Risks of Prometryn Use to Federally Threatened California Red-legged Frog
(*Rana aurora draytonii*)

Pesticide Effects Determination

Environmental Fate and Effects Division
Office of Pesticide Programs
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Primary Authors:
Jim Hetrick Ph.D., Senior Scientist
Silvia Termes Ph.D, Chemist
Tanja Crk, Biologist
Stephanie Syslo, Environmental Scientist/RAPL

Secondary Review:

Branch Chief, Environmental Risk Assessment Branch 3: Dana Spatz

Table of Contents

List of Tables

List of Figures

List of Appendices

1.0 Executive Summary

The purpose of this assessment is to evaluate potential direct and indirect effects on the California red-legged frog *(Rana aurora draytonii*) (CRLF) arising from Federal Insecticide, Fungicide, Rodenticide Act (FIFRA) regulatory actions regarding use of prometryn on agricultural sites. In addition, this assessment evaluates whether these actions can be expected to result in modification of the species' designated critical habitat. This assessment was completed in accordance with the U.S. Fish and Wildlife Service (U.S. FWS) and National Marine Fisheries Service (NMFS) *Endangered Species Consultation Handbook* (U.S. FWS/NMFS 1998) and procedures outlined in the Agency's Overview Document (U.S. EPA 2004).

The CRLF was listed as a threatened species by U.S. FWS in 1996. The species is endemic to California and Baja California (Mexico) and inhabits both coastal and interior mountain ranges. A total of 243 streams or drainages are believed to be currently occupied by the species, with the greatest numbers in Monterey, San Luis Obispo, and Santa Barbara counties (U.S. FWS 1996) in California.

Prometryn is a symmetric triazine (*s*-triazine) herbicide. Formulation types registered include liquids and wettable granules; therefore, all formulations are designed for liquid application. Currently, labeled uses of prometryn include uses on celery, fennel, dill, parsley, and cotton. These uses are considered as part of the federal action evaluated in this assessment.

Prometryn is stable to abiotic hydrolysis and direct photolysis in water and soil. Biotransformation has been identified as a major dissipation process, albeit slow. There are no data on the persistence, transformation, or partitioning in water-sediment systems. It is very mobile to mobile in soils (average K_{oc} = 244 L/kg-OC). Based on the value of the vapor pressure and Henry's Law Constant it is not expected to volatilize from soil or water. The low *n*-octanol-water partition coefficient (Log $K_{ow} < 4$) of prometryn suggests low bioaccumulation potential. The major biotransformation product is a hydroxy-triazine that is potentially a biotransformation product in common with hydroxyl degradates of other *s*-triazine herbicides. Based on the persistence and mobility of prometryn, major routes of transport are likely to be runoff to surface water bodies or to adjacent fields and leaching to ground water. Prometryn could also reach non-target sites by drift and/or wind erosion. Water monitoring data from California has identified that the concentrations of prometryn in surface water range from 0.0054 to 0.621 µg/L. Although prometryn has been detected in rain and wet deposition, the vapor pressure suggests low potential for volatility.

Since CRLFs exist within aquatic and terrestrial habitats, exposure of the CRLF, its prey, and its habitats to prometryn are assessed separately for the two habitats. The PRZM/EXAMS aquatic exposure model was used to estimate high-end exposures of prometryn in aquatic habitats resulting from runoff and spray drift from different uses. The 1-in-10-year peak model-estimated environmental concentrations (EECs) for selected CA scenarios range from 37.6 to 377.3 µg/L. This residue accumulation is the result of high persistence of prometryn in conjunction with the static hydrology (no flow) of the standard pond. These estimates are supplemented with analysis of available California surface water monitoring data from U. S. Geological Survey's National Water Quality Assessment (NAWQA) program and the California Department of Pesticide Regulation. The maximum concentration of prometryn reported by NAWQA for California surface waters with agricultural watersheds is 0.621µg/L. This value is approximately 607 times *less than* the maximum model-estimated environmental concentration.

The T-REX model is used to estimate prometryn exposures to the terrestrial-phase CRLF and its potential prey resulting from uses involving prometryn applications. The AgDRIFT model is also used to estimate deposition of prometryn on terrestrial and aquatic habitats from spray drift. The TerrPlant model is used to estimate prometryn exposures to terrestrial-phase CRLF habitat, including plants inhabiting semi-aquatic and dry areas, resulting from uses involving foliar prometryn applications.

The effects determination assessment endpoints for the CRLF include direct toxic effects on the survival, reproduction, and growth of the CRLF itself, as well as indirect effects, such as reduction of the prey base or modification of its habitat. Direct effects to the CRLF in the aquatic habitat are based on toxicity information for freshwater fish, which are generally used as a surrogate for aquatic-phase amphibians. In the terrestrial habitat, direct effects are based on toxicity information for birds, which are used as a surrogate for terrestrial-phase amphibians. Given that the CRLF's prey items and designated critical habitat requirements in the aquatic habitat are dependant on the availability of freshwater aquatic invertebrates and aquatic plants, toxicity information for these taxonomic groups is also discussed. In the terrestrial habitat, indirect effects due to depletion of prey are assessed by considering effects to terrestrial insects, small terrestrial mammals, and frogs. Indirect effects due to effects to the terrestrial habitat are characterized by available data for terrestrial monocots and dicots.

Degradation products of prometryn in soil metabolism studies are 2,4-bis(isopropylamino)-6-hydroxy-s-triazine (CS-11526) at 27% of the applied radioactivity after one year post-treatment and 2-amino-4-isopropylamino-6-methylthio-s-triazine (GS-11354) at less than 10% after one year post-treatment. There are no available ecotoxicity data and limited environmental fate data for these degradation products. Based on EPA's human health assessment conducted to support the prometryn RED (Wassell 1998), the only residue of concern is prometryn. Therefore, the degradation products of prometryn are not considered in this assessment.

Risk quotients (RQs) are derived as quantitative estimates of potential high-end risk. Acute and chronic RQs are compared to the Agency's levels of concern (LOCs) to identify instances where prometryn use within the action area has the potential to adversely affect the CRLF and its designated critical habitat via direct effects or indirectly via direct effects to its food supply (i.e., freshwater invertebrates, algae, fish, frogs, terrestrial invertebrates, and mammals) or habitat (i.e., aquatic plants and terrestrial upland and riparian vegetation). When RQs for each particular type of effect are below

LOCs, the pesticide is determined to have “no effect” on the CRLF. Where RQs exceed LOCs, a potential to cause adverse effects is identified, leading to a conclusion of “may affect.” If a determination is made that use of prometryn use within the action area “may affect” the CRLF or its designated critical habitat, additional information is considered to refine the potential for exposure and effects, and the best available information is used to distinguish those actions that “may affect, but are not likely to adversely affect” (NLAA) from those actions that are “likely to adversely affect” (LAA) the CRLF. Similarly for critical habitat, additional information is considered to refine the potential for exposure and effects to distinguish those actions that do or do not result in effects to its critical habitat.

Based on the best available information, the Agency makes a Likely to Adversely Affect determination for the CRLF from the use of prometryn. Additionally, the Agency has determined that there is a potential for effects to the CRLF designated critical habitat from the use of the chemical. A summary of the risk conclusions and effects determinations for the CRLF and its critical habitat is presented in **Table 1-1** and **Table 1-2** . Use-specific determinations for direct and indirect effects to the CRLF are provided in
Table 1-3 and **Table 1-4**. Further information on the results of the effects determination is included as part of the Risk Description in Section 5.2. Given the LAA determination for the CRLF and potential effects to designated critical habitat, a description of the baseline status and cumulative effects for the CRLF is provided in **Attachment 2**.

Table 1-1 Effects Determination Summary for Prometryn Use and the CRLF

Assessment Endpoint	Effects Determination [1]	Basis for Determination
Survival, growth, and/or reproduction of CRLF individuals	LAA[1]	**Potential for Direct Effects** ***Aquatic-phase (Eggs, Larvae, and Adults)***: Acute risk to freshwater fish LOCs are exceeded for all parsley uses (Spring, Fall: 2 lbs a.i./A) and most cotton uses (Fall, pre-emergence ground spray and pre-emergence aerial spray: 2.4 lbs a.i./A). No chronic risk LOCs are exceeded based on the most sensitive toxicity data for freshwater fish across any of the evaluated uses (Table 5-1). ***Terrestrial-phase (Juveniles and Adults)***: Prometryn is practically non-toxic to birds on an acute oral and sub-acute dietary exposure basis and as such, the studies did not provide definitive toxicity endpoints. No mortality was observed in the acute oral study and only a single mortality was observed in the sub-acute dietary toxicity study and the mortality was not considered treatment-related. There are no chronic risk LOC exceedances for birds consuming small insects at the maximum annual application rate for prometryn (2.4 lbs a.i./A to cotton) in T-REX using dietary-based RQs.

Assessment Endpoint	Effects Determination [1]	Basis for Determination
		Potential for Indirect Effects
		Aquatic prey items, aquatic habitat, cover and/or primary productivity Risk to aquatic plant LOC exceedances are observed for non-vascular and vascular aquatic plants for all uses. For a complete list see Tables 5-2, 5-4. Acute risk LOC exceedances are not observed for freshwater invertebrates. The same finding applies here for fish and frog prey items as in the direct effects (aquatic-phase CRLF) component above.
		Terrestrial prey items, riparian habitat Risk to terrestrial invertebrate LOC exceedances are observed for small insects at the maximum annual application rate for cotton (2.4 lbs a.i./A). Small terrestrial mammals are also potentially affected by prometryn as acute and chronic risk LOC exceedances are observed for small mammals. The same description applies here for frog prey items as in the direct effects (terrestrial-phase CRLF) component above. Risk to terrestrial plant LOCs are exceeded for monocots in dry and semi-aquatic areas for cotton uses (ground: 2.4 lbs a.i./A) and in dry and semi-aquatic areas as well as spray drift alone for cotton uses (aerial: 2.4 lbs a.i./A). Dicots are more sensitive as LOC exceedances are estimated in dry and semi-aquatic areas for both application types on cotton (ground and aerial spray at 2.4 lbs a.i./A).
[1] No effect (NE); May affect, but not likely to adversely affect (NLAA); May affect, likely to adversely affect (LAA)		

Table 1-2 Effects Determination Summary for Prometryn Use and CRLF Critical Habitat Impact Analysis

Assessment Endpoint	Effects Determination	Basis for Determination
Modification of aquatic-phase PCE	Habitat Effects	Risk to terrestrial plant LOCs are exceeded for monocots in dry and semi-aquatic areas for cotton uses (ground: 2.4 lbs a.i./A) and in dry and semi-aquatic areas as well as spray drift alone for cotton uses (aerial: 2.4 lbs a.i./A). Dicots are more sensitive as LOC exceedances are estimated in dry and semi-aquatic areas for both application types on cotton (ground and aerial spray at 2.4 lbs a.i./A). Risk to aquatic plant LOC exceedances are observed for non-vascular and vascular plants for all uses. Acute risk to freshwater fish LOCs are exceeded for all parsley uses (Spring, Fall: 2 lbs a.i./A) and most cotton uses (Fall, pre-emergence ground spray and pre-emergence aerial spray: 2.4 lbs a.i./A). No chronic risk LOCs are exceeded based on the most sensitive toxicity data for freshwater fish and for any evaluated use.

Assessment Endpoint	Effects Determination	Basis for Determination
		Acute and chronic risk LOC exceedances are not observed for freshwater invertebrates.
Modification of terrestrial-phase PCE		Risk to terrestrial plant LOCs are exceeded for monocots in dry and semi-aquatic areas for cotton uses (ground: 2.4 lbs a.i./A) and in dry and semi-aquatic areas as well as spray drift alone for cotton uses (aerial: 2.4 lbs a.i./A). Dicots are more sensitive as LOC exceedances are estimated in dry and semi-aquatic areas for both application types on cotton (ground and aerial spray at 2.4 lbs a.i./A). No definitive acute RQs could be derived because the acute avian effects data shows no mortality at the highest test concentrations. Chronic risk LOC exceedances are not observed for birds consuming small insects when considering a cotton application (2.4 lbs a.i./A) in T-REX using dietary based RQs. This direct effects description applies for frog prey items as an indirect effect on the CRLF. Risk to terrestrial invertebrate LOC exceedances are observed for small insects at the maximum annual application rate for cotton (2.4 lbs a.i./A). Small terrestrial mammals are also affected by prometryn as acute and chronic risk LOC exceedances are observed for small mammals.

Table 1-3 Prometryn Use-specific Direct Effects Determinations[1] for the CRLF

Use(s)	Aquatic Habitat		Terrestrial Habitat	
	Acute	Chronic	Acute	Chronic
Cotton	LAA	NE	NLAA	NLAA

[1] NE = No effect; NLAA = May affect, but not likely to adversely affect; LAA = Likely to adversely affect

Table 1-4 Prometryn Use-specific Indirect Effects Determinations[1] Based on Effects to Prey

Use(s)	Algae	Aquatic Invertebrates		Terrestrial Invertebrates (Acute)	Aquatic-phase frogs and fish		Terrestrial-phase frogs		Small Mammals	
		Acute	Chronic		Acute	Chronic	Acute	Chronic	Acute	Chronic
Cotton	LAA	NE	NE	LAA	LAA	NE	NLAA	NLAA	LAA	LAA

[1] NE = No effect; NLAA = May affect, not likely to adversely affect; LAA = Likely to adversely affect

Based on the conclusions of this assessment, a formal consultation with the U. S. Fish and Wildlife Service under Section 7 of the Endangered Species Act should be initiated. When evaluating the significance of this risk assessment's direct/indirect and adverse habitat modification effects determinations, it is important to note that pesticide exposures and predicted risks to the species and its resources (i.e., food and habitat) are not expected to be uniform across the action area. In fact, given the assumptions of drift and downstream transport (i.e., attenuation with distance), pesticide exposure and associated risks to the species and its resources are expected to decrease with increasing distance away from the treated field or site of application. Evaluation of the implication of this non-uniform distribution of risk to the species would require information and assessment techniques that are not currently available. Examples of such information and methodology required for this type of analysis would include the following:

- Enhanced information on the density and distribution of CRLF life stages within specific recovery units and/or designated critical habitat within the action area. This information would allow for quantitative extrapolation of the present risk assessment's predictions of individual effects to the proportion of the population extant within geographical areas where those effects are predicted. Furthermore, such population information would allow for a more comprehensive evaluation of the significance of potential resource impairment to individuals of the species.
- Quantitative information on prey base requirements for individual aquatic- and terrestrial-phase frogs. While existing information provides a preliminary picture of the types of food sources utilized by the frog, it does not establish minimal requirements to sustain healthy individuals at varying life stages. Such information could be used to establish biologically relevant thresholds of effects on the prey base, and ultimately establish geographical limits to those effects. This information could be used together with the density data discussed above to characterize the likelihood of adverse effects to individuals.
- Information on population responses of prey base organisms to the pesticide. Currently, methodologies are limited to predicting exposures and likely levels of direct mortality, growth or reproductive impairment immediately following exposure to the pesticide. The degree to which repeated exposure events and the inherent demographic characteristics of the prey population play into the extent to which prey resources may recover is not predictable. An enhanced understanding of long-term prey responses to pesticide exposure would allow for a more refined determination of the magnitude and duration of resource impairment, and together with the information described above, a more complete prediction of effects to individual frogs and potential modification to critical habitat.

2.0 Problem Formulation

Problem formulation provides a strategic framework for the risk assessment. By identifying the important components of the problem, it focuses the assessment on the most relevant life history stages, habitat components, chemical properties, exposure routes, and endpoints. The structure of this risk assessment is based on guidance contained in U.S. Environmental Protection Agency's (EPA's) Guidance for Ecological Risk Assessment (U.S. EPA 1998), the Services' Endangered Species Consultation Handbook (U.S. FWS/NMFS 1998) and is consistent with procedures and methodology outlined in the Overview Document (U.S. EPA 2004) and reviewed by the U.S. Fish and Wildlife Service and National Marine Fisheries Service (U.S. FWS/NMFS 2004).

2.1 Purpose

The purpose of this endangered species assessment is to evaluate potential direct and indirect effects on individuals of the federally threatened California red-legged frog (*Rana aurora draytonii*) (CRLF) arising from FIFRA regulatory actions regarding use of prometryn on celery, fennel, dill, parsley, and cotton. In addition, this assessment evaluates whether use on these sites is expected to result in effects to the species' designated critical habitat. This ecological risk assessment has been prepared consistent with a settlement agreement in the case Center for Biological Diversity (CBD) vs. EPA *et al.* (Case No. 02-1580-JSW(JL)) entered in Federal District Court for the Northern District of California on October 20, 2006.

In this assessment, direct and indirect effects to the CRLF and potential effects to its designated critical habitat are evaluated in accordance with the methods described in the Agency's Overview Document (U.S. EPA 2004). Screening level methods include use of standard models such as PRZM-EXAMS, T-REX, TerrPlant, and AgDRIFT. Use of such information is consistent with the methodology described in the Overview Document (U.S. EPA 2004), which specifies that "the assessment process may, on a case-by-case basis, incorporate additional methods, models, and lines of evidence that EPA finds technically appropriate for risk management objectives" (Section V, page 31 of U.S. EPA 2004).

In accordance with the Overview Document, provisions of the Endangered Species Act (ESA), and the Services' Endangered Species Consultation Handbook, the assessment of effects associated with registrations of prometryn is based on an action area. The action area is the area directly or indirectly affected by the federal action. It is acknowledged that the action area for a national-level FIFRA regulatory decision associated with a use of prometryn may potentially involve numerous areas throughout the United States and its Territories. However, for the purposes of this assessment, attention will be focused on relevant sections of the action area including those geographic areas associated with locations of the CRLF and its designated critical habitat within the state of California. As part of the "effects determination," one of the following three conclusions will be reached regarding the potential use of prometryn in accordance with current labels:

- “No effect”;
- “May affect, but not likely to adversely affect”; or
- “May affect and likely to adversely affect”.

Designated critical habitat identifies specific areas that have the physical and biological features, known as primary constituent elements, or PCEs, essential to the conservation of the listed species. The PCEs for CRLFs are aquatic and upland areas where suitable breeding and non-breeding aquatic habitat is located, interspersed with upland foraging and dispersal habitat.

If the results of initial screening-level assessment methods show no direct or indirect effects (no LOC exceedances) upon individual CRLFs or upon the PCEs of the species’ designated critical habitat, a “no effect” determination is made for use of prometryn as it relates to this species and its designated critical habitat. If, however, potential direct or indirect effects to individual CRLFs are anticipated or effects may impact the PCEs of the CRLF’s designated critical habitat, a preliminary “may affect” determination is made for the FIFRA regulatory action regarding prometryn.

If a determination is made that use of prometryn within the action area(s) associated with the CRLF “may affect” this species or its designated critical habitat, additional information is considered to refine the potential for exposure and for effects to the CRLF and other taxonomic groups upon which these species depend (*e.g.*, aquatic and terrestrial vertebrates and invertebrates, aquatic plants, riparian vegetation, etc.). Additional information, including spatial analysis (to determine the geographical proximity of CRLF habitat and prometryn use sites) and further evaluation of the potential impact of prometryn on the PCEs is also used to determine whether effects to designated critical habitat may occur. Based on the refined information, the Agency uses the best available information to distinguish those actions that “may affect, but are not likely to adversely affect” from those actions that “may affect and are likely to adversely affect” the CRLF or the PCEs of its designated critical habitat. This information is presented as part of the Risk Characterization in Section 5 of this document.

The Agency believes that the analysis of direct and indirect effects to listed species provides the basis for an analysis of potential effects on the designated critical habitat. Because prometryn is expected to directly impact living organisms within the action area (defined in Section 2.7), critical habitat analysis for prometryn is limited in a practical sense to those PCEs of critical habitat that are biological or that can be reasonably linked to biologically mediated processes (i.e., the biological resource requirements for the listed species associated with the critical habitat or important physical aspects of the habitat that may be reasonably influenced through biological processes). Activities that may modify critical habitat are those that alter the PCEs and appreciably diminish the value of the habitat. Evaluation of actions related to use of prometryn that may alter the PCEs of the CRLF’s critical habitat form the basis of the critical habitat impact analysis. Actions that may affect the CRLF’s designated critical habitat have been identified by the Services and are discussed further in Section 2.6.

2.2 Scope

Prometryn is used nationally as a herbicide on a variety of terrestrial food and feed crops and terrestrial non-food crops. Currently registered uses of prometryn in California are: cotton, celery, parsley, dill, and fennel; cotton accounted for approximately 73% of the total pounds applied in California in 2006, with the remainder used on celery (22%), parsley (4%), fennel (1%) and dill (<1%).

The end result of the EPA pesticide registration process (*i.e.*, the FIFRA regulatory action) is an approved product label. The label is a legal document that stipulates how and where a given pesticide may be used. Product labels (also known as end-use labels) describe the formulation type (*e.g.*, liquid or granular), acceptable methods of application, approved use sites, and any restrictions on how applications may be conducted. Thus, the use or potential use of prometryn in accordance with the approved product labels for California is "the action" relevant to this ecological risk assessment.

Although current registrations of prometryn allow for use nationwide, this ecological risk assessment and effects determination addresses currently registered uses of prometryn in portions of the action area that are reasonably assumed to be biologically relevant to the CRLF and its designated critical habitat. Further discussion of the action area for the CRLF and its critical habitat is provided in Section 2.7 and 2.6, respectively.

Degradation products of prometryn in soil metabolism studies are 2,4-bis(isopropylamino)-6-hydroxy-s-triazine (CS-11526) at 27% of the applied radioactivity after one year post-treatment and 2-amino-4-isopropylamino-6-methylthio-s-triazine (GS-11354) at less than 10% after one year post-treatment. There are no available ecotoxicity data and limited environmental fate data for these degradation products. Based on the human health assessment (Wassell 1998), the only residue of concern is prometryn. Therefore, the degradation products of prometryn are not considered further in this assessment.

The Agency does not routinely include in its risk assessments an evaluation of mixtures of active ingredients, either those mixtures of multiple active ingredients in product formulations or those in the applicator's tank (e.g., additives such as surfactants). In the case of the product formulations of active ingredients (*i.e.*, a registered end-use product containing more than one active ingredient), each active ingredient is subject to an individual risk assessment for regulatory decision regarding the active ingredient on a particular use site. If effects data are available for a formulated product containing more than one active ingredient, they may be used qualitatively or quantitatively in accordance with the Agency's Overview Document and the Services' Evaluation Memorandum (U.S. EPA 2004; U.S. FWS/NMFS 2004).

Two registered end-use products (Suprend Herbicide and Prometryne +MSMA) contain multiple active ingredients. Given that the two formulated products for prometryn do not have LD_{50} values with associated 95% confidence intervals, it is not possible to undertake a quantitative or qualitative analysis for potential interactive effects (for a fuller description and summary table see **Appendix B**). Analysis of the available open literature data for multiple active ingredient products relative to the single active ingredient is provided in Section 4.0.

2.3 Previous Assessments

Below is a summary of previous ecological risk assessments for prometryn.

2.3.1 Section 18 CA Department of Pesticide Regulation (December 4, 1998)

EFED's Ecological Effects Branch (EEB) reviewed the proposed renewal emergency exemption for the use of prometryn on parsley in the following eight southern California counties (Monterey, Orange, Riverside, San Benito, San Luis Obispo, Santa Barbara, Stanislaus, and Ventura). EEB concluded that the proposed use would not result in acute risks to non-target birds, mammals, and fish, based on the available toxicity data. The likelihood of adverse chronic effects on small mammals and birds was unclear, because some studies were incomplete.

Available toxicity data on terrestrial and aquatic plants indicated that prometryn is highly toxic to some plant species, hence sensitive non-target crops and non-target plants adjacent to parsley fields might be adversely affected. There is general concern for adverse effects on non-target plants. The risk quotients for non-target plants near treated-parsley fields from runoff ranged from less than 1 up to 294 for aquatic species, up to 24 for terrestrial plants, and up to 29 for wetland plants. Many endangered and threatened plant species had been identified in the eight parsley-growing counties covered by this petition. Information on the location of parsley fields with respect to critical habitat for plants were unavailable, hence it was not possible to assess potential risks for listed plants. The U.S. Fish and Wildlife Service had indicated that a 20 yard buffer zone should be maintained around listed plant species.

2.3.2 Reregistration Eligibility Document for Prometryn (1996) – EPA 738-R-95-033

The ecological risk assessment was conducted in support of the reregistration eligibility decision for prometryn. Maximum single application rates for use of prometryn on celery, cotton, dill and pigeon peas ranged from 0.8 lbs ai/A to 3.2 lbs ai/A [4.0 lbs a.i./A in Hawaii]. Prometryn applications were evaluated as pre- or post-emergent, at plant, post-plant herbicide applied via ground or aerial spray.

PRZM/EXAMS modeling predicted peak prometryn concentrations from 179.9 to 276.8 µg/L for single application of 2.8 lbs ai/a via aerial and ground spray on cotton.
The acute endangered species LOC was exceeded for freshwater fish. Chronic risk to freshwater fish was not evaluated because of the lack of valid data; therefore, risk to listed aquatic vertebrate species was presumed. No LOCs were exceeded for acute or chronic effects to freshwater invertebrates. The restricted LOC was exceeded for marine/estuarine invertebrates (mollusks) for single applications of 2.8 lbs ai/A on cotton and 3.2 lbs ai/A on celery. Additionally, the endangered species LOC was exceeded for

marine/estuarine invertebrates and fish. Prometryn was expected to cause adverse effects to non-target aquatic plants.

Terrestrial EECs for prometryn ranged from 22 mg/kg on fruits to 960 mg/kg [Hawaii application at 4 lbs ai/A] on short range grasses. No acute LOCs were exceeded for birds. However, the chronic LOC was exceeded for birds consuming short range grass or fruit/vegetable leaves using 4 lb ai/A application rate of prometryn. The endangered species LOC was exceeded for small mammals consuming grasses within prometryn treated areas. Terrestrial plant RQs ranged from 19.9 for monocot (vigor) to 533.3 for dicot (vigor). Therefore, prometryn was expected to cause adverse effects to non-target terrestrial plants as well.

2.3.3 Effects Determination for Pacific Salmonids

On November 29, 2002, EPA initiated formal consultation with the National Marine Fisheries Service relative to potential effects of prometryn on listed Pacific salmon and steelhead. Of the 26 species of salmon and steelhead to which effects were assessed, EPA found prometryn would have no effects on 17 and may affect 9. On November 5, 2007, a suit was filed against the National Marine Fisheries Service (NMFS) seeking a judgement that NMFS' failure to complete section 7 consultation on 37 pesticides for which EPA initiated consultation, violates section 7(b)(1) of the Endangered Species Act and section 706(1) of the Administrative Procedure Act. On July 30, 2008 a settlement was filed in that case which among other things, establishes a schedule for issuing biological opinions for the 37 actions on which EPA initiated consultation. The biological opinion that will address the consultation initiated for prometryn, is scheduled to be issued by NMFS in February, 2012.

2.3.4 Special Local Need Registrations

Special Local Need registrations for California were granted for use of Prometryne 4L Herbicide (EPA Reg. No. 34704-692) on fennel (CA960025); and Caparol 4L Herbicide (EPA Reg. No. 100-620) on celery (CA980017). While these SLNs have not expired, the products are now fully registered for use on these crops as of 3/18/1997 and 6/15/1999, respectively.

