each grouped rationale to ensure that all vulnerability, exposure, and toxicity assumptions made are applicable for each species within the group and are expected to result in a similar determination for each species. We do not include any species in the grouped rationales when certain assumptions for each grouping are not applicable, require additional information to make a determination, or otherwise present unique circumstances that warrant additional discussion elsewhere in this Opinion. For these species, we provide individual *Integration and Synthesis* summaries that further describe the information we considered to inform our rankings, as well as incorporate any additional, species-specific information that would be relevant to its final determination.

Categories of species that are grouped together because we generally expect them to be at a low risk of jeopardy include the following:

- Species with low exposure, and as such, only small number of individuals are likely to be exposed. We group these species together based on the metric we use to conclude they have low exposure (e.g., the amount of agricultural overlap with the action area or low usage within the range of the species). However, certain species with low exposure that are especially vulnerable to extinction (e.g., severely small population numbers, pesticides listed as a major threat to the species) may not be included in these groups, as even low levels of exposure with adverse effects can have significant consequences for highly vulnerable species.

- If applicable for a particular pesticide, species in taxonomic groups that we expect will have a low level of direct and indirect adverse effects from expected environmental concentrations of the pesticide being assessed. Where relevant, we group these species together as we expect direct and indirect effects, if any, will be low, regardless of the extent of overlap and usage.

- Species that have medium or high overlap, but we expect to have a low level of direct and indirect adverse effects resulting from conservation measures (i.e., measures on the general label or from Herbicide Strategy mitigations implemented through a species-specific PULA).

As stated above, species that do not meet the assumptions of a group are assessed in individual *Integration and Synthesis* summaries. For this consultation, these include species with low overlap that may be especially vulnerable to effects, as discussed above in this section, or species that may be exposed in a manner that is not addressed by spray draft and runoff mitigations (e.g., on-field exposure, or exposure resulting from non-agricultural usage). Where applicable, we modify initial exposure and toxicity rankings in our individual *Integration and Synthesis* summaries according to additional information regarding exposure and effects for individual species.

Plant Groupings

Because there is a large number of plant species considered in this consultation, we further divided plants into five groups for ease of analysis and clarity of presentation based on the plant's habitat type. We first divided plants into those that occur in nonflowing wetlands; terrestrial plants that occur near citrus, avocado, or almond orchards; and all other plants. We based this division on EPA's analysis that plants in nonflowing wetlands and those that are exposed near the specified types of orchards would be exposed to the highest concentrations of simazine, and, when applicable, would require a greater degree of mitigation to reduce concentrations in their habitat (i.e., six total runoff points, to be implemented through PULAs). We further divided the nonflowing wetland and all other plant groups based upon whether species were monocots, dicots, or non-flowering plants. Though we expect similar effects to monocots and dicots from simazine exposure, this further division allowed us to present information for these species in smaller groups for the reader's convenience. Thus, plants are grouped into the following five Integrations and Synthesis appendices:

- Terrestrial plants with risk of exposure from citrus, avocados, or almonds (Integration and Synthesis Appendix C-B5)
- Dicots and non-flowering plants in nonflowing wetlands (Integration and Synthesis Appendix C-B4)
- Monocots in nonflowing wetlands (Integration and Synthesis Appendix C-B3)
- All other dicots and non-flowering plants (Integration and Synthesis Appendix C-B2)
- All other monocots (Integration and Synthesis Appendix C-B1)

Within each plant appendix, we apply the grouping categories described above (i.e., overlap, usage, and conservation measures), and provide individual Integration and Synthesis summaries for plants that may found on-field, within non-agricultural simazine use sites, or may otherwise require further analysis.

Effects of the Action on Animals

In the Integration and Synthesis summaries (Appendix C), we evaluate the results of exposure to simazine for each taxa group (as described in the *Effects of the Action* section of this Opinion). Generally, we anticipate relatively low levels of direct (i.e., growth and reductions) and indirect effects to animals exposed to simazine from off-site transport as a result of conservation measures incorporated into the action. For animals occurring on agricultural or non-agricultural simazine use sites, or animals occurring in low-flow or low-volume aquatic environments, we anticipate variable levels of growth and reproductive effects, based on their sensitivity to simazine, diet, habitat preferences, and other life history characteristics. We summarize these results and related conclusion rationales for the species in the sections below.

For each animal species, we considered the information described above and developed a rationale for the conclusion. Within each taxa group, we documented our determinations for each endangered and threatened species and critical habitat. Proposed species and critical habitat are included in the taxa group tables, and determinations for each are provided as part of our conference biological opinion. Our analyses for species are provided in the sub-appendices of Appendix C and for critical habitats in Appendix D. Each taxa group and associated assumptions and narratives are included in the sections below. Where rationales for conclusions could be written broadly enough to apply to multiple species within a taxa or geographic group (e.g., snails, mussels), we streamlined reporting to the different exposure groupings as discussed earlier, for clarity and to avoid redundancy. Conclusions for all species addressed in this Opinion are in Table 3 above.

Amphibians

This taxa group includes species from the orders Anura and Caudata, including frogs, salamanders, and toads. All amphibians are ectothermic and have skin that is permeable to air and water. Frogs and toads share many similar life history characteristics.

Frogs (family Ranidae) and toads (family Bufonidae) generally have both an aquatic and terrestrial phase; although adults of some species may spend more time on land (e.g., Yosemite toad, California red-legged frog), others may spend most of their time in their aquatic environment (e.g., mountain yellow-legged frog), only moving onto land to occasionally forage along the water's edge. Both frog and toad families lay eggs in an aquatic environment, which develop into tadpoles and eventually metamorphose into adults. Metamorphosis may occur within a single breeding season or over one to three breeding seasons depending on environmental conditions.

Salamanders exhibit a diverse array of life history characteristics. For instance, the family Plethodontidae (lungless salamanders) includes fully terrestrial species (e.g., Jemez Mountains salamander) which breathe entirely through their skin, lay eggs in a underground burrow, and have hatchlings that resemble small adults compared to fully aquatic species (e.g., Georgetown salamanders) that retain their gills throughout adulthood. Mole salamanders (family Ambystomatidae) have adults that are fully terrestrial, have fully developed lungs, and spend most of their time in underground burrows, but return to their natal breeding habitat to lay eggs which become tadpoles with gills until undergoing metamorphosis. The vast majority of amphibians that have an aquatic phase tend to spawn large numbers of eggs with limited or no parental care after laying (e.g., Oregon spotted frog). Terrestrial salamanders spawn far fewer eggs (typically under 20) in which the parent often guards the eggs until hatching (e.g., Shenandoah salamander). Both aquatic and terrestrial amphibians typically remain within or very close to their natal habitat (e.g., Texas blind salamander, Shenandoah salamander), while amphibians that have both an aquatic and terrestrial phase may remain close to their natal breeding habitat (e.g., Wyoming toad, Houston toad) or may travel several miles in search of suitable upland habitat or even new breeding habitats (e.g., California red-legged frog, Houston toad).

Effects to Amphibian Species

As described in the *Approach* section above, we considered species-specific information (e.g., environmental baseline, cumulative effects, status of the species, exposure, and toxicity) to reach our determinations as to whether the proposed action is likely to jeopardize the continued existence of all the species within this taxon. More detail on the approach for the subsets of species and usage categories is provided in the amphibian *Integration and Synthesis* summary (Appendix C). Information on the status of the species can be found in Appendix B and information on all species vulnerability, exposure, and toxicity (including species summarized in grouped rationales) can be found in Appendix E.

Because some amphibians can have both a terrestrial and aquatic phase, we considered the risk of adverse effects in both habitats in our analysis for these species (e.g., California tiger salamander, Houston toad, mountain yellow-legged frog).

Use areas for simazine overlap with and occur adjacent to habitats used within the ranges of all the listed amphibian species in this consultation. Exposure to this herbicide can result in sublethal effects to aquatic and aquatic-phase amphibians from exposure to simazine residues dissolved in water, and the loss of important plant resources for breeding or sheltering that can lead to or other detrimental effects. The effects can vary greatly by species depending on the degree of overlap between herbicide uses and the species range, the species' preferred habitats, exposure concentrations (i.e., dose), and the relationship to plant-based resources of the species. Amphibian tadpoles generally feed on algae and detritus, while adults eat aquatic and terrestrial invertebrates, and in the case of larger frogs and toads, small terrestrial vertebrates. Plant-based food resources are susceptible to contamination by herbicides as direct adverse effects that can in turn temporarily reduce these resources available to amphibians. The anticipated exposures and herbicide effects on amphibians and their plant-based resources, as well as the status of the species and factors related to their vulnerabilities, were considered when evaluating the effects of the proposed action on each amphibian species.

As discussed in the *Approach to the Effects Analysis* section, we grouped species based on their vulnerability, exposure, and toxicity, as species with the same ranking combinations likely have a similar risk profile and final determination. We do not include any species in the grouped rationales when certain assumptions are not applicable, require additional information to make a determination, or unique circumstances are otherwise present that warrant additional discussion elsewhere in the Opinion. For these species, we provide individual Integration and Synthesis summaries that further describe the information we considered to inform our rankings, as well as incorporate any additional, species-specific information that would be relevant to its final determination.

Terrestrial-phase Amphibians

Few toxicity studies are available for terrestrial amphibians exposed to simazine. The available toxicity data and thresholds for birds are used as a surrogate for terrestrial amphibians (see the *Assumptions and Uncertainties for All Species* section below for additional details on our use of surrogate toxicity data). As discussed in the *General Effects*, dietary exposure was determined to be the primary driver of effects for terrestrial vertebrates for simazine, and thus we focus our discussion on that. Based on EPA's modeling results, we do not anticipate that most amphibians are likely to accumulate more than low levels of simazine from dietary exposures, with concentrations below which we expect adverse effects. However, we expect that some amphibians exposed on use sites after recent treatment with simazine will be exposed to estimated dosages that lead to sublethal effects to growth and reproduction, depending on the food item consumed and amount of time foraging on-site.

We anticipate nearly all listed terrestrial-phase amphibian species will experience indirect adverse effects resulting from impacts to affected plant-based resources. Given that most amphibians consume arthropod prey (in at least one part of their life cycle) and given that that terrestrial arthropod prey are not sensitive to simazine but aquatic arthropod prey are sensitive to simazine exposure at estimated environmental concentrations, we anticipate listed amphibian species are likely to experience some impacts to their prey resources, even at low levels of exposure. However, we do not anticipate all arthropod species are equally sensitive to simazine as variations in features like physiology, life history traits, and behaviors, will result in different prey species exhibiting different levels of effects in response to exposure. Thus, we anticipate a range of indirect adverse effects are likely depending on an individual species' life history traits and dietary preferences as these factors can influence the level of indirect adverse effects a species is likely to experience.

Aquatic and aquatic-phase amphibians

Few toxicity studies are available for aquatic amphibians exposed to simazine. The available toxicity data and thresholds for fish are used as a surrogate for aquatic amphibians (see the *Assumptions and Uncertainties for All Species* section below for additional details on our use of surrogate toxicity data). Similar to our assessment of other listed aquatic species, we anticipate aqueous exposure to simazine residues dissolved in water is the primary route of exposure to aquatic and aquatic-phase amphibians.

Risk of adverse effects to aquatic amphibians is a function of the level of anticipated exposure and the estimated exposure concentration. In general, aquatic and aquatic-phase amphibian species that either have very little simazine use and usage within their range or species that occur in habitats that are not likely to accumulate more than low levels of simazine are at low risk of mortality and sublethal adverse effects. For instance, while species like the Texas blind salamander, Barton Springs salamander, Georgetown salamander, and Austin blind salamander, which are fully aquatic species that live entirely in subterranean aquifers, may be sensitive to simazine exposure, but they are not likely to experience any significant levels of exposure as we expect simazine residues in surface waters are not likely to remain in concentrations high enough to penetrate the soil column and enter the subterranean aquifers where these species live due to the flashiness of this karst aquifer system. Thus, we anticipate these species are not likely to be exposed to more than low levels of simazine and are not likely to experience adverse effects from exposure. Similarly, species like the Neuse River waterdog (which is fully aquatic) or the reticulated flatwoods salamander (which has an aquatic tadpole stage and semi-aquatic adult stage) occur in areas where there is extensive simazine use and usage within their ranges, but occupy habitats that are not likely to accumulate more than low levels of dissolved simazine in their aquatic habitats, indicating that, while exposure is likely to occur, no more than low levels of sublethal adverse effects are reasonably certain to result from that exposure. In contrast, aquatic and aquatic-phase amphibians at the greatest risk of adverse effects, are those that occur in areas with high levels of simazine use sites within their ranges and occur in habitats that accumulate high levels of simazine. For example, we anticipate the western spadefoot is at high risk of the effects of simazine when individuals are exposed in small waterbodies or waterbodies with low flow rates as estimated environmental concentrations in these areas are predicted to be above levels where adverse effects are observed in reference toxicity studies.

In addition to direct adverse effects, we anticipate aquatic amphibians are likely to experience indirect adverse effects resulting from impacts to affected dietary items. We do not anticipate more than low levels of adverse effects to aquatic plant resources (like periphyton, algae, or detritus), or widespread loss of arthropod prey. We do not anticipate all aquatic plant resources will be impacted from simazine exposure, as in most aquatic systems, these resources are replenished over time based on several microcosm and mesocosm studies discussed in the Effects to Aquatic Plant Communities section of this Opinion. Most amphibians rely heavily on arthropod prey. However, we do not anticipate all arthropod species are equally sensitive to simazine as variations in features like physiology, life history traits, behaviors, will result in different prey species exhibiting different levels of mortality in response to exposure. Thus, we anticipate a range of indirect adverse effects are likely depending on an individual species' life history traits and dietary preferences as these factors can influence the level of indirect adverse effects a species is likely to experience.

As discussed in the *Approach to the Effects Analysis* section, we grouped species based on their vulnerability, exposure, and toxicity rankings, as species with the same ranking combinations likely have a similar risk profile and final determination. We do not include species in the grouped rationales when certain assumptions are not applicable, require additional information to make a determination, or otherwise present unique circumstances that warrant additional discussion elsewhere in the Opinion. For these species, we provide individual Integration and

Synthesis summaries that further describe the information we considered to inform our rankings, as well as incorporate any additional, species-specific information that would be relevant to its final determination.

The amphibian species included in this Opinion, and our conclusions for each, are presented in Table 3. Species-specific information (e.g., environmental baseline, cumulative effects, status of the species, exposure, and toxicity) was considered for all species, including those species in the grouped analyses, and are presented in full in Appendices B and E. Our determination as to whether the proposed action is likely to jeopardize the continued existence of all the species within this taxon is discussed in Appendix C. In this appendix, we present our grouped and individual integration and synthesis summaries for all amphibians considered in this Opinion.

Bivalves (Mussels)

The mussel species in this taxa group includes individuals from the families Margaritiferidae and Unionidae. Of the approximately 105 species in this taxon, only the Alabama pearlshell and the spectaclecase occur in the family Margaritiferidae; the rest occur in the family Unionidae. In general, threats to bivalves are associated with habitat alteration and degradation (e.g., sedimentation, river channelization, river impoundment, drought, nutrient enrichment, chemical contamination) and introductions of non-native species (Master 1993, Neves, Bogan, et al. 1997, Neves 1999, Havlik and Marking 1987, Schloesser and Nalepa 1995, Schloesser, Nalepa and Mackie 1996, Stewart and Swinford 1995). Impacts from past and ongoing threats have left many species in these taxa with one or few remaining populations that are typically fragmented and isolated from one another. Population status is generally characterized as declining or unknown.

Almost all the mussel species in this analysis use a fish host to complete their reproduction cycle, with the exception of the green floater which is able to reproduce at times without a fish host, and the salamander mussel which uses the mud puppy salamander as a host. Both Unionidae and Margaritiferidae mussels vary in their host specificity. Some mussel species can use a variety of fish species as hosts, but they are usually limited to one or two families of fishes. A small number of mussels appear to be limited to a single fish host (obligate host); for example, the scaleshell appears to utilize the freshwater drum (*Aplodinotus grunniens*) exclusively as a host for its larvae. The reproductive life cycle involving the fish host begins when glochidia (i.e., parasitic larvae) are released from the female mussel and attach to the appropriate fish host and the fish host’s epithelial cells form a cyst around the glochidia. The glochidia have a parasitic relationship with the host, deriving all their nutrients from the host for several weeks or months as they transform into juvenile mussels. After transformation, the juvenile mussel drops from the host fish and buries into the sediment.

<u>Effects to Mussel Species</u>

As described in the *Approach* section above, we considered species-specific information (e.g., environmental baseline, cumulative effects, status of the species, exposure, and toxicity) to reach our determinations as to whether the proposed action is likely to jeopardize the continued existence of all the species within this taxon. More detail on the approach for the subsets of species and usage categories is provided in the bivalves (mussels) *Integration and Synthesis* summary (Appendix C). Information on the status of the species can be found in Appendix B and information on all species vulnerability, exposure, and toxicity (including species summarized in grouped rationales) can be found in Appendix E.

For all uses of simazine, we do not anticipate direct mortality to the mussels themselves, however there is the potential for sublethal effects to mussels (reduction in fecundity). We observe the sublethal effects to mussels in some lower flow or lower volume aquatic habitats. We do not anticipate use of simazine will cause mortality to any individuals of host species directly exposed either through exposure to runoff or spray drift from applications based on conservation measures incorporated into the action. However, we anticipate some sublethal effects to some fish host species (e.g., reductions in growth). This exposure may vary depending on waterbody type as described previously in the *General Effects* section. For example, for host fish with some or all life stages in small flowing or static waterbodies (e.g., some darters, sculpins, mosquito fish, stonerollers, some minnow), sublethal effects are generally likely to be higher than those in larger water bodies, like larger rivers or lakes (e.g., large and smallmouth bass, logperch, catfish, and bullhead). We anticipate variable degrees of effects to host fish. Particularly near smaller waterbodies, exposures are likely to result in high levels of sublethal effects where exposure occurs.

For host fish species that prey on invertebrates or fish, we anticipate simazine exposure will cause some alteration in their forage base but only temporarily. Reduced food availability to the host fish could result in some effects on individual host fish or their populations, particularly in habitats where food resources may already be relatively scarce. Where localized effects to zooplankton prey occur from applications of simazine, we anticipate these to be relatively short-term, whereas additional food resources from upstream sources would quickly recolonize or host fish would seek out other areas of available prey, where sufficient habitat is present to do so. In static water bodies, such has larger lakes, we anticipate localized effects to reductions in zooplankton prey would also occur from applications of simazine. However, these invertebrate prey resources are also likely to be replenished over a short period of time from within or close to the habitat. However, where unaffected areas are limited due to fragmented habitat, and during the time in which prey resources have adequately re-established to provide a sufficient prey base, we anticipate some reduced ability of host fish to forage or reduced body condition for these fish. Such effects would result in lower growth of affected host fish. Mussels generally consume phytoplankton and detritus, which is not anticipated to be impacted by simazine applications.

Overall, based on the general regions of the country where listed bivalve species occur and the relevant crops growing in these regions, we do not anticipate more than low levels of adverse effects to a small subset of the mussels or their host fish are likely to occur. Based on available toxicity data in fish, we anticipate, even at maximum estimated environmental concentrations, no host fish mortality or mussel mortality is likely as estimated environmental concentrations are typically well below the fish mortality and mussel mortality thresholds. However, depending on habitat and simazine use sites present within the range, we expect sublethal effects to occur for a subset of listed mussels and their host fish. As such, while we expect most listed bivalves are not likely to experience more than low levels of direct or indirect adverse effects, some may experience a higher degree of adverse effects in certain parts of their range.

As discussed in the *Approach to the Effects Analysis* section, we grouped species based on their vulnerability, exposure, and toxicity rankings, as species with the same ranking combinations likely have a similar risk profile and final determination. We do not include any species in the grouped rationales when certain assumptions for the grouping were not applicable, require additional information to make a determination, or otherwise present unique circumstances that warrant additional discussion elsewhere in this Opinion. For these species, we provide individual Integration and Synthesis summaries that further describe the information we considered to inform our rankings, as well as incorporate any additional, species-specific information that would be relevant to its final determination.

The bivalve species included in this Opinion, and our conclusions for each, are presented in Table 3. Species-specific information (e.g., environmental baseline, cumulative effects, status of the species, exposure, and toxicity) was considered for all species, including those species in the grouped analyses, and are presented in full in Appendices B and E. Our determination as to whether the proposed action is likely to jeopardize the continued existence of all the species within this taxon is discussed in Appendix C. In this appendix, we present our grouped and individual integration and synthesis summaries for all bivalves considered in this Opinion.

Birds

Birds are a diverse group in the class Aves, which is divided into 23 taxonomic orders based on the similarity of their characteristics: ducks, geese, and swans (Anseriformes); grouse, quail, and allies (Galliformes); grebes (Podicipediformes); pigeons and doves (Columbiformes); cuckoos (Cuculiformes); nightjars (Caprimulgiformes); swifts and hummingbirds (Apodiformes); cranes and rails (Gruiformes); plovers, sandpipers, and allies (Charadriiformes); loons (Gaviiformes); tubenoses (Procellariiformes); storks (Ciconiiformes); frigatebirds, boobies, cormorants, darters, and allies (Suliformes); pelicans, herons, ibises, and allies (Pelecaniformes); New World vultures (Cathartiformes); hawks, kites, eagles, and allies (Accipitriformes); owls (Strigiformes); trogons and quetzals (Trogoniformes); kingfishers and allies (Coraciiformes); woodpeckers (Piciformes); caracaras and falcons (Falconiformes); parrots (Psittaciformes); and perching birds (Passeriformes).

Birds are ubiquitous throughout the landscape, as they can be found using virtually every type of habitat and land use across the full spectrum of terrestrial and aquatic environments. Each bird species generally occurs within certain habitat types and specific geographical areas, although ranges for many bird species are expansive, especially for species that migrate. Resident species stay in the same area year-round, although they may make seasonal movements between local habitat areas. Migratory birds tend to have complex and extensive habitat needs, requiring networks of appropriate habitats in key locations across large geographical areas that include most available land uses. They require suitable habitats in different places for breeding and overwintering, as well as flyways and stopover sites for travelling, resting, and refueling during migration. Effects of reductions in habitat quantity and quality, the primary causes of negative population trends for many species, are often exacerbated by the direct loss of bird life from environmental hazards. Clean air, clean water, and abundant, diverse, and healthy habitats are essential for listed bird species to survive and recover.

<u>Effects to Bird Species</u>

As described in the *Approach* section above, we used exposure and toxicity data, in combination with relevant life history information, to assess all birds for effects to the proposed action. Species-specific information (e.g., environmental baseline, cumulative effects, status of the species, exposure, and toxicity) was considered for all species to reach our determinations as to whether the proposed action is likely to jeopardize the continued existence of all the species within this taxon. More detail on the approach for the subsets of species and usage categories is provided in the birds *Integration and Synthesis* summary (Appendix C). Information on the status of the species can be found in Appendix B and information on all species vulnerability, exposure, and toxicity (including species summarized in grouped rationales) can be found in Appendix E.

Exposure varied among the species based on the habitats in which they forage, breed, and shelter from low to high. While species like the Least Bell's vireo and Mississippi sandhill crane are likely to experience adverse effects from simazine exposure, there is a low level of overlap between the action area and their ranges, indicating that adverse effects would be limited to a very small number of individuals, which would not likely result in significant adverse effects to the species overall. In contrast, some species, like the whooping crane or Gunnison sage grouse, have extensive simazine use sites within their range, and while not likely to die, are expected to experience sublethal adverse effects, including reproductive effects such as reduction in number of eggs laid, viable 3-week embryos, hatchling survival, and 4-day old chick survival if exposed to food on sites recently treated with simazine. However, as these effects are only expected under certain conditions (i.e., feeding exclusively on certain dietary items from recently treated simazine use sites), we anticipate these effects will occur infrequently.

Similarly, we anticipate listed bird species will only experience indirect adverse effects in limited circumstances. Due to conservation measures incorporated into the action, listed bird species exposed to simazine off use sites are not expected to experience declines in food resources or alteration to habitat structure or function. While we expect plants and some animals exposed to simazine on use sites will experience effects to growth or reproduction, we do not anticipate that any listed birds forage on simazine use sites to the extent that these effects will result in a loss of food resources to the species.

As discussed in the *Approach to the Effects Analysis* section, we grouped species based on their vulnerability, exposure, and toxicity rankings, as species with the same ranking combinations likely have a similar risk profile and final determination. We do not include any species in the grouped rationales when certain assumptions for the grouping are not applicable, additional information is required to make a determination, or unique circumstances are otherwise present that warrant additional discussion elsewhere in this Opinion. For these species, we provide individual Integration and Synthesis summaries that further describe the information we considered to inform our rankings, as well as incorporate any additional, species-specific information that would be relevant to its final determination. For birds, we included species that have been proposed for delisting (i.e., wood stork) in these groups and provided rationales for our determinations.