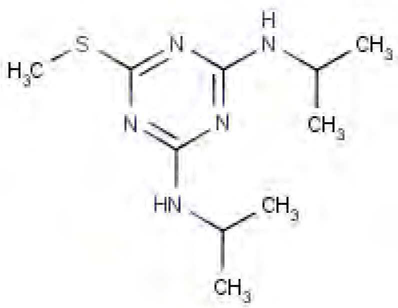
2.4 Stressor Source and Distribution

2.4.1 Environmental Fate and Physical-Chemical Properties

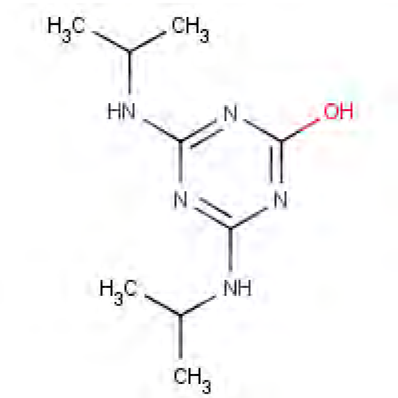
Prometryn is a methylthio-(symmetric) triazine. The chemical names (CAS and IUPAC) are presented below together with the CAS Registry Number and chemical structure:

U.S. EPA PC Code: 080805
CA DPR Chemical Code: 502
IUPAC: N^2,N^4-diisopropyl-6-methylthio-1,3,5-triazine-2,4-diamine
CAS: N^2,N^4-diisopropyl-6-methylthio-1,3,5-triazine-2,4-diamine

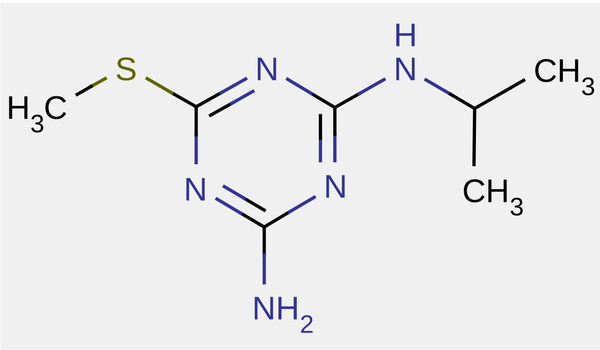
CAS Reg. No.: 7287-19-6
Chemical Formula: $C_{10}H_{19}N_5S$
SMILES: CSc1nc(NC(C)C)nc(NC(C)C)n1
Chemical Structures:



NAME: Prometryn [ANSI:BSI:ISO]
RN: 7287-19-6



Name: Prometryn aerobic soil metabolite (27% after one year incubation)
CAS Name: 1,3,5-Triazin-2(1H)-one, 4,6-bis((1-methylethyl)amino)-
IUPAC: 2,4-bis(isopropylamino)-6-hydroxy-s-triazine
CAS Reg No. 7374-53-0



Name: Prometryn aerobic soil metabolite (1.1% after one year incubation)
IUPAC: 2-amino-4isopropylamino-6-methylthio-s-triazine (GS-1154)

The physical and chemical properties of prometryn relevant to its environmental fate and transport behavior appear in **Table 2-1**. The environmental fate properties of prometryn, along with the major and minor degradates detected in the submitted environmental fate and transport studies are presented in **Table 2-2**.

Table 2-1. Physical and Chemical Properties of Prometryn

Molecular Weight, g/mole	241.4
Solubility in Water, mg/L, 25°C	33
Vapor Pressure, 25°C.	1.6 x 10^{-6} mmHg 1.3 x 10^{-4} Pa
Henry's Law Constant*, atm m^3 $mole^{-1}$	9 x 10^{-9}
n-octanol/water partition coefficient, Log Kow	3.46
*EPI Suite (Ver. 4) estimation	

Table 2-2 Environmental Fate Properties of Prometryn

Study	Value	Major Degradates *Minor Degradates*	MRID #	Study Classification
Abiotic Hydrolysis	Stable at pH 5, 7, and 9	None	40573704	Acceptable
Direct Aqueous Photolysis	Stable		40573705	Acceptable
Soil Photolysis	Stable		40573706	Acceptable
Aerobic Soil Metabolism	$t_{½}$ 270 days; (One soil) Sandy Soil	2,4-bis(isopropylamino)-6-hydroxy-s-triazine (GS-11526, 27% 0f the applied radioactivity after 360 days 2-amino-4isopropylamino-6-methylthio-s-triazine (Gs-1154) 1.1% at 30 days	00148338	Acceptable
Anaerobic Soil Metabolism	$t_{½}$ 90 days Stable		41155509	Acceptable
Anaerobic Aquatic Metabolism	No data submitted	No data	-	N/A
Aerobic Aquatic Metabolism	No data submitted	No data	-	N/A
163- Mobility in Soil Batch-equilibrium Adsorption/ Desorption	$K_{Freundlich, Adsorpotion}$ 0.86 (agricultural sand) to 3.18 (silty clay loam); add other soils	GS 11526 mobile to very mobile (Freudlich K_{ads} 7.1 to 0.5) GS-11354 very mobile (Freudlich K_{ads} 0.63-1.9)	40573713 41875901 41875902 41875903 41875904 41875905	Acceptable

Study	Value	Major Degradates *Minor Degradates*	MRID #	Study Classification
163-2 Volatility from Soil (Laboratory)	Sand soil Volatility was minimal, 0.99% prometryn volatilized by 30 days	No	41875906	Acceptable
Terrestrial Field Dissipation	t ½ Cotton CA, 105 days t ½ Cotton CA 71 days t ½ Cotton TX 14-30 days t ½ Cotton TX 14 days	Prometryn was not detected below 45 cm CS-11354 detected at 120 cm soil depth		Acceptable (for flowable concentrate)
Aquatic Field Dissipation	No data	Not applicable	Not applicable	N/A
Bioaccumulation in Fish	Residues did not accumulate in fish even though the Log K_{ow} 0f 3.46 is above the Log 3 trigger value			

According to the submitted physical-chemical and environmental fate data, the degradation of prometryn is driven by microbial (biotic) activity and not by abiotic processes; however, biotransformation is slow. The major biotransformation product is GS-11534 (see Table 2-2b). The low Freundlich adsorption coefficient of prometryn suggests that the chemical has the potential to run off and/or leach to ground water. Although no guideline studies are available to evaluate the role of indirect photolysis, indirect photolysis in natural water has been reported for prometryn (Connell *et al.* 2004; Cessna 2008; Kontantinous 2001; Garbini 2007).

Other possible transformation products of prometryn have been reported recently (2008-2009). These transformation products originate through oxidation of the thioether linkage in the parent compound, in which the sulfide sulfur S(-II) oxidizes to S(IV) and S(VI) and generates the sulfoxide and/or the sulfone. Some bacteria are capable of oxidizing the thioether sulphur to form sulfoxide and/or sulfone in prometryn (Harada *et al.* 2006; Yamazaki *et al.* 2008), but their formation in environmental media has not been established.

Degradation products of prometryn in soil metabolism studies are 2,4-bis(isopropylamino)-6-hydroxy-s-triazine (CS-11526) at 27% of the applied radioactivity after one year post-treatment and 2-amino-4-isopropylamino-6-methylthio-s-triazine (GS-11354) at less than 10% after one year post-treatment. There are no available ecotoxicity data and limited environmental fate data for these degradation products. Based on the human health assessment (Wassell 1998), the only residue of concern is prometryn. Therefore, the degradation products of prometryn are not considered further in this assessment.

Prometryn is persistent and mobile in terrestrial and aquatic environments.

2.4.2 Environmental Transport Assessment

Potential transport mechanisms include surface water runoff, leaching to ground water, spray drift, and secondary drift of volatilized or soil-bound residues leading to deposition onto nearby or more distant ecosystems.

A number of studies have documented atmospheric transport and re-deposition of pesticides from the Central Valley to the Sierra Nevada Mountains (Fellers *et al.* 2004; Sparling *et al.* 2001; LeNoir *et al.* 1999; McConnell *et al.* 1998). Prevailing winds blow across the Central Valley eastward to the Sierra Nevada Mountains, transporting airborne industrial and agricultural pollutants into the Sierra Nevada ecosystems (Fellers *et al,* 2004; LeNoir *et al,* 1999; McConnell *et al,* 1998). Several sections of critical habitat for the CLRF are located east of the Central Valley. The magnitude of transport via secondary drift depends on prometryn's ability to mobilize into air and its eventual removal through wet and dry deposition of gases/particles and photochemical reactions in the atmosphere. Very recently (2008-2009) the US Geological Survey (USGS) has reported data on the presence of pesticides in rain and wet deposition in four USA agricultural watersheds. Among other pesticides, prometryn was detected in rain and wet deposition in California (Vogel *et al.*, 2008)[1]. The basin studied in California was San Joaquin -Tulare- Merced River[2], which contains habitat for the California RLF.

2.4.3. Mechanism of Action

Prometryn is a systemic herbicide that acts as a photosynthesis inhibitor. Prometryn binds to the D-1 quinone-binding protein (Q_8), thereby blocking photosynthetic electron transport.

2.4.4 Use Characterization

Prometryn is used as an herbicide on a variety of terrestrial food and feed crops and terrestrial non-food crops. Food crops include: cotton, celery, parsley, dill, fennel, pigeon peas and parsnips; non-food uses include: crops grown for seed (carrot, parsley, parsnips, and coriander); and kenaf. Prometryn can be applied at the following times: preplant; preemergence; post emergence directed; post-transplanting (for celery and fennel); and at lay-by and for winter weed control (for cotton). As shown in **Figure 2-1**, U.S. Geological Survey (USGS) National Water Quality Assessment Program (NAWQA) data indicate that in 2002, prometryn was used on agricultural crops predominantly in California, west Texas, the southeastern states, and the Mississippi Delta region. At that time, the use of prometryn on cotton represented about 98% of the national use.

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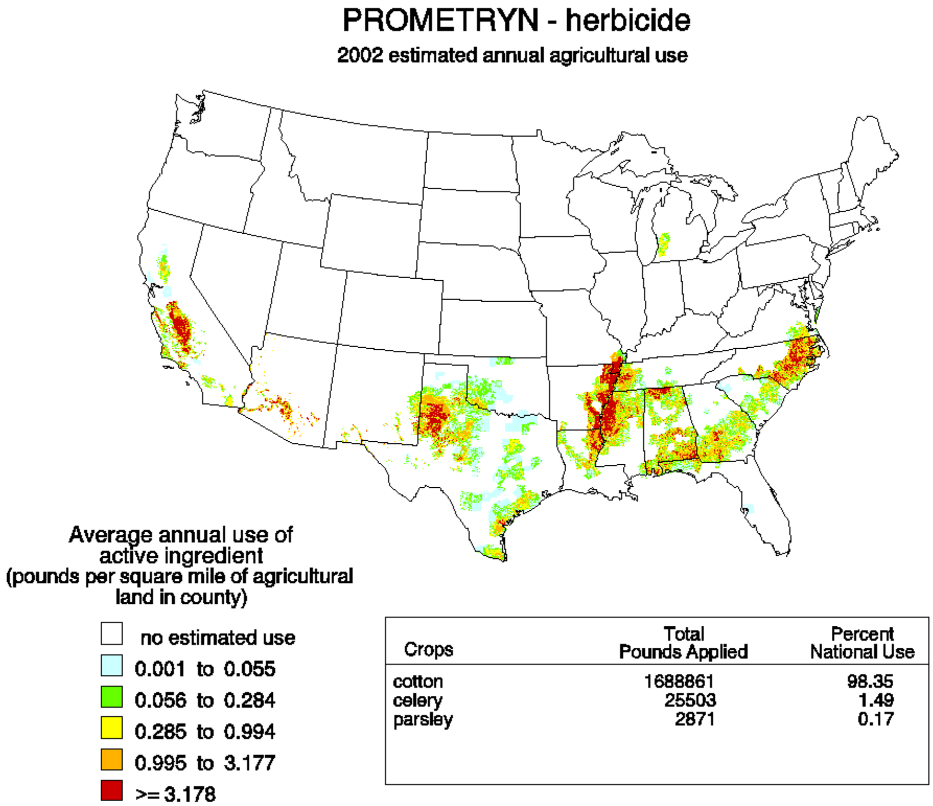
[1] The cropland in West California (samples collected) is dominated by almonds and vineyards. In addition to the California studies, other studies where conducted in agricultural watersheds in Maryland, Indiana, and Nebraska. Atrazine was detected in all samples. Simazine was the most frequently detected triazine in California.

[2] For description of the California's Merced River Basin see Vogel *et al.* (2008) and pertinent references therein. The environmental setting of the Basin is described in Gronberg & Kratzer (2007).



Crops	Total Pounds Applied	Percent National Use
cotton	1688861	98.35
celery	25503	1.49
parsley	2871	0.17

Figure 2-1. Map of Estimated Annual Agricultural Use of Prometryn in 2002.
Source: http://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=02&map=m1987

The food use on pigeon peas and parsnips and the non food uses (carrot, parsley, parsnips, and coriander grown for seed; and kenaf) are not being assessed because the use on pigeon peas is restricted to Puerto Rico only; the food use on parsnips is a SLN for Florida only; and the non-food uses of prometryn are geographically restricted to areas outside of California (**Table 2-3**). If use patterns indicate that pigeon peas and/or the non-food crops are grown in California in the future, the conclusions of this assessment may need to be revisited.

Table 2-3 Labels for Products Containing Prometryn for Uses in States Other Than California

Registration #	Product name	Crops	Geographical restriction
NON-FOOD/NON-FEED USES			
OR04000200	Caparol 4L (100-620)	For seed only: Carrot, Coriander, Parsley, Parsnip	OR only
WA96001400		For seed only: Carrot, Coriander, Parsley, Parsnip	WA only
AR96000600 LA98000500 MS96001300	Prometryne 4L	Kenaf	AR, LA, MS only
FOOD/FEED USES			
FL97001100	Caparol 4L (100-620)	Parsnip	FL only
100-620	Caparol 4L	Pigeon peas	Puerto Rico only
66222-15	Prometrex 4L	Pigeon peas	Puerto Rico only

Table 2-4 Labels for Products Containing Prometryn Registered for Use in California

Registration #	Product name	Crops
100-620	Caparol 4L Herbicide	Cotton, celery
100-1163	Suprend Herbicide	Cotton
9779-297	Prometryne 4L Herbicide	Cotton, celery
9779-317	Prometryne + MSMA	Cotton
10163-94	Gowan Prometryne 4L Herbicide	Cotton, dill, parsley
34704-692	Prometryne 4L Herbicide	Cotton, celery, parsley, dill, fennel
66222-15	Prometrex 4L	Cotton, celery, parsley, dill, fennel
66222-116	Cotton-Pro	Cotton, celery, parsley
CA960025	Prometryne 4L Herbicide	Fennel
CA980017	Caparol 4L Herbicide	Celery

Analysis of labeled use information is the critical first step in evaluating the federal action. The current label for prometryn represents the FIFRA regulatory action; therefore, labeled use and application rates specified on the label form the basis of this assessment. The assessment of use information is critical to the development of the action area and selection of appropriate modeling scenarios and inputs.

For uses permitted in California, prometryn is formulated as a flowable concentrate (8.4-45.41% active ingredient), as a wettable granular (79.3% active ingredient), as a liquid (44-44.4% active ingredient), or as an emulsifiable concentrate (44.4% active ingredient). Application rates range from 0.5 to 2.4 lbs active ingredient/acre (or lbs a.i./A). Labels have additional requirements in order to reduce spray drift. These include the buffers and other mitigation actions listed in Section 3.2.5 as well as a requirement for a spray with medium to coarse droplet size applied at a maximum boom height of 4 ft above the ground or canopy for ground spray and of 10 ft for aerial spray.

The uses and corresponding application rates and methods considered in this assessment are presented in **Table 2-5**.

Table 2-5 Prometryn Uses Assessed for the CRLF

Use	Max. Single Appl. Rate (lb ai/A)	Max. Number of Applications per Year	Incorporation Depth (cm)	Application Method (s)
Celery	2	1	4.0	Soil broadcast, soil band treatment, soil incorporated, band treatment, basal spray treatment, directed spray; sprinkler irrigation
Cotton	2.4	Varies[a]	10[b]	Aerial // Fixed wing aircraft; sprinkler irrigation; Soil broadcast, soil band treatment, soil incorporated, band treatment, basal spray treatment, directed spray
Dill	1.6	1	4.0	Soil broadcast, soil band treatment, soil incorporated, band treatment, basal spray treatment, directed spray;
Fennel	2	1	4.0	Soil broadcast, soil band treatment, soil incorporated, band treatment, basal spray treatment, directed spray;
Parsley	2	1	4.0	Preplant broadcast incorporated or shielded applicator post-transplant

[a] Can apply up to 5.5 lbs a.i. /A total per year over the following main application times: Pre-plant (max application of 2.4 lb a.i. /A); post-emergence (up to 3 applications totaling 2 lbs a.i./A); lay-by (maximum 1.6 lbs a.i./A); and winter weed control (maximum 2.4 lbs a.i./A).
[b] Ground application depth is 4 inches (~10cm); this value was used in modeling

Application equipment includes: fixed wing aircraft (cotton only); band sprayer or sprayer; ground boom (high volume [cotton only] and low pressure [cotton only] and low volume [celery and cotton only]); shielded applicator (cotton only); sprinkler irrigation (celery and cotton only); and low-pressure hand wand or backpack sprayer.

The Agency's Biological and Economic Analysis Division (BEAD) can provide an analysis of county-level usage using state-level usage data obtained from USDA-NASS[3],

[3] United States Depart of Agriculture (USDA), National Agricultural Statistics Service (NASS) Chemical Use Reports provide summary pesticide usage statistics for select agricultural use sites by chemical, crop and state. See http://www.usda.gov/nass/pubs/estindx1.htm#agchem.

Doane (www.doane.com; the full dataset is not provided due to its proprietary nature) and the California's Department of Pesticide Regulation Pesticide Use Reporting (CDPR PUR) database[4]. However, for this assessment, no national usage data were provided, so the usage data reported for prometryn by county in this California-specific assessment were generated using CDPR PUR data. Eight years (1999-2006) of usage data were included in this analysis. Data from CDPR PUR were obtained for every pesticide application made on every use site at the section level (approximately one square mile) of the public land survey system. BEAD summarized these data to the county level by site, pesticide, and unit treated. Calculating county-level usage involved summarizing across all applications made within a section and then across all sections within a county for each use site and for each pesticide. The county level usage data that were calculated include: average annual pounds applied, average annual area treated, and average and maximum application rate across all eight years. The units of area treated are also provided where available.

Based on this analysis, an average of 188,863 lbs of prometryn was applied in California to an average of 131,775 acres per year. From 1999-2006, prometryn was used in a total of 21 counties for the five registered uses (**Figure 2-2a**). Use was at a maximum of 282,295 lbs in 2000 and then steadily dropped to less than half that by 2006, to 103,113 lbs/year. Use on cotton steadily decreased from 1999 to 2006; use on the other crops was generally uniform over the reporting period. Four counties accounted for 80% of the total pounds applied per county in 2006 [Fresno (43%), Kern (20%), Ventura (10%), Monterey (7%)] (**Figure 2-2b**). Cotton accounted for approximately 73% of the total lbs applied in CA in 2006, with celery (22%), parsley (4%), fennel (1%) and dill (<1%). This analysis may not be entirely representative of current use rates because application intensity may have changed since the 2006 data were reported, and because it may also include misreporting.

An evaluation of usage information was conducted to determine the area where use of prometryn may impact the CRLF. This analysis is used to characterize where predicted exposures are most likely to occur, but does not preclude use in other portions of the action area. A more detailed review of the county-level use information was also completed. These data suggest that prometryn has historically been applied in areas used by the CRLF (Figures 2-2a and 2-2b), such as in the southern Central Valley (one of the recovery units) where there is high prometryn use on cotton.

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[4] The California Department of Pesticide Regulation's Pesticide Use Reporting (CDPR PUR) database provides a census of pesticide applications in the state. See http://www.cdpr.ca.gov/docs/pur/purmain.htm.

Figure 2-2a Average Annual Prometryn Use in Total Pounds per County

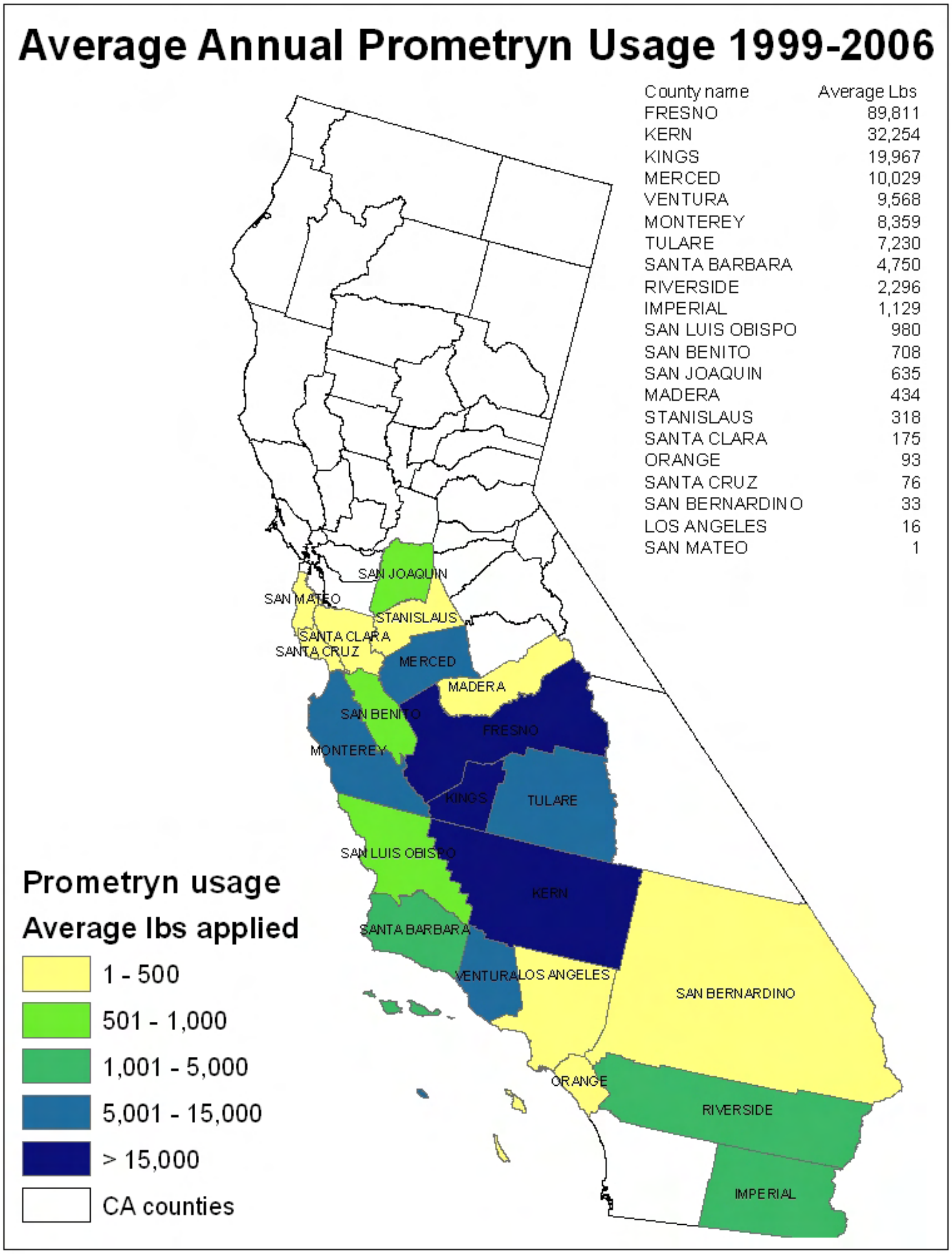
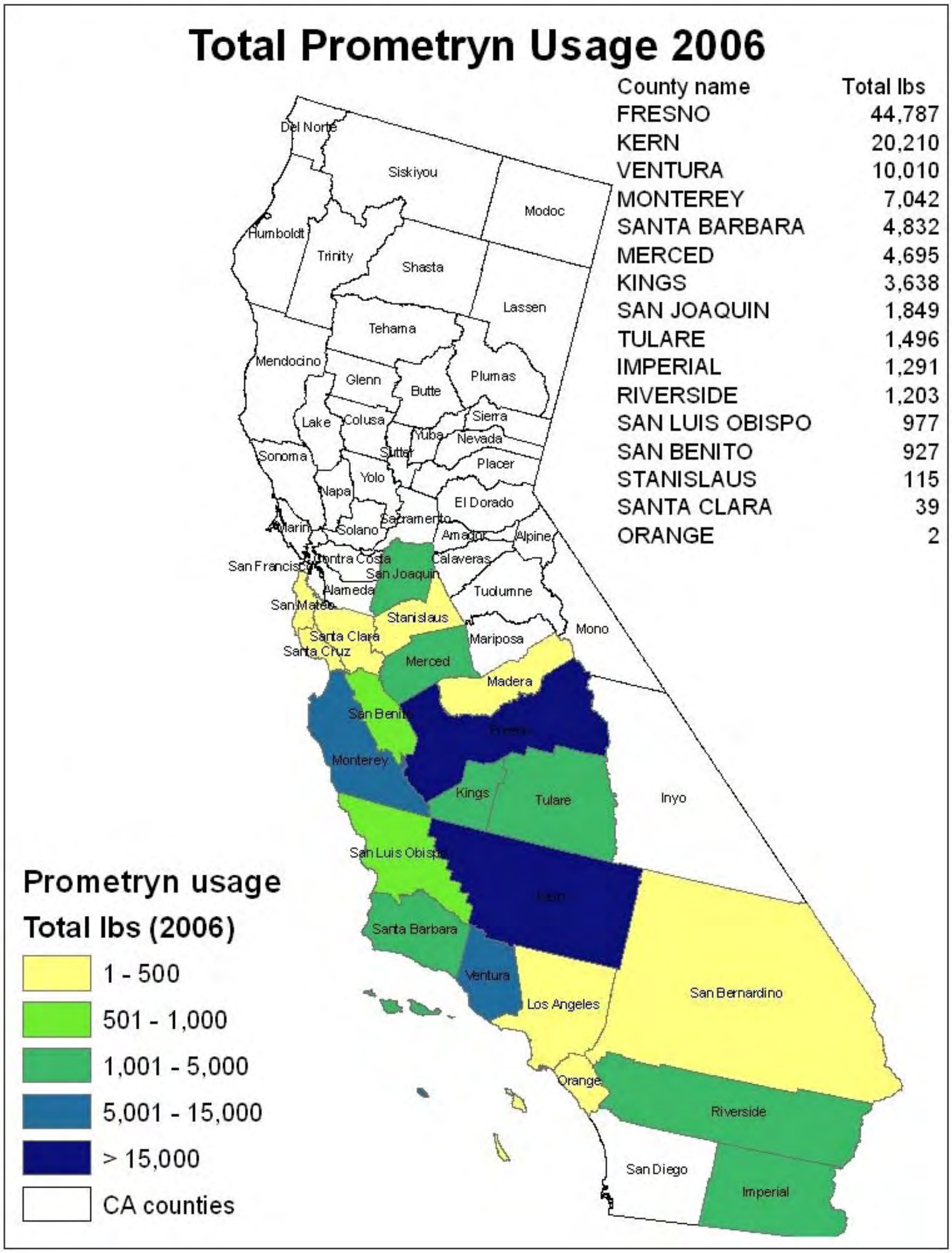


Figure 2-2b 2006 Prometryn Use in Total Pounds per County



A summary of prometryn usage for all California use sites is provided in **Table 2-6**

Table 2-6 Summary of California Department of Pesticide Registration (CDPR) Pesticide Use Reporting (PUR) Data from 1999 to 2006 for Currently Registered Prometryn Uses

Site Name	Average Pounds All Uses[1]	Avg App Rate All Uses [1] (lbs per acre)	Avg 95th% App Rate[1] (lbs per acre)	Avg 99th% App Rate[1] (lbs per acre)
Cotton	161775	1.51	1.94	2.17
Celery	22831	1.26	1.98	2.07
Parsley	3620	1.42	1.95	2.18
Fennel	573	0.75	1.80	1.92
Dill	64	1.27	1.59	1.72
[1] Weighted average				

2.5 Assessed Species

The CRLF was federally listed as a threatened species by U.S. FWS effective June 24, 1996 (U.S. FWS 1996). It is one of two subspecies of the red-legged frog and is the largest native frog in the western United States (U.S. FWS 2002). A brief summary of information regarding CRLF distribution, reproduction, diet, and habitat requirements is provided in Sections 2.5.1 through 2.5.4, respectively. Further information on the status, distribution, and life history of and specific threats to the CRLF is provided in Attachment I.

Final critical habitat for the CRLF was designated by U.S. FWS on April 13, 2006 (U.S. FWS 2006; 71 FR 19244-19346). Further information on designated critical habitat for the CRLF is provided in Section 2.6.

2.5.1 Distribution

The CRLF is endemic to California and Baja California (Mexico) and historically inhabited 46 counties in California including the Central Valley and both coastal and interior mountain ranges (U.S. FWS 1996). Its range has been reduced by about 70%, and the species currently resides in 22 counties in California (U.S. FWS 1996). The species has an elevational range of near sea level to 1,500 meters (5,200 feet) (Jennings and Hayes 1994); however, nearly all of the known CRLF populations have been documented below 1,050 meters (3,500 feet) (U.S. FWS 2002).