The bird species included in this Opinion, and our conclusions for each are presented in Table 3. In addition to the species vulnerability assessments and summarized Environmental Baseline and Cumulative Effects information relevant to the analysis, we further discuss the effects of the action, and our determination as to whether the proposed action is likely to jeopardize the continued existence of all the species within this taxon in Appendix C.

Crustaceans

The crustaceans taxa group includes the following orders: Amphipoda (amphipods); Anostraca (fairy shrimp), Decapoda (shrimp, crayfish), Isopoda (isopods), and Notostraca (fairy shrimp, tadpole shrimp). Most are aquatic and dwell in streams, vernal pools, or subterranean habitats. Several partially terrestrial species live in ephemeral habitats (i.e., vernal pools), and are adapted to survive periodic dry conditions (e.g., cyst phase of fairy shrimp and tadpole shrimp).

Effects to Crustacean Species

As described in the *Approach* section above, we considered species-specific information (e.g., environmental baseline, cumulative effects, status of the species, exposure, and toxicity) to reach our determinations as to whether the proposed action is likely to jeopardize the continued existence of all the species within this taxon. More detail on the approach for the subsets of species and usage categories is provided in the crustacean *Integration and Synthesis* summary (Appendix C). Information on the status of the species can be found in Appendix B and information on all species vulnerability, exposure, and toxicity (including species summarized in grouped rationales) can be found in Appendix E.

We anticipate all crustacean species will be directly affected from exposure through concentrations in water. We do not anticipate mortality from simazine where individuals are exposed, however, sublethal effects are anticipated for crustaceans. For species in streams, wetlands, and non-subterraneous aquatic habitats, we anticipate that drift or runoff from nearby applications are mitigated from the conservation measures incorporated into the action to levels below which where we would observe effects for most species. Effects to invertebrate prey or invertebrate constituents of detritus in the forage base were considered in the analysis based on the assumption that additional indirect effects may occur to these species via temporary reductions in prey resources after applications.

We anticipate that the crustaceans considered in this Opinion will not die from simazine uses where exposure occurs but sublethal effects (reduction in number of offspring) will result from exposure to simazine at some estimated environmental concentrations. For many narrow endemics, any adverse effects could result in species-level effects due to isolation and low population numbers. Risk to some crustaceans was observed but overlap and usage varied from (<0.1-49.6%), and sublethal effects were anticipated based on available reference toxicity data.

Indirect effects were analyzed for crustaceans and are discussed in each individual crustacean grouping or individual Integration and Synthesis write up. We assumed that other aquatic invertebrates taken as dietary items would experience similar adverse sublethal effects as listed aquatic invertebrates. Other aquatic plant-based dietary items such as algae or phytoplankton were not considered to be adversely impacted from exposure to simazine long-term as these dietary items would only be temporarily lost within their habitats and thus not limit the availability of the listed crustacean to forage or reproduce.

However, we expect a number of listed crustacean species are not likely to experience more than low levels of exposure for a variety of reasons. Species like the Hay's spring amphipod or Shasta crayfish have very little simazine use sites within their watersheds. For cave-dwelling crustaceans (i.e., cave crayfish, Madison cave isopod, Alabama cave shrimp, Kentucky cave shrimp, Illinois cave amphipod, Squirrel Chimney cave shrimp), we do not anticipate that direct application or drift are likely pathways of exposure. As such, we anticipate only low levels of simazine exposure and effects, if any, for these species. In contrast, species like the slenderclaw crayfish have high amounts of simazine use sites within their watersheds and are likely to be exposed to concentrations of simazine that we expect to cause high levels of sublethal effects to growth and reproduction. As discussed in the *Approach to the Effects Analysis* section, we grouped species based on their vulnerability, exposure, and toxicity rankings, as species with the same ranking combinations likely have a similar risk profile and final determination. We did not include any species in the grouped rationales when certain assumptions for the grouping are not applicable, additional information was required to make a determination, or unique circumstances were otherwise present that warrant additional discussion in the Opinion elsewhere. For these species, we provide individual Integration and Synthesis summaries that further describe the information we considered to inform our rankings, as well as incorporate any additional, species-specific information that would be relevant to its final determination.

The crustacean species included in this Opinion, and our conclusions for each, are presented in Table 3. Species-specific information (e.g., environmental baseline, cumulative effects, status of the species, exposure, and toxicity) was considered for all species, including those species in the grouped analyses, and are presented in full in Appendices B and E. Our determination as to whether the proposed action is likely to jeopardize the continued existence of all the species within this taxon is discussed in Appendix C. In this appendix, we present our grouped and individual integration and synthesis summaries for all crustaceans considered in this Opinion.

Fish

The fish species in this taxa group include a wide variety of families: sturgeon (Acipenseridae), cavefish (Amblyopsidae), a silverside (Atherinidae), suckers (Catostomidae), sunfish (Centrarchidae), sculpins (Cottidae), dace, minnows, and other cyprinids (Cyprinidae), goby (Gobidae), madtoms (Ictaluridae), smelt (Osmeridae), darters and logperch (Percidae), mosquitofish and topminnows (Poeciliidae), and salmonids (Salmonidae). Most are freshwater species, with a few species of sturgeon, salmonids, and smelt using freshwater, estuarine, and/or marine waters at different stages in their life cycles.

Effects to Fish Species

As described in the *Approach* section above, we considered species-specific information (e.g., environmental baseline, cumulative effects, status of the species, exposure, and toxicity) to reach our determinations as to whether the proposed action is likely to jeopardize the continued existence of all the species within this taxon. More detail on the approach for the subsets of species and usage categories is provided in the fish *Integration and Synthesis* summary (Appendix C). Information on the status of the species can be found in Appendix B and information on all species vulnerability, exposure, and toxicity (including species summarized in grouped rationales) can be found in Appendix E.

Effects to fish from simazine uses vary depending on the extent of simazine use sites within the species' watersheds, anticipated usage in the species' watershed, specific life history traits, and dietary items consumed. In general, we anticipate adverse effects will be in the form of sublethal effects to reductions in growth from contact with simazine residues dissolved in waterbodies. We also anticipate there will be adverse effects resulting from some temporary reductions in the abundance of aquatic plant-based dietary items and some reductions in arthropod prey, which may result in reduced fitness. In general, listed fish species that we expect to have a low risk of adverse effects from the proposed action are those that prefer or exclusively occupy areas of high flow or waterbodies with large volumes, or that occur in areas with very little pesticide usage. For instance, while species like the Arkansas River shiner, Neosho madtom, and boulder darter have high overlap with agricultural use sites, these species occupy waterbodies that are not likely to accumulate high levels of simazine and conservation measures incorporated into the action will reduce simazine exposure even further to levels that we expect to result in no more than low levels of sublethal adverse effects to growth.

Listed fish species that are at the greatest risk of adverse effects are those that occupy habitats that are likely to accumulate high levels of simazine, such as small waterbodies with low flow rates or small water volume and occur in areas containing extensive simazine use sites or areas of high usage.

The fish species included in this Opinion, and our conclusions for each, are presented in Table 3. Species-specific information (e.g., environmental baseline, cumulative effects, status of the species, exposure, and toxicity) was considered for all species, including those species in the grouped analyses, and are presented in full in Appendices B and E. Our determination as to whether the proposed action is likely to jeopardize the continued existence of all the species within this taxon is discussed in Appendix C. In this appendix, we present our grouped and individual integration and synthesis summaries for all fishes considered in this Opinion.

Insects (Aquatic and Terrestrial)

This taxa group includes several orders of insects, including Coleopterans (beetles), Dipterans (flies), Hemipterans (true bugs), Hymenopterans (bees), Lepidopterans (butterflies and moths), Odonates (dragonflies and damselflies), and Orthopterans (grasshoppers). These species exhibit a variety of life history characteristics. All are generally short-lived, although some may live multiple years (e.g., at a larval stage). Some adult life stages may be very short, as brief as a few weeks. Most insect species considered in this Opinion are terrestrial. As a group, they inhabit numerous habitat types within the action area, depending on the species' life history requirements. The terrestrial insects are generally capable of flight, at least in adult life stages. Some adults are not able to or naturally expected to move large distances and are restricted to small habitat patches separated by unsuitable habitat. Some aquatic insects are fully aquatic, such as riffle beetles. Others have both aquatic and terrestrial life stages, including dragonflies, damselflies, stoneflies and similar species. For species with both terrestrial and aquatic life stages, juvenile and subadult (i.e., eggs, larvae, pupae) individuals generally live in aquatic habitats, while the adult life stage either exclusively or primarily occupies terrestrial habitats, depending on the species.

As described in the *Approach* section above, we considered species-specific information (e.g., environmental baseline, cumulative effects, status of the species, exposure, and toxicity) to reach our determinations as to whether the proposed action is likely to jeopardize the continued existence of all the species within this taxon. More detail on the approach for the subsets of species and usage categories is provided in the mammals *Integration and Synthesis* summary (Appendix C). Information on the status of the species can be found in Appendix B and information on all species vulnerability, exposure, and toxicity (including species summarized in grouped rationales) can be found in Appendix E.

The terrestrial and aquatic insect species included in this Opinion, and our conclusions for each, are presented in Table 3. Species-specific information (e.g., environmental baseline, cumulative effects, status of the species, exposure, and toxicity) was considered for all species, including those species in the grouped analyses, and are presented in full in Appendices B and E. Our determination as to whether the proposed action is likely to jeopardize the continued existence of all the species within this taxon is discussed in Appendix C. In this appendix, we present our grouped and individual integration and synthesis summaries for all terrestrial and aquatic insect species considered in this Opinion.

Effects to Terrestrial Insect Species

We do not anticipate that terrestrial insects will die where exposure occurs, but are likely to experience sublethal effects at certain concentrations. Indirect effects for terrestrial insects that rely on plant resources such as butterflies needing host plants for nectaring or larval development or beetles relying on plant resources for food were analyzed based on the relationship to the plant resource such as being an obligate to a plant resource or if the listed terrestrial insect was able to rely on a variety of plant-based resources. We anticipate that risk will be high for terrestrial insects that rely on and obligate to plant-based foods (e.g., nectar, leaves, berries) from exposure to simazine versus terrestrial insects that can rely on a variety of plant based resources will experience less risk.

We do not anticipate any adverse effects to listed insects that consume other terrestrial invertebrate prey (e.g., American burying beetle, northeastern beach tiger beetle, and Puritan tiger beetle) and species that are reliant on other invertebrates for survival (e.g., myrmecophilous butterflies like the Fender's blue butterfly). This information was provided in the discussion for the species, and a similar effect was noted for the dietary item or obligate relationship.

Effects to Aquatic Insect Species and Life Stages

There is only one partial aquatic insect considered in this opinion, the Hine's emerald dragonfly, and one fully aquatic insect considered in this Opinion, Comal Springs riffle beetle. Simazine will cause a reduction in offspring survival of individuals exposed to some estimated environmental concentrations but we do not anticipate any mortality to aquatic insects from simazine exposure. We anticipate exposed individuals during the aquatic phase could experience some indirect adverse effects as they rely on other aquatic invertebrates for prey. Indirect effects were assessed for aquatic insects that consumed other insects, based on the assumption that most invertebrate dietary items that would experience similar adverse effects to listed aquatic invertebrate species. We do not anticipate any adverse effects to detritus from exposure to simazine. We anticipate some temporary reductions in plant-based resources for this dragonfly such as it would use for breeding or sheltering

As discussed in the *Approach to the Effects Analysis* section, we grouped species based on their vulnerability, exposure, and toxicity rankings, as species with the same ranking combinations likely have a similar risk profile and final determination. We do not include species in the grouped rationales when certain assumptions are not applicable, require additional information to make a determination, or otherwise present unique circumstances that warrant additional discussion elsewhere in the Opinion. For these species, we provide individual Integration and Synthesis summaries that further describe the information we considered to inform our rankings, as well as incorporate any additional, species-specific information that would be relevant to its final determination.

The terrestrial and aquatic insect species included in this Opinion, and our conclusions for each, are presented in Table 3. Species-specific information (e.g., environmental baseline, cumulative effects, status of the species, exposure, and toxicity) was considered for all species, including those species in the grouped analyses, and are presented in full in Appendices B and E. Our determination as to whether the proposed action is likely to jeopardize the continued existence of all the species within this taxon is discussed in Appendix C. In this appendix, we present our grouped and individual integration and synthesis summaries for all terrestrial and aquatic insect species considered in this Opinion.

Mammals

All mammals are vertebrate endotherms distinguished from other animal taxa by possessing hair or fur and mammary glands for milk production in females. Terrestrial mammals in this Opinion include species from the orders Carnivora (carnivores), Chiroptera (bats), Eulipotyphla (shrews), Lagomorpha (rabbits), and Rodentia (rodents). Mammal species exhibit a variety of life history characteristics. Some species hibernate, such as the Virginia big-eared bat, and others like the northern long-eared bat migrate. Some species live in underground burrows, such as kangaroo rats and beach mice, while others spend most of the day in trees, like the ocelot. Species' ranges vary from only one location (e.g., riparian brush rabbit) to only a few locations (e.g., southeastern beach mouse), but others occur across many states (e.g., gray wolf, gray bat). Diet varies among species greatly as well. Some species are carnivores like the ocelot; the Buena Vista Lake ornate shrew and many bats are insectivores; pocket gophers and the Columbia Basin pygmy rabbit are herbivores; and other species, like beach mice, consume insects and vegetation.

Effects to Mammal Species

As described in the *Approach* section above, we considered species-specific information (e.g., environmental baseline, cumulative effects, status of the species, exposure, and toxicity) to reach our determinations as to whether the proposed action is likely to jeopardize the continued existence of all the species within this taxon. More detail on the approach for the subsets of species and usage categories is provided in the mammals *Integration and Synthesis* summary (Appendix C). Information on the status of the species can be found in Appendix B and information on all species vulnerability, exposure, and toxicity (including species summarized in grouped rationales) can be found in Appendix E.

Effects to mammals from simazine uses vary depending on the amount of overlap with simazine uses, anticipated usage in the species' range, specific life history traits, and dietary items consumed. In general, we anticipate adverse effects will be in the form of sublethal adverse effects, including adverse effects to growth (e.g., reduced body weight and body weight gain) and potential reproductive effects, including altered reproductive hormone levels from the consumption of contaminated food items. Additionally, we anticipate indirect adverse effects, through the loss of plant food and habitat resources, are likely to occur.

In general, individuals of listed mammal species that forage on simazine use sites will be exposed to the highest levels of simazine and will experience the greatest levels of direct adverse effects. Individuals that primarily forage directly on either agricultural or non-agricultural use sites will accumulate high levels of simazine. While we do not anticipate these individuals are likely to accumulate levels of simazine that will cause any mortality, we anticipate these individuals will experience high levels of sublethal adverse effects, including reduced growth and impacts to reproduction. While different dietary items will accumulate different levels of simazine, we anticipate any mammal that primarily forages on simazine use sites, regardless of their dietary preferences, will experience sublethal adverse effects. In contrast, individuals that do not occur on agricultural or non-agricultural use sites or individuals that infrequently occur in use sites but are not likely to use these areas as their primary foraging areas, are not likely to accumulate more than low levels of simazine and are not likely to experience any mortality and no more than low levels of sublethal adverse effects.

Indirect effects in the form of reduced abundance of plant-based food items may occur for obligate herbivores (e.g., riparian woodrat, Columbia Basin pygmy rabbit, pocket gophers) as available toxicity data indicate that adverse effects to plant growth and survival are likely to occur with simazine exposure. However, we anticipate required conservation measures on product labels (e.g., spray drift buffers, three runoff mitigation points) for all agricultural uses will greatly minimize impacts to plants that listed mammals depend on for food or habitat.

Similarly, we anticipate existing pesticide use practices and conditions associated with non-agricultural uses (e.g., use of coarse droplet sizes, application of specific areas within use sites, continuous vegetative cover, no tillage) will greatly limit the extent of off-site transport and similarly minimize the impacts to plants in areas adjacent to non-agricultural simazine use sites. While we anticipate sensitive plant species will still be impacted by simazine exposure, we generally anticipate that this impact to sensitive plant species will not significantly change the overall composition of necessary plant assemblages and communities or reduce the overall availability to serve as food or habitat resources to listed mammals. In contrast, we do not anticipate a reduction in the abundance of insect species with exposure to simazine, indicating that obligate insectivores (e.g., Indiana bat, gray bat, northern long-eared bat, Buena Vista Lake ornate shrew) are not likely to experience high levels of prey loss.

As discussed in the *Approach to the Effects Analysis* section, we grouped species based on their vulnerability, exposure, and toxicity rankings, as species with the same ranking combinations likely have a similar risk profile and final determination. We do not include any species in the grouped rationales when certain assumptions for the grouping are not applicable, additional information is required to make a determination, or unique circumstances are otherwise present that warrant additional discussion in the Opinion elsewhere. For these species, we provide individual Integration and Synthesis summaries that further describe the information we considered to inform our rankings, as well as incorporate any additional, species-specific information that would be relevant to its final determination.

The mammal species included in this Opinion, and our conclusions for each, are presented in Table 3. In addition to the species vulnerability assessments and summarized Environmental Baseline and Cumulative Effects information relevant to the analysis, we further discuss the effects of the action, and our determination as to whether the proposed action is likely to jeopardize the continued existence of all the species within this taxon in Appendix C. In this appendix, we present our grouped and individual integration and synthesis summaries for all mammals considered in this Opinion. Additional information on the status of the species can be found in Appendix B and additional information on the vulnerability, exposure, and toxicity for all species can be found in Appendix E.

Reptiles

The reptile taxa group includes species from the orders Crocodilia (crocodiles), Squamata (lizards and snakes), and Testudines (turtles). Reptiles are tetrapod vertebrates, creatures that either have four limbs or, like snakes, are descended from four-limbed ancestors. Reptiles are ectothermic, relying on external heat sources (e.g., sunlight, warm surfaces) to regulate their body temperatures. Most reptiles are oviparous (egg layers; e.g., Alameda whipsnake, American crocodile, Plymouth redbelly turtle), although several species of squamates are viviparous (give live birth; e.g., giant garter snake). Reptiles do not have an aquatic larval stage. For those species that are oviparous, eggs usually have a soft leathery shell, although some eggs may have a hard shell. Eggs are usually laid on land in a nest covered with a layer of soil or vegetative debris or laid in some form of burrow. Most reptiles do not care for eggs once they have been deposited. However, American crocodiles for example, will guard their nests until the eggs hatch. Reptiles can be found in a variety of habitats from sea level to mountainous terrain. Terrestrial and freshwater/estuarine reptiles can be found living along coastlines in mangrove swamps (e.g., American crocodile), in freshwater streams (e.g., yellow-blotched map turtle) and ponds or wetlands (e.g., bog turtle), to forests (e.g., Louisiana pine snake) and to drier environments including creosote bush scrub (e.g., desert tortoise) and wind-blown sandy environments (e.g., Coachella Valley fringe-toed lizard). Most listed reptiles have relatively small current ranges and are limited to one to a few counties within a single state (e.g., blue-tailed mole skink), while a few tend to have larger ranges (e.g., gopher tortoise). Reptiles face numerous threats including habitat destruction, fragmentation, land-use changes, changes in habitat suitability (e.g., timber practices, invasive species), disease, predation, loss of natural processes (e.g., fire suppression), and climate change. In addition, chemicals and pollution can alter the suitability of a species environment (e.g., water quality), and can affect the species itself by reducing its survival and reproduction. Clean air and clean water, and abundant, diverse, and healthy habitats are essential for listed reptile species to survive and recover in the wild.

Effects to Reptile Species

As described in the Approach section above, we considered species-specific information (e.g., environmental baseline, cumulative effects, status of the species, exposure, and toxicity) to reach our determinations as to whether the proposed action is likely to jeopardize the continued existence of all the species within this taxon. More detail on the approach for the subsets of species and usage categories is provided in the reptiles Integration and Synthesis summary (Appendix C). Information on the status of the species can be found in Appendix B and information on all species vulnerability, exposure, and toxicity (including species summarized in grouped rationales) can be found in Appendix E. Use areas for simazine overlap with and/or occur adjacent to habitats within the ranges of nearly all the listed reptile species in this consultation. Exposure to this herbicide at high concentrations can result in indirect effects from the loss of important plant-based food resources that can lead to detrimental effects. The effects can vary greatly by species depending on the degree of overlap between simazine's uses and the species range, usage patterns, the species' preferred habitats, and the diet of the species considering how their food resources may be affected. Reptiles have a highly varied diet, from those species that are generally herbivorous (e.g., desert tortoise) to those species that eat primarily aquatic and terrestrial invertebrates, fish, and/or small mammals. Crocodiles are opportunistic feeders and will eat whatever they can catch, including snakes, fish, crabs, small mammals, turtles, and birds. The majority of reptiles have high vulnerabilities due to small and isolated populations (e.g., blunt-nosed leopard lizard, San Francisco garter snake, New Mexican ridge-nosed rattlesnake, flattened musk turtle, and many others); they are limited to one or a few populations, one or more populations are declining, and they face continuing threats such as habitat loss and exposure to environmental contaminants.

Listed reptiles are likely to be exposed to simazine through the consumption of contaminated plant-based food resources. Expected usage within the species' range varied for reptiles from extremely low levels (<1% range treated annually) to high levels (49.9% range treated annually). One factor that influenced the likelihood of adverse effects from simazine exposure was whether the species was expected to forage on simazine use sites. When available information indicated individuals would not be exposed on use sites, the effects anticipated for these species were lower as estimated environmental concentrations are much lower in adjacent areas than concentrations within use sites. For example, available information regarding the bog turtle's propensity to travel through and possibly rarely feed but not shelter or breed in agricultural areas was noted to reduce but not eliminate the possibility of individuals in the population to be exposed. Other species found to feed primarily on-field on plant-based resources were likely to have greater exposures (e.g., Eastern indigo snake or the Eastern Massasauga rattlesnake). We do not anticipate reptiles that forage on-field will die from simazine exposure but expect these species to experience reproductive effects such as reduction in number of eggs laid, viable 3-week embryos, hatchling survival, and 4-day old survival if exposed to food on sites recently treated with simazine. However, as these effects are only expected under certain conditions (i.e., feeding exclusively on certain dietary items from recently treated simazine use sites), we anticipate these effects will occur infrequently.

Similarly, we anticipate listed reptile species will only experience indirect adverse effects in limited circumstances. Due to conservation measures incorporated into the action, listed reptiles exposed to simazine off use sites are not expected to experience declines in food resources or alteration to habitat structure or function. While we expect plants and some animals exposed to simazine on use sites will experience effects to growth or reproduction, we do not anticipate that any listed reptiles will forage on simazine use sites to the extent that these effects will result in a loss of food resources to the species.

As discussed in the Approach to the Effects Analysis section, we grouped species based on their vulnerability, exposure, and toxicity rankings, as species with the same ranking combinations likely have a similar risk profile and final determination. We do not include any species in the grouped rationales when certain assumptions are not applicable, additional information is required to make a determination, or unique circumstances are otherwise present that warrant additional discussion elsewhere in this Opinion. For these species, we provide individual Integration and Synthesis summaries that further describe the information we considered to inform our rankings, as well as incorporate any additional, species-specific information that would be relevant to its final determination.

The reptile species included in this Opinion, and our conclusions for each, are presented in Table 3. In addition to the species vulnerability assessments and summarized Environmental Baseline and Cumulative Effects information relevant to the analysis, we further discuss the effects of the action, and our determination as to whether the proposed action is likely to jeopardize the continued existence of all the species within this taxon in Appendix C. In this appendix, we present our grouped and individual integration and synthesis summaries for all reptiles considered in this Opinion. Additional information on the status of the species can be found in Appendix B and additional information on the vulnerability, exposure, and toxicity for all species can be found in Appendix E.

Snails

For this Opinion, only aquatic snails were determined to be likely to be adversely affected from simazine exposure.

Effects to Aquatic Snail Species

We reviewed listed and proposed freshwater aquatic snails that occur within the U.S. There are two non-essential experimental populations for the Anthony's riversnail (Entity IDs 9507 and 3842). The life history and distribution information vary substantially by species. Freshwater snails inhabit a range of water bodies, from cave pools, springs, and small tributaries, up to large rivers. A threat common among many of the listed aquatic snails are the effects posed by dams (e.g., reduced ability to expand range and exchange genetic information between populations, and alternation of flow and water quality). For additional information, see the *Status of the Species and Critical Habitat* (Appendix B) for these species and *Environmental Baseline*. Relevant life history traits are discussed below for a general understanding of ecology of each species.

In general, we expect that aquatic snails will have a low risk of mortality or sublethal effects as a result of exposure to simazine based on acute and chronic toxicity data for freshwater snails to triazine herbicides (see section *Effects to Aquatic Invertebrates)*. As there is a paucity of data from aquatic mollusks for simazine, we use atrazine data to address sublethal effects for aquatic freshwater snails.