Populations currently exist along the northern California coast, northern Transverse Ranges (U.S. FWS 2002), foothills of the Sierra Nevada (5-6 populations), and in southern California south of Santa Barbara (two populations) (Fellers 2005a). Relatively larger numbers of CRLFs are located between Marin and Santa Barbara Counties (Jennings and Hayes 1994). A total of 243 streams or drainages are believed to be currently occupied by the species, with the greatest numbers in Monterey, San Luis Obispo, and Santa Barbara counties (U.S. FWS 1996). Occupied drainages or watersheds include all bodies of water that support CRLFs (i.e., streams, creeks, tributaries, associated natural and artificial ponds, and adjacent drainages), and habitats through which CRLFs can move (i.e., riparian vegetation, uplands) (U.S. FWS 2002).

The distribution of CRLFs within California is addressed in this assessment using four categories of location including recovery units, core areas, designated critical habitat, and known occurrences of the CRLF reported in the California Natural Diversity Database (CNDDB) that are not included within core areas and/or designated critical habitat (see Figure 2-3). Recovery units, core areas, and other known occurrences of the CRLF from the CNDDB are described in further detail in Attachment I, and designated critical habitat is addressed in Section 2.6. Recovery units are large areas defined at the watershed level that have similar conservation needs and management strategies. The recovery unit is primarily an administrative designation, and land area within the recovery unit boundary is not exclusively CRLF habitat. Core areas are smaller areas within the recovery units that comprise portions of the species' historic and current range and have been determined by U.S. FWS to be important in the preservation of the species. Designated critical habitat is generally contained within the core areas, although a number of critical habitat units are outside the boundaries of core areas, but within the boundaries of the recovery units. Additional information on CRLF occurrences from the CNDDB is used to cover the current range of the species not included in core areas and/or designated critical habitat, but within the recovery units.

Other Known Occurrences from the CNDBB

The CNDDB provides location and natural history information on species found in California. The CNDDB serves as a repository for historical and current species location sightings. Information regarding known occurrences of CRLFs outside of the currently occupied core areas and designated critical habitat is considered in defining the current range of the CRLF. See: http://www.dfg.ca.gov/bdb/html/cnddb_info.html for additional information on the CNDDB.

Recovery Units

1. Sierra Nevada Foothills and Central Valley
2. North Coast Range Foothills and Western Sacramento River Valley
3. North Coast and North San Francisco Bay
4. South and East San Francisco Bay
5. Central Coast
6. Diablo Range and Salinas Valley
7. Northern Transverse Ranges and Tehachapi Mountains
8. Southern Transverse and Peninsular Ranges

Core Areas

1. Feather River
2. Yuba River- S. Fork Feather River
3. Traverse Creek/ Middle Fork/ American R. Rubicon
4. Cosumnes River
5. South Fork Calaveras River*
6. Tuolumne River*
7. Piney Creek*
8. Cottonwood Creek
9. Putah Creek – Cache Creek*
10. Lake Berryessa Tributaries
11. Upper Sonoma Creek
12. Petaluma Creek – Sonoma Creek
13. Pt. Reyes Peninsula
14. Belvedere Lagoon
15. Jameson Canyon – Lower Napa River
16. East San Francisco Bay
17. Santa Clara Valley
18. South San Francisco Bay
19. Watsonville Slough-Elkhorn Slough
20. Carmel River – Santa Lucia
21. Gablan Range
22. Estero Bay
23. Arroyo Grange River
24. Santa Maria River – Santa Ynez River
25. Sisquoc River
26. Ventura River – Santa Clara River
27. Santa Monica Bay – Venura Coastal Streams
28. Estrella River
29. San Gabriel Mountain*
30. Forks of the Mojave*
31. Santa Ana Mountain*
32. Santa Rosa Plateau
33. San Luis Ray*
34. Sweetwater*
35. Laguna Mountain*

* Core areas that were historically occupied by the California red-legged frog are not included in the map

Figure 2-3 Recovery Unit, Core Area, Critical Habitat, and Occurrence Designations for CRLF

2.5.2 Reproduction

CRLFs breed primarily in ponds; however, they may also breed in quiescent streams, marshes, and lagoons (Fellers 2005a). According to the Recovery Plan (U.S. FWS 2002), CRLFs breed from November through late April. Peaks in spawning activity vary geographically; Fellers (2005b) reports peak spawning as early as January in parts of coastal central California. Eggs are fertilized as they are being laid. Egg masses are typically attached to emergent vegetation, such as bulrushes (*Scirpus* spp.) and cattails (*Typha* spp.) or roots and twigs, and float on or near the surface of the water (Hayes and Miyamoto 1984). Egg masses contain approximately 2000 to 6000 eggs ranging in size between 2 and 2.8 mm (Jennings and Hayes 1994). Embryos hatch 10 to 14 days after fertilization (Fellers 2005a) depending on water temperature. Egg predation is reported to be infrequent and most mortality is associated with the larval stage (particularly through predation by fish); however, predation on eggs by newts has also been reported (Rathburn 1998). Tadpoles require 11 to 28 weeks to metamorphose into juveniles (terrestrial-phase), typically between May and September (Jennings and Hayes 1994, U.S. FWS 2002); tadpoles have been observed to over-winter (delay metamorphosis until the following year) (Fellers 2005b; U.S. FWS 2002). Males reach sexual maturity at 2 years, and females reach sexual maturity at 3 years of age; adults have been reported to live 8 to 10 years (U.S. FWS 2002). **Figure 2-4** depicts CRLF annual reproductive timing.

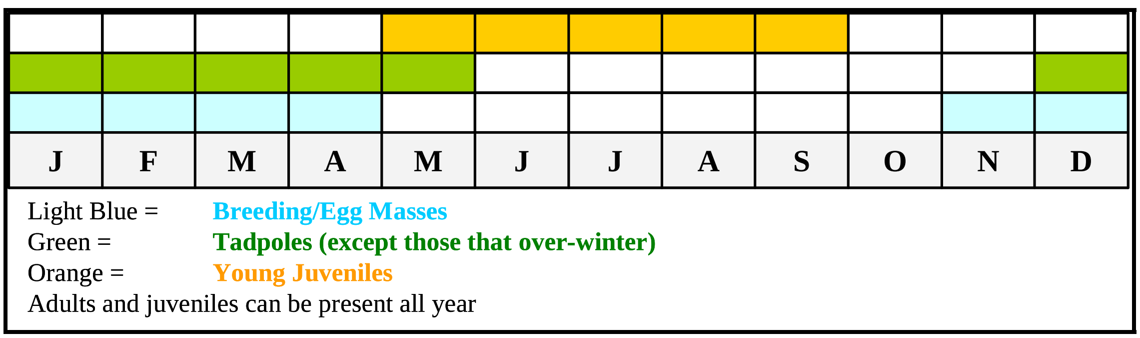


Figure 2-4 CRLF Reproductive Events by Month

2.5.3 Diet

Although the diet of CRLF aquatic-phase larvae (tadpoles) has not been studied specifically, it is assumed that their diet is similar to that of other frog species, with the tadpoles feeding exclusively in water and consuming diatoms, algae, and detritus (U.S. FWS 2002). Tadpoles filter and entrap suspended algae (Seale and Beckvar 1980) via mouthparts designed for effective grazing of periphyton (Wassersug 1984; Kupferberg *et al.* 1994; Kupferberg 1997; Altig and McDiarmid 1999).

Juvenile and adult CRLFs forage in aquatic and terrestrial habitats, and their diet differs greatly from that of larvae. The main food source for juvenile aquatic- and terrestrial-phase CRLFs is thought to be aquatic and terrestrial invertebrates found along the shoreline and on the water surface. Hayes and Tennant (1985) report, based on a study examining the gut content of 35 juvenile and adult CRLFs, that the species feeds on as many as 42 different invertebrate taxa, including Arachnida, Amphipoda, Isopoda, Insecta, and Mollusca. The most commonly observed prey species were larval alderflies (*Sialis* cf. *californica*), pillbugs (*Armadilliadrium vulgare*), and water striders (*Gerris* sp). The preferred prey species, however, was the sowbug (Hayes and Tennant 1985). This study suggests that CRLFs forage primarily above water, although the authors note other data reporting that adults also feed under water, are cannibalistic, and consume fish. For larger CRLFs, over 50% of the prey mass may consists of vertebrates such as mice, frogs, and fish, although aquatic and terrestrial invertebrates were the most numerous food items (Hayes and Tennant 1985). For adults, feeding activity takes place primarily at night; for juveniles feeding occurs during the day and at night (Hayes and Tennant 1985).

2.5.4 Habitat

CRLFs require aquatic habitat for breeding, but also use other habitat types including riparian and upland areas throughout their life cycle. CRLF use of their environment varies; they may complete their entire life cycle in a particular habitat or they may utilize multiple habitat types. Overall, populations are most likely to exist where multiple breeding areas are embedded within varying habitats used for dispersal (U.S. FWS 2002). Generally, CRLFs utilize habitat with perennial or near-perennial water (Jennings *et al.* 1997). Dense vegetation close to water, shading, and water of moderate depth are habitat features that appear especially important for CRLF (Hayes and Jennings 1988). Breeding sites include streams, deep pools, backwaters within streams and creeks, ponds, marshes, sag ponds (land depressions between fault zones that have filled with water), dune ponds, and lagoons. Breeding adults have been found near deep (0.7 m) still or slow moving water surrounded by dense vegetation (U.S. FWS 2002); however, the largest number of tadpoles have been found in shallower pools (0.26 – 0.5 m) (Reis 1999). Data indicate that CRLFs do not frequently inhabit vernal pools, as conditions in these habitats generally are not suitable (Hayes and Jennings 1988).

CRLFs also frequently breed in artificial impoundments such as stock ponds, although additional research is needed to identify habitat requirements within artificial ponds (U.S. FWS 2002). Adult CRLFs use dense, shrubby, or emergent vegetation closely associated with deep-water pools bordered with cattails and dense stands of overhanging vegetation (http://ecos.fws.gov/speciesProfile/SpeciesReport.do?spcode=D02D).

In general, dispersal and habitat use depends on climatic conditions, habitat suitability, and life stage. Adults rely on riparian vegetation for resting, feeding, and dispersal. The foraging quality of the riparian habitat depends on moisture, composition of the plant community, and presence of pools and backwater aquatic areas for breeding. CRLFs can be found living within streams at distances up to 3 km (2 miles) from their breeding site and have been found up to 30 m (100 feet) from water in dense riparian vegetation for up to 77 days (U.S. FWS 2002).

During dry periods, the CRLF is rarely found far from water, although it will sometimes disperse from its breeding habitat to forage and seek other suitable habitat under downed trees or logs, industrial debris, and agricultural features (U.S. FWS 2002). According to Jennings and Hayes (1994), CRLFs also use small mammal burrows and moist leaf litter as habitat. In addition, CRLFs may also use large cracks in the bottom of dried ponds as refugia; these cracks may provide moisture for individuals avoiding predation and solar exposure (Alvarez 2000).

2.6 Designated Critical Habitat

In a final rule published on April 13, 2006, 34 separate units of critical habitat were designated for the CRLF by U.S. FWS (U.S. FWS 2006; FR 51 19244-19346). A summary of the 34 critical habitat units relative to U.S. FWS-designated recovery units and core areas (previously discussed in Section 2.5.1) is provided in Attachment I.

'Critical habitat' is defined in the ESA as the geographic area occupied by the species at the time of the listing where the physical and biological features necessary for the conservation of the species exist, and there is a need for special management to protect the listed species. It may also include areas outside the occupied area at the time of listing if such areas are 'essential to the conservation of the species.' All designated critical habitat for the CRLF was occupied at the time of listing. Critical habitat receives protection under Section 7 of the ESA (Section 7) through prohibition against destruction or adverse modification with regard to actions carried out, funded, or authorized by a federal Agency. Section 7 requires consultation on federal actions that are likely to result in the destruction or adverse modification of critical habitat.

To be included in a critical habitat designation, the habitat must be 'essential to the conservation of the species.' Critical habitat designations identify, to the extent known using the best scientific and commercial data available, habitat areas that provide essential life cycle needs of the species or areas that contain certain primary constituent elements (PCEs) (as defined in 50 CFR 414.12(b)). PCEs include, but are not limited to, space for individual and population growth and for normal behavior; food, water, air, light, minerals, or other nutritional or physiological requirements; cover or shelter; sites for breeding, reproduction, rearing (or development) of offspring; and habitats that are protected from disturbance or are representative of the historic geographical and ecological distributions of a species. The designated critical habitat areas for the CRLF are considered to have the following PCEs that justify critical habitat designation:

- Breeding aquatic habitat;
- Non-breeding aquatic habitat;
- Upland habitat; and
- Dispersal habitat.

Further description of these habitat types is provided in **Attachment 1**.

Occupied habitat may be included in the critical habitat only if essential features within the habitat may require special management or protection. Therefore, U.S. FWS does not include areas where existing management is sufficient to conserve the species. Critical habitat is designated outside the geographic area presently occupied by the species only when a designation limited to its present range would be inadequate to ensure the conservation of the species. For the CRLF, all designated critical habitat units contain all four of the PCEs, and were occupied by the CRLF at the time of the Final Rule (FR) listing notice in April 2006 (71 FR 19243, 2006). The FR notice designating critical habitat for the CRLF includes a special rule exempting routine ranching activities associated with livestock ranching from incidental take prohibitions. The purpose of this exemption is to promote the conservation of rangelands, which could be beneficial to the CRLF, and to reduce the rate of conversion to other land uses that are incompatible with CRLF conservation. Please see Attachment I for a full explanation on this special rule.

U.S. FWS has established adverse modification standards for designated critical habitat (U.S. FWS 2006). Activities that may destroy or adversely modify critical habitat are those that alter the PCEs and jeopardize the continued existence of the species. Evaluation of actions related to use of prometryn that may alter the PCEs of the CRLF's critical habitat form the basis of the critical habitat impact analysis. According to U.S. FWS (2006), activities that may affect critical habitat and therefore result in adverse effects to the CRLF include, but are not limited to the following:

(1) Significant alteration of water chemistry or temperature to levels beyond the tolerances of the CRLF that result in direct or cumulative adverse effects to individuals and their life-cycles.
(2) Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs.
(3) Significant increase in sediment deposition within the stream channel or pond or disturbance of upland foraging and dispersal habitat that could result in elimination or reduction of habitat necessary for the growth and reproduction of the CRLF by increasing the sediment deposition to levels that would adversely affect their ability to complete their life cycles.
(4) Significant alteration of channel/pond morphology or geometry that may lead to changes to the hydrologic functioning of the stream or pond and alter the timing, duration, water flows, and levels that would degrade or eliminate the CRLF and/or its habitat. Such an effect could also lead to increased sedimentation and degradation in water quality to levels that are beyond the CRLF's tolerances.
(5) Elimination of upland foraging and/or aestivating habitat or dispersal habitat.
(6) Introduction, spread, or augmentation of non-native aquatic species in stream segments or ponds used by the CRLF.
(7) Alteration or elimination of the CRLF's food sources or prey base (also evaluated as indirect effects to the CRLF).

As previously noted in Section 2.1, the Agency believes that the analysis of direct and indirect effects to listed species provides the basis for an analysis of potential effects on the designated critical habitat. Because prometryn is expected to directly impact living organisms within the action area, critical habitat analysis for prometryn is limited in a practical sense to those PCEs of critical habitat that are biological or that can be reasonably linked to biologically mediated processes.

2.7 Action Area

For listed species assessment purposes, the action area is considered to be the area affected directly or indirectly by the federal action and not merely the immediate area involved in the action (50 CFR 402.02). It is recognized that the overall action area for the national registration of prometryn is likely to encompass considerable portions of the United States based on the large array of agricultural uses. However, the scope of this assessment limits consideration of the overall action area to those portions that may be applicable to the protection of the CRLF and its designated critical habitat within the state of California. The Agency's approach to defining the action area under the provisions of the Overview Document (U.S. EPA 2004) considers the results of the risk assessment process to establish boundaries for that action area with the understanding that exposures below the Agency's defined Levels of Concern (LOCs) constitute a no-effect threshold. For the purposes of this assessment, attention will be focused on the footprint of the action (i.e., the area where pesticide application occurs), plus all areas where offsite transport (i.e., spray drift, downstream dilution, etc.) may result in potential exposure within the state of California that exceeds the Agency's LOCs.

Deriving the geographical extent of this portion of the action area is based on consideration of the types of effects that prometryn may be expected to have on the environment, the exposure levels to prometryn that are associated with those effects, and the best available information concerning the use of prometryn and its fate and transport within the state of California. Specific measures of ecological effect for the CRLF that define the action area include any direct and indirect toxic effect to the CRLF and any potential modification of its critical habitat, including reduction in survival, growth, and fecundity as well as the full suite of sublethal effects available in the effects literature. Therefore, the action area extends to a point where environmental exposures are below any measured lethal or sublethal effect threshold for any biological entity at the whole organism, organ, tissue, and cellular level of organization. In situations where it is not possible to determine the threshold for an observed effect, the action area is not spatially limited and is assumed to be the entire state of California.

The definition of action area requires a stepwise approach that begins with an understanding of the federal action. The federal action is defined by the currently labeled uses for prometryn. An analysis of labeled uses and review of available product labels was completed. Several of the currently labeled uses are special local needs (SLN) uses or are restricted to specific states and are excluded from this assessment. In particular, the food use on pigeon peas and parsnips and the non food uses (carrot, parsley, parsnips, and coriander grown for seed, and kenaf) are not being assessed because the use on pigeon peas is restricted to Puerto Rico only; the food use on parsnips is restricted to Florida only; and the non-food uses of prometryn are geographically restricted to areas outside of California (**Table 2-3**). For those uses relevant to the CRLF, the analysis indicates that, for prometryn, the following agricultural food uses are considered as part of the federal action evaluated in this assessment: cotton, celery, parsley, dill, and fennel.

Due to the lack of a defined no effect concentration for given studies in the ECOTOX data (October 31, 2008), the spatial extent of the action area (i.e., the boundary where exposures and potential effects are less than the Agency's LOC) for prometryn cannot be determined. Therefore, it is assumed that the action area encompasses the entire state of California, regardless of the spatial extent (*i.e.*, initial area of concern or footprint) of the pesticide use(s). The following ECOTOX studies reported LOAELs without a corresponding NOAEL: Kozlowski & Torrie 1965 (Ref. #41006) on Norway pine, Isakeit & Lockwood 1989 (Ref. #70027) on fungi, El-Abyad *et al*. 1988 (Ref. #104611) on fungi, Osman & El-Khadem 1989 (Ref. #106174) on fungi, and Tezak *et al.* 1992 (Ref. #105506) on the Norway rat.

2.8 Assessment Endpoints and Measures of Ecological Effect

Assessment endpoints are defined as "explicit expressions of the actual environmental value that is to be protected."[5] Selection of the assessment endpoints is based on valued entities (*e.g.,* CRLF, organisms important in the life cycle of the CRLF, and the PCEs of its designated critical habitat), the ecosystems potentially at risk (*e.g.*, waterbodies, riparian vegetation, and upland and dispersal habitats), the migration pathways of prometryn (*e.g.,* runoff, spray drift, etc.), and the routes by which ecological receptors are exposed to prometryn (*e.g.*, direct contact, etc.).

2.8.1 Assessment Endpoints for the CRLF

Assessment endpoints for the CRLF include direct toxic effects on the survival, reproduction, and growth of the CRLF, as well as indirect effects, such as reduction of the prey base or effects to its habitat. In addition, potential effects to critical habitat is assessed by evaluating potential effects to PCEs, which are components of the habitat areas that provide essential life cycle needs of the CRLF. Each assessment endpoint requires one or more "measures of ecological effect," defined as changes in the attributes of an assessment endpoint or changes in a surrogate entity or attribute in response to exposure to a pesticide. Specific measures of ecological effect are generally evaluated based on acute and chronic toxicity information from registrant-submitted guideline tests that are performed on a limited number of organisms. Additional ecological effects data from the open literature are also considered. It should be noted that assessment endpoints are limited to direct and indirect effects associated with survival, growth, and fecundity, and do not include the full suite of sublethal effects used to define the action area. According the Overview Document (U.S. EPA 2004), the Agency relies on acute and chronic effects endpoints that are either direct measures of impairment of survival, growth, or fecundity or endpoints for which there is a scientifically robust, peer reviewed relationship that can quantify the impact of the measured effect endpoint on the assessment endpoints of survival, growth, and fecundity.

A complete discussion of all the toxicity data available for this risk assessment, including resulting measures of ecological effect selected for each taxonomic group of concern, is included in Section 4.0 of this document. A summary of the assessment endpoints and measures of ecological effect selected to characterize potential assessed direct and indirect CRLF risks associated with exposure to prometryn is provided in **Table 2-7**.

[5] U.S. EPA (1992). *Framework for Ecological Risk Assessment.* EPA/630/R-92/001.

Table 2-7 Assessment Endpoints and Measures of Ecological Effects

Assessment Endpoint[c]	Measures of Ecological Effects[6]
Aquatic-Phase CRLF (Eggs, larvae, juveniles, and adults)[a]	
Direct Effects	
1. Survival, growth, and reproduction of CRLF	1a. Rainbow Trout (*Oncorhynchus mykiss)* $LC_{50,}$ 1b. Fathead Minnow (*Pimephales promelas)* NOAEC
Indirect Effects and Critical Habitat Effects	
2. Survival, growth, and reproduction of CRLF individuals via indirect effects on aquatic prey food supply (*i.e.*, fish, freshwater invertebrates, non-vascular plants)	2a. Rainbow Trout LC_{50}, Waterflea (*Daphnia magna)* EC_{50}, Freshwater diatom (*Naviculla pelliculosa)* EC_{50}, Duckweed (*Lemna gibba)* EC_{50} 2b. Fathead Minnow NOAEC, Waterflea NOAEC
3. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat, cover, food supply, and/or primary productivity (*i.e.*, aquatic plant community)	3a. Duckweed EC_{50} 3b. Freshwater diatom EC_{50}
4. Survival, growth, and reproduction of CRLF individuals via effects to riparian vegetation	4a. Oat EC_{25} (seedling emergence); Onion EC_{25} (vegetative vigor) 4b. Cabbage EC_{25} (seedling emergence); Cucumber EC_{25} (vegetative vigor)
Terrestrial-Phase CRLF (Juveniles and adults)	
Direct Effects	
5. Survival, growth, and reproduction of CRLF individuals via direct effects on terrestrial phase adults and juveniles	5a. Mallard Duck[b] (*Anas platyrhynchos*) LD_{50}, Northern Bobwhite Quail (*Colunus virginianus*) LC_{50} 5b. Mallard Duck NOAEC
Indirect Effects and Critical Habitat Effects	
6. Survival, growth, and reproduction of CRLF individuals via effects on terrestrial prey (*i.e.,*terrestrial invertebrates, small mammals , and frogs)	6a. Honey bee (*Apis mellifera*) LD_{50}, Rat (*Ratus norvegicus*) LD_{50} 6b. None, Rat NOAEC
7. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat (*i.e.*, riparian and upland vegetation)	7a. Oat EC_{25} (seedling emergence); Onion EC_{25} (vegetative vigor) 7b. Cabbage EC_{25} (seedling emergence); Cucumber EC_{25} (vegetative vigor)

[a] Adult frogs are no longer in the "aquatic phase" of the amphibian life cycle; however, submerged adult frogs are considered "aquatic" for the purposes of this assessment because exposure pathways in the water are considerably different that exposure pathways on land.
[b] Birds are used as surrogates for terrestrial phase amphibians.
[c] All endpoints are based on guideline studies

[6] All registrant-submitted toxicity data reviewed for this assessment are included in **Appendix A**. Reviewed open literature data is included in **Appendices F-1** and **F-2.**

2.8.2 Assessment Endpoints for Designated Critical Habitat

As previously discussed, designated critical habitat is assessed to evaluate actions related to the use of prometryn that may alter the PCEs of the CRLF's critical habitat. PCEs for the CRLF were previously described in Section 2.6. Actions that may modify critical habitat are those that alter the PCEs and jeopardize the continued existence of the CRLF. Therefore, these actions are identified as assessment endpoints. It should be noted that evaluation of PCEs as assessment endpoints is limited to those of a biological nature (i.e., the biological resource requirements for the listed species associated with the critical habitat) and those for which prometryn effects data are available.

Measures of such possible effects by labeled use of prometryn on critical habitat of the CRLF are described in **Table 2-8.** Some components of these PCEs are associated with physical abiotic features (*e.g.*, presence and/or depth of a water body, or distance between two sites), which are not expected to be measurably altered by use of pesticides. Assessment endpoints used for the analysis of designated critical habitat are based on the adverse modification standard established by U.S. FWS (2006).

Table 2-8 Summary of Assessment Endpoints and Measures of Ecological Effect for Primary Constituent Elements of Designated Critical Habitat[a]

Assessment Endpoint	Measures of Ecological Effect
Aquatic-Phase CRLF PCEs (Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)	
Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile and adult CRLFs.	a. Freshwater diatom EC_{50} b. Oat EC_{25} (seedling emergence); Onion EC_{25} (vegetative vigor) c. Cabbage EC_{25} (seedling emergence); Cucumber EC_{25} (vegetative vigor)
Alteration in water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	a. Freshwater diatom EC_{50} b. Oat EC_{25} (seedling emergence); Onion EC_{25} (vegetative vigor) c. Cabbage EC_{25} (seedling emergence); Cucumber EC_{25} (vegetative vigor)
Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.	a. Rainbow Trout LC_{50}, Waterflea. EC_{50} b. Fathead Minnow NOAEC, Waterflea NOAEC
Reduction and/or modification of aquatic-based food sources for pre-metamorphs (*e.g.*, algae)	a. Freshwater diatom EC_{50}
Terrestrial-Phase CRLF PCEs (Upland Habitat and Dispersal Habitat)	
Elimination and/or disturbance of upland habitat; ability of habitat to support food source of CRLFs: Upland areas within 200 ft of the edge of the riparian vegetation or dripline surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance	a. Oat EC_{25} (seedling emergence); Onion EC_{25} (vegetative vigor) b. Cabbage EC_{25} (seedling emergence); Cucumber EC_{25} (vegetative vigor) c. Rat LD_{50}, Rat NOAEC, Honey Bee LD_{50}, Mallard Duck LD_{50}, Northern Bobwhite Quail LC_{50}, Mallard Duck NOAEC, Rainbow Trout LC_{50}, Fathead Minnow NOAEC
Elimination and/or disturbance of dispersal habitat: Upland or riparian dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal	
Reduction and/or modification of food sources for terrestrial phase juveniles and adults	
Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	
[a] Physico-chemical water quality parameters such as salinity, pH, and hardness are not evaluated because these processes are not biologically mediated and, therefore, are not relevant to the endpoints included in this assessment.	

2.9 Conceptual Model

2.9.1 Risk Hypotheses

Risk hypotheses are specific assumptions about potential adverse effects (i.e., changes in assessment endpoints) and may be based on theory and logic, empirical data, mathematical models, or probability models (U.S. EPA 1998). For this assessment, the risk is stressor-linked, where the stressor is the release of prometryn to the environment. The following risk hypotheses are presumed for this endangered species assessment:

The labeled use of prometryn within the action area may:

- directly affect the CRLF by causing mortality or by adversely affecting growth or fecundity;
- indirectly affect the CRLF by reducing or changing the composition of food supply;
- indirectly affect the CRLF or affect designated critical habitat by reducing or changing the composition of the aquatic plant community in the ponds and streams comprising the species' current range and designated critical habitat, thus affecting primary productivity and/or cover;
- indirectly affect the CRLF or affect designated critical habitat by reducing or changing the composition of the terrestrial plant community (i.e., riparian habitat) required to maintain acceptable water quality and habitat in the ponds and streams comprising the species' current range and designated critical habitat;
- affect the designated critical habitat of the CRLF by reducing or changing breeding and non-breeding aquatic habitat (via modification of water quality parameters, habitat morphology, and/or sedimentation);
- affect the designated critical habitat of the CRLF by reducing the food supply required for normal growth and viability of juvenile and adult CRLFs;
- affect the designated critical habitat of the CRLF by reducing or changing upland habitat within 200 ft of the edge of the riparian vegetation necessary for shelter, foraging, and predator avoidance;
- affect the designated critical habitat of the CRLF by reducing or changing dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal; or
- affect the designated critical habitat of the CRLF by altering chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs.

2.9.2 Diagram

The conceptual model is a graphic representation of the structure of the risk assessment. It specifies the prometryn release mechanisms, biological receptor types, and effects endpoints of potential concern. The conceptual models for terrestrial and aquatic exposures are shown in **Figure 2-5** and **Figure 2-6**, respectively, which include the conceptual models for the aquatic and terrestrial PCE components of critical habitat.

Exposure routes shown in dashed lines are not quantitatively considered because the contribution of those potential exposure routes to potential risks to the CRLF and effects to designated critical habitat is expected to be negligible.

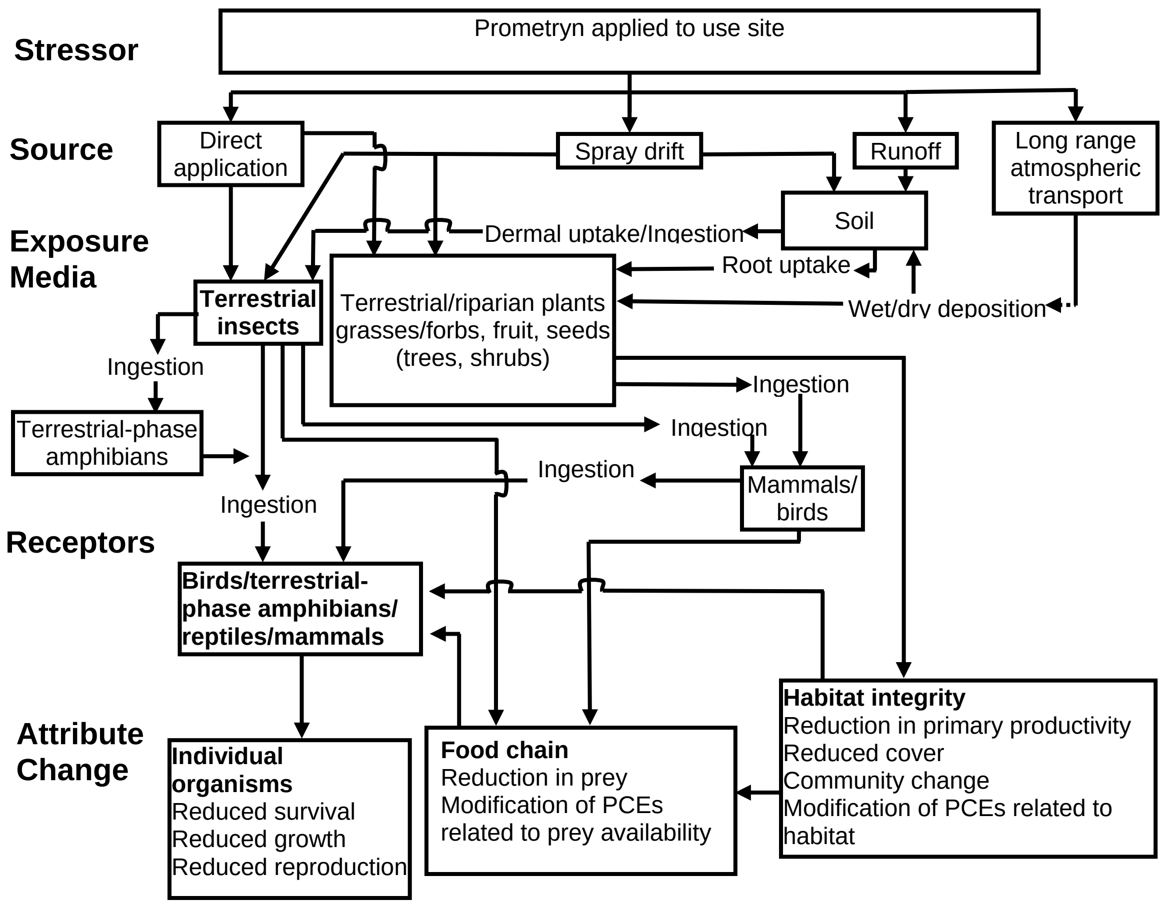
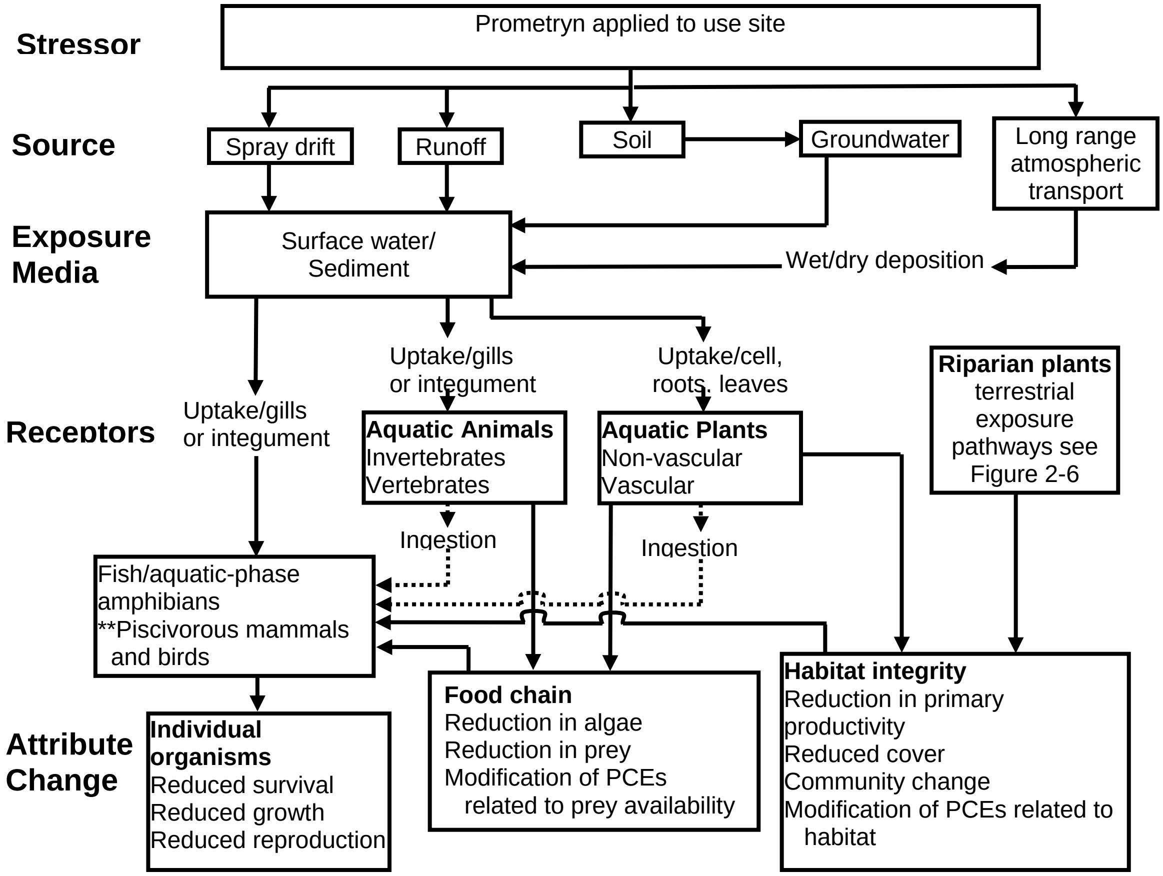


Figure 2-5 Conceptual Model for Prometryn Effects on Terrestrial-Phase of the CRLF



** Route of exposure includes only ingestion of aquatic fish and invertebrates

Figure 2-6 Conceptual Model for Prometryn Effects on Aquatic-Phase of the CRLF

2.10 Analysis Plan

In order to address the risk hypothesis, the potential for direct and indirect effects were estimated for the CRLF, its prey, and its habitat. In the following sections, the use, environmental fate, and ecological effects of prometryn are characterized and integrated to assess the risks. This is accomplished using a risk quotient (or RQ, ratio of exposure concentration to effects concentration) approach. Although risk is often defined as the likelihood and magnitude of adverse ecological effects, the risk quotient-based approach does not provide a quantitative estimate of likelihood and/or magnitude of an adverse effect. However, as outlined in the Overview Document (U.S. EPA 2004), the likelihood of effects to individual organisms from particular uses of prometryn is estimated using the probit dose-response slope and either the level of concern (discussed below) or actual calculated risk quotient value.

2.10.1 Measures to Evaluate the Risk Hypothesis and Conceptual Model

2.10.1.1 Measures of Exposure

The environmental fate and physical-chemical properties of prometryn along with available monitoring data indicate that runoff and leaching to ground water (surface water/ground water interactions) are the principal potential transport mechanisms of prometryn to the aquatic and terrestrial habitats of the CRLF. In this assessment, transport of prometryn through runoff and spray drift is considered in deriving quantitative estimates of prometryn exposure to the CRLF, its prey, and its habitat. The laboratory volatility study (MRID 41875906) as well as the vapor pressure (product chemistry data) and Henry's Law constant (estimated from EPI Suite[7]) suggest low volatilization potential. However, recent data have shown the presence of prometryn in rain and wet deposition (Vogel *et al.* 2008), which suggests that atmospheric transport can occur.

Measures of exposure are based on aquatic and terrestrial models that predict estimated environmental concentrations (EECs) of prometryn using maximum labeled application rates, target crop, time of application, and methods of application. The model used to predict aquatic EECs is a Pesticide Root Zone Model coupled with the Exposure Analysis Model System (PRZM/EXAMS). The model used to predict terrestrial EECs on food items is T-REX. The model used to derive EECs relevant to terrestrial and wetland plants is TerrPlant. These models are parameterized using relevant reviewed registrant-submitted environmental fate data.

PRZM (version 3.12.2, May 2005) and EXAMS (version 2.98.4.6, April 2005) are screening simulation models coupled with the input shell pe5.pl (Aug 2007) to generate daily exposures and 1-in-10 year EECs of prometryn that may occur in surface water bodies adjacent to application sites receiving prometryn through runoff and spray drift for specific scenarios. PRZM simulates pesticide application, movement and transformation on an agricultural field and the resultant pesticide loadings to a receiving water body via runoff, erosion and spray drift. EXAMS simulates the fate of the pesticide and resulting concentrations in the water body. The standard scenario used for ecological pesticide assessments assumes application to a 10-hectare agricultural field that drains into an adjacent 1-hectare water body, 2-meters deep (20,000 m^3 volume) with no outlet. PRZM/EXAMS was used to estimate screening-level exposure of aquatic organisms to prometryn. The measure of exposure for aquatic species is the 1-in-10 year return peak or rolling mean concentration. The 1-in-10 year peak is used for estimating acute exposures of direct effects to the aquatic phase of the CRLF, as well as indirect effects to the CRLF through effects to potential prey items, including: algae, aquatic invertebrates, fish and frogs. The 1-in-10-year 60-day mean is used for assessing chronic exposure to the CRLF and fish and frogs serving as prey items; the 1-in-10-year 21-day mean is used for assessing chronic exposure for aquatic invertebrates, which are also potential prey items. In addition, GENEEC (Version 2.0, August 2001) estimates pesticide

[7] Estimation Programs Interface (EPI) Suite TM, Version 4.0

concentrations immediately after application followed by a single run-off event (peak concentration) as well as the concentration in water (pond) as a function of time (21 day average for chronic exposure concentration to aquatic invertebrates and 60 day average chronic exposure concentration for fish).

Exposure estimates for the terrestrial-phase CRLF and terrestrial invertebrates and mammals (serving as potential prey) assumed to be in the target area or in an area exposed to spray drift are derived using the T-REX model (version 1.3.1, December 7, 2006). This model incorporates the Kenega nomograph, as modified by Fletcher *et al.* (1994), which is based on a large set of actual field residue data. The upper limit values from the nomograph represent the 95th percentile of residue values from actual field measurements (Hoerger and Kenega 1972). For modeling purposes, direct exposures of the CRLF to prometryn through contaminated food are estimated using the EECs for the small bird (20 g) which consumes small insects. Dietary-based and dose-based exposures of potential prey (small mammals) are assessed using the small mammal (15 g) which consumes short grass. The small bird (20g) consuming small insects and the small mammal (15g) consuming short grass are used because these categories represent the largest RQs of the size and dietary categories in T-REX that are appropriate surrogates for the CRLF and one of its prey items. Estimated exposures of terrestrial insects to prometryn are bound by using the dietary based EECs for small insects and large insects.

Birds are currently used as surrogates for terrestrial-phase CRLF. However, amphibians are poikilotherms (body temperature varies with environmental temperature) while birds are homeotherms (temperature is regulated, constant, and largely independent of environmental temperatures). Therefore, amphibians tend to have much lower metabolic rates and lower caloric intake requirements than birds or mammals. As a consequence, birds are likely to consume more food than amphibians on a daily dietary intake basis, assuming similar caloric content of the food items. Therefore, the use of avian food intake allometric equation as a surrogate to amphibians is likely to result in an over-estimation of exposure and risk for reptiles and terrestrial-phase amphibians. Therefore, T-REX (version 1.4.1, October 8, 2008) has been refined to the T-HERPS model (version 1.0, May 15, 2007), which allows for an estimation of food intake for poikilotherms using the same basic procedure as T-REX to estimate avian food intake.

EECs for terrestrial plants inhabiting dry and wetland areas are derived using TerrPlant (version 1.2.2, 12/26/2006). This model uses estimates of pesticides in runoff and in spray drift to calculate EECs. EECs are based upon solubility, application rate and minimum incorporation depth.

The AgDRIFT model (Ver. 2.1.03) is used to assess exposures of terrestrial phase CRLF and its prey to prometryn deposited on terrestrial habitats by spray drift. AGDISP (version 8.13; dated 12/14/2004) (Teske and Curbishley 2003) is used to simulate aerial and ground applications using the Gaussian far-field extension. In addition to the buffered area from the spray drift analysis, the downstream extent of prometryn that exceeds the LOC for the effects determination is also considered.

2.10.1.2 Measures of Effect

Data identified in Section 2.8 are used as measures of effect for direct and indirect effects to the CRLF. Data were obtained from registrant submitted studies or from literature studies identified by ECOTOX (OPP 2004) [8]. The ECOTOXicology database (ECOTOX) was searched in order to provide more ecological effects data and in an attempt to bridge existing data gaps. ECOTOX is a source for locating single chemical toxicity data for aquatic life, terrestrial plants, and wildlife. ECOTOX was created and is maintained by the U.S. EPA, Office of Research and Development, and the National Health and Environmental Effects Research Laboratory's Mid-Continent Ecology Division.

The assessment of risk for direct effects to the terrestrial-phase CRLF makes the assumption that toxicity of prometryn to birds is similar to or less than the toxicity to the terrestrial-phase CRLF. The same assumption is made for fish and aquatic-phase CRLF. Algae, aquatic invertebrates, fish, and amphibians represent potential prey of the CRLF in the aquatic habitat. Terrestrial invertebrates, small mammals, and terrestrial-phase amphibians represent potential prey of the CRLF in the terrestrial habitat. Aquatic, semi-aquatic, and terrestrial plants represent habitat of CRLF.

The acute measures of effect used for animals in this screening-level assessment are the LD_{50}, LC_{50} and EC_{50}. LD stands for "Lethal Dose", and LD_{50} is the amount of a material, given all at once, that is estimated to cause the death of 50% of the test organisms. LC stands for “Lethal Concentration” and LC_{50} is the concentration of a chemical that is estimated to kill 50% of the test organisms. EC stands for “Effective Concentration” and the EC_{50} is the concentration of a chemical that is estimated to produce a specific effect in 50% of the test organisms. Endpoints for chronic measures of exposure for listed and non-listed animals are the NOAEL/NOAEC and NOEC. NOAEL stands for “No Observed-Adverse-Effect-Level” and refers to the highest tested dose of a substance that has been reported to have no harmful (adverse) effects on test organisms. The NOAEC (*i.e.*, “No-Observed-Adverse-Effect-Concentration”) is the highest test concentration at which none of the observed effects were statistically different from the control. The NOEC is the No-Observed-Effects-Concentration. For non-listed plants, only acute exposures are assessed (*i.e.*, EC_{25} for terrestrial plants and EC_{50} for aquatic plants).

It is important to note that the measures of effect for direct and indirect effects to the CRLF and its designated critical habitat are associated with impacts to survival, growth, and fecundity, and do not include the full suite of sublethal effects used to define the action area. According to the Overview Document (USEPA 2004), the Agency relies on effects endpoints that are either direct measures of impairment of survival, growth, or fecundity or endpoints for which there is a scientifically robust, peer reviewed relationship that can quantify the impact of the measured effect endpoint on the assessment endpoints of survival, growth, and fecundity.

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[8] Office of Pesticide Programs, U.S. EPA. July 16, 2004. Interim Guidance of the Evaluation Criteria for Ecological Toxicity Data in the Open Literature: Phases I and II. Procedures for Identifying, Selecting, and Acquiring Toxicity Data Published in the Open Literature For Use in Ecological Risk Assessments.

2.10.1.3 Integration of Exposure and Effects

Risk characterization is the integration of exposure and ecological effects characterization to determine the potential ecological risk from agricultural uses of prometryn, and the likelihood of direct and indirect effects to CRLF in aquatic and terrestrial habitats. The exposure and toxicity effects data are integrated in order to evaluate the risks of adverse ecological effects on non-target species. For the assessment of prometryn risks, the risk quotient (RQ) method is used to compare exposure and measured toxicity values. EECs are divided by acute and chronic toxicity values. The resulting RQs are then compared to the Agency's levels of concern (LOCs) (U.S. EPA 2004) (see **Appendix C**)

For this endangered species assessment, listed species LOCs are used for comparing RQ values for acute and chronic exposures of prometryn directly to the CRLF. If estimated exposures directly to the CRLF of prometryn resulting from a particular use are sufficient to exceed the listed species LOC, then the effects determination for that use is "may affect". When considering indirect effects to the CRLF due to effects to animal prey (aquatic and terrestrial invertebrates, fish, frogs, and mice), the listed species LOCs are also used. If estimated exposures to CRLF prey of prometryn resulting from a particular use are sufficient to exceed the listed species LOC, then the effects determination for that use is a "may affect." If the RQ being considered also exceeds the non-listed species acute risk LOC, then the effects determination is a LAA. If the acute RQ is between the listed species LOC and the non-listed acute risk species LOC, then further lines of evidence (*i.e*. probability of individual effects, species sensitivity distributions) are considered in distinguishing between a determination of NLAA and a LAA. When considering indirect effects to the CRLF due to effects to algae as dietary items or plants as habitat, the non-listed species LOC for plants is used because the CRLF does not have an obligate relationship with any particular aquatic and/or terrestrial plant. If the RQ being considered for a particular use exceeds the non-listed species LOC for plants, the effects determination is "may affect". Further information on LOCs is provided in **Appendix C**.

2.10.1.4 Data Gaps

Environmental Fate

Anaerobic and aerobic aquatic metabolism studies are not available for prometryn. The aerobic aquatic metabolism half-life of a chemical is an important input parameter in PRZM-EXAMS simulations. The aerobic soil metabolism was used to estimate the aerobic aquatic metabolism rate in accordance with the PRZM-EXAMS Input Parameters Selection Guidelines (February 28, 2001).

Environmental Effects

Environmental effects data gaps that are identified do not affect the CRLF assessment. The following studies are required for future risk assessments where it is expected that marine/estuarine ecosystems are likely to be affected by prometryn use:

- 850.1350 Aquatic invertebrate life cycle (saltwater): mysid shrimp (*Mysidopsis bahia*)
- 850.1400 Fish early-life stage (saltwater): recommended sheepshead minnow (*Cyprinodon variegatus*)

3.0 Exposure Assessment

Prometryn is formulated as liquid, emulsifiable concentrate, flowable concentrate, and water dispersible granules. Application equipment includes ground and aerial spray applications.

3.1 Label Application Rates and Intervals

Prometryn labels may be categorized into two types: labels for manufacturing uses (including technical grade prometryn and its formulated products) and end-use products. While technical products, which contain prometryn of high purity, are not used directly in the environment, they are used to make formulated products, which can be applied in specific areas to control weeds. The formulated product labels legally limit prometryn's potential use to only those crops and sites that are specified on the labels.

Prometryn is used only on agricultural crops. Currently registered agricultural uses of prometryn within California include celery, fennel (anise, sweet anise), dill, parsley, and cotton. The uses being assessed are summarized in **Table 3-1**.

Table 3-1 Prometryn Use Input Parameters[1]

Agricultural Commodity	Number of crop cycles per year	Maximum Single application rate, lb a.i./acre	Number of Applications per crop cycle	Interval between applications	Maximum Application per crop/year lb a.i./acre	Method of Application
Celery	2.5[2]	2	1	n/a	5	Ground
Fennel	2[2]	2	1	n/a	4	Ground
Parsley	1[3]	2	1	n/a	2	Ground
Dill	1[3]	1.6	1	n/a	1.6	Ground
Cotton	1	2.4	1-3*		5.5	Ground/Aerial

[1] Product label for Caparol® 4L; 44.4% prometryn; liquid; EPA Reg No. 100-620 is representative of typical application instructions for prometryn herbicide use on cotton in California.
[2] U.S. EPA. 2007. Memo from Monisha Kaul (BEAD) to Melissa Panger (EFED). Subject: Maximum Number of Crop Cycles Per Year in California for Methomyl Use Sites. Dated February 28.
[3] Number of crop cycles per year as assumed by EFED
* Applications on cotton were modeled using single applications for pre-plant and post-harvest. Also, the labels allow 3 applications: as an aerial spray application of 2.4 lbs ai/A at pre-plant; ground spray application of 0.7 lbs ai/A at post-plant; and ground spray application of 2.4 lbs ai/A at post-harvest.

3.2 Aquatic Exposure Assessment

3.2.1 Modeling Approach

Aquatic exposures are quantitatively estimated for all of assessed uses using scenarios that represent high exposure sites for prometryn use. Each of these sites represents a 10 hectare field that drains into a 1-hectare pond that is 2 meters deep and has no outlet.

Exposure estimates generated using the standard pond are intended to represent a wide variety of vulnerable water bodies that occur at the top of watersheds including prairie pot holes, playa lakes, wetlands, vernal pools, man-made and natural ponds, and intermittent and first-order streams. As a group, there are factors that make these water bodies more or less vulnerable than the standard surrogate pond. Static water bodies that have larger ratios of drainage area to water body volume would be expected to have higher peak EECs than the standard pond. These water bodies will be either shallower or have large drainage areas (or both). Shallow water bodies tend to have limited additional storage capacity, and thus, tend to overflow and carry pesticide in the discharge whereas the standard pond has no discharge. As watershed size increases beyond 10 hectares, at some point, it becomes unlikely that the entire watershed is planted to a single crop, which is all treated with the pesticide. Headwater streams can also have peak concentrations higher than the standard pond, but they tend to persist for only short periods of time and are then carried downstream.

Crop-specific management practices for all of the assessed uses of prometryn were used for modeling, including application rates, number of applications per year, application intervals, application methods and the first application date for each crop. The date of first application was developed based on several sources of information including data provided by BEAD, a summary of individual applications from the CDPR PUR data, and Crop Profiles maintained by the USDA. Additionally, seasonal high rainfall distribution patterns were used to select pesticide application dates when CDPR PUR data did not indicate any clear seasonal trend in prometryn application timing. This situation was observed for prometryn applications to fennel, parsley, and celery. A sample distribution of prometryn applications to parsley is shown in **Figure 3-1**.

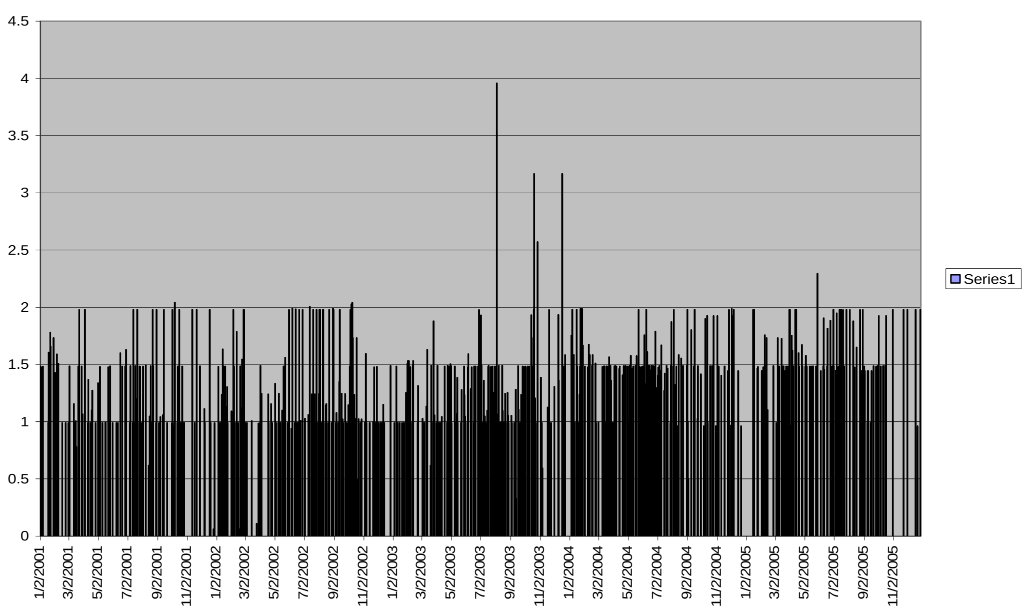

Figure 3-1: CDPR PUR Data on Prometryn Applications on Parsley. Application rate in lbs/A is on the y-axis and date is on the x-axis.

Because there is no temporal pattern for prometryn use on parsley, a spring and fall application timing was selected to provide a conservative aquatic exposure assessment for prometryn. These application timings correspond with seasonal high events (**Figure 3-2**).

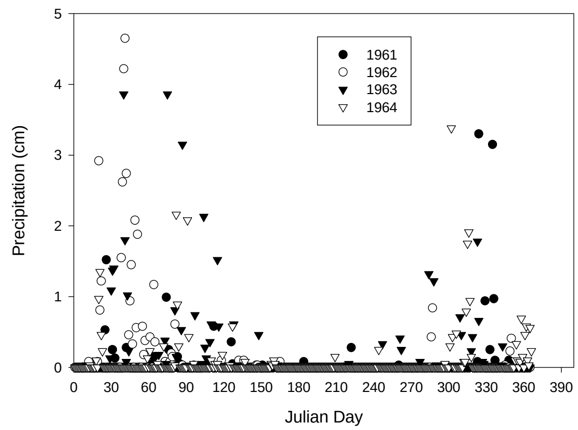

Figure 3-2: Precipitation Data from 1961 to 1964 for Surrogate Parsley Scenario (CA Lettuce Scenario)

3.2.2 Model Inputs

PRZM and EXAMS

Prometryn environmental fate and physical-chemical data used as PRZM/EXAMS input parameters are listed in **Table 3-2**.

Table 3-2 Physical and Chemical Properties and Environmental Fate Data Used as Input Parameters for PRZM and EXAMS [1]

Physical-chemical Properties and Environmental Fate Input parameters (PRZM – EXAMS)	Available Data	Selected Value	Source
Molecular Weight, grams/mole	241.35	241.35	Product chemistry
Solubility in Water, mg/L	33	33	Product chemistry
Vapor Pressure	1.0×10^{-3} torr	1.0×10^{-3} torr	Product chemisty
Henry's Law Constant, atm-m3-mole-1	No; Estimated	9.1×10^{-9}	Estimated (EPIWin, Version 4.0)
[Abiotic] Hydrolysis Half-life, days	Stable	0	161-1 MRID495737-04
[Direct] Photolysis in Water, days	Stable	0	161-2 MRID495737-04

Aerobic Soil Metabolism Half-life. Days	270 days (1-soil)	3x270 **810**	162-1 MRID00148338
Anaerobic Aquatic Metabolism Half-life	No	0	Not applicable
Aerobic Aquatic Metabolism Half-life	No	2x810 **1620**	Not applicable
Sorption Coefficients, Koc Dependent	Sand 246 Loamy sand 169 Silt loam 117 Silty clay loam 448	**244**	163-1 MRID 00148338
[1] Inputs determined in accordance with EFED "Guidance for Chemistry and Management Practice Input Parameters for Use in Modeling the Environmental Fate and Transport of Pesticides" dated February 28, 2002			

The rationale for PRZM/EXAMS scenarios selection is shown in **Table 3-3.** When available, scenarios developed for the California RLF were used. Otherwise, standard scenarios for California were used. For each agricultural commodity, spring and fall applications were considered in the assessment to account for seasonal precipitation patterns.