The endangered and threatened freshwater snails live in springs (e.g., Alamosa spring snail, Koster's spring snail, Chupadera spring snail, Lacy elimia, magnificent ramshorn) or flowing waters such as streams and rivers (e.g., Anthony's river snail, Snake River physa snail, Bliss Rapids snail, Tulotoma snail) and require pristine water quality with specific levels of temperature, rates of water flow, oxygenation, and pH in order to thrive. While we anticipate simazine use sites occur in or near the ranges of most listed snail species (indicating that exposure is reasonably certain to occur), because of the relative tolerance of aquatic snails to simazine, we expect there is a low risk of adverse effects from exposure to simazine. In addition, all listed aquatic snails have low overlap or low Census of Agriculture data (all less than 5%) indicating exposure is likely to be low. We anticipate that in addition to low usage in the use areas of simazine within the species ranges, exposure will be low for both those aquatic snails that inhabit rivers and streams or other water bodies with higher flow and those in lower flow and lower volume aquatic habitats such as springs, seeps, ponds, or creeks. EECs are not expected to reach levels that will cause mortality or sub-lethal effects in any of these aquatic habitats. As such, we do not anticipate mortality or sublethal effects will occur from exposure for any of the listed aquatic snails. Available toxicity data in plants indicate that the food resources most aquatic snails require (e.g., algae, periphyton, detritus) are not likely to experience more than low levels of adverse effects at estimated environmental concentrations of simazine. Any loss of plant-based resources for aquatic snails are temporary in their aquatic environments and we do not anticipate any impacts to detritus from simazine exposure. As such, we generally do not anticipate listed aquatic snail species are likely to experience indirect adverse effects from simazine use.

In conclusion, we anticipate that over the duration of the proposed action, simazine exposure is reasonably certain to occur for the listed and proposed aquatic snail species and their food sources. However, we anticipate no more than low level temporary effects, if any, to food resources.

The snail species included in this Opinion, and our conclusions for each, are presented in Table 3. In addition to the species vulnerability assessments and summarized Environmental Baseline and Cumulative Effects information relevant to the analysis, we further discuss the effects of the action, and our determination as to whether the proposed action is likely to jeopardize the continued existence of all the species within this taxon in Appendix C. In this appendix, we present our grouped and individual integration and synthesis summaries for all snails considered in this Opinion. Additional information on the status of the species can be found in Appendix B and additional information on the vulnerability, exposure, and toxicity for all species can be found in Appendix E.

Effects of the Action on Plants

In the Integration and Synthesis summaries (Appendix C), we evaluate the results of exposure to simazine to plants based on conservation measures incorporated into the action and the habitats they occupy (as described in the *Toxicity* and *Effects of the Action* section of this Opinion). As described in the *Approach* section above, we used exposure and toxicity data, in combination with relevant life history information, to assess all plants for effects to the proposed action. Information on the status of the species can be found in Appendix B and information on all species vulnerability, exposure, and toxicity (including species summarized in grouped rationales) can be found in Appendix E. As discussed in the *Approach to the Effects Analysis* section, we grouped species based on their vulnerability, exposure, and toxicity rankings, as species with the same ranking combinations likely have a similar risk profile and final determination. We do not include any species in the grouped rationales when certain assumptions upon which the grouping is based are not applicable, additional information is required to make a determination, or unique circumstances are otherwise present that warrant additional discussion elsewhere in the Opinion. For these species, we provide an integration and synthesis summary where we discuss the necessary details needed to make a final determination. In situations where the combination of rankings indicates that additional information was analyzed before a jeopardy determination is made, we provide a species-specific narrative that outlines the information that informed the rankings the species was assigned, as well as incorporates any additional, species-specific information that is relevant to its final determination.

Effects to plants (monocot or dicot) reported from studies of seedling emergence exposed to simazine indicate that adverse effects to growth occur at low levels of exposure (see Plant Toxicity data in the General Effects to Plants section in this Opinion). While the general approach taken to assess these effects is the same as for all listed species, for our assessment of listed plants, we broke species into five appendices as described in detail previously. In short, we divided species into different appendices based on characteristics we expected to result in exposure to higher concentrations of simazine from off-site transport (i.e., habitat or proximity to certain simazine use sites). However, after considering the conservation measures incorporated into the action, we now expect no more than low levels of exposure and adverse effects to growth for all plant species exposed to simazine off-site. We expect plants that occur on simazine use sites to be exposed to higher concentrations of simazine and result in high levels of effects to growth and mortality. However, we do not expect simazine use sites to provide suitable habitat for most listed plant species, and as such, expect exposure on use sites to be infrequent.

For most plant species, we do not expect that simazine use will result in any indirect adverse effects to individual plants as we do not anticipate simazine to reduce the abundance and availability of the pollinators and seed dispersers necessary to support reproduction. For plants that are obligate to biotic pollinators or seed dispersers, we consider potential effects to these species individually, as applicable.

For each plant species, we considered all the information described above, and developed a rationale for the conclusion. We documented our determinations for each endangered and threatened species. Proposed species are included in the tables and individual rationales, although determinations for these species are provided as part of our conference biological opinion. Our analyses for these species are provided in the sub-appendices of Appendix C. Information on the status of the species can be found in Appendix B and information on all species vulnerability, exposure, and toxicity (including species summarized in grouped rationales) can be found in Appendix E. All plants addressed in this Biological Opinion can be found in Table 3 above.

Critical Habitat Assessment

We assessed whether the registration of simazine is likely to result in destruction or adverse modification of designated or proposed critical habitat. Destruction or adverse modification means a direct or indirect alteration that appreciably diminishes the value of critical habitat as a whole for the conservation of a listed species (50 CFR 402.02). We analyze effects to critical habitat separately from effects to the species. Our analysis of destruction or adverse modification is centered around the exposure and adverse effects to the physical and biological features (PBFs) of designated and proposed critical habitat. The effects to PBFs are related to but are not always the same as effects to the species, and the species does not have to be present in critical habitat for adverse effects to the critical habitat to occur.

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

When designating critical habitat, we first evaluate areas currently occupied by the species and consider what PBFs a species needs for life processes and successful reproduction. For an unoccupied area to be designated as critical habitat, we must determine that there is a reasonable certainty that the area will contribute to the conservation of the species and that the area contains one or more of the PBFs essential to the conservation of the species. These areas may require special management considerations or protection, as described in designation rules. General PBFs of critical habitats include space for individual and population growth and for normal behavior; cover or shelter; food, water, air, light, minerals, or other nutritional or physiological requirements; sites for breeding and rearing offspring; habitats that are protected from disturbance or are representative of the historical, geographic, and ecological distributions of a species; and other features. Specific PBFs are also often included in critical habitat rules to describe habitat elements that are essential for the species based on the best scientific data available about the species' habitat, ecology, and life history. A feature may be a single habitat characteristic, or a more complex combination of habitat characteristics and functions.

For purposes of assessing whether a destruction or adverse modification determination is appropriate, the effects of the action, together with the status of critical habitat, the environmental baseline, and any cumulative effects, are evaluated to determine whether any direct or indirect alteration would appreciably diminish the value of critical habitat as a whole for the conservation of a listed species. Such alterations may include, but are not limited to, those that alter the PBFs essential to the conservation of a species. To facilitate our analysis of the large number of critical habitat proposals and designations in this Opinion, we identified the types of PBFs that we anticipate will be negatively affected by the proposed action. We identified four categories of PBFs that are likely susceptible to the effects of simazine:

(1) water quality,

(2) host plants,

(3) plant assemblages,

(4) plant functions, and

(5) animals, including prey, pollinators, seed dispersers, and host fish.

These types of PBFs are collectively referred to herein as the "relevant PBFs." We reviewed each critical habitat designation to determine if any relevant PBFs are identified as essential features of critical habitat for a listed or proposed species. For those critical habitats with rules that do not include specific PBFs, we assigned any relevant PBFs based on available information regarding specific needs of the listed species. Any critical habitats that do not contain relevant PBFs are given "no destruction or adverse modification" determinations as there are no links between simazine exposure and impacts to critical habitat function. For each critical habitat containing at least one relevant PBF, we assessed the overall exposure of critical habitat to simazine, the expected impact of simazine exposure to each relevant PBF, and the expected overall impact to the conservation value of the critical habitat as a whole. We use this process to determine if a critical habitat is likely to experience destruction or adverse modification.

Exposure of Critical Habitat to Simazine

Similar to the assessment of exposure to listed species, we consider the extent of agricultural overlap, the level of past simazine usage on use sites and the resultant exposure to adjacent habitat areas, the likelihood of exposure from non-agricultural uses, and any additional exposure information pertinent to a given critical habitat (e.g., USDA Census of Agriculture all herbicide usage data, past usage from the California Department of Pesticide Regulation, specific habitat characteristics that result in higher or lower levels of simazine accumulation).

<u>Overlap</u>

Exposure to Agricultural Uses

Similar to our analysis of listed species, we use the agricultural overlap between the action area and designated critical habitat units as a metric of exposure. The EPA provided the overlap between simazine use sites and designated or proposed critical habitats (i.e., on-field overlap) and the overlap between simazine use sites buffered out to 305-meters (which is the maximum distance at which EPA determined adverse effects are likely to occur to listed species) and designated or proposed critical habitat units (i.e., off-field overlap). We determine the total overlap between critical habitat and the action area by summing the on- and off-field area overlaps for each relevant use type. Critical habitats with greater than 10% total overlap are considered to have a high level of overlap. Critical habitats with 5-10% overlap are considered to have a medium level of overlap, and critical habitats with less than 5% total overlap are considered to have a low level of overlap. This assignment of high, medium, or low to these specific percentages of overlap are used for purposes of organizing our analyses along assumptions that generally apply with all the critical habitats evaluated in this Opinion. While we generally expect critical habitats with high overlap with use sites and spray drift areas will have greater exposure to simazine, available information may indicate that general assumption does not apply to certain critical habitats. For example, even if the critical habitat of a species has a high overlap with use and spray drift sites, the Service may have information indicating that areas of critical habitat that contain or produce the necessary PBFs are not located on or near use sites.

For critical habitats designated for aquatic species, the EPA uses the HUC-12 watersheds that contain the designated critical habitat units to calculate the extent of overlap and past simazine usage. Unlike the ranges for listed aquatic species, critical habitat units for listed aquatic species are typically designated or proposed as highly refined areas demarcating specific (often small) waterbodies or smaller areas or reaches of larger waterbodies. Spray drift and runoff entering waterbodies can be transported far downstream in relatively short periods of time within watersheds, indicating that critical habitat for aquatic species can be exposed to simazine used in areas far away from the border of the designated units. Thus, the EPA calculated overlap and past usage using the broader watershed in order to better capture the potential exposure to critical habitat designated or proposed for aquatic species. As such, on-field overlap for aquatic critical habitat represents that amount of agriculture occurring within the watershed containing critical habitat. Thus, we only assess this watershed-level overlap for the critical habitats for listed aquatic species instead of separating out on-field and off-field overlap metrics like for critical habitats proposed or designated for terrestrial species as this watershed overlap metric already encompasses exposure from off-site transport.

Exposure to Non-Agricultural Uses

Similar to our assessment process for listed species, we assess exposure of critical habitat to non-agricultural uses of simazine in a qualitative manner by considering whether non-agricultural use sites are likely to contain or produce many of the PBF requirements, the life history of species, methods of pesticide application, available past usage data, and any existing conservation measures to reduce drift and runoff or otherwise limit exposure to critical habitat.

<u>Usage</u>

Similar to our analysis of listed species, we use past simazine usage data in our assessment of exposure to critical habitat. The EPA applied the level of past simazine usage, as summarized by the State Summary and Usage Matrix (SUUM) in the BE, to calculate the percent of a critical habitat that is likely treated with simazine annually. We determine the total portion of the critical habitat treated with simazine annually by aggregating the percent critical habitat treated of all non-highly redundant crop groups. Unlike in our analysis for listed species, the percent of a critical habitat likely to be treated annually is almost always the same as the percent overlap as the conservative assumptions used in the application of SUUM data coupled with the small area covered by critical habitat relative to the species' ranges often results in the suggestion of high levels of simazine usage across critical habitats.

Similar to our analysis of listed species, we use past simazine usage data in our assessment of exposure to critical habitat. The EPA applied the level of past simazine usage, as summarized by the State Summary and Usage Matrix (SUUM) in the BE, to calculate the percent of a critical habitat that is likely treated with simazine annually. We determine the total portion of the critical habitat treated with simazine annually by aggregating the percent critical habitat treated of all non-highly redundant crop groups (i.e., we sum all relevant crop type adjusted overlaps with either corn or soybean and either citrus, grapes, or other orchards in our total usage calculations)crop use data layer categories. Unlike in our analysis for listed species, the percent of a critical habitat likely to be treated annually is almost always the same as the percent overlap as the conservative assumptions used in the application of SUUM data coupled with the small area covered by critical habitat relative to the species' ranges often results in the suggestion of high levels of simazine usage across critical habitats.