Table 3-3. Scenarios for PRZM and EXAMS and Application Dates

Agricultural Commodity	Selected Scenario	Meteorological Station	Metfile
Celery/Fennel	CA RLF, Row Crop	Monterrey (Santa Maria)	W23234
Parsley	CA Lettuce STD	Monterrey (Santa Maria)	W23273
Dill	CA RLF Row Crop	See celery	See celery
Cotton	CA Cotton STD	Bakersfield	W23155

3.2.3 Results

Aquatic EECs for the various scenarios and application practices are listed in **Table 3-4**. Inputs and outputs for PRZM-EXAMS are contained in **Appendix J-1.** Inputs and outputs for GENEEC are also contained in **Appendix J-1** as well as **Appendix J-2.**

Table 3-4: PRZM/EXAMS Estimated Exposure Concentrations (µg/L) for Prometryn

Crop	Application Timing	Incorporation Depth (cm)	1-in-10-Year		
			Peak	21-Day	60- Day
Celery/ Fennel	Spring (April 1) Fall (December 1)	4.0 4.0	102.9 152.9	102 142.8	101.9 142.5
Parsley	Spring(April 1) Fall (December 1)	4.0 4.0	209.7 377.3	208.8 370.0	206.9 368.7
Cotton	Spring (April 1)-Ground Spray Spring (April 1)-Aerial Spray Fall (December 1) – Ground Premg(grd spray)-Post Ememg-Post Har[1] Premg(air spray)-Post Ememg-Post Har[1]	4.0 10.2 4.0 10.2-4.0-4.0 10.2-4.0-4.0	49.2 88.8 93.2 187.0 240.0	48.4 88.2 88.8 182.4 235.4	47.3 87.7 86.3 176.9 231.7
[1]Pre-emergent application date = April 1; Post-emergent application date = May 1; Post harvest application date = December 1					

It is important to note that the 1-in-10 year estimated environmental concentrations represent accumulated concentrations over a 27 year period. Because of the year to year accumulation in the standard pond, the 1-in-10 year EEC has no probabilistic meaning regarding return frequency of concentrations. However, the reported EECs are highly conservative due to the extensive residue accumulation in the pond.

3.2.4 Existing Surface Water Monitoring Data

A critical step in the process of characterizing EECs is comparing the modeled estimates with available surface water monitoring data. Monitoring data were evaluated to assess measured concentrations of prometryn in surface water and ground water. Monitoring data were obtained from USGS NAWQA (http://water.usgs.gov/nawqa) and the California Department of Pesticide Regulation (CDPR).

3.2.4.1 USGS NAWQA Surface Water Data

The USGS surface water monitoring data for prometryn in CA are based on 14 sampling sites in 5 counties. The minimum reporting limit (MRL) for prometryn ranged from 0.0054 to 0.011 μg/L with a median MRL of 0.0054 μg/L. The detection frequency of prometryn among surface water monitoring sites is 32.6% (105 detects/322 total samples). The maximum prometryn concentration detected was 0.621 μg/L (**Table 3-5**). The maximum average prometryn concentration is 0.032593 μg/L. The sampling site with the highest prometryn concentrations (Site # 11274538) was identified as Stanislaus County with a watershed size of 1,276 square miles. The land use in the watershed is classified as mixed.

Table 3-5: USGS NAWQA Surface Water Data for Prometryn in California

County	USGS Station ID	Number of Samples	Maximum Conc. (μg/L)
MERCED	11273500	38	0.006
MERCED	372323120481700	5	0.014
MERCED	372829120420801	7	0.023
MERCED	372839120413901	19	0.010
MERCED	373012120393401	4	0.012
MERCED	373020120385201	1	0.005
MERCED	373112120382901	23	0.014
MERCED	373115120382801	26	0.013
RIVERSIDE	11074000	30	0.006
SACRAMENTO	11447360	30	0.008
SACRAMENTO	11447650	52	0.006
SAN JOAQUIN	11303500	57	0.285
STANISLAUS	11274538	28	0.621
STANISLAUS	374111121000301	1	0.005
STANISLAUS	374115120591601	1	0.005

3.2.4.2 USGS NAWQA Groundwater Data

The USGS ground water monitoring data for prometryn in CA are based on 247 sample sites in Fresno, Kern, Kings, Madera, Merced, Orange, and Sacramento Counties. The minimum reporting limit (MRL) for prometryn ranged from 0.0059 to 0.054 µg/L. There were no detections of prometryn in ground water. The land use in the ground water recharge areas were classified as cropland, mixed, orchard/vineyard, and residential/commerical.

3.2.4.3 California Department of Pesticide Regulation (CDPR) Data

The CDPR surface water monitoring data for prometryn is based on samples from Butte, Colusa, Sacramento, Solano, Sutter, Tehama, Yolo, Yuba, and Shasta Counties. The limit of quantification (LOQ) was 0.5 µg/L. The CDPR data for surface water shows no detections of prometryn. All samples had prometryn concentrations below the LOQ.

3.2.4.4 Atmospheric Monitoring Data

The US Geological Survey (USGS) has reported the presence of pesticides in rain and wet deposition in four USA agricultural watersheds (Maryland, Indiana, Nebraska and California) at a 0.05 µg/L laboratory reporting limit (Vogel *et al.*, 2008)[9] during the 2003-2004 sampling period.

In California, prometryn was detected in rain in the San Joaquin -Tulare- Merced River[10] basin, where a wide variety of crops are grown. Wet depositions were related to storm events during the rainy season (December-March). Of 13 detections, a maximum concentration of 0.031 µg/L was detected in rain on December 11, 2003 and a maximum wet deposition of 0.735 µg/m^2 on December 16, 2003. Because data on detection of prometryn in rain and wet deposition is limited, spray drift estimates are used as high-end estimates for potential atmospheric transport contributions.

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9 The cropland in West California (samples collected) is dominated by almonds and vineyards. In addition to the California studies, other studies where conducted in agricultural watersheds in Maryland, Indiana, and Nebraska. Atrazine was detected in all samples. Simazine was the most frequently detected triazine in California.

10 For description of the California's Merced River Basin see Vogel *et al.* (2008) and pertinent references therein. The environmental setting of the Basin is described in Gronberg & Kratzer (2007).

3.2.5 Spray Drift Buffer

Aerial and ground spray application are recommended for prometryn [Caparol (EPA Reg No. 100-620), Cotton Pro (EPA Reg No. 1812-274), Prometryn 4L Herbicide (EPA Reg. No. 9779-297), and Prometryne + MSMA (EPA Reg, No. 9779-317). There are limited mitigation measures for ground spray applications. For aerial applications, spray drift management options are as follows:

- Applications should be made a maximum height of 10 feet.
- Applications should not be made at wind speeds exceeding 10 mph
- A 400-feet upwind spray drift buffer should be employed when sensitive non-target plants are near the application site
- Coarse to Medium Droplet Size Spectrum should be used.

Tier 1 AgDrift (ver 2.1.03) modeling was used to estimate the impact of the 400 feet buffer on terrestrial and aquatic EECs. Input parameters for Tier I AgDrift spray drift modeling are shown in **Table 3-6**. Spray drift deposition concentrations for Tier I AgDrift modeling are shown in **Table 3-7**. Provided the pesticide travels a distance of 400 ft from a point source, a 0.011 spray fraction of the applied concentration is expected for aerial applications and a 0.0021 spray fraction of applied concentration is expected for ground applications (according to Tier 1 AgDrift). The terrestrial plant EECs calculated in TerrPlant based on the given aerial and ground spray fractions at 400 ft are shown in **Table 3-8.**

Table 3-6: Input Parameters for Tier I AgDrift Modeling

Parameter	Input Value
Droplet Spectrum	Coarse to Medium
Application Rate	2.4 lbs/A

Table 3-7: Tier 1 AgDrift Terrestrial and Aquatic EECs from Spray Drift Alone

Buffer Distance (feet)	Spray Method	Maximum Application Rate (lbs ai/A)	EECs	
			Terrestrial (lbs ai/A)	Aquatic (µg/L)
0	Aerial	2.4	1.200	11.993
400	Aerial	2.4	0.026	1.207
997	Aerial	2.4	0.013	0.699
0	Ground	2.4	2.43	2.218
400	Ground	2.4	0.005	0.227
997	Ground	2.4	0.002	0.092

Table 3-8: Terrestrial Plant EECs given aerial and ground spray fractions of the maximum applied concentration (2.4 lbs a.i./A on cotton) at 400 feet

Use	Application rate (lbs a.i./A)	Application method	Spray Fractions at 400 ft	Spray drift EEC (lbs a.i./A)	Dry area EEC (lbs a.i./A)	Semi-aquatic area EEC (lbs a.i./A)
Cotton	2.4	Aerial[1]	0.011	0.0264	0.0384	0.1464
Cotton	2.4	Ground[2]	0.0021	0.005	0.053	0.485

[1] Incorporation depth is 4 inches (10.2 cm) based on the label.
[2] Incorporation depth is 1.6 inches (4 cm) based on PRZM/EXAMS default. The TerrPlant default value of 1 was used.

Tier II AgDisp (ver 8.13) modeling was conducted to assess the impact of spray drift mitigation recommendations for aerial spray applications. Input parameters for Tier II AgDisp spray drift modeling are shown in **Table 3-9**. Spray drift deposition concentrations from Tier II AgDisp modeling are shown in **Table 3-10**.

Table 3-9: Input Parameters for Tier II AgDisp Modeling

Parameter	Input Value
Aircraft	Air tractor AT-401
Release Height	10 ft
DSD	Coarse to Medium
Wind Speed	10 mph
Temp	650F
Relative Humidity	50%
Spray Volume Rate	5 gal/A
Active Fraction	0.1056
Nonvol. Fraction	0.24

Table 3-10: Tier II AgDisp Terrestrial and Aquatic EECs from Aerial Spray Drift Alone

Buffer Distance (feet)	Spray Method	Maximum Application Rate (lbs ai/A)	EECs	
			Terrestrial (lbs ai/A)	Aquatic (µg/L)
0	Aerial	2.4	6.1	3.546
400	Aerial	2.4	0.04	0.611
1000	Aerial	2.4	0.0094	0.083

3.3 Terrestrial Animal Exposure Assessment

T-REX (Version 1.4.1) is used to calculate dietary and dose-based EECs of prometryn for the CRLF and its potential prey (*e.g.* small mammals and terrestrial insects) inhabiting terrestrial areas. EECs used to represent the CRLF are also used to represent exposure values for frogs serving as potential prey of CRLF adults. T-REX simulates a 1-year time period. For this assessment, ground and aerial spray applications of prometryn are considered, as discussed below.

Terrestrial EECs for foliar formulations of prometryn were derived for the uses summarized in **Table 3-4**. Given that no data on interception and subsequent dissipation from foliar surfaces is available for prometryn, a default foliar dissipation half-life of 35 days is used based on the work of Willis and McDowell (1987). Although prometryn can be applied to cotton at pre-plant (2.4 lbs a.i./A), post-emergence (0.7 lbs a.i./A), and post harvest (2.4 lbs a.i./A), all three uses were not included in T-REX because the model does not account for variable application rates during the year. Therefore, the highest application rate (2.4 lbs a.i./A) on cotton at pre-plant incorporation (Reg No. 100-620, 9779-297, 10163-94, 34704-692, 66222-15) was used in T-REX to calculate the most conservative estimates of the risk quotients and the lowest application rate (0.7 lbs a.i./A) on cotton was used to bracket the risk quotient estimates at the lower bound values. Celery, fennel, parsley and dill all have application rates below the highest application rate on cotton. Therefore, the risk quotient calculations on the highest application rate on cotton are representative of the upper bound estimates of risk to the CRLF. Use specific input values, including number of applications, application rate and application interval are provided in **Table 3-11**. An example output from T-REX is available in **Appendix E**.

Table 3-11 Input Parameters for Foliar Applications Used to Derive Terrestrial EECs for Prometryn with T-REX

Use (Application method)	Application rate (lbs ai/A)	Number of Applications
Cotton	2.4	1
Cotton	0.7	1
Celery/Fennel/Parsley	2.0	1
Dill	1.6	1

T-REX is also used to calculate EECs for terrestrial insects exposed to prometryn. Dietary-based EECs calculated by T-REX for small and large insects (units of a.i./g) are used to bound an estimate of exposure to terrestrial insects. Available acute contact toxicity data for bees exposed to prometryn (in units of µg a.i./bee), are converted to µg a.i./g (of bee) by multiplying by 1 bee/0.128 g. The EECs are later compared to the adjusted acute contact toxicity data for bees in order to derive RQs.

For modeling purposes, exposures of the CRLF to prometryn through contaminated food are estimated using the EECs for the small bird (20 g) which consumes small insects. Dietary-based and dose-based exposures of potential prey are assessed using the small mammal (15 g) which consumes short grass. Upper-bound Kenega nomogram values reported by T-REX for these two organism types are used for derivation of EECs for the CRLF and its potential prey (**Table 3-12**). Dietary-based EECs for small and large insects reported by T-REX as well as the resulting adjusted EECs are available in **Table 3-13**. An example output from T-REX v. 1.4.1 is available in **Appendix E**.

Table 3-12 Upper-bound Kenega Nomogram EECs for Dietary- and Dose-based Exposures of the CRLF and its Prey to Prometryn

Use	Application Rate (lbs a.i./A)	EECs for CRLF (small avian, 20g) Residues on small insects		EECs for Prey (small mammals, 15g) Residues on short grass	
		Dietary-based EEC (ppm)	Dose-based EEC (mg/kg-bw)	Dietary-based EEC (ppm)	Dose-based EEC (mg/kg-bw)
Cotton	2.4	324	369	576	549.2
Cotton	0.7	94.5	107.6	168	160.2
Celery/Fennel/Parsley	2.0	270	307.5	480	457.6
Dill	1.6	216	246	384	366.1

Table 3-13 EECs (ppm) for Indirect Effects to the Terrestrial-Phase CRLF via Effects to Terrestrial Invertebrate Prey Items

Use	Application Rate (lbs a.i./A)	Small Insect	Large Insect
Cotton	2.4	324	36
Cotton	0.7	94.5	10.5
Celery/Fennel/Parsley	2.0	270	30
Dill	1.6	216	24

3.4 Terrestrial Plant Exposure Assessment

TerrPlant (Version 1.2.2) is used to calculate EECs for non-target plant species inhabiting dry and semi-aquatic areas. Parameter values for application rate, drift assumption and incorporation depth are based upon the use and related application method (**Table 3-14**). A runoff value of 0.02 is utilized based on prometryn's solubility in water (33 mg/L), which is classified by TerrPlant. For aerial and ground application methods, drift is assumed to be 5% and 1%, respectively. EECs relevant to terrestrial plants consider pesticide concentrations in drift and in runoff. These EECs are listed by use in Table 3-14. An example output from TerrPlant v.1.2.2 is available in **Appendix M.**

Table 3-14 TerrPlant Inputs and Resulting EECs for Plants Inhabiting Dry and Semi-aquatic Areas Exposed to Prometryn via Runoff and Drift

Use	Application rate (lbs a.i./A)	Application method	Drift Value (%)	Spray drift EEC (lbs a.i./A)	Dry area EEC (lbs a.i./A)	Semi-aquatic area EEC (lbs a.i./A)
Cotton	2.4	Aerial (Winter)[1]	5	0.12	0.132	0.24
Cotton	2.4	Ground Spray (Spring)[2]	1	0.024	0.072	0.504
Cotton	0.7	Aerial (Winter)[1]	5	0.035	0.038	0.07
Cotton	0.7	Ground Spray (Spring)[2]	1	0.007	0.021	0.147
Celery/Fennel/Parsley	2.0	Ground Spray[2]	1	0.02	0.06	0.42
Dill	1.6	Ground Spray[2]	1	0.016	0.048	0.336

[1] Incorporation depth is 4 inches (10.2 cm) based on the label.
[2] Incorporation depth is 1.6 inches (4 cm) based on PRZM/EXAMS default. The TerrPlant default value of 1 was used.

4.0 Effects Assessment

This assessment evaluates the potential for prometryn to directly or indirectly affect the CRLF or its designated critical habitat. As discussed in Section 2.7, assessment endpoints for the CRLF effects determination include direct toxic effects on the survival, reproduction, and growth of CRLF, as well as indirect effects, such as reduction of the prey base or effects to its habitat. In addition, potential effects to critical habitat are assessed by evaluating effects to the PCEs, which are components of the critical habitat areas that provide essential life cycle needs of the CRLF. Direct effects to the aquatic-phase of the CRLF are based on toxicity information for freshwater fish, while terrestrial-phase effects are based on avian toxicity data, given that birds are generally used as a surrogate for terrestrial-phase amphibians. Because the frog's prey items and habitat requirements are dependent on the availability of freshwater fish and invertebrates, small mammals, terrestrial invertebrates, and aquatic and terrestrial plants, toxicity information for these taxa are also discussed. Acute (short-term) and chronic (long-term) toxicity information is characterized based on registrant-submitted studies and a comprehensive review of the open literature on prometryn.

As described in the Agency's Overview Document (U.S. EPA 2004), the most sensitive endpoint for each taxon is used for risk estimation. For this assessment, evaluated taxa include freshwater fish, freshwater invertebrates, aquatic plants, birds (surrogate for terrestrial-phase amphibians), mammals, terrestrial invertebrates, and terrestrial plants.

Toxicity endpoints are established based on data generated from guideline studies submitted by the registrant, and from open literature studies that meet the criteria for inclusion into the ECOTOX database maintained by EPA/Office of Research and Development (ORD) (U.S. EPA 2004). Open literature data presented in this assessment were obtained from ECOTOX information obtained on October 31, 2008. In order to be included in the ECOTOX database, papers must meet the following minimum criteria:

(1) the toxic effects are related to single chemical exposure;[11]
(2) the toxic effects are on an aquatic or terrestrial plant or animal species;
(3) there is a biological effect on live, whole organisms;
(4) a concurrent environmental chemical concentration/dose or application rate is reported; and
(5) there is an explicit duration of exposure.

Data that pass the ECOTOX screen are evaluated along with the registrant-submitted data, and may be incorporated qualitatively or quantitatively into this endangered species assessment. In general, effects data in the open literature that are more conservative than the registrant-submitted data are considered. The degree to which open literature data are quantitatively or qualitatively characterized for the effects determination is dependent on whether the information is relevant to the assessment endpoints (*i.e.*, maintenance of

[11] The studies that have information on mixtures are listed in the bibliography as rejected due to the presence of mixtures. These studies are evaluated by EFED when applicable to the assessment; however, the data is not used quantitatively in the assessment.

CRLF survival, reproduction, and growth) identified in Section 2.8. For example, endpoints such as behavior modifications are likely to be qualitatively evaluated, because quantitative relationships between modifications and reduction in species survival, reproduction, and/or growth are not available. Although the effects determination relies on endpoints that are relevant to the assessment endpoints of survival, growth, or reproduction, it is important to note that the full suite of sublethal endpoints potentially available in the effects literature (regardless of their significance to the assessment endpoints) are considered to define the action area for prometryn.

Citations of all open literature not considered as part of this assessment because they were either rejected by the ECOTOX screen or accepted by ECOTOX but not used (*e.g.*, the endpoint is less sensitive) are included in **Appendices G** and **H**. **Appendix H** also includes a rationale for rejection of those studies that did not pass the ECOTOX screen and those that were not evaluated as part of this endangered species risk assessment. A detailed spreadsheet of the available ECOTOX open literature data, including the full suite of lethal and sublethal endpoints is presented in **Appendix F-1**. **Appendix F-2** represents the available ECOTOX open literature data as a bibliographic listing with rationale for use or rejection in the risk assessment. **Appendix L** also includes a summary of the human health effects data for prometryn.

In addition to registrant-submitted and open literature toxicity information, other sources of information, including use of the acute probit dose-response relationship to establish the probability of an individual effect and reviews of the Ecological Incident Information System (EIIS), are conducted to further refine the characterization of potential ecological effects associated with exposure to prometryn. A summary of the available aquatic and terrestrial ecotoxicity information, use of the probit dose-response relationship, and the incident information for prometryn are provided in Sections 4.1 through 4.4, respectively.

Degradation products of prometryn in soil metabolism studies are 2,4-bis(isopropylamino)-6-hydroxy-s-triazine (CS-11526) at 27% of the applied radioactivity after one year post-treatment and 2-amino-4-isopropylamino-6-methylthio-s-triazine (GS-11354) at less than 10% after one year post-treatment. There are no available ecotoxicity data and limited environmental fate data for these degradation products. Based on EPA's human health assessment conducted to support the prometryn RED (Wassell 1998), the only residue of concern is prometryn. Therefore, the degradation products of prometryn are not considered in this assessment.

Two acceptable ecotoxicity studies on mixtures are available for two freshwater fish species using Primaze 80W, which contains 40% prometryn a.i. and 38% atrazine a.i. The acute toxicity study (MRID 00040692) on the warmwater fish bluegill sunfish (*Lepomis macrochirus*) yielded a 96-hour LC_{50} of 21 mg/L, which indicates that the formulated product is slightly toxic to bluegill. The acute toxicity study (MRID 00024738) on the coldwater fish rainbow trout (*Oncorhynchus mykiss*) yielded a 96-hour LC_{50} of 9.6 mg/L, which indicates that the formulated product is moderately toxic to rainbow trout.

4.1 Evaluation of Aquatic Ecotoxicity Studies

Table 4-1 summarizes the most sensitive aquatic toxicity endpoints for the CRLF, based on an evaluation of both the submitted studies and the open literature, as previously discussed. A brief summary of submitted and open literature data considered relevant to this ecological risk assessment for the CRLF is presented below. Additional information is provided in **Appendix A.**

Table 4-1 Freshwater Aquatic Toxicity Profile for Prometryn

Assessment Endpoint	Species	Toxicity Value Used in Risk Assessment	Describe effect (i.e. mortality, growth, reproduction)	Citation MRID # (Author & Date)	Study Classification
Acute Direct Toxicity to Aquatic-Phase CRLF	Rainbow Trout[1]	LC_{50} = **2.9** mg a.i./L Slope = 3.4 (95% C.I. 1.99-4.83) TGAI, 99% a.i.	Mortality	00070686 (Beliles, Scott, & Knott 1965)	Acceptable
Chronic Direct Toxicity to Aquatic-Phase CRLF	Fathead Minnow[1]	NOAEC = **0.62** mg a.i./L TGAI, 98.4%	Most sensitive endpoint is growth (specifically, dry and wet weight)	43801702 (Graves, Mank, & Swigert 1995)	Acceptable
Indirect Toxicity to Aquatic-Phase CRLF via Acute Toxicity to Freshwater Invertebrates (i.e. prey items)	*Daphnia magna*	EC_{50} = **18.59** mg a.i./L Slope = 5.24 (95% C.I. 3.34-7.14) TGAI, 98.9% a.i.	Mortality	00070146 (Vilkas 1977)	Acceptable
Indirect Toxicity to Aquatic-Phase CRLF via Chronic Toxicity to Freshwater Invertebrates (i.e. prey items)	*Daphnia magna*	NOAEC = **1.0** mg a.i./L TGAI, 98.1% a.i.	Most sensitive biological parameter is daphnid length	40573720 (Surprenant 1988)	Acceptable
Indirect Toxicity to Aquatic-Phase CRLF via Toxicity to **Non-vascular** Aquatic Plants	*Navicula pelliculosa* (freshwater diatom)	EC_{50} = **0.001** mg a.i./L TGAI, 98.4 % a.i.	Mortality (percent inhibition) based on cell count data (or, mean standing crop, cells/mL) on day 5	42620201 (Hughes & Alexander 1992a)	Acceptable

Indirect Toxicity to Aquatic-Phase CRLF via Toxicity to **Vascular** Aquatic Plants	*Lemna gibba*	EC_{50} = **.0118** mg a.i./L TGAI, 98.4 % a.i.	Mortality (percent inhibition) based on mean frond counts on day 14	42520901 (Hughes & Alexander 1992b)	Acceptable
[1] Used as surrogate for the aquatic-phase CRLF					

Toxicity to aquatic fish and invertebrates is categorized using the system shown in **Table 4-2** (U.S. EPA 2004). Toxicity categories for aquatic plants have not been defined.

Table 4-2 Categories of Acute Toxicity for Fish and Aquatic Invertebrates

LC_{50} (ppm)	Toxicity Category
< 0.1	Very highly toxic
> 0.1 – 1	Highly toxic
> 1 – 10	Moderately toxic
> 10 – 100	Slightly toxic
> 100	Practically nontoxic

4.1.1 Toxicity to Freshwater Fish

Ecotoxicity data for freshwater fish are generally used as surrogates for aquatic-phase amphibians when amphibian toxicity data are not available (U.S. EPA, 2004). A comprehensive search of the open literature provided no valid toxicity information on lethal or sublethal effects of prometryn to amphibians.

Given that no prometryn toxicity data are available for aquatic-phase amphibians, freshwater fish data are used as a surrogate to estimate direct acute and chronic risks to the CRLF. Freshwater fish toxicity data are also used to assess potential indirect effects of prometryn to the CRLF. Effects to freshwater fish resulting from exposure to prometryn could indirectly affect the CRLF via reduction in available food. As discussed in Section 2.5.3, over 50% of the prey mass of the CRLF may consist of vertebrates such as mice, frogs, and fish (Hayes and Tennant 1985).

A summary of acute and chronic freshwater fish data is provided below in Sections 4.1.1.1 through 4.1.1.3.

4.1.1.1 Freshwater Fish: Acute Exposure (Mortality) Studies

The acute freshwater fish, rainbow trout (*Onchorynchus mykiss* formerly *Salmo gairdneri*), study (MRID 00070686) reports a LC_{50} of 2.9 mg a.i./L based on mortality over a 96 hour period. The endpoint is based on six nominal concentration levels (8.73, 4.88, 2.78, 1.57, 0.87, and 0.56 ppm), a solvent (acetone) control, and negative control. The water temperature varied quite a bit starting from 12.8 to 17.2^{o}C. The study did not indicate if the concentration of the solvent control is the same concentration used in the test levels. The outcome of the positive control (DDT) gave an LC_{50} estimate that was

10x higher than a previous study estimate. Assuming that the difference in the two studies may come as a result of differing methodologies, the latter suggests that the endpoint cited from this study, which is used in this assessment, may be underestimating toxicity of prometryn to freshwater fish. Despite these and several minor guideline deviations, the study is considered acceptable for 99% a.i. prometryn.

Based on the available data, prometryn is categorized as moderately toxic to freshwater fish on an acute exposure basis.

In addition, two acceptable ecotoxicity studies on formulations are available for two freshwater fish species using Caparol 80W, containing 80% prometryn a.i. The acute toxicity study (MRID 00121155) on the warmwater fish bluegill sunfish (*L. macrochirus*) yielded a 96-hour LC_{50} of 10 mg/L (*i.e.*, the lowest concentration tested) (8 mg a.i./L), which indicates that the formulated product is moderately toxic to bluegill. The acute toxicity study (MRID 00121154) on the coldwater fish rainbow trout (*O. mykiss*) yielded a 96-hour LC_{50} of 7.2 mg/L (5.8 mg a.i./L), which indicates that the formulated product is moderately toxic to rainbow trout. The endpoints generated by the formulation studies were not lower than those obtained from the technical study. As a result, the endpoints generated by the formulated product studies are not used in risk estimation.

Additional acute effects data for freshwater fish were not available for degradates of prometryn from either the registrant-submitted guideline studies or the open literature. No additional open literature studies were available which would provide effects information based on the parent compound.

4.1.1.2 Freshwater Fish: Chronic Exposure (Early Life Stage and Reproduction) Studies

The chronic freshwater fish, fathead minnow (*Pimephales promelas*), early life-stage study (MRID 43801702) reports a NOAEC of 0.62 mg a.i./L based on decreased growth (17% reduced wet weight at 2.4 mg a.i./L relative to the average of the negative and solvent controls;16.7% reduced dry weight at 1.2 mg a.i./L relative to the average of the negative and solvent controls) over a 28-day period. The endpoint is based on six mean-measured concentration levels (2.4, 1.2, 0.62, 0.31, 0.16, and 0.081 mg a.i./L), a solvent (acetone) control, and negative control. All fish appeared normal throughout the test. The most sensitive endpoint was growth, specifically dry and wet weight. The study is considered acceptable for 98.4% a.i. prometryn.