Similar to our analysis of listed species occurring entirely in California, we use data from the California Department of Pesticide Regulation's California Pesticide Use Report to determine the percent of critical habitat treated annually with simazine in place of SSUM data when available and applicable. For critical habitats in California, we report the percent of critical habitat that has been treated with any pesticides, percent of critical habitat treated with any insecticide, and the percent of critical habitat treated with simazine over a 10-year period (2013-2022). The EPA also provides estimates of the average number of growers/applicators that report pesticide usage within sections containing critical habitat in that same 10-year period, which we use as a surrogate metric for the potential variability in pesticide usage over time (e.g., a large number of growers reporting pesticide usage in a section containing critical habitat indicates less variability in the total area treated each year as changes in pesticide usage of a few growers is not likely to affect the proportion of the range treated).

We evaluate total usage based on the total percent area that is likely to be treated with simazine annually. Critical habitats for which data indicate will have more than 10% area treated with simazine each year are considered to have a high level of usage. Critical habitats that will have 5-10% area treated with simazine each year are considered to have to have a medium level of usage, and critical habitats that data indicate will have less than 5% area treated with simazine each year are considered to have a low level of usage. Similar to overlap scores, this assignment of high, medium, or low usage to these specific percentages are used for purposes of organizing our analyses along assumptions that generally apply with all the critical habitats evaluated in this Opinion. While we generally expect high past usage can be indicative of increased exposure of critical habitat to simazine, available information may indicate that general assumption does not apply to certain critical habitats. For example, even in areas of high usage, the Service may have information indicating that the specific areas required by a listed species will not accumulate more than low levels of simazine (e.g., waterbodies with high flow and clearance rates).

Additional Exposure Considerations

When information on a specific species' use of critical habitat areas indicates that exposure assumptions are not likely true (e.g., for species where use sites are not likely to contain or produce many of the PBF requirements or critical habitats located in protected areas where agricultural or non-agricultural pesticide usage is not expected), we qualitatively incorporate that information into our exposure rankings. Some examples of relevant information include knowledge of a species' preferred habitat characteristics (e.g., species that only occupy waterbodies with high flow rates, species that only consume certain taxa of prey) or additional sources of usage data, such as the USDA CoA. We use the percent of a critical habitat treated with any insecticide as an additional line of evidence to characterize the level of exposure a critical habitat will experience. Given that these data are more spatially specific than usage data provided by the SUUM (with the exception of California, where data are available at a sub-county level) and covers all herbicides used (not just simazine), we consider instances where the CoA reports low levels of usage for all herbicides within a species' range as strong evidence that simazine usage is unlikely to exceed low levels of usage throughout the course of the action. When additional exposure considerations are available, we qualitatively adjust our exposure assessment to reflect this additional information as appropriate.

Adverse Effects to Critical Habitat PBFs

We characterize the expected impacts to critical habitats based on the anticipated level of adverse effects to PBFs. Our analysis of toxicity assumes critical habitats are exposed to simazine at levels estimated by EPA's environmental exposure modeling and is focused on determining the level of adverse effect expected to occur once exposure has taken place. We compare estimated concentrations of simazine in critical habitat to toxic effects reported in available toxicity studies of various taxa of organisms to determine the level of impact to relevant PBFs. We also include any additional considerations regarding a listed species' life history that provides additional context to the specific parameters that PBFs need to meet to maintain their function (e.g., how sensitive a listed species is to simazine may influence the level of impact to a water quality PBF relative to another species).

Water Quality

Critical habitats that list water quality as a relevant PBF (e.g., low levels of chemical contaminants, high quality water) are likely to experience adverse effects from the presence of simazine within waterbodies found inside critical habitat boundaries (whether through direct application or exposure through spray drift deposition or runoff). If a listed species is sensitive to chemical pollutants, exposure to simazine could result in toxic effects to individuals. Thus, the presence of simazine will likely result in adverse effects to the water quality PBF as individuals of the listed species may not be able to fully use or occupy critical habitat. The level of impact to water quality is dependent on the expected environmental concentration of simazine likely to occur in critical habitat and the sensitivity of a listed species to simazine.

We compare estimated environmental concentrations of simazine provided by the EPA to available reference toxicity data for the most appropriate surrogate taxa or species to assess the anticipated impact of simazine use on critical habitat water quality. The EPA models simazine concentrations using a variety of models to provide estimates that generally correspond with different types of waterbodies required by listed species in critical habitat (i.e., waterbodies with high flow rates, large volume waterbodies, low volume/low flow rate waterbodies) at a national scale. We use the maximum estimated environmental concentrations of simazine corresponding to the use sites that overlap with critical habitat from the most appropriate HUC-2 region from the EPA models that best correspond to the types of waterbodies relevant to the species to estimate impacts to water quality in critical habitat. We compared these estimated environmental concentrations to the most sensitive toxic endpoint reported in EPA's BE to assess the anticipated impacts to water quality in critical habitats proposed or designated for listed animal species. We compared estimated environmental concentrations to plant HC_{05} reported in EPA's BE to assess impacts to water quality in critical habitats proposed or designated for listed plant species.

We qualitatively assess the impact to the water quality PBF as high, medium, or low. In cases where the predicted level of simazine in critical habitat waterbodies would cause high levels of direct adverse effects to individuals, we anticipate high impacts to the water quality PBF. We anticipate low levels of impact in cases where predicted simazine concentrations are not likely to cause more than low levels of mortality. When a range of adverse effects are likely to occur (e.g., for species that can use habitats with a wide range of flow rates and depth profiles), we anticipate a medium level of impacts to indicate that a range of adverse effects are likely to occur and to emphasize that impacts to water quality are likely dependent on the specific areas within critical habitat where exposure occurs.

If available life history information indicates a listed aquatic species prefers a particular type of waterbody, we qualitatively adjust our assessment of adverse effects to weigh impacts to the waterbodies preferred by the species more heavily. While simazine is moderately persistent in the environment, we do anticipate water quality will recover over time as residues degrade, are taken up into the sediment, or otherwise transported out of critical habitat. The time to recovery depends on many factors (e.g., how much simazine accumulates in a waterbody, variations in temperature, flow rate and other environmental conditions, if repeated exposures are likely). We incorporate this information when available and relevant in our critical habitat determination rationales.

Host plants

Critical habitats that list a specific host plant or plants as a relevant PBF (e.g., a particular species of plant required to rear larvae, specific set of plants that adults require for foraging) are likely to experience adverse effects from the presence of simazine within critical habitat boundaries (whether through direct application or exposure through spray drift deposition or runoff). Given that simazine is an herbicide designed to kill plant pest species, we anticipate most host plants are highly sensitive to simazine exposure and are likely to be adversely affected by simazine exposure. Thus, the presence of simazine will likely result in adverse effects to the host plant PBF as the critical habitat may not contain the necessary host plants to support the species.

We qualitatively assess the anticipated impact to the host plant PBF by considering available information regarding the nature of the host plant/listed species relationship. While we broadly anticipate host plants are likely to experience high levels of adverse effects at most estimated environmental concentrations of simazine, additional factors could modify and adjust our level of anticipated impact. Information such as the number of host plants that the species can use, how common or abundant the host plant is, and where the host plant grows (e.g., directly on simazine use sites or only in off-site areas), and whether the availability of host plants is a limiting factors in the recovery of the species can all modify the anticipated level of impact to the host plant PBF.

Plant Assemblages

Many critical habitat designations list particular plant species assemblages as indicative of necessary habitat to support the recovery of a listed species. Given that simazine is an herbicide designed to control plant pest species, we anticipate most component species within a plant assemblage will experience adverse effects from simazine exposure (whether through direct application in critical habitat or through spray drift and runoff from nearby use sites). However, we do not anticipate all plants are equally sensitive to simazine as variations in size, growth pattern, natural sensitivity, recovery potential, and many other factors across different species will likely result in differential responses to simazine exposure. Thus, we anticipate a range of adverse effects to the plant assemblage PBF is likely.

We qualitatively assess the anticipated impact to the plant assemblage PBF by considering available information regarding the composition of plant assemblages required by the species, including the general taxonomic diversity of the plant assemblages, whether the species requires a series of specific species or a general type of plant community or assemblage, and the variations in features represented by the plant communities. In general, we anticipate more diverse plant assemblages (e.g., species from a variety of plant families and genera, assemblages that are a mixture of herbaceous and woody plants) are more likely to be comprised of species that have a broader range of responses and more robust to simazine exposure than communities consisting of few plant species, communities with only the same types of plants or plants from the same taxonomic groups. Similarly, we anticipate PBFs that only specify a general type of plant community (e.g., grassland prairies, old growth forests) are likely more robust to simazine exposure as we do not anticipate the listed species requires any one particular sensitive plant species and we do not anticipate simazine residues are likely to completely destroy an entire plant community at estimated environmental concentrations.

Plant Functions

Many critical habitat designations list plants and their specific functions relative to the listed species as a necessary feature that supports the recovery of a listed species rather than discuss any specific plant species. Common plant functions listed in PBFs include herbaceous plants or tall grasses for cover from predators, plants to support the necessary prey base for the listed species, plants that serve as food for listed species, and plants that serve as other critical habitat-specific features, such as substrate for egg laying. We anticipate plant function PBFs will experience adverse effects with exposure to simazine residues in critical habitat as simazine is specifically designed to target plant species. However, we do not anticipate all plant function PBFs are equally sensitive to simazine exposure and expect that there will be a range of adverse effects likely to occur that will vary across critical habitats.

We qualitatively assess the anticipated impact to plant function PBFs by considering available information regarding the nature of the plant function in critical habitat (i.e., what specific function are the plants serving?), whether only a single (or a select few) species or an entire plant community is required to fulfill the specified function, and what habitats the needed plants occupy, among other potentially relevant critical habitat-specific factors. For instance, plant function PBFs that specify the presence of plants as a structure component of habitat (e.g., physical structure to act as nesting sites, substrate for eggs) are more robust to simazine exposure than other plant functions as we do not anticipate simazine exposure will decrease the availability of existing, large, woody plants to provide this type of habitat function. In contrast, plant function PBFs that are focused on food availability may be more sensitive to simazine exposure as small changes in the availability of these plants may immediately impact the listed species' ability to use critical habitat. Similarly, critical habitats that specify a necessary plant function that can be fulfilled by a variety of plant species are likely more robust to simazine exposure as we anticipate there will be a natural variation in sensitivity to simazine across plant species, and that more plants that can fulfill a particular function would indicate a greater likelihood that the critical habitat could still provide a particular function for the listed species despite adverse effects to one or two sensitive plant species. Plants in certain habitats are likely to experience higher exposure concentrations than others, such as plants in non-flowing wetlands. Thus, available information on the types of habitats where these plant functions are required may impact the overall assessment of effects to the PBF.

Animals as Prey, Pollinators/Seed Dispersers, and Host Fish

Critical habitats that list animal species as a relevant PBF (e.g., mollusk and annelid prey, vertebrate prey, fish hosts) are likely to experience adverse effects from simazine exposure. Available toxicity data indicate that simazine is practically non-toxic to terrestrial insect species. As such, we generally do not include insects in this PBF assessment as we do not anticipate any level of simazine exposure in critical habitat is likely to impact insect prey, pollinator, or seed disperser availability. If a listed species is highly dependent on an animal species that is sensitive to simazine at estimated environmental concentrations, then the presence of simazine within critical habitat will result in high levels adverse effects to the non-arthropod PBF as individuals of the listed species may not have the necessary prey or host fish resources necessary for survival and recovery. The overall impact of simazine to non-arthropod prey will vary greatly between the different taxa included in this PBF category.

Our assessment of impacts to the animal PBF is based on available information regarding what types of animals are required, the nature of a listed species' relationship with its required animals, and where within critical habitat (relative to simazine use sites) exposure is likely to occur. We compare estimated environmental concentrations generated by the EPA to available toxicity data provided in the BE to determine the overall effect to the non-arthropod PBF. Based on available toxicity data, we generally do not anticipate animal species are likely to die with exposure to simazine at estimated environmental concentrations. However, we anticipate animals are likely to experience a range of sublethal adverse effects with exposure to simazine. Aquatic non-mollusk invertebrates, fish, and aquatic-phase amphibians are likely to experience adverse effects to growth. Mollusks are not likely to experience any sublethal effects at estimated environmental concentrations. Terrestrial vertebrates are likely to experience adverse effects to both growth and reproduction, but only when individuals are exposed directly on simazine use sites. Terrestrial vertebrate species exposed to simazine in areas adjacent to use sites (e.g., areas only exposed through spray drift and runoff) are not likely to experience any direct adverse effects. We anticipate critical habitats with animal PBFs that specify very specific animal species are more sensitive to simazine exposure as impacts to a small number of animal species may result in large impacts in critical habitat's ability to support its listed species. For example, listed bivalve species who require a singly or a select few fish species to act as hosts for their larvae or listed plants that rely on a specific species of bird or bat for pollination and seed dispersal, may experience more severe impacts from simazine exposure as reductions in the availability of just one or two species can severely impact the listed species that are dependent on these animals. In contrast, critical habitats with animal PBFs that describe a general resource, such as a general fish assemblage to act as hosts for a listed bivalve's larvae or a general prey base of species across numerous taxa, are likely to be more robust to simazine exposure as we anticipate a wider range of sensitivities across more animal species, which results in a higher likelihood that there will still be sufficient numbers of required animals to support the listed species despite potential impacts to a few sensitive species.