Additional chronic effects data for freshwater fish were not available for formulated products or degradates of prometryn from either the registrant-submitted guideline studies or the open literature. No additional open literature studies were available which would provide effects information based on the parent compound.

4.1.1.3 Freshwater Fish: Sublethal Effects and Additional Open Literature Information

No additional information is available that indicates greater acute freshwater fish sensitivity to prometryn than the submitted data. In addition, no laboratory freshwater fish early life-stage or life-cycle tests using prometryn and/or its formulated products or degradates were located in the open literature.

4.1.2 Toxicity to Freshwater Invertebrates

Freshwater aquatic invertebrate toxicity data were used to assess potential indirect effects of prometryn to the CRLF. Effects to freshwater invertebrates resulting from exposure to prometryn could indirectly affect the CRLF via reduction in available food items. As discussed in Section 2.5.3, the main food source for juvenile aquatic- and terrestrial-phase CRLFs is thought to be aquatic invertebrates found along the shoreline and on the water surface, including aquatic sowbugs, larval alderflies and water striders.

A summary of acute and chronic freshwater invertebrate data is provided below in Sections 4.1.2.1 through 4.1.2.3.

4.1.2.1 Freshwater Invertebrates: Acute Exposure (Mortality) Studies

The acute freshwater invertebrate, water flea (*Daphnia magna*), study (MRID 00070146) reports an EC_{50} of 18.59 mg a.i./L based on mortality over a 48 hour period. The endpoint is based on five nominal concentration levels (100, 56, 32, 18, and 10 ppm), a solvent (acetone) control, and negative control. Despite several minor guideline deviations, the study is considered acceptable for the 98.9% a.i. prometryn.

Based on the available data, prometryn is categorized as slightly toxic to freshwater invertebrates on an acute exposure basis. Additional acute effects data for freshwater invertebrates were not available for formulated products or degradates of prometryn from either the registrant-submitted guideline studies or the open literature. No additional open literature studies using the parent compound were available which would provide more sensitive effects information than the current registrant-submitted studies.

4.1.2.2 Freshwater Invertebrates: Chronic Exposure (Reproduction) Studies

The chronic freshwater invertebrate, water flea (*Daphnia magna*), life-cycle study (MRID 40573720) reports a NOAEC of 1.0 mg a.i./L based on decreased growth (16.7% reduced body length at 2.0 mg a.i./L from the control) over a 21-day exposure period. The endpoint is based on five mean-measured concentration levels (2, 1, 0.46, 0.27, and 0.10 mg a.i./L) and a dilution water control. There was no significant effect of prometryn concentration on daphnid survival, the number of days to first brood, or the number of offspring produced. Despite several minor guideline deviations, the study is considered acceptable for the 98.1% a.i. prometryn.

Additional chronic effects data for freshwater invertebrates were not available for formulated products or degradates of prometryn from either the registrant-submitted guideline studies or the open literature. No additional open literature studies were available which would provide effects information based on the parent compound.

4.1.2.3 Freshwater Invertebrates: Sublethal Effects and Open Literature Data

No additional information is available that indicates greater acute freshwater invertebrate sensitivity to prometryn than the submitted data. In addition, no laboratory freshwater invertebrate life-cycle tests using prometryn and/or its formulated products or degradates were located in the open literature.

4.1.3 Toxicity to Aquatic Plants

Aquatic plant toxicity studies were used as one of the measures of effect to evaluate whether prometryn may affect primary production and the availability of aquatic plants as food for CRLF tadpoles. Primary productivity is essential for indirectly supporting the growth and abundance of the CRLF.

Laboratory studies were used to determine whether prometryn may cause direct effects to aquatic plants. A summary of the laboratory data for aquatic plants is provided in Section 4.1.3.1.

4.1.3.1 Aquatic Plants: Laboratory Data

Non-vascular plants

The non-vascular freshwater diatom (*Navicula pelliculosa*) toxicity test (MRID 42620201) reports an EC_{50} of 0.001 mg a.i./L based on the percent inhibition of plant cell count (in cells/mL) on the final day (*i.e.*, the fifth day) of exposure. The endpoint is based on mean-measured concentration levels of 8.02, 3.85, 1.87, 0.962, 0.562, and 0.288 μg a.i./L), a solvent (dimethylformamide) control, and a negative control. Increasing amounts of prometryn technical had an increasingly inhibitory effect on cell growth. Five-day responses ranged from 19.6 to 98.9% inhibition across the concentrations tested. Despite a couple minor guideline deviations, the study is considered acceptable for the 98.4% a.i. prometryn.

Further examination of toxicity data for other non-vascular plants indicated EC_{50} values for green algae (*Pseudokirchneriella subcapitata* formerly *Selenastrum capricornutum*) of 0.012 mg a.i./L, marine diatom (*Skeletonema costatum*) of 0.0076 mg a.i./L, freshwater cyanobacteria (*Anabaena flos-aquae*) of 0.0401 mg a.i./L; these other nonvascular plant test species are approximately seven to forty times less sensitive to prometryn than the freshwater diatom. Additional effects data for non-vascular plants were not available for formulated products or degradates of prometryn from either the registrant-submitted guideline studies or the open literature. No additional open literature studies using the parent compound were available which would provide more sensitive effects information than the current registrant-submitted studies.

Vascular plants

The vascular aquatic plant, duckweed (*Lemna gibba* G3) toxicity test (MRID 42520901) reports an EC_{50} of 0.0118 mg a.i./L based on mortality (*i.e.*, percent inhibition) via mean frond counts on the final day (*i.e.,* the fourteenth day) of exposure. The endpoint is based on mean-measured concentration levels of 40.2, 18.1, 8.42, 3.99, 1.76, and 1.01 µg a.i./L, a solvent (dimethylformamide) control, and a negative control Percent inhibition of frond formation across treatment concentrations ranged from 1.29 to 91.9%. Fronds in the two highest concentration solutions appeared smaller in size and lighter in color than in the controls. Root length also appeared reduced for plants exposed to the highest concentration solution. Despite several minor guideline deviations, the study is considered acceptable for the 98.4% a.i. prometryn.

Additional effects data for vascular plants were not available for formulated products or degradates of prometryn from either the registrant-submitted guideline studies or the open literature. No additional open literature studies were available which would provide effects information based on the parent compound.

4.2 Toxicity of Prometryn to Terrestrial Organisms

Table 4-3 summarizes the most sensitive terrestrial toxicity endpoints for the CRLF, based on an evaluation of the registrant-submitted studies. A brief summary of registrant-submitted data considered relevant to this ecological risk assessment for the CRLF is presented below.

Table 4-3 Terrestrial Toxicity Profile for Prometryn

Assessment Endpoint	Species	Toxicity Value Used in Risk Assessment	Describe effect (i.e. mortality, growth, reproduction)	Citation MRID # (Author & Date)	Study Classification
Acute Dose-based Direct Toxicity to Terrestrial-Phase CRLF	Mallard Duck	LD_{50} > **4640** mg/kg Slope = N.A. TGAI, 98.8 % a.i.	There was no mortality at any dosage level. Reduction in feed consumption and body weight gain at the 4640 mg/kg dose level (highest of five)	00082966 (Fink, Beavers & Brown 1977)	Acceptable

Assessment Endpoint	Species	Toxicity Value Used in Risk Assessment	Describe effect (i.e. mortality, growth, reproduction)	Citation MRID # (Author & Date)	Study Classification
Acute Dietary-based Direct Toxicity to Terrestrial-Phase CRLF	Northern Bobwhite Quail	LC_{50} > **5000** mg/kg diet Slope = N.A. TGAI, 98.1% a.i.	Only one out of 10 individuals was observed dead at the 5000 mg/kg diet concentration (highest of five) on day 6 (out of 8 days). Reduction in body weight gain and food consumption at two highest treatment levels.	40457502 (Fletcher 1984)	Acceptable
Chronic Direct Toxicity to Terrestrial-Phase CRLF	Mallard Duck	NOAEC = **500** mg /kg diet TGAI, 98.1% a.i.	Only one adult mortality at the 250 mg/kg diet level. No other measures were statistically significant from the control	41035901 (Fletcher & Pedersen 1989)	Acceptable
Indirect Toxicity to Terrestrial-Phase CRLF (via acute toxicity to mammalian prey items)	Rat	LD_{50} = **1802** mg/kg bw	Oral toxicity	00060314 (Kapp 1975)	---
Indirect Toxicity to Terrestrial-Phase CRLF (via chronic toxicity to mammalian prey items)	Rat	NOAEC = **10** mg/kg diet NOAEL = **0.65** mg/kg bw TGAI	Reproductive endpoints based on decreased pup weight	41445101 (Giknis & Yau 1990)	**---**
Indirect Toxicity to Terrestrial-Phase CRLF (via acute toxicity to terrestrial invertebrate prey items)	Honey bee	LD_{50} > 96.69 µg/bee TGAI	10% mortality at the tested limit dose	00036935 (Atkins, Greywood & Macdonald 1975)	Acceptable

Assessment Endpoint	Species	Toxicity Value Used in Risk Assessment	Describe effect (i.e. mortality, growth, reproduction)	Citation MRID # (Author & Date)	Study Classification
Indirect Toxicity to Terrestrial- and Aquatic-Phase CRLF (via toxicity to terrestrial plants)	Seedling Emergence Monocots (Oat)	EC_{25} = **0.049** lbs a.i./A NOAEC = 0.011[§] lbs a.i./A TGAI, 98.1% a.i.	Oat: decreased dry weight	41035904 (Canez 1988a)	Acceptable
	Seedling Emergence Dicots (Cabbage)	EC_{25} = **0.02** lbs a.i./A NOAEC = 0.008[†] lbs a.i./A TGAI, 98.1% a.i.	Cabbage: decreased dry weight	41035904 (Canez 1988a)	Acceptable
	Vegetative Vigor Monocots (Onion)	EC_{25} = **0.18** lbs a.i./A NOAEC = 0.1 lbs a.i./A TGAI, 98.1% a.i.	Onion: decreased dry weight	41035903 (Canez 1988b)	Acceptable
	Vegetative Vigor Dicots (Lettuce*)	EC_{25} = **0.01** lbs a.i./A NOAEC = 0.003[§] lbs a.i./A TGAI, 98.1% a.i.	Lettuce: decreased dry weight	41035903 (Canez 1988b)	Acceptable

[§] The original NOAEC is undefined; the NOAEC cited is the EC_{05}
[†] The original NOAEC ≥ EC_{25} ; the NOAEC cited is the EC_{05}
* The cucumber is potentially the more sensitive dicot tested; however, the endpoint values obtained for it are considered invalid.

Acute toxicity to terrestrial animals is categorized using the classification system shown in **Table 4-4** (U.S. EPA 2004). Toxicity categories for terrestrial plants have not been defined.

Table 4-4 Categories of Acute Toxicity for Avian and Mammalian Studies

Toxicity Category	Oral LD_{50}	Dietary LC_{50}
Very highly toxic	< 10 mg/kg	< 50 ppm
Highly toxic	10 - 50 mg/kg	50 - 500 ppm
Moderately toxic	51 - 500 mg/kg	501 - 1000 ppm
Slightly toxic	501 - 2000 mg/kg	1001 - 5000 ppm
Practically non-toxic	> 2000 mg/kg	> 5000 ppm

4.2.1 Toxicity to Birds

As specified in the Overview Document, the Agency uses birds as a surrogate for terrestrial-phase amphibians when amphibian toxicity data are not available (U.S. EPA 2004). No terrestrial-phase amphibian data are available for prometryn; therefore, acute and chronic avian toxicity data are used to assess the potential direct effects of prometryn to terrestrial-phase CRLFs.

4.2.1.1 Birds: Acute Exposure (Mortality) Studies

The avian acute dose-based (gavage) study with mallard duck (*Anas platyrhynchos*) (MRID 00082966) reports a LD_{50} of >4640 mg/kg-bw over an 8-day period. The endpoint is based on five nominal concentration levels (4640, 2150, 1000, 464, and 215 mg/kg-bw) a negative control and a positive control (dieldren) were also used. However, prometryn did not cause any signs of toxicity at the dosage levels tested. Instead, reductions in feed consumption and body weight gain were observed at the 4640 mg/kg-bw dose level. Several guideline deviations were observed in this study. The birds were only 14 days old instead of the recommended 16 weeks and the observation period lasted 8 days instead of the recommended 14 days. In addition, the mean body weight food consumption was only measured on the first and last days. Despite these and a couple other minor guideline deviations, the study is considered acceptable for 98.8% a.i. prometryn.

The avian subacute dietary study with, Northern bobwhite quail (*Colinus virginianus*) (MRID 40457502) reports a LC_{50} of >5000 mg/kg-diet over an 8-day exposure period. The endpoint is based on five nominal dietary exposure levels (5000, 2500, 1250, 625, and 312 mg/kg-diet) and a negative control. A single mortality was observed in the 5000 mg/kg-diet level on the sixth day. Birds receiving prometryn displayed concentration-related food avoidance during the test period. There was a reduction in body weight gain and food consumption at the two highest treatment levels compared to the controls. Yet, body weight measurements were not made on day 5, the final day of the fasting phase of the test. After receiving plain feed for days 6-8, feeding resumed to normal. Although failure to obtain day 5 body weights may lead to a masking of toxicological effects, the reductions in body weight at day 8 are evidence of a treatment effect at the two highest test concentrations. Despite this and a couple other minor guideline deviations, the study is considered acceptable for 98.1% a.i. prometryn.

Based on the available data, prometryn is categorized as practically non-toxic to avian species tested on an acute oral and sub-acute dietary exposure basis. Additional acute effects data for avian species were not available for formulated products or degradates of prometryn from either the registrant-submitted guideline studies or the open literature. No additional open literature studies were available which would provide effects information based on the parent compound.

4.2.1.2 Birds: Chronic Exposure (Growth, Reproduction) Studies

A chronic avian reproduction study (MRID 41035901) with 26.5 week old mallard ducks reports a NOAEC of 500 mg/kg-diet based on adult mortality over a 20 week period. The endpoint is based on three nominal concentration levels (500, 250, and 50 mg/kg-diet) and a negative control. Mortality was observed in one adult female duck at the 250 mg/kg-diet level. Necropsy revealed clotted blood present in the lungs, oral cavity, esophagus, and around the heart. A nodule was found on the lobe of the right lung. There were no abnormal behavioral effects noted during the study. Gross pathological examinations conducted on half of the birds surviving to terminal sacrifice revealed abnormalities in 7 birds. Measures of adult body weight, food consumption, reproductive parameters, and egg shell thickness were not significantly different between the control and treatment groups. Inconsistent differences in offspring body weights were not considered to be treatment-related. Despite a small series of guideline deviations, the study is considered acceptable for 98.1% a.i. prometryn.

Additional chronic effects data for avian species were not available for formulated products or degradates of prometryn from either the registrant-submitted guideline studies or the open literature. No additional open literature studies were available which would provide effects information based on the parent compound.

4.2.1.3 Terrestrial-phase Amphibian Acute and Chronic Studies

No additional information is available on terrestrial-phase amphibian acute and/or chronic sensitivity to prometryn. In addition, no laboratory acute or chronic tests using prometryn and/or its formulated products or degradates were located in the open literature.

4.2.2 Toxicity to Mammals

Mammalian toxicity data are used to assess potential indirect effects of prometryn to the terrestrial-phase CRLF. Effects to small mammals resulting from exposure to prometryn could also indirectly affect the CRLF via reduction in available food. As discussed in Section 2.5.3, over 50% of the prey mass of the CRLF may consist of vertebrates such as mice, frogs, and fish (Hayes and Tennant 1985). Sections 4.2.2.1 and 4.2.2.2 contain additional information on the mammalian acute and chronic guideline studies, respectively, used in the assessment.

4.2.2.1 Mammals: Acute Exposure (Mortality) Studies

The available data indicate that prometryn is slightly toxic to small mammals on an acute oral basis (MRID 00060314). The endpoint used in the assessment is based on the results from male rats (LD_{50} = 1802 mg/kg-bw). The study is classified as category III.

Additional acute effects data for small mammal species were not available for formulated products or degradates of prometryn from either the registrant-submitted guideline studies or the open literature. No additional open literature studies were available which would provide effects information based on the parent compound.

4.2.2.2 Mammals: Chronic Exposure (Growth, Reproduction) Studies

In a two generation reproductive toxicity study (MRID 41445101), prometryn technical was administered in the diet to groups of 30 male and 30 female Sprague-Dawley rats at levels of 0, 10 ppm (0.6 mg/kg-bw/day in males, 0.7 mg/kg-bw/day in females), 750 ppm (47.8 mg/kg-bw/day in males, 53.6 mg/kg-bw/day in females) or 1500 ppm (96.7 mg/kg-bw/day in males, 105.6 mg/kg-bw/day in females). Body weight gain in F0 males decreased significantly at 1500 ppm (11-40%) and 750 ppm (11-18%). Body weight gain decreased in F0 females by up to 50% at 1500 ppm and 750 ppm. Similar changes in body weight gain were seen in F1 males. Corresponding decreases in food consumption were also observed. The Parental Systemic Toxicity NOAEC was 10 ppm; the LOAEC was 750 ppm, based on decreased food consumption, body weight and body weight gain. Statistically significant decreases in F1 pup body weights were observed at 1500 and 750 ppm (up to 12%) during lactation. A similar, though less marked, profile was seen in F2 generation pups. While actual weight loss was small (5-12%), and may be artifactual to maternal weight losses, it is considered of toxicological significance because of its potential negative impact on postnatally developing systems such as the neuro- and immune systems. Reproductive NOAEC was 10 ppm (0.65 mg/kg-bw/day); the LOAEC was 750 ppm (~50 mg/kg-bw/day), based on decreased pup weight.

Additional chronic effects data for small mammal species were not available for formulated products or degradates of prometryn from either the registrant-submitted guideline studies or the open literature. No additional open literature studies were available which would provide effects information based on the parent compound.

4.2.3 Toxicity to Terrestrial Invertebrates

Terrestrial invertebrate toxicity data are used to assess potential indirect effects of prometryn to the terrestrial-phase CRLF. Effects to terrestrial invertebrates resulting from exposure to prometryn could also indirectly affect the CRLF via reduction in available food.

4.2.3.1 Terrestrial Invertebrates: Acute Exposure (Mortality) Studies

The use of prometryn on cotton and other crops that require pollination may result in exposure to non-target beneficial insects, such as the honey bee (*Apis mellifera*). The results of a laboratory acute contact toxicity test (MRID 00036935) of prometryn on the honey bee indicate 10 % mortality after direct treatment at a limit test dose of 96.69 µg/bee; therefore, the LD_{50} value for the contact test is greater than 96.69 µg/bee. As a result, prometryn is categorized as practically non-toxic to honeybees on an acute contact exposure basis.

Additional acute effects data for terrestrial invertebrate species were not available for formulated products or degradates of prometryn from either the registrant-submitted guideline studies or the open literature. No additional open literature studies using the parent compound were available which would provide more sensitive effects information than the current registrant-submitted studies.

4.2.4 Toxicity to Terrestrial Plants

Terrestrial plant toxicity data are used to evaluate the potential for prometryn to affect riparian zone and upland vegetation within the action area for the CRLF. Impacts to riparian and upland (i.e., grassland, woodland) vegetation could result in indirect effects to both aquatic- and terrestrial-phase CRLFs, as well as effects to designated critical habitat PCEs via increased sedimentation, alteration in water quality, and reduction in upland and riparian habitat that provides shelter, foraging, predator avoidance and dispersal for juvenile and adult CRLFs.

Plant toxicity data from registrant-submitted studies were reviewed for this assessment. One open literature terrestrial plant study was reviewed for determining effects to woody plants. The study was not used in risk estimation but is summarized in the risk description (Section 5.2.3.2). Registrant-submitted studies are conducted under conditions and with species defined in EPA toxicity test guidelines. Sub-lethal endpoints such as plant growth, dry weight, and biomass are evaluated for both monocotyledonous (monocots) and dicotyledonous (dicots) species, and effects are evaluated at both seedling emergence and vegetative life stages.

The results of the Tier II seedling emergence and vegetative vigor toxicity tests on non-target plants are summarized below in **Table 4-5.**

Table 4-5 Non-target Terrestrial Plant Seedling Emergence and Vegetative Vigor Toxicity (Tier II) Data

Crop	Type of Study Species	NOAEC (lb ai/A)	EC_{25} (lb ai/A)	Most sensitive parameter*
Seedling Emergence				
Monocots	Oat	0.011§	0.049	Dw
	Ryegrass	0.07†	0.18	Ht
	Corn	0.2	0.35	Dw
	Onion	0.038	0.08	Ht
Dicots	Soybean	0.043†	0.23	Dw
	Lettuce	0.001†	0.025	dw
	Carrot		0.173	pe
	Tomato	0.063†	0.18	dw
	Cucumber	0.004§	0.026	dw
	Cabbage	0.008†	0.02	dw
Vegetative Vigor				
Monocots	Oat	0.8	1.4	Dw
	Ryegrass	≥1.6	>1.6	dw, ht, su
	Corn	0.4	0.79	Dw
	Onion	0.1	0.18	Dw
Dicots	Soybean	0.044§	0.17	Dw
	Lettuce	0.003§	0.01	Dw
	Carrot	≥ 1.6	>1.6	dw, ht, su
	Tomato	0.051§	0.12	Dw
	Cucumber$^{\Delta}$	0.019	0.1	Ht
	Cabbage	0.05	0.10	Dw

*Sensitive parameters: ht – plant height measurements, pe – percentage of seedlings emerged, dw – dry weight determination, su – survival
† The original NOAEC ≥ EC_{25} ; the NOAEC cited is the EC_{05}
§ The original NOAEC is undefined; the NOAEC cited is the EC_{05}
$^{\Delta}$ The cucumber dry weight is potentially the more sensitive parameter; however, the endpoint values obtained for it are considered invalid.

4.3 Use of Probit Slope Response Relationship to Provide Information on the Endangered Species Levels of Concern

The Agency uses the probit dose-response relationship as a tool for providing additional information on the potential for acute direct effects to individual listed species and aquatic animals that may indirectly affect the listed species of concern (U.S. EPA 2004). As part of the risk characterization, an interpretation of acute RQ for listed species is discussed. This interpretation is presented in terms of the chance of an individual event (i.e., mortality or immobilization) should exposure at the EEC actually occur for a species with sensitivity to prometryn on par with the acute toxicity endpoint selected for RQ calculation. To accomplish this interpretation, the Agency uses the slope of the dose response relationship available from the toxicity study used to establish the acute toxicity measures of effect for each taxonomic group that is relevant to this assessment. The individual effects probability associated with the acute RQ is based on the mean estimate of the slope and an assumption of a probit dose-response relationship. In addition to a single effects probability estimate based on the mean, upper and lower estimates of the effects probability are also provided to account for variance in the slope, if available.

Individual effect probabilities are calculated based on an Excel spreadsheet tool IECV1.1 (Individual Effect Chance Model Version 1.1) developed by the U.S. EPA, OPP, Environmental Fate and Effects Division (June 22, 2004). The model allows for such calculations by entering the mean slope estimate (and the 95% confidence bounds of that estimate) as the slope parameter for the spreadsheet. In addition, the acute RQ is entered as the desired threshold.

4.4 Incident Database Review

A review of the EIIS database for ecological incidents involving prometryn was completed on March 25, 2009. The results of this review for terrestrial, plant, and aquatic incidents are discussed below in Sections 4.4.1 through 4.4.3, respectively. A complete list of the incidents involving prometryn including associated uncertainties is included as **Appendix I**.

4.4.1 Terrestrial Animal Incidents

No prometryn incidents have been reported involving terrestrial organisms.

4.4.2 Plant Incidents

Six prometryn incidents have been reported involving terrestrial plants. The first incident (#I019130-056) is on corn (*Zea mays*) where prometryn was classified as a 'probable' cause via broadcast application on 86 acres in New Madrid County, Missouri (date 7.31.2007). The route of exposure was through carryover, or the presence of residues from an application made in a previous growing season, that resulted in plant damage. The remaining five incidents involve applications to cotton (*Gossypium*). One incident (#I007796-005) classified as 'possibly' attributable to prometryn included 600 acres of cotton in Hale County, Texas (date 8.25.1998). The chemical was applied directly to cotton and resulted in the loss of all the treated cotton plants. The next incident (#I007796-006) is also classified as 'possibly' attributable to prometryn resulted in the loss of 1,424 acres of cotton in Hale County, Texas (date 8.17.1998). As in the previous incident involving cotton, the chemical was applied directly to the cotton plants. The legality of each of the aforementioned uses is not specified in the incident report. Another incident (#I009573-014) that is classified as 'probably' attributable to prometryn occurred as a result of a broadcast application to 48 acres in Caldwell County, Texas (date 11.12.1999). The chemical (formulation code 'F') was applied directly to the cotton plants and resulted in plant damage in the form of chlorotic yellowing. The following incident (#I016903-008) is classified as ‘possibly’ attributable to prometryn occurred following a band application to 60 acres of cotton in Uvalde County, Texas (date 7.21.2005). The chemical (formulation code ‘EC’) was applied directly to the cotton field and resulted in plant damage; the herbicide caused chlorotic yellowing of the cotton plant leaves. The final incident (#I016903-009) is classified as ‘possibly’ attributable to prometryn and occurred as a result of a band application to 26 acres of cotton in Evans County, Georgia (date 9.8.2005). The chemical (formulation code ‘EC’) was applied directly to cotton fields and resulted in plant damage; the herbicide caused chlorotic yellowing, necrotic browning, and death of the leaves. Each of the latter three incidents was categorized as a ‘registered use’ of prometryn.

4.4.3 Aquatic Animal Incidents

Three freshwater aquatic incidents involving fish kills have been reported for prometryn in August 1996 in Richland County, Louisiana; it is possible that the three incidents are related. However, prometryn is classified as an ‘unlikely’ cause; instead, exposure to profenofos and/or azinphos-methyl as well as low dissolved oxygen levels brought on by death of oxygen producing plants may have contributed to the multiple fish deaths. The incidents (dated 8.6.1996) include runoff from a ‘registered use’ of prometryn on cotton on land adjacent to surface water and resulted in the loss of “thousands” of common carp (*Cyprinus carpio*) and 25 shad (Clupeidae) in Crew Lake (#I004021-004) and a use of ‘unknown’ legality killed “hundreds” of buffalo (Catostomidae) and “thousands” of shad in Little Lake LaFourche (#I004021-005). The third incident (dated 8.7.1996) is classified as a ‘registered use’ on cotton that killed hundreds of buffalo, gar (*Lepisosteus* spp.), and shad in Dave’s Bayou, south of Charlieville (#I004668-011).

5.0 Risk Characterization

Risk characterization is the integration of the exposure and effects characterizations. Risk characterization is used to determine the potential for direct and/or indirect effects to the CRLF or for modification to its designated critical habitat from the use of prometryn in CA. The risk characterization provides an estimation (Section 5.1) and a description (Section 5.2) of the likelihood of adverse effects; articulates risk assessment assumptions, limitations, and uncertainties; and synthesizes an overall conclusion regarding the likelihood of adverse effects to the CRLF or its designated critical habitat (i.e., "no effect," "likely to adversely affect," or "may affect, but not likely to adversely affect").

5.1 Risk Estimation

Risk is estimated by calculating the ratio of exposure to toxicity. This ratio is the risk quotient (RQ), which is then compared to pre-established acute and chronic levels of concern (LOCs) for each category evaluated (**Appendix C**). For acute exposures to the CRLF and its animal prey in aquatic habitats, as well as terrestrial invertebrates, the LOC is 0.05. For acute exposures to the CRLF and mammals, the LOC is 0.1. The LOC is 1.0 for chronic exposures to CRLF and its prey, as well as acute exposures to plants.

Risk to the aquatic-phase CRLF is estimated by calculating the ratio of exposure to toxicity using 1-in-10 year EECs based on the label-recommended prometryn usage scenarios summarized in Table 3-4 and the appropriate aquatic toxicity endpoint from Table 4-1. Risks to the terrestrial-phase CRLF and its prey (*e.g*. terrestrial insects, small mammals and terrestrial-phase frogs) are estimated based on exposures resulting from applications of prometryn (Table 3-11 and Table 3-12) and the appropriate toxicity endpoint from Table 4-3. Exposures are also derived for terrestrial plants, as discussed in Section 3.4 and toxicity summarized in Section 4.2.4, based on the highest application rates of prometryn use within the action area.