Critical Habitat Determinations

To determine the overall impact of the proposed action to designated or proposed critical habitat, we assess the anticipated impacts to each relevant PBF alongside the anticipated level of exposure to determine both the overall adverse effect of simazine exposure and the footprint of the anticipated adverse effect across the entire critical habitat. Our results can be found in Appendix D. To streamline our discussion in this Opinion, we group critical habitats that have the same or very similar rationales for their conclusions to increase efficiency and avoid repetition. We considered relevant information and data unique to each individual species when assigning species to groups, which we incorporated into the rationales as appropriate. Species- and critical habitat-specific information (e.g., environmental baseline, cumulative effects, status of the critical habitat, exposure, and toxicity) was considered for all critical habitats, including those in the grouped analyses, and are presented in full in Appendices B and E. In cases where a combination of rankings and additional considerations provides a clear narrative for a determination that the proposed action is not likely to destroy or adversely modify a critical habitat, we provide a group rationale that outlines how the combination of species vulnerability, critical habitat exposure, anticipated effects to PBFs, and additional considerations result in this conclusion for all relevant critical habitats. Within these grouped rationales, we add additional information, when relevant, to support our conclusions. We review each grouped rationale to ensure that all assumptions made are applicable for each critical habitat within the group and are expected to result in a similar determination for each critical habitat. We do not include any critical habitats in the grouped rationales when certain assumptions upon which the grouping is based are not applicable, additional information is required to make an adverse modification determination, or unique circumstances are otherwise present that warrant additional discussion elsewhere in the Opinion. For those critical habitats, we provide an additional discussion for this specific designation to convey the necessary details supporting our determination. For instance, we did not include critical habitats in grouped rationales when we determined that CalPUR data did not have a sufficient sample size for the respective critical habitat in order for us to confidently conclude that exposure was unlikely to occur. In other cases, we had information suggesting that there may be impacts from simazine to certain areas of a designation that are biologically significant to the species, even if there is low overlap between critical habitat and the action area or if data from the Census of Agriculture indicated low levels of past usage. These critical habitats have an individualized discussion in Appendix D.

Assumptions and Uncertainties for All Species in this Consultation

There are many uncertainties and assumptions that accompany an analysis of this size and scope. The manner in which chemicals can move through the environment and interact with other biotic and non-biotic stressors is highly complex and necessitates that we focus our analysis on those factors that are identifiable, reasonably predictable, likely to influence whether species are affected, and for which we have data to characterize those effects. As such, we have made assumptions about certain elements of the analysis for which we have limited abilities to address directly due to lack of relevant data or appropriate models. In all circumstances, we based our ESA section 7 analyses on the best scientific and commercial data available.

Below we identify several assumptions and uncertainties we have considered in our analysis for the overall approach, as well as specific to the effects analyses. In some instances, we are aware that certain assumptions, when considered alone, may under-predict effects to listed species. However, by using conservative assumptions in other areas that may overestimate effects in some instances, we expect that we are capturing the overall breadth of effects to species and critical habitat in evaluating whether EPA's action is likely to jeopardize listed species or destroy or adversely modify critical habitat. For example, we lack data to quantitatively assess the effects of simazine to individual species in combination with other stressors in the environment (e.g., temperature, other chemicals; exposure to multiple stressors). However, by making conservative assumptions about exposure to simazine at maximum environmental concentrations and looking at the full extent of lethal and sublethal effects, we expect that we are capturing the breadth of effects to species, including those that may manifest at sub-maximal concentrations, but in combination with other environmental stressors. In some cases, we are unable to predict whether individual assumptions will under- or over-predict effects to listed species and critical habitats. Overall, we expect that when considered together, the assumptions we have made are based upon the best scientific and commercial data available, capture the magnitude and extent of the effects of the action, and are otherwise consistent with the ESA and its implementing regulations.

Surrogate Data

In the *General Effects* section, we briefly discuss how we used toxicity data to analyze effects to listed species. Very few listed species have toxicity data specifically addressing effects from simazine. We therefore discuss toxicity data that are available for the taxa groups and the decision process we employed to arrive at the toxicity values we use for our effects analyses. Where toxicity data are lacking, such as for reptiles and amphibians, we discuss the use of toxicity data from other taxonomic groups in the *Effects to Reptiles*, *Effects to Terrestrial Amphibians, and Effects to Fish and Aquatic-Phase Amphibians* sections. More specifically, we use fish and bird data for aquatic and terrestrial amphibians, respectively and bird data for reptiles.

For aquatic plants, toxicity data are reported as mg a.i./L, which are differing units from how terrestrial plant toxicity data are provided (lbs a.i./acre). Aquatic plant toxicity data are most often based on studies on non-vascular algae which may or may not be applicable to listed aquatic vascular plants to assess effects. For many plants, often the only correlation between tested species and the listed species is that they share a seed growth mechanism, such as if both the listed and test species are dicots. However, there are several listed ferns and other allies, conifers/cycads, and some lichens that would not be comparable to any tested species, and we use available toxicity data from dicot species for these non-flowering plants.

In addition, there are several data gaps for basic biology for plant and animal species covered under this consultation that add additional complexity to this analysis. For example, there is often little to no available data regarding different types of effects (e.g., sub-lethal, effects to prey base, effects to pollinators, direct impacts to flowering plants) of pesticides on species that are rare, highly specialized, and occur in specialized habitats. The toxicity data we have chosen to use, and have discussed in depth in the general effects to taxa sections, is the best available information we have regarding the impacts of this herbicide to listed species. These data often represent one or more species within a taxa group that are applied to all species within that taxa group (e.g., honey bee toxicity data to address effects to all insects) or a taxa group for which data are lacking (e.g., fish toxicity data to address effects to aquatic-phase amphibians).

Estimated Environmental Concentrations

For the analysis of the effects of simazine to different taxonomic groups in this Opinion, we assume that individuals will be exposed to the modeled annual maximum pesticide concentrations. We use the maximum EECs in our Integration and Synthesis analyses to address the effects to species in the most conservative way that captures the breadth of effects to species or critical habitat, though where adequate information exists to assess the likelihood of exposure to maximum EECs, we present that information for individual species or groups of species. We assess effects based on a single exposure of simazine, when, in reality, individuals may be exposed more than one time to concentrations that could cause effects; thus, this assumption may also underestimate effects.

Specifically for aquatic species, exposures are based on pesticide crop use scenarios that generate the highest EECs, which also may overestimate effects. We assume that individuals will be exposed to a range of modeled annual maximum pesticide concentrations for a species that inhabits higher flow/volume waterbodies or if they inhabit low flow/volume waterbodies or both, the range of EECs always provides for a conservative assumption for the concentrations of the pesticide in the given waterbody. We do not expect listed aquatic species will occur on-field, and thus expect exposure will only result from off-field transport via spray drift or runoff. Given that the ranges for listed aquatic species are generally delineated using the relevant HUC 12 watersheds, we anticipate that all residues that leave use sites will be collected in the waterbodies within the species range where individuals occur regardless of how residues leave treated sites or where in the range they are deposited. As such, we do not extend overlap metrics off-field as the species' range already incorporates off-field areas and any off-site transport of simazine into the species' habitat is captured by the 0-m overlap. For aquatic species, distribution within aquatic habitats is assessed based on very generic habitat flow volumes and rates and may over- or underestimate exposures to listed fishes, crustaceans, aquatic insects, aquatic snails, and mussels.

This Opinion operates on the assumption that all use sites will be treated at the same time, and all individual members of a listed species within the use overlap will be exposed to peak applications, once a year. In reality, we do not expect all use sites will be treated at the same time, resulting in every individual member of a species that overlaps the area being exposed to peak applications and, therefore, we acknowledge this approach will overestimate exposure. On the other hand, some areas may have additional peak events occurring in a year, and, therefore, the above assumption may underestimate exposure. The assumption that use area represents where a given pesticide will be applied, for a small ranging species, may over- or underestimate the exposure. The assumption that the use scenario generating the highest combined application rates should represent exposures resulting from a given UDL (e.g., Vegetables and Ground Fruit) may overestimate effects. These assumptions vary in whether they over or underestimate exposures depending on the analysis being done. However, overall, our analysis in this Opinion contains reasonable assumptions in determining whether the proposed action is likely to jeopardize listed species or destroy or adversely modify critical habitat.

Species-specific Information

Where more life history information was available for a species, it allowed us to make fewer assumptions about how the species may be exposed to simazine. Specifically, knowledge of the types of habitats used by individuals of a species and their tendency to be found near and within use sites allowed us to better predict whether individuals would be exposed to simazine and, if so, the magnitude of that exposure. However, the extent of this information, and our ability to project the likelihood of exposure in this manner varied across species. This lack of information could result in an overestimation or underestimation.

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

Effects to Critical Habitat

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

Species Range Maps

One of the main uncertainties within the analysis for this consultation is the reliance on current ranges for each species that may not accurately reflect the species' actual distribution within those mapped ranges. Often these ranges are defined as entire counties or smaller subunits (e.g., quads, HUCs) within which the species is known to occur but do not identify actual areas of suitable habitat where the species is likely to be found. Through internal Service efforts to refine species ranges, we actively work to continuously refine and improve many of the existing current range maps, either by reducing the number of overall counties or by mapping at a sub-county level (e.g., by habitat associations for some plants), based on the best scientific and commercial data available at the time. However, even refined range maps may include areas not specific to species' habitat requirements.

Without detailed information on where a species can be found, our assumption for this assessment is that each species analyzed is uniformly distributed within its range. This may overestimate or underestimate our understanding of where a species is found. Exceptions to this assumption were for species where information is known based on specific data from Service Recovery Plans or 5-Year Reviews, or from Service species leads directly (e.g., Moapa dace, Lange's metalmark butterfly, least Bell's vireo). Some species will have information where specific segments of the range have been identified for recovery, for critical habitat, or for other specified uses, and the locations of populations of the species are known within these areas.

Use sites

For terrestrial and aquatic species, we assume the GIS information we have for all simazine use sites is accurately represented within the species' range because this is the best information available to us. This may over or underestimate the presence of use sites.

Crop Sequence Boundary Data

With the use of GIS data that are raster based, there has always been the question of how certain pixels may potentially misrepresent agricultural areas (spurious pixels; see further discussion in the *Action Area* Section of this Opinion) within the range of listed species or critical habitats. This can lead to over or under predicting the extent of overlap of use sites within a species range or critical habitat. The Service and EPA are working together to assess the usefulness of addressing this issue with utilizing the USDA's crops sequence boundary data layer. These data produce estimates of field boundaries, crop acreage, and crop rotations across CONUS. It uses satellite imagery with other public data to conduct area and statistical analysis of planted U.S. commodities[34]using polygon-based GIS data. The Service and EPA are determining if and how CSB based UDLs can be used to support ESA assessments of pesticides.

EPA and the Service evaluated this method in an on-going effort utilizing ground truthing tools (e.g., aerial photography) and find it to be a promising tool providing increased accuracy to minimize under or over representing agricultural areas within a species range or critical habitat. In general, utilization of the CSB data results in more accurate representation of agricultural land uses and improved resolution of field-level remote sensing compared to the CDL data and greatly diminishes the "noise" of spurious pixels in the raster CDLs.

[34]https://www.nass.usda.gov/Research_and_Science/Crop-Sequence-Boundaries/index.php

Pesticide Usage Information

Pesticide usage data is derived from a variety of sources that inherently vary with respect to the reliability, accuracy, and specificity of the data being reported. We assume these data may over- or underestimate the actual pesticide usage based on the source. Kynetec agricultural data may over- or underestimate actual usage due to the methodology behind how these data are collected, how they are applied within a given state where a crop may be grown, and how they are statistically analyzed. The Census of Agriculture data includes all pesticides that are either herbicides or insecticides, as appropriate, and thus inherently overestimates usage for most individual pesticides. However, this information is available at the county level and thus provides more geographic specificity than state-level data; thus we consider this a reliable upper-limit of exposure for an individual active ingredient. The California pesticide use reporting data from California's pesticide use reporting program is a very comprehensive pesticide usage database (CDPR 2020). Under the program, all agricultural pesticide use must be reported monthly, and all agricultural uses can be evaluated on a scale as precise as a county-township range section (a section being a land unit which constitutes one square mile or 2.6 square kilometers, containing 640 acres) and as broad as the county level. These data are generally very reliable, but even section-level analysis may include areas that are not within the species' range, and uncertainties in the reporting exist. As such, while we have greater confidence in these data, we acknowledge that it may still over- or under-estimate exposure to listed species.

Spray Drift Effects

Spray drift is a primary route of offsite transport of pesticides when applied to use areas. For all species, spray drift will increase the area of overlap with the species range and is particularly important for species that are not anticipated to enter use sites (i.e., plants), as it may represent the only exposure to simazine that is likely to occur. However, it is important to note that spray drift areas and areas for different uses can overlap with one another, depending on their proximity on the landscape. For this reason, combining areas from different uses where spray drift exposure could occur without accounting for this proximity is likely to overestimate the total overlap with the species range.

Endocrine Disrupting Compounds

As discussed previously in the *Evaluating the effects of endocrine disrupting chemicals* section of this Opinion, endocrine disrupting compounds or EDCs are chemicals that bind to hormone receptors inside of an organism's cells and can interfere with hormone signaling, eliciting changes in hormone production, affecting sex steroids, thyroid hormones and neurotransmitters. . For our analysis of effects to listed species, we rely on growth and reproduction endpoints, which are commonly used to assess the deleterious consequences of the molecular effects of endocrine disrupting compounds on listed species. We acknowledge that there is uncertainty in the use of growth and reproductive endpoints to capture the molecular effects of endocrine disrupting compounds, and in doing this, may underestimate or overestimate effects to listed species.

Many of the uncertainties previously discussed regarding surrogacy or extrapolation from field studies also apply to EDCs. For examples, the use of growth and reproductive endpoints as measured in a laboratory may not capture certain effects that are not measured in these studies or cannot be fully evaluated in a laboratory environment, such as immunosuppression or altered sex ratios. While our analysis may under-estimate effects by not accounting for these endpoints, we do not currently have adequate data to link many sublethal responses to individual or population level effects. Individuals may compensate for molecular-level effects at the cellular, organismal, or behavioral level with ultimately no detectable effects to fitness. Or, conversely, these responses may manifest in response to stressors that individuals face in their natural habitat and result in effects of a higher magnitude than reported. For all effects, there is inherent intra-species variation to any stressor in the environment, and as such, one individual's response within a population may differ from another individual's response. For any endpoint, scaling effects to population level impacts can be highly nuanced and influenced by environmental factors and species life history.

Evaluation of EDCs may also introduce novel uncertainties to our analysis. Use of growth and reproduction endpoints may under-estimate effects to species by not capturing potential effects to populations on a long-term scale. There is some evidence that exposure to low levels of EDCs can impact populations for generations through maternal offloading to offspring, exposure to developing oocytes, or from offspring inheriting genetic or molecular (i.e., epigenetic) modifications induced by initial exposure that occurred in a previous generation (Major, et al. 2020). These long-term, multigenerational or transgenerational effects suggest that EDC exposure could have lasting impacts on population of exposed organisms; however, it is unknown if organisms will be able to adapt to the effects of EDC exposure over time or if effects will become additive over generations.

However, the use of growth and reproductive endpoints may also over-estimate effects of EDCs. Studies that test these endpoints often rely on chronic, repeated exposure to a chemical over periods of time (e.g., days, weeks, or months) that may not be representative of an individual's exposure in the wild. Factors such as degradation of the pesticide, movement of individuals across landscapes, or opportunistic use of available food sources may result in exposure to pesticides that are variable and, at times, below the levels of sustained exposure in toxicity tests. As such, toxicity testing may aggregate multiple effects to an organism that may result from this long-term repeated exposure, leading to a greater effect than would occur from a shorter-term exposure or individual responses. Using growth and reproductive endpoints can also overestimate effects by failing to recognize an individual or population's ability to compensate for these effects. We consider this information for individual species, however, acknowledging where appropriate that listed species may be particularly vulnerable to environmental stressors and less able to withstand perturbations than a more robust population.

In general, we acknowledge the uncertainty in using growth and reproductive endpoints, while also acknowledging that we currently lack data to assess the linkage of these responses directly to an individual listed species or to a listed species population level response. Furthermore, other environmental factors and stressors (including other EDCs) are also likely influencing the same molecular and physiological systems impacted by an EDC. Co-exposure between EDCs and other stressors can result in complex interactions (including magnification of endocrine disrupting effects or attenuating/counteracting effects on endocrine systems), further limiting our ability to assess the impacts of a single EDC, in isolation, to a population or a species. As such, reliance on these data may over- or under- predict the scope of the endocrine disrupting effects of simazine to listed species.

Summary

We acknowledge that many of the assumptions we have made in this analysis have the potential to under- or overestimate the extent of effects to listed resources. However, we have provided an explanation of why we made the assumptions and addressed uncertainties and have endeavored to clarify and frame our assumptions to adequately support our understanding of the effects of the action below provides a summary of our main assumptions and uncertainties, including whether there is an underestimate, overestimate, or an unknown risk of overestimating or underestimating effects to the species associated with each. However, overall, our analysis in this Opinion contains reasonable assumptions in determining whether the proposed action is likely to jeopardize listed species or destroy or adversely modify critical habitat.

Table 18. Assumptions and Uncertainties for the Effects Analysis.[35]

Assumptions and Uncertainties_1.xlsx

CONCLUSION

Our draft Opinion considers 696 species (see individual taxa/group tables in the *Integration and Synthesis* section of this Opinion) and finds that the proposed registration that is being reviewed for simazine is not likely to jeopardize the continued existence of 665 species. Of these, 30 species are subject to conference opinions (19 are proposed endangered and 11 are proposed threatened). These species that are not likely to be jeopardized by the proposed action have vulnerabilities ranging from low to high, represented by a single, few or many populations, with populations that may be declining, stable or increasing. While most listed species have isolated and fragmented populations, some of these species are generally less vulnerable to overall threats. We expect these species to experience low levels of direct and indirect adverse effects due to 1) conservation measures incorporated into the action to reduce off-site transport from spray drift or runoff, 2) lower overlaps with use sites and lower levels of past simazine usage, indicating only small numbers of individuals are likely to be exposed, or 3) low toxicological response to simazine. While we do anticipate that for many species, a small number of individuals are likely to experience reduced growth or reproduction, or have a reduction in food

[35] To view the spreadsheet in MS Excel from the MS Word Opinion, double click on the Excel icon. To view the spreadsheet in MS Excel from the portable document format (pdf) Opinion, open the Opinion in the desktop version of Adobe Acrobat or Reader and open the embedded attachment corresponding to the numbered table.

resources, we do not anticipate species-level adverse effects, and, therefore, we do not anticipate that the registration of simazine is likely to jeopardize the continued existence of these species.

We have identified 32 species that require further coordination before issuance of our final Opinion. These species generally have high vulnerabilities (e.g., they are represented by a single or a few populations, their populations are declining, populations are small or isolated and fragmented across their range) and may be subject to more than low levels of adverse effects from simazine exposure, even after incorporating conservation measures into the action that aim to reduce off-site transport. Further analysis is required to determine the extent of effects, if any, and the resultant risk to these listed species. We anticipate that individuals exposed on use sites or from concentrations in water will experience adverse effects to growth, reproduction or survival depending on their taxonomic group and level of exposure. We intend to continue coordinating with EPA and simazine registrants between the release of this draft Opinion and the transmission of the final Opinion to gain information regarding the exposure and effects of each of these species to simazine.

Destruction or adverse modification means a direct or indirect alteration that appreciably diminishes the value of critical habitat as a whole for the conservation of a listed species. Through this consultation, we determined pertinent elements of the PBFs of proposed and designated critical habitats that are susceptible to effects from simazine. These elements fall within the following categories: (1) water quality, (2) host plants, (3) plant assemblages, (4) plant functions, and (5) animals, including prey, pollinators, seed dispersers, and host fish. The degree to which these PBFs would be affected by simazine and the consequences for each critical habitat was evaluated, and our assessments and conclusions are included in Appendix D.

The Opinion covers designated or proposed critical habitats for 265 species (see individual taxa/group tables in the *Integration and Synthesis* section of this Opinion). Our draft Opinion finds that the proposed registration of simazine is not likely to destroy or adversely modify 252 proposed or designated critical habitats. Based on the critical habitat analysis described above, although some adverse effects are anticipated for certain critical habitats, we do not anticipate that the proposed action would adversely impact these critical habitats to a level that would appreciably diminish the value of those critical habitats for the conservation of their respective species, as a whole. Therefore, it is the Opinion of the Service that the proposed action is not likely to result in the destruction or adverse modification of these critical habitats.

We have identified 13 critical habitats that require further coordination before issuance of our final Opinion. Based on the critical habitat analysis described above and presented in Appendix D, adverse effects are anticipated for some critical habitats. These critical habitats may be subject to more than low levels of adverse effects from simazine exposure, even after incorporating conservation measures into the action that aim to reduce off-site transport. We anticipate that PBFs exposed on use sites or from concentrations in water will experience adverse effects to growth, reproduction or survival depending on their taxonomic group and level of exposure. However, further analysis is required to determine the extent of effects, if any, and the resultant risk to these critical habitats. We intend to continue coordinating with EPA and simazine registrants between the release of this draft Opinion and the transmission of the final Opinion to gain information regarding the exposure and effects of each of these critical habitats to simazine.

INCIDENTAL TAKE STATEMENT

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

AMOUNT OR EXTENT OF TAKE

We are deferring to a finer-scale description of the amount or extent of take, as well as any related Reasonable and Prudent Measures and Terms and Conditions, until we finalize the biological opinion, pending further coordination with the EPA and registrants. We generally described the types of anticipated incidental take in the *Integration and Synthesis* section and its appendices and our *Conclusion* section above. Briefly, we anticipate the proposed action will result in sublethal effects (i.e., impacts to growth or reproduction) to individual animals, the numbers of which will vary by species. Some species will also experience impacts to their prey or forage base, or to other species or habitat on which they depend, which will impact their growth, reproduction, and/or survival. As with mortality and sublethal effects, the numbers of individuals affected by impacts to their prey or forage base and the anticipated degree of such effects will also vary by species.

CONFERENCE REPORT

CONFERENCING ON PROPOSED AND CANDIDATE SPECIES AND PROPOSED CRITICAL HABITAT

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

In this case, because the duration of the proposed action is 15 years, the Agencies agreed it would be prudent to use this opportunity for EPA to conference with the Service on the effects to species that are proposed for listing and critical habitats proposed for designation. By conferencing now, any future consultation required under 7(a)(2) when a species listing or critical habitat designation is finalized may be streamlined, and in some cases, conferences can satisfy the consultation requirements under 7(a)(2). Using this approach, in this conference, we found the proposed action is not likely to jeopardize most proposed species or result in the destruction or adverse modification of most proposed critical habitat designations that were analyzed in this conference opinion. For some proposed species and proposed critical habitats, we intend to continue coordination with EPA and simazine registrants between the draft Opinion and transmission of the final Opinion.

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

CONSERVATION RECOMMENDATIONS

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

EPA's implementation of the following conservation recommendations would provide information and support for future consultations involving upcoming FIFRA registrations authorizing use of pesticide active ingredients that may affect ESA-listed species and critical habitats:

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

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

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

4. Work with other appropriate federal, state, and local partners to study the efficacy of conservation practices in reducing pesticide loading to streams, lakes, wetlands, sinkholes, and other terrestrial and aquatic habitats from off-site transport. Topics may include the width, structure and complexity of buffer strips, swales, riparian areas, other vegetation types, use of in-field native vegetation buffers and cover crops, precision agriculture technologies and other strategies that have the potential to reduce adverse impacts to listed species.

5. Develop methods and models that better describe and quantify pesticide persistence and fate and transport to assist in analyses for future pesticide consultations. For example, models may be used to better quantify pesticide persistence in freshwater and terrestrial environments that correlate to mortality or sublethal effects. Similarly, improving capabilities to model pesticide fate and transport at the watershed scale would help to inform future analyses.

6. Develop methods to better understand and quantify pesticide exposure from non-agricultural uses.

7. Develop criteria that address when pesticide-contaminated sediment is an important route of exposure to aquatic or terrestrial organisms.

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

9. Work with stakeholders and other interested parties to develop conservation guidelines.

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

REINITIATION NOTICE

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

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