5.1.1 Exposures in the Aquatic Habitat

5.1.1.1 Direct Effects to Aquatic-Phase CRLF

Direct effects to the aquatic-phase CRLF are based on peak EECs in the standard pond and the lowest acute toxicity value for freshwater fish. In order to assess potential direct chronic risks to the CRLF, 60-day EECs calculated via the PRZM/EXAMS model and the lowest chronic toxicity value for freshwater fish are used. The Agency's acute LOCs are exceeded for all parsley, a fall celery/fennel, and two pre-emergence cotton uses (**Table 5-1**). Based on these results, prometryn may directly affect the aquatic-phase of the CRLF.

Table 5-1 Summary of Direct Effect RQs for the Aquatic-phase CRLF

Use Type	Application rate (lb ai/A) and type	Peak EEC (µg/L)[a]	60-Day EEC (µg/L)[a]	Direct Effects Acute RQs[b]	Probability of Individual Effect at RQ (confidence interval)	Direct Effects Chronic RQs[b]
Celery/Fennel (Spring, Apr 1)	2 (liquid)	102.9	101.9	0.04[c]	1 in 9.98 x 10^5 (1 in 370 to 1 in 1.37 x 10^{11})	0.16[d]
Celery/Fennel (Fall, Dec 1)	2 (liquid)	152.9	142.5	**0.05**	1 in 2.06 x 10^5 (1 in 208 to 1 in 6.06 x 10^9)	0.23
Parsley (Spring, Apr 1)	2 (liquid)	209.7	206.9	**0.07**	1 in 2.32 x 10^4 (1 in 93 to 1 in 8.23 x 10^7)	0.33
Parsley (Fall, Dec 1)	2 (liquid)	377.3	368.7	**0.13**	1 in 772 (1 in 26 to 1 in 1.07 x 10^5)	0.59
Cotton (Spring, Apr 1)-Ground spray	2.4 (liquid)	49.2	47.3	0.02	1 in 2.62 x 10^8 (1 in 2.77 x 10^3 to 1 in 8.75 x 10^{15})	0.08
Cotton (Spring, Apr 1)-Aerial spray	2.4 (liquid)	88.8	87.7	0.03	1 in 8.91 x 10^6 (1 in 819 to 1 in 1.05 x 10^{13})	0.14
Cotton (Fall, Dec 1)	2.4 (liquid)	93.2	86.3	0.03	1 in 8.91 x 10^6 (1 in 819 to 1 in 1.05 x 10^{13})	0.14
Cotton (Pre-emergence)-ground spray	Seasonal 5.5[1] (liquid) Single App 2.4 (liquid)	187.0	176.9	**0.06**	1 in 6.13 x 10^4 (1 in 133 to 1 in 5.55 x 10^8)	0.29
Cotton (Pre-emergence)-Aerial spray	Seasonal 5.5[1] (liquid) Single App 2.4 (liquid)	240.0	231.7	**0.08**	1 in 1.04 x 10^4 (1 in 69 to 1 in 1.71 x 10^7)	0.37

[a] The highest EEC (acute) and 60-day EEC (chronic) based on given use type of prometryn (see Table 3-4). The exposure estimates are based on PRZM/EXAMS model.
[b] RQs associated with acute (LC_{50} = 2.9 mg/L, rainbow trout) and chronic (NOAEC = 0.62 mg/L, fathead minnow) direct toxicity to the CRLF are also used to assess potential indirect effects to the CRLF based on a reduction in freshwater fish and frogs as food items.
[c] RQ < acute risk to endangered species LOC of 0.05. The LOC exceedances are in bold.
[d] RQ < chronic LOC of 1.0.
Note: The probability of individual effect at ES LOC for acute direct toxicity is 1 in 2.06 x 10^5 (95% C.I. 1 in 208 to 1 in 6.06 x 10^9)

5.1.1.2 Indirect Effects to Aquatic-Phase CRLF via Reduction in Prey (non-vascular aquatic plants, aquatic invertebrates, fish, and frogs)

5.1.1.2.1 Non-vascular Aquatic Plants

Indirect effects of prometryn to the aquatic-phase CRLF (tadpoles) via reduction in non-vascular aquatic plants in its diet are based on peak EECs from the standard pond (*i.e.*, calculated via the PRZM/EXAMS model) and the lowest toxicity value (EC_{50}) for aquatic non-vascular plants. In this case the lowest EC_{50} (0.001 mg a.i./L) was based on toxicity to *N. pelliculosa*. The Agency's non-listed plant LOCs are exceeded for all prometryn uses (**Table 5-2**). Based on these results, prometryn may indirectly affect the CRLF via reduction in non-vascular aquatic plants.

Table 5-2 Summary of RQs Used to Estimate Indirect Effects to the CRLF via Effects to Non-Vascular Aquatic Plants (diet of CRLF in tadpole life stage and habitat of aquatic-phase CRLF). RQs based on EECs from PRZM/EXAMS and a freshwater non-vascular plant EC_{50} of 0.001 mg a.i./L

Uses	Application rate (lb ai/A) and type	Peak EEC (µg/L)	Indirect effects RQ* (food and habitat)
Celery/Fennel (Spring, Apr 1)	2 (liquid)	102.9	**102.9**
Celery/Fennel (Fall, Dec 1)	2 (liquid)	152.9	**152.9**
Parsley (Spring, Apr 1)	2 (liquid)	209.7	**209.7**
Parsley (Fall, Dec 1)	2 (liquid)	377.3	**377.3**
Cotton (Spring, Apr 1)-Ground spray	2.4 (liquid)	49.2	**49.2**
Cotton (Spring, Apr 1)-Aerial spray	2.4 (liquid)	88.8	**88.8**
Cotton (Fall, Dec 1)	2.4 (liquid)	93.2	**93.20**
Cotton (Pre-emergence)- Ground spray	Seasonal 5.5[1] (liquid) Single App 2.4 (liquid)	187.0	**187.0**
Cotton (Pre-emergence)-Aerial spray	Seasonal 5.5[1] (liquid) Single App 2.4 (liquid)	240.0	**240.0**

* LOC exceedances (RQ $\geq$ 1) are bolded and shaded. RQ = use-specific peak EEC/ 0.001 mg/L.
[1]The labels allow 3 applications as an aerial/ground spray application of 2.4 lbs ai/A at pre-plant, ground spray application of 0.7 lbs ai/A at post-plant, and ground spray application of 2.4 lbs ai/A at post-harvest.

5.1.1.2.2 Aquatic Invertebrates

Indirect acute effects to the aquatic-phase CRLF via effects to prey (invertebrates) in aquatic habitats are based on peak EECs in the standard pond (*i.e.*, calculated via the PRZM/EXAMS model) and the lowest acute toxicity value for freshwater invertebrates, *i.e.*, waterflea EC_{50} = 18.6 mg a.i./L. For chronic risks, 21-day EECs and the lowest chronic toxicity value for invertebrates, *i.e.*, waterflea NOAEC = 1.0 mg a.i./L, are used to derive RQs. A summary of the acute and chronic RQ values for exposure to aquatic invertebrates (as prey items of aquatic-phase CRLFs) is provided in **Table 5-3**. However, no LOC exceedances were observed. Based on acute and chronic RQ values that are below the Agency's LOCs as well as supporting low values (*i.e.*, on the order of approximately 1 in a quintillion to over 1 in a septillion) for the probability of individual effect (based on acute data only), prometryn is expected to have no indirect effect on the CRLF via reduction in freshwater invertebrate prey items.

Table 5-3 Summary of Acute and Chronic RQs Used to Estimate Indirect Effects to the CRLF via Direct Effects on Aquatic Invertebrates as Dietary Food Items (prey of CRLF juveniles and adults in aquatic habitats) RQs based on EECs from PRZM/EXAMS. Acute and chronic toxicity endpoints are based on waterflea data at 18.6 and 1.0 mg a.i./L, respectively.

Uses	Application rate (lb ai/A) and type	Peak EEC (μg/L)	21-day EEC (μg/L)	Indirect Effects Acute RQ*	Probability of Individual Effect at RQ (confidence interval)	Indirect Effects Chronic RQ*
Celery/Fennel (Spring, Apr 1)	2 (liquid)	102.9	102.0	0.01	1 in 1.87×10^{25} (1 in 8.37×10^{10} to 1 in 6.86×10^{45})	0.10
Celery/Fennel (Fall, Dec 1)	2 (liquid)	152.9	142.8	0.01	1 in 1.87×10^{25} (1 in 8.37×10^{10} to 1 in 6.86×10^{45})	0.14
Parsley (Spring, Apr 1)	2 (liquid)	209.7	208.8	0.01	1 in 1.87×10^{25} (1 in 8.37×10^{10} to 1 in 6.86×10^{45})	0.21
Parsley (Fall, Dec 1)	2 (liquid)	377.3	370.0	0.02	1 in 3.67×10^{18} (1 in 1.44×10^{8} to 1 in 2.75×10^{33})	0.37
Cotton (Spring, Apr 1)-Ground spray	2.4 (liquid)	49.2	48.4	<0.01	---	0.05
Cotton (Spring, Apr 1)-Aerial spray	2.4 (liquid)	88.8	88.2	<0.01	---	0.09
Cotton (Fall, Dec 1)	2.4 (liquid)	93.2	88.8	0.01	1 in 1.87×10^{25} (1 in 8.37×10^{10} to 1 in 6.86×10^{45})	0.09
Cotton (Pre-emergence)-Ground spray	Seasonal 5.5[1] (liquid) Single App 2.4 (liquid)	187.0	182.4	0.01	1 in 1.87×10^{25} (1 in 8.37×10^{10} to 1 in 6.86×10^{45})	0.18
Cotton (Pre-emergence)-Aerial spray	Seasonal 5.5[1] (liquid) Single App 2.4 (liquid)	240.0	235.4	0.01	1 in 1.87×10^{25} (1 in 8.37×10^{10} to 1 in 6.86×10^{45})	0.24

* Risk to listed species LOC exceedances (acute RQ $\geq$ 0.05; chronic RQ $\geq$ 1.0) are not observed for aquatic invertebrates (indirect effects to CRLF). Acute RQ = use-specific peak EEC / 18.6 mg a.i. per liter. Chronic RQ = use-specific 21-day EEC / 1.0 mg a.i. per liter.

[1]The labels allow 3 applications as an aerial/ground spray application of 2.4 lbs ai/A at pre-plant, ground spray application of 0.7 lbs ai/A at post-plant, and ground spray application of 2.4 lbs ai/A at post-harvest.

NOTE: Probability of Individual Effect at ES acute LOC is 1 in 2.16×10^{11} (95% C.I. 1 in 1.44×10^{5} to 1 in 1.29×10^{20})

5.1.1.2.3 <u>Fish and Frogs</u>

Fish and frogs also represent potential prey items of adult aquatic-phase CRLFs. RQs associated with direct acute and chronic risk to the CRLF (Table 5-1) are used to assess potential indirect effects to the CRLF based on a reduction in freshwater fish and frogs as food items. Based on LOC exceedances observed in all parsley, a fall celery/fennel, and two pre-emergence cotton uses (Table 5-1) for acute mortality, prometryn may indirectly affect the CRLF via reduction in freshwater fish and frogs as food items.

5.1.1.3 Indirect Effects to CRLF via Reduction in Habitat and/or Primary Productivity (Freshwater Aquatic Plants)

Indirect effects to the CRLF via direct toxicity to aquatic plants are estimated using peak EECs from the standard pond (*i.e.*, calculated via the PRZM/EXAMS model) and the most sensitive vascular and non-vascular plant toxicity endpoints. Because there are no obligate relationships between the CRLF and any aquatic plant species, the most sensitive EC_{50} value (0.012 mg a.i./L), rather than NOAEC values, were used to derive RQs. RQs for non-vascular plants are presented in Section 5.1.1.2.1 and Table 5-2. The Agency's non-listed plant LOCs are exceeded for all prometryn uses (**Table 5-4**). Based on these results and those of section 5.1.1.2.1, prometryn may indirectly affect the CRLF via reduction in both vascular and non-vascular aquatic plants.

Table 5-4 Summary of RQs Used to Estimate Indirect Effects to the CRLF via Effects to Vascular Aquatic Plants (habitat of aquatic-phase CRLF)[a] RQs based on EECs from PRZM/EXAMS and a freshwater vascular plant EC_{50} of 0.012 mg a.i./L.

Uses	Application rate (lb ai/A) and type	Peak EEC (µg/L)	Indirect effects RQ* (food and habitat)
Celery/Fennel (Spring, Apr 1)	2 (liquid)	102.9	**8.7**
Celery/Fennel (Fall, Dec 1)	2 (liquid)	152.9	**12.9**
Parsley (Spring, Apr 1)	2 (liquid)	209.7	**17.8**
Parsley (Fall, Dec 1)	2 (liquid)	377.3	**32.0**
Cotton (Spring, Apr 1)-Ground spray	2.4 (liquid)	49.2	**4.2**
Cotton (Spring, Apr 1)-Aerial spray	2.4 (liquid)	88.8	**7.5**
Cotton (Fall, Dec 1)	2.4 (liquid)	93.2	**7.9**
Cotton (Pre-emergence)- Ground spray	Seasonal 5.5[1] (liquid) Single App 2.4 (liquid)	187.0	**15.8**
Cotton (Pre-emergence)-Aerial spray	Seasonal 5.5[1] (liquid) Single App 2.4 (liquid)	240.0	**20.3**

[a] RQs used to estimate indirect effects to the CRLF via toxicity to non-vascular aquatic plants are summarized in Table 5-2
* Risk to non-listed species LOC exceedances (RQ $\geq$ 1) are bolded and shaded. RQ = use-specific peak EEC/ 0.012 mg/L.
[1]The labels allow 3 applications as an aerial/ground spray application of 2.4 lbs ai/A at pre-plant, ground spray application of 0.7 lbs ai/A at post-plant, and ground spray application of 2.4 lbs ai/A at post-harvest.

5.1.2 Exposures in the Terrestrial Habitat

5.1.2.1 Direct Effects to Terrestrial-phase CRLF

As discussed in Section 3.3, potential direct effects to terrestrial-phase CRLFs are based on ground spray and aerial applications of prometryn.

Potential direct acute effects to the terrestrial-phase CRLF are derived by considering dose- and dietary-based EECs modeled in T-REX for a small bird (20 g) consuming small invertebrates (Table 3-11) and acute oral and subacute dietary toxicity endpoints for avian species. The acute avian effects data show no mortality in the acute oral toxicity study (LD_{50} > 4,640 mg/kg-bw) and a single mortality in the highest treatment level of prometryn in the subacute dietary toxicity study (LC_{50} > 5,000 mg/kg-diet). As a result, the calculated RQs (not reported) are indefinite (see discussion in the risk description section 5.2.1.2 below).

Potential direct chronic risk from prometryn use to the terrestrial-phase CRLF are derived by considering dietary-based exposures modeled in T-REX for a small bird (20g) consuming small invertebrates (**Table 5-5)**. Chronic risks are estimated using the lowest available toxicity data for birds. EECs are divided by toxicity values to estimate chronic dietary-based RQs. The chronic RQ calculated in T-REX does not exceed the Agency's chronic risk LOC. Although prometryn is classified as practically non-toxc to birds on an acute oral and subacute dietary exposure basis, there is uncertainty associated with the indefinite acute oral LD_{50} and the subacute dietary LC_{50} values; as such there is uncertainty regarding the extent to which prometryn may directly affect the terrestrial-phase of the CRLF. Further discussion is provided in the Risk Description (Section 5.2.1.2).

Table 5-5 Summary of Chronic RQs* Used to Estimate Direct Effects to the Terrestrial-phase CRLF (non-granular application). Based on a Mallard Duck NOAEC of 500 mg/kg-diet.

Use	Application Rate (lbs a.i./A)	Dietary-based Chronic RQ[1]
Cotton	2.4	0.65
Cotton	0.7	0.19
Celery/ Fennel /Parsley	2.0	0.54
Dill	1.6	0.43
* Chronic risk LOC exceedances (chronic RQ $\geq$ 1) are bolded and shaded. [1] Based on NOAEC = 500 ppm		

5.1.2.2 Indirect Effects to Terrestrial-Phase CRLF via Reduction in Prey (terrestrial invertebrates, mammals, and frogs)

5.1.2.2.1 Terrestrial Invertebrates

In order to assess the risks of prometryn to terrestrial invertebrates, which are considered prey of CRLF in terrestrial habitats, the honey bee is used as a surrogate for terrestrial invertebrates. The toxicity value for terrestrial invertebrates is calculated by multiplying the lowest available acute contact LD_{50} (> 96.7 µg a.i./bee at which 10% mortality was reported) by 1 bee/0.128g, which is based on the weight of an adult honey bee. EECs (µg a.i./g of bee or ppm) calculated by T-REX for small and large insects are divided by the calculated toxicity value for terrestrial invertebrates, which is >755µg a.i./g bee. A probit slope value for the acute honey bee toxicity test is not available; therefore, the effect probability was calculated based on a default slope assumption of 4.5 with upper and lower 95% confidence intervals of 2 and 9 (Urban and Cook, 1986). Since the LD_{50} is an indeterminate endpoint, however, the calculated effect probability and associated RQs (not reported) are indefinite (see discussion in the risk description section 5.2.2.4). As a result, indirect effects to the CRLF via reduction in terrestrial invertebrate prey items is uncertain

5.1.2.2.2 Mammals

Risks associated with ingestion of small mammals by large terrestrial-phase CRLFs are derived for dose-based exposures modeled in T-REX for a small mammal (15g) consuming short grass. Potential acute and chronic risks are estimated using the most sensitive mammalian toxicity data. EECs are divided by the toxicity value to estimate acute and chronic dose-based RQs as well as chronic dietary-based RQs. A probit slope value for the acute oral toxicity in rat test is not available; therefore, the effect probability was calculated based on a default slope assumption of 4.5 with upper and lower 95% confidence intervals of 2 and 9 (Urban and Cook, 1986). **Table 5-6** provides a summary of acute and chronic RQs. Based on acute and chronic risk LOC exceedances, prometryn may indirectly affect the CRLF via reductions in small mammal prey items.

Table 5-6 Summary of Acute and Chronic RQs* Used to Estimate Indirect Effects to the Terrestrial-phase CRLF via Direct Effects on Small Mammals as Dietary Food Items (non-granular application). Based on the maximum annual application rate to cotton.

Use	Application Rate (lbs a.i./A)	Chronic RQ		Acute RQ	
		Dose-based Chronic RQ[1]	Dietary-based Chronic RQ[2]	Dose-based Acute RQ[3]	Probability of Individual Effect at RQ
Cotton	2.4	**384.4**	**57.6**	**0.14**	1 in 1.64 x 10^{4} (1 in 23 to 1 in 1.31 x 10^{14})
Cotton	0.7	**112.1**	**16.8**	**0.04**	1 in 6.33 x 10^{9} (1 in 386 to 1 in 7.49 x 10^{35})
Celery/ Fennel/ Parsley	2.0	**320.4**	**48**	**0.12**	1 in 5.85 x 10^{4} (1 in 30.5 to 1 in 1.73 x 10^{16})
Dill	1.6	**256.3**	**38.4**	**0.09**	1 in 7.91 x 10^{5} (1 in 54.8 to 1 in 4.10 x 10^{20})

* LOC exceedances (acute RQ $\geq$ 0.1 and chronic RQ $\geq$ 1) are bolded and shaded.
[1] Based on dose-based EEC and prometryn rat NOAEL = 0.65 mg/kg-bw.
[2] Based on dietary-based EEC and prometryn rat NOAEC = 10 mg/kg-diet.
[3]Based on dose-based EEC and prometryn rat acute oral LD_{50} = 1802 mg/kg-bw.
NOTE: Probability of Individual Effect at risk to ES acute LOC is 1 in 2.94 x 10^{5} (95% C.I. 1 in 44 to 1 in 8.86 x 10^{18}).

5.1.2.2.3 Frogs

An additional prey item of the adult terrestrial-phase CRLF is other species of frogs. In order to assess risks to these organisms, dietary-based and dose-based exposures modeled in T-REX for a small bird (20g) consuming small invertebrates are used. See Section 5.1.2.1 and associated table (Table 5-5) for results. Based on the uncertainty associated with the indefinite LD_{50}/LC_{50} values in the avian acute oral and subacute dietary studies, prometryn may indirectly affect the CRLF via reduction in frogs as prey items. Further discussion is provided in the Risk Description (Section 5.2.1.2).

5.1.2.3 Indirect Effects to CRLF via Reduction in Terrestrial Plant Community (Riparian and Upland Habitat)

Potential indirect effects to the CRLF resulting from direct effects on riparian and upland vegetation are assessed using terrestrial plant seedling emergence and vegetative vigor EC_{25} data. See **Table 5-7** and **Table 5-8,** respectively, for monocot and dicot RQs. Based on terrestrial plant risk LOC exceedances observed in both monocots and dicots, prometryn may indirectly affect the CRLF via reduction in terrestrial plants. Example output from TerrPlant v.1.2.2 is provided in **Appendix M.**

Table 5-7 RQs* for Monocots Inhabiting Dry and Semi-Aquatic Areas Exposed to Prometryn via Runoff and Drift

Use	Application rate (lbs a.i./A)	Application method	Drift Value (%)	Spray drift RQ Non-Listed	Dry area RQ Non-Listed	Semi-aquatic area RQ Non-Listed
Cotton	2.4	Aerial[1]	5	**2.45**	**2.69**	**4.90**
Cotton	2.4	Ground Spray[2]	1	0.49	**1.47**	**10.29**
Cotton	0.7	Aerial[1]	5	0.71	0.79	**1.43**
Cotton	0.7	Ground Spray[2]	1	0.14	0.43	**3.00**
Celery/ Fennel/ Parsley	2.0	Ground Spray[2]	1	0.41	**1.22**	**8.57**
Dill	1.6	Ground Spray[2]	1	0.33	0.98	**6.86**

* Risk to non-listed terrestrial plant LOC exceedances (RQ $\geq$ 1) are bolded and shaded.
[1] Incorporation depth is 4 inches (10.2 cm) based on the label.
[2] Incorporation depth is 1.6 inches (4 cm) based on PRZM/EXAMS default. The TerrPlant default value of 1 was used.

Table 5-8 RQs* for Dicots Inhabiting Dry and Semi-Aquatic Areas Exposed to Prometryn via Runoff and Drift

Use	Application rate (lbs a.i./A)	Application method	Drift Value (%)	Spray drift RQ Non-Listed	Dry area RQ Non-Listed	Semi-aquatic area RQ Non-Listed
Cotton	2.4	Aerial[1]	5	**12**	**6.6**	**12**
Cotton	2.4	Ground Spray[2]	1	**2.40**	**3.60**	**25.2**
Cotton	0.7	Aerial[1]	5	**3.50**	**1.93**	**3.50**
Cotton	0.7	Ground Spray[2]	1	0.70	**1.05**	**7.35**
Celery/ Fennel/ Parsley	2.0	Ground Spray[2]	1	**2**	**3**	**21**
Dill	1.6	Ground Spray[2]	1	**1.6**	**2.4**	**16.8**

* Risk to non-listed terrestrial plant LOC exceedances (RQ $\geq$ 1) are bolded and shaded.
[1] Incorporation depth is 4 inches (10.2 cm) based on the label.
[2] Incorporation depth is 1.6 inches (4 cm) based on PRZM/EXAMS default. The TerrPlant default value of 1 was used.

5.1.3 Primary Constituent Elements of Designated Critical Habitat

5.1.3.1 Aquatic-Phase CRLF (Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)

Three of the four assessment endpoints for the primary constituent elements (PCEs) of designated critical habitat for the aquatic-phase CRLF are related to potential effects to aquatic and/or terrestrial plants:

- Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile (aquatic-phase) and adult (terrestrial-phase) CRLFs.
- Alteration in water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs and their food source.
- Reduction and/or modification of aquatic-based food sources for pre-metamorphs (*e.g.*, algae).

Based on the risk estimation for potential effects to aquatic non-vascular and vascular plants as well as terrestrial plants provided in Sections 5.1.1.2.1, 5.1.1.3, and 5.1.2.3, respectively, where plant risk LOC exceedances are observed in all aquatic non-vascular plant scenarios (Table 5-2), aquatic vascular plant scenarios (Table 5-4), and terrestrial plants (Tables 5-7, 5-8) prometryn may affect aquatic-phase CRLF PCEs of designated habitat related to effects on aquatic and terrestrial plants.

The remaining aquatic-phase PCE is "alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source." To assess the impact of prometryn on this PCE (*i.e.*, alteration of food sources), acute and chronic freshwater fish and invertebrate toxicity endpoints, as well as endpoints for aquatic non-vascular plants, are used as measures of potential effects. RQs for these endpoints were calculated in Sections 5.1.1.1, 5.1.1.2, and 5.1.1.3. Based on exceedences observed for most acute freshwater fish scenarios (Table 5-1) and all non-vascular plant scenarios (Table 5-2), prometryn may affect aquatic-phase PCEs of designated habitat related to effects of alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.

5.1.3.2 Terrestrial-Phase (Upland Habitat and Dispersal Habitat)

The first two assessment endpoints for PCEs of designated critical habitat for the terrestrial-phase CRLF are related to potential effects to terrestrial plants:

- Elimination and/or disturbance of upland habitat; ability of habitat to support food source of terrestrial-phase CRLFs: Upland areas within 200 ft of the edge of the riparian vegetation or dripline surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance

- Elimination and/or disturbance of dispersal habitat: Upland or riparian dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal

The risk estimation for terrestrial-phase PCEs of designated habitat related to potential effects on terrestrial plants is provided in Section 5.1.2.3. Based on risk to terrestrial plant LOC exceedances observed in monocots and dicots (Tables 5-8, 5-9), prometryn may affect the first and second terrestrial-phase CRLF PCEs.

The third terrestrial-phase PCE is "reduction and/or modification of food sources for terrestrial phase juveniles and adults." To assess the potential impact of prometryn on this PCE, acute and chronic toxicity endpoints for birds, mammals, and terrestrial invertebrates are used as measures of effects. RQs for these endpoints were calculated in Section 5.1.2. Based on LOC exceedances observed for small mammals (Table 5-6) and small insects (not reported), prometryn may affect the third terrestrial–phase CRLF PCE.

The fourth terrestrial-phase CRLF PCE is based on alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source. Direct chronic RQs for terrestrial-phase CRLFs are presented in Section 5.1.2.1. Based on acute and chronic LOC exceedances estimated for small mammal RQs (Table 5-6) and potential effect on frogs as prey items (Section 5.1.2.2.3), prometryn may affect the forth terrestrial–phase CRLF PCE.

5.1.4 Spatial Extent of Potential Effects

An LAA effects determination applies to those areas where it is expected that the pesticide's use will directly or indirectly affect the CRLF or its designated critical habitat. To determine this area, the footprint of prometryn's use pattern is identified, using land cover data that correspond to prometryn's use pattern. The spatial extent of the effects determination also includes areas beyond the initial area of concern that may be impacted by runoff and/or spray drift. The identified direct and indirect effects and/or effects to critical habitat are anticipated to occur only for those currently occupied core habitat areas, CNDDB occurrence sections, and designated critical habitat for the CRLF that overlap with the initial area of concern plus a distance of 997 ft for aerial spray applications (and 190 ft for ground spray applications) from its boundary. It is assumed that non-flowing waterbodies (or potential CRLF habitat) are included within this area.

In addition to the spray drift buffer, the results of the downstream dilution extent analysis result in a distance of 285 km which represents the maximum continuous distance of downstream dilution from the edge of the initial area of concern. If any of these streams reaches flow into CRLF habitat, there is potential to affect either the CRLF or affect its habitat. These lotic aquatic habitats within the CRLF core areas and critical habitats potentially contain concentrations of prometryn sufficient to result in LAA determination or effects to critical habitat.

The determination of the buffer distance and downstream dilution for spatial extent of the effects determination is described below.

5.1.4.1 Spray Drift

In order to determine terrestrial and aquatic habitats of concern due to prometryn exposures through spray drift, it is necessary to estimate the distance that spray applications can drift from the treated area and still be present at concentrations that exceed levels of concern. An analysis of spray drift distances was completed using all available tools, including AgDrift, AGDISP, and the Gaussian extension to AGDISP.

Aerial and ground spray application are recommended for prometryn [Caparol (EPA Reg No. 100-620), Cotton Pro (EPA Reg No. 1812-274), Prometryn 4L Herbicide (EPA Reg. No. 9779-297) Prometryne + MSMA (EPA Reg, No. 9779-317). There are limited mitigation measures for ground spray applications. For aerial applications, spray drift management options are as follows:

- Applications should be made a maximum height of 10 feet.
- Applications should not be made at wind speeds exceeding 10 mph
- A 400-feet upwind spray drift buffer should be employed when sensitive non-target plants are near the application site
- Coarse to Medium Droplet Size Spectrum should be used.

Tier 1 AgDrift (ver 2.1.03) modeling was used to estimate the impact of the 400 feet buffer on terrestrial and aquatic EECs. Input parameters for Tier I AgDrift spray drift modeling are shown in **Table 5-9**. Spray drift deposition concentrations for Tier I AgDrift modeling are shown in **Table 5-10**. Provided the pesticide travels a distance of 400 ft from a point source, a 0.011 spray fraction of the applied concentration is expected for aerial applications and a 0.0021 spray fraction of applied concentration is expected for ground applications (according to Tier 1 AgDrift). The terrestrial plant RQs calculated in TerrPlant based on the given aerial and ground spray fractions at 400 ft are shown in **Table 5-11.** Non-listed terrestrial plant (dicot) LOCs are exceeded (for spray drift only) for aerial spray applications of the maximum applied concentration (2.4 lbs a.i./A) at 400 ft. Non-listed terrestrial plant LOCs are exceeded (for dry and semi-aquatic areas given spray drift and runoff) for both aerial spray and ground spray applications of the maximum applied concentration (2.4 lbs a.i./A) at 400 ft. Given the most sensitive terrestrial plant endpoint (0.01 lbs a.i./A), the spray drift buffers beyond which LOC exceedences are not expected are 997 ft for aerial spray applications and 190 ft for ground spray applications. The calculated aerial spray buffer distance (997 ft) exceeds the currently prescribed buffer distance (400 ft).

Table 5-9: Input Parameters for Tier I AgDrift Modeling

Parameter	Input Value
Droplet Spectrum	Coarse to Medium
Application Rate	2.4 lbs/A

Table 5-10: Tier 1 AgDrift Terrestrial and Aquatic EECs from Spray Drift Alone

Buffer Distance (feet)	Spray Method	Maximum Application Rate (lbs ai/A)	EECs	
			Terrestrial (lbs ai/A)	Aquatic (μg/L)
0	Aerial	2.4	1.200	11.993
400	Aerial	2.4	0.026	1.207
997	Aerial	2.4	0.013	0.699
0	Ground	2.4	2.43	2.218
400	Ground	2.4	0.005	0.227
997	Ground	2.4	0.002	0.092

Table 5-11: Terrestrial Plant RQs given aerial and ground spray fractions of the maximum applied concentration (2.4 lbs a.i./A on cotton) at 400 feet

Use	Application rate (lbs a.i./A)	Application method	Spray Fractions at 400 ft	Plant Type	Spray drift RQ	Dry area RQ	Semi-aquatic area RQ
Cotton	2.4	Aerial[1]	0.011	Monocot	0.54	0.78	**2.99**
Cotton	2.4	Aerial[1]	0.011	Dicot	**2.64**	**1.92**	**7.32**
Cotton	2.4	Ground[2]	0.0021	Monocot	0.10	**1.08**	**9.90**
Cotton	2.4	Ground[2]	0.0021	Dicot	0.50	**2.65**	**24.25**

* Risk to non-listed terrestrial plant LOC exceedances (RQ ≥ 1) are bolded and shaded.
[1] Incorporation depth is 4 inches (10.2 cm) based on the label.
[2] Incorporation depth is 1.6 inches (4 cm) based on PRZM/EXAMS default. The TerrPlant default value of 1 was used.
Note: see Table 3-8 for EECs

Tier II AgDisp (ver 8.13) modeling was conducted to assess the impact of spray drift mitigation recommendations for aerial spray applications. Input parameters for Tier II AgDisp spray drift modeling are shown in **Table 5-12**. Spray drift deposition concentrations from Tier II AgDisp modeling are shown in **Table 5-13**.

Table 5-12: Input Parameters for Tier II AgDisp Modeling

Parameter	Input Value
Aircraft	Air tractor AT-401
Release Height	10 ft
DSD	Coarse to Medium
Wind Speed	10 mph
Temp	650F
Relative Humidity	50%
Spray Volume Rate	5 gal/A
Active Fraction	0.1056
Nonvol. Fraction	0.24

Table 5-13: Tier II AgDisp Terrestrial and Aquatic EECs from Aerial Spray Drift Alone

Buffer Distance (feet)	Spray Method	Maximum Application Rate (lbs ai/A)	EECs	
			Terrestrial (lbs ai/A)	Aquatic (µg/L)
0	Aerial	2.4	6.1	3.546
400	Aerial	2.4	0.04	0.611
1000	Aerial	2.4	0.0094	0.083

5.1.4.2 Downstream Dilution Analysis

The downstream extent of exposure in streams and rivers is where the EEC could potentially be above levels that would exceed the most sensitive LOC. To complete this assessment, the greatest ratio of aquatic RQ to LOC was estimated. Using an assumption of uniform runoff across the landscape, it is assumed that streams flowing through treated areas (i.e. the initial area of concern) are represented by the modeled EECs; as those waters move downstream, it is assumed that the influx of non-impacted water will dilute the concentrations of prometryn present.

Using a EC_{50} value of 1 ug/L for non-vascular aquatic plants (the most sensitive species) and a maximum peak EEC of 377.3 ug/L for fall application to parsley yields an RQ/LOC ratio of 377.3 (377.3/1). Using the downstream dilution approach (described in more detail in **Appendix D**) yields a target percent crop area (PCA) of 0.27. This value has been input into the downstream dilution approach and results in a distance of kilometers which represents the maximum continuous distance of downstream dilution from the edge of the initial area of concern. Similar to the spray drift buffer described above, the LAA/NLAA determination is based on the area defined by the point where concentrations exceed the EC_{50} value.

5.1.4.3 Overlap between CRLF habitat and Spatial Extent of Potential Effects

An LAA effects determination is made to those areas where it is expected that the pesticide's use will directly or indirectly affect the CRLF or its designated critical habitat and the area overlaps with the core areas, critical habitat and available occurrence data for CRLF.

For prometryn, the use pattern in the land cover classes including cotton, celery, fennel, parsley, and dill also includes areas beyond the initial area of concern that may be impacted by runoff and/or spray drift overlaps with CRLF habitat. **Appendix D** provides maps of the initial area of concern, along with CRLF habitat areas, including currently occupied core areas, CNDDB occurrence sections, and designated critical habitat. It is expected that any additional areas of CRLF habitat that are located 997 ft for aerial spray applications (and 190 ft for ground spray applications) from boundary (to account for offsite migration via spray drift) and 285 kilometers of stream reach (to account for downstream dilution) outside the initial area of concern may also be impacted and are part of the full spatial extent of the LAA/effects to critical habitat effects determinations.

5.2 Risk Description

The risk description synthesizes an overall conclusion regarding the likelihood of adverse impacts leading to an effects determination (*i.e.*, "no effect," "may affect, but not likely to adversely affect," or "likely to adversely affect") for the CRLF and its designated critical habitat.

Based on the RQs presented in the Risk Estimation (Section 5.1) a preliminary overall effects determination is "may affect" for the CRLF and "may affect" critical habitat.

The direct or indirect risk LOCs are exceeded or use may modify the PCEs of the CRLF's critical habitat, the Agency concludes a preliminary "may affect" determination for the FIFRA regulatory action regarding prometryn. A summary of the risk estimation results are provided in **Table 5-** for direct and indirect effects to the CRLF and in **Table 5-1** for the PCEs of designated critical habitat for the CRLF.

Table 5-14 Risk Estimation Summary for Prometryn - Direct and Indirect Effects to CRLF

Assessment Endpoint	LOC Exceedances (Y/N)	Description of Results of Risk Estimation
Aquatic-Phase (eggs, larvae, tadpoles, juveniles, and adults)		
Direct Effects Survival, growth, and reproduction of CRLF individuals via direct effects on aquatic phases	Acute: Y	Acute risk to freshwater fish (and by extension to aquatic-phase amphibians) LOCs are exceeded based on exposure estimates in all parsley uses (Spring, Fall: 2 lbs a.i./A) and most cotton uses (Fall, pre-emergence ground spray and pre-emergence aerial spray: 2.4 lbs a.i./A).
	Chronic: N	No chronic risk LOCs are exceeded based on the most sensitive toxicity data for freshwater fish across any of the uses evaluated (Table 5-1).
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects to food supply (*i.e.*, freshwater invertebrates, non-vascular plants)	Non-vascular plants: Y	Risk to aquatic plant LOC are exceeded for non-vascular aquatic plants in all uses. For a complete list see Table 5-2.
	Freshwater invertebrates: N	No acute nor chronic risk LOC exceedances are observed for freshwater invertebrates.
	Fish and frogs: Y	The same description applies here for fish and frog prey items as in the direct effects (aquatic-phase CRLF) component above.
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects on habitat, cover, and/or primary productivity (*i.e.*, aquatic plant community)	Y	Risk to aquatic plant LOCs are exceeded for vascular aquatic plants in all uses. For a complete list see Table 5-4.
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects to riparian vegetation, required to maintain acceptable water quality and habitat in ponds and streams comprising the species' current range.	Y	Risk to terrestrial plant LOCs are exceeded for monocots in dry and semi-aquatic areas for cotton uses (ground: 2.4 lbs a.i./A) and in dry and semi-aquatic areas as well as spray drift alone for cotton uses (aerial: 2.4 lbs a.i./A). Dicots are more sensitive as LOC exceedances are estimated in dry and semi-aquatic areas for both application types on cotton (ground and aerial spray at 2.4 lbs a.i./A).
Terrestrial-Phase (Juveniles and adults)		
Direct Effects Survival, growth, and reproduction of CRLF individuals via direct effects	Acute: N	No definitive acute RQs could be derived because the acute avian effects data shows no mortality at the highest test concentrations.

Assessment Endpoint	LOC Exceedances (Y/N)	Description of Results of Risk Estimation
on terrestrial phase adults and juveniles	Chronic: N	Chronic risk LOC exceedances are not observed for birds consuming small insects when considering the maximum annual application rate to cotton (2.4 lbs a.i./A) in T-REX using dietary based RQs.
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects on prey (*i.e.*, terrestrial invertebrates, small terrestrial mammals and terrestrial phase amphibians)	Y	Acute risk to terrestrial invertebrate LOC exceedances are observed for small insects for cotton use (2.4 lbs a.i./A). Small terrestrial mammals may also be affected by prometryn as acute and chronic risk LOC exceedances are observed for small mammals serving as prey. The same description applies here for frog prey items as in the direct effects (terrestrial-phase CRLF) component above.
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects on habitat (*i.e.*, riparian vegetation)	Y	The same description applies here as in the indirect effects to riparian vegetation for the aquatic-phase CRLF component above.

Table 5-15 Risk Estimation Summary for Prometryn – PCEs of Designated Critical Habitat for the CRLF

Assessment Endpoint	Habitat Effects (Y/N)	Description of Results of Risk Estimation
Aquatic-Phase PCEs (Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)		
Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator	Non-vascular aquatic plants: Y	Risk to aquatic plant LOC exceedances are observed for non-vascular aquatic plants in all uses. For a complete list see Table 5-2.

Assessment Endpoint	Habitat Effects (Y/N)	Description of Results of Risk Estimation
avoidance, and aquatic dispersal for juvenile and adult CRLFs.	Terrestrial plants: Y	Risk to terrestrial plant LOCs are exceeded for monocots in dry and semi-aquatic areas for cotton uses (ground: 2.4 lbs a.i./A) and in dry and semi-aquatic areas as well as spray drift alone for cotton uses (aerial: 2.4 lbs a.i./A). Dicots are more sensitive as LOC exceedances are estimated in dry and semi-aquatic areas for both application types on cotton (ground and aerial spray at 2.4 lbs a.i./A).
Alteration in water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	Same as above	Same as above
Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.	Acute: Y	Acute risk to freshwater fish LOCs are exceeded for all parsley uses (Spring, Fall: 2 lbs a.i./A) and most cotton uses (Fall, pre-emergence ground spray and pre-emergence aerial spray: 2.4 lbs a.i./A).
	Chronic: N	No chronic risk LOCs are exceeded based on the most sensitive toxicity data for freshwater fish and for any use type (Table 5-1).
	Freshwater Invertebrates: N	Invertebrate risk LOC exceedances are not observed for freshwater invertebrates.
Reduction and/or modification of aquatic-based food sources for pre-metamorphs (e.g., algae)	Non-vascular plants: Y	Risk to aquatic plant LOC exceedances are observed for non-vascular aquatic plants in all uses. For a complete list see Table 5-2.
Terrestrial-Phase PCEs (Upland Habitat and Dispersal Habitat)		
Elimination and/or disturbance of upland habitat; ability of habitat to support food source of CRLFs: Upland areas within 200 ft of the edge of the riparian vegetation or dripline surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance	Terrestrial plants: Y	Risk to terrestrial plant LOCs are exceeded for monocots in dry and semi-aquatic areas for cotton uses (ground: 2.4 lbs a.i./A) and in dry and semi-aquatic areas as well as spray drift alone for cotton uses (aerial: 2.4 lbs a.i./A). Dicots are more sensitive as LOC exceedances are estimated in dry and semi-aquatic areas for both application types on cotton (ground and aerial spray at 2.4 lbs a.i./A).
Elimination and/or disturbance of dispersal habitat: Upland or riparian	Same as above	Same as above

Assessment Endpoint	Habitat Effects (Y/N)	Description of Results of Risk Estimation
dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal		
Reduction and/or modification of food sources for terrestrial phase juveniles and adults	Y	Acute risk LOC exceedances are observed for small insects for cotton use (2.4 lbs a.i./A).
Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	Y	Acute and chronic risk LOC exceedances are observed (Table 5-6) for small mammals serving as prey.

Following a "may affect" determination, additional information is considered to refine the potential for exposure at the predicted levels based on the life history characteristics (*i.e.*, habitat range, feeding preferences, etc.) of the CRLF. Based on the best available information, the Agency uses the refined evaluation to distinguish those actions that "may affect, but are not likely to adversely affect" from those actions that are "likely to adversely affect" the CRLF and its designated critical habitat.

The criteria used to make determinations that the effects of an action are "not likely to adversely affect" the CRLF and its designated critical habitat include the following:

- Significance of Effect: Insignificant effects are those that cannot be meaningfully measured, detected, or evaluated in the context of a level of effect where "take" occurs for even a single individual. "Take" in this context means to harass or harm, defined as the following:
    - Harm includes significant habitat modification or degradation that results in death or injury to listed species by significantly impairing behavioral patterns such as breeding, feeding, or sheltering.
    - Harass is defined as actions that create the likelihood of injury to listed species to such an extent as to significantly disrupt normal behavior patterns which include, but are not limited to, breeding, feeding, or sheltering.
- Likelihood of the Effect Occurring: Discountable effects are those that are extremely unlikely to occur.
- Adverse Nature of Effect: Effects that are wholly beneficial without any adverse effects are not considered adverse.

A description of the risk and effects determination for each of the established assessment endpoints for the CRLF and its designated critical habitat is provided in Sections 5.2.1 through 5.2.3.

5.2.1 Direct Effects

5.2.1.1 Aquatic-Phase CRLF

The aquatic-phase includes life stages of the frog that are obligatory aquatic organisms, including eggs and larvae. It also includes submerged terrestrial-phase juveniles and adults, which spend a portion of their time in water bodies that may receive runoff and spray drift containing prometryn.

As shown in Table 5-1, acute RQs based on the highest modeled EECs for prometryn use on all parsley (2.0 lbs a.i./A), a fall celery/fennel (2.0 lbs a.i./A), and two pre-emergence cotton uses (2.4 lb a.i./A) and the most sensitive acute freshwater fish data (*i.e.*, rainbow trout; used as a surrogate for aquatic-phase amphibians) exceed the Agency's acute risk to listed species LOC. Acute RQs for other modeled uses did not exceed Agency's acute risk to listed species LOC. Similarly, chronic RQs based on parallel information (using data on another aquatic-phase amphibian surrogate, *i.e.*, the fathead minnow) are well below the Agency's chronic risk LOC. The RQs are based on PRZM/EXAMS EECs. Comparison of the highest modeled surface water EEC (peak = 377 µg/L on parsley fall application representing acute effects) with available NAWQA surface water monitoring data from California indicates that the peak modeled EEC is approximately 607 times higher than the maximum concentration of prometryn (0.621 µg/L) detected in Stanislaus County. The lowest level at which the acute risk to listed species LOC exceedances were observed was at a peak (acute effects) of 152.9 µg/L, which is 246 times higher than the maximum concentration detected. Therefore, use of modeled EECs is assumed to provide a conservative measure of prometryn exposures for aquatic-phase CRLFs.

The probability of an individual effect to aquatic-phase CRLFs was calculated based on the acute rainbow trout study (MRID 00070686) reported slope, 3.4 (95% C.I. 1.99-4.83). The corresponding estimated chance of an individual acute mortality to the aquatic-phase CRLF at the highest RQ level of 0.13, for example, is 1 in 772 (with respective lower and upper bounds of 1 in 26 to 1 in 1.07 x 10^5). Given the relatively high probability of an individual mortality occurrence and acute RQs that are above the Agency's acute risk LOC, prometryn is likely to cause direct adverse effects to aquatic-phase CRLFs.

There were no acceptable amphibian studies available in the ECOTOX open literature for prometryn. Three freshwater aquatic incidents involving fish kills (carp, shad, buffalo, and car) have been reported in 1996 in Richland County of Louisiana for which prometryn is an 'unlikely' cause; additional chemicals were detected with presumably higher toxicity to freshwater fish.

In summary, the Agency concludes a "likely to adversely affect" or "LAA" determination for direct acute effects to the aquatic-phase CRLF, based on risk of acute mortality and all available lines of evidence. A “no effect” determination applies to direct chronic effects to the aquatic-phase CRLF, via growth, based on all available lines of evidence.

5.2.1.2 Terrestrial-Phase CRLF

As stated in the risk estimation section (Section 5.1.2.1), the avian acute oral and subacute dietary RQs were not estimated because the LD_{50} and LC_{50} values exceed the maximum limit concentrations tested. The data indicate that prometryn is practically non-toxic to birds on both an acute oral and subacute dietary exposure basis with no mortalities at acute oral doses up to and including 4,640 mg/kg-bw (highest level tested) in the acute oral study with mallard ducks (MRID 00082966) and one mortality at the highest concentration level of 5,000 mg/kg-diet in the subacute dietary study with Northern bobwhite quail (MRID 40457502). Observed sublethal effects for these studies include reductions in feed consumption and body weight gain at the highest dose level of the acute oral study (4,640 mg/kg-bw; NOAEL: 2,150 mg/kg-bw) and at the two highest concentration levels (2,500 mg/kg-diet and 5,000 mg/kg-diet; NOAEC: 1,250 mg/kg-diet) in the subacute dietary study.

Using the terrestrial model, T-REX (v. 1.4.1) and the highest dose level tested in the acute oral study (4,640 mg/kg-bw with mallard ducks), the adjusted dose for a 20 g bird would be 2,409 mg/kg-bw. For the use on cotton with a single application rate of 2.4 lbs a.i./A, the dose-based EEC for small birds feeding on small insects is 369 mg/kg-bw. Comparing the two values, the highest dose tested in the acute oral avian study is 6.5 times higher than the estimated dose-based EEC for cotton (*e.g.*, an estimated upper bound RQ would be 0.15). The acute risk to listed birds LOC is 0.1. Therefore, on a dose-basis, there is an uncertainty associated with potential mortality to endangered species at levels above 2,409 mg/kg-bw for a 20 g bird consuming small insects. For the uncertainty to be diminished (*e.g.*, for the upper bound RQ to be less than the acute risk to listed species LOC of 0.1), either the highest dose level tested or the acute LD_{50} in the acute oral study would have to be greater than 7,000 mg/kg-bw (3,635 mg/kg-bw for a 20 g bird).

In an effort to refine the acute dose-based risk estimates, the T-REX model was modified to account for the lower metabolic rate and lower caloric requirement of amphibians (compared to birds). Acute dose-based RQs were recalculated using the T-HERPS (Version 1.0) model for small (1 g), medium (37 g), and large (238 g) frogs. For sample T-HERPS output refer to **Appendix K.** Using this refinement, the highest acute dose-based RQ is 0.05 for 37 g frogs eating small herbivorous mammals. This is less than the acute LOC of 0.1 for listed species.

The T-REX model was used again to interpret potential sublethal effects. For sublethal effects on a dose-basis, the NOAEL is 2,150 mg/kg-bw. This corresponds to an adjusted dose of 1,116.3 mg/kg-bw for a 20 g bird. The dose-based EEC is 369 mg/kg-bw, which is 3 times lower than the NOAEL for sublethal effects. Therefore, sublethal effects on a dose-basis are not expected.

In the subacute dietary study of Bobwhite quail, one mortality was observed at the highest concentration level of 5,000 mg/kg-diet. The estimated dietary-based EEC for cotton is 324 mg/kg, which is roughly 15 times lower than 5,000 mg/kg (*e.g.*, the upper bound subacute dietary based RQ would be 0.06). This value is less than the avian acute risk to listed species LOC of 0.1. Sublethal effects were observed at concentration levels of 2,500 mg/kg-diet and above with a NOAEC of 1,250 mg/kg-diet. It was noted in the study that food avoidance was observed at the two highest concentration levels. The dietary-based EEC is nearly 4 times less than the NOAEC for sublethal effects. Therefore, based on the reported food avoidance at the levels with decreased body weight and the fact that the EEC is 4 times lower than the NOAEC, sublethal effects on a sub-acute dietary exposure basis are not considered likely.

The probabilities of an individual effect to terrestrial-phase CRLFs were not calculated for the same reasons that RQs were not calculated.

The chronic toxicity study (MRID 41035901) with mallard ducks had one adult (parental) mortality at the 250 mg/kg-diet level. Since this mortality did not appear to be dose-related, it is not considered to be treatment-related. As stated in the risk estimation section, the chronic RQ for birds following use on cotton is 0.65, which does not exceed the Agency's avian chronic risk LOC of 1.

There were no acceptable amphibian studies available in the ECOTOX open literature for prometryn. Similarly, no prometryn incidents have been reported involving terrestrial organisms.

In summary, the Agency concludes a "may effect, not likely to adversely affect" or "NLAA" determination for acute and chronic direct effects to the terrestrial-phase CRLF, via mortality, growth, and fecundity, based on all available lines of evidence. The effects are insignificant for the following reasons:

- There were no mortalities at the highest dose level tested in the avian acute oral toxicity study. Using the highest dose tested in the model, T-HERPS, provides an upper bound RQ that does not exceed the acute avian LOC for listed species.
- There was one mortality at the highest dietary concentration in the avian subacute dietary toxicity study. Using the highest concentration tested to estimate an upper bound RQ provides an RQ that does not exceed the acute risk to listed species LOC for birds.
- Observed sublethal effects related to growth in both the acute oral study and the subacute dietary study were observed at significantly higher dose and concentration levels than the EECs provided in the T-REX model following use at the maximum annual application rate used on cotton.
- The chronic RQ for birds following use on cotton does not exceed the chronic risk to birds LOC.

5.2.2 Indirect Effects (via Reductions in Prey Base)

5.2.2.1 Algae (non-vascular plants)

As discussed in Section 2.5.3, the diet of CRLF tadpoles is composed primarily of unicellular aquatic plants (*i.e*., algae and diatoms) and detritus. RQs were calculated using the most sensitive non-vascular plant EC_{50} (0.001 mg a.i./L) taken from a freshwater diatom study (MRID 42620201) and EECs generated from PRZM/EXAMS. Further examination of toxicity data for other non-vascular plants: green algae (0.012 mg a.i./L), diatom (0.0076 mg a.i./L) , and blue-green algae (0.0401 mg a.i./L) indicates that they are approximately seven to forty times less sensitive to prometryn than the freshwater diatom. As shown in Table 5-2, non-vascular plant RQs based on *N. pelliculosa* exceed the Agency's risk to non-listed aquatic plants LOC for all uses. Therefore, prometryn is likely to indirectly affect the CRLF via reduction in non-vascular aquatic plants.

Not unlike the outcome for the direct effect to the aquatic-phase CRLF, comparison of the highest modeled surface water EEC (peak = 377 µg/L on parsley fall application) with available NAWQA surface water monitoring data from California indicates that the peak modeled EEC is approximately 607 times higher than the maximum concentration of prometryn (0.621 µg/L) detected in Stanislaus County. The lowest modeled surface water EEC (peak = 49.2 µg/L on fall application on celery/fennel) is 79 times higher than the maximum concentration. LOC exceedences were observed at all modeled EEC levels. Therefore, use of modeled EECs is assumed to provide a conservative measure of prometryn exposures for aquatic non-vascular plants.

Open literature data were not suitable for use in this assessment. No prometryn incidents have been reported involving aquatic non-vascular plants.

In summary, based on all available lines of evidence the Agency concludes a "likely to adversely affect" determination for indirect effects of prometryn to CRLF tadpoles, via reductions in non-vascular plants.

5.2.2.2 Aquatic Invertebrates

The potential for prometryn to elicit indirect effects to the CRLF via effects on freshwater invertebrate food items is dependent on several factors including: (1) the potential magnitude of effect on freshwater invertebrate individuals and populations; and (2) the number of prey species potentially affected relative to the expected number of species needed to maintain the dietary needs of the CRLF. Together, these data provide a basis to evaluate whether the number of individuals within a prey species is likely to be reduced such that it may indirectly affect the CRLF.

RQs were calculated using the most sensitive freshwater invertebrate EC_{50} (18.59 mg a.i./L) and NOAEC (1.0 mg a.i./L) values taken from an acute toxicity to *D. magna* study

(MRID 00070146) and a chronic toxicity to *D. magna* study (MRID 40573720), respectively, as well as EECs generated from PRZM/EXAMS. The probability of an individual effect to aquatic-phase CRLFs was calculated despite low RQs for all uses modeled in PRZM/EXAMS (Table 5-3). The slope, 5.24 (95% C.I. 3.34-7.14), of the dose-response curve used in calculating the probability was based on the acute toxicity study with daphnids. Given that RQs were far below Agency's acute and chronic risk LOCs as well as the low probabilities of individual effect, prometryn is not likely to indirectly affect the CRLF via reduction in aquatic invertebrate prey items.

The range of EECs modeled for acute effects on aquatic invertebrates is 49.2-377.3 μg/L; the range of EECs modeled for chronic effects is 48.4- 370 μg/L. These concentrations are far above the maximum concentration found in surface water data (0.621 μg/L) and yet no acute risk LOC exceedances were observed.

In summary, based on all available lines of evidence, the Agency concludes a "no effect" determination for indirect effects to the CRLF via direct acute (mortality) and chronic (growth, reproductive and/or survival) effects on freshwater invertebrates as prey.

5.2.2.3 Fish and Aquatic-phase Frogs

No endangered species chronic risk LOCs were exceeded for freshwater fish, which are used as a surrogate for aquatic-phase amphibians. However, acute risk to listed species LOCs is exceeded for freshwater fish. Given the relatively high probability of an individual mortality occurrence and exceedance of acute RQs (refer to Section 5.2.1.1), prometryn is likely to cause direct acute mortality to aquatic-phase CRLFs. Therefore, indirect effects to the CRLF via a reduction in freshwater fish and other aquatic-phase frog species as prey items may occur, and the effects determination for indirect acute effects is "likely to adversely affect" or "LAA" while the determination for indirect chronic effects is "no effect".

5.2.2.4 Terrestrial Invertebrates

When the terrestrial-phase CRLF reaches juvenile and adult stages, its diet is mainly composed of terrestrial invertebrates. As previously discussed in Section 5.1.2.2, indirect effects to the CRLF via reduction in terrestrial invertebrate prey items is uncertain because the LD_{50} is an indeterminate endpoint (*i.e.*, >96.7 μg a.i./bee). The reported mortality is 10% at this concentration. The indefinite RQs calculated for small insects indicate exceedances of the Agency's LOC for terrestrial invertebrates for all modeled uses (cotton 2.4 lbs a.i./L; cotton 0.7; celery/fennel/parsley 2.0; dill 1.6) from <0.13 to < 0.43; the upper bound RQ for large insects is less than the LOC of 0.05. Small insects are, therefore, more likely to be affected by prometryn as the probability of individual effect for the highest application rate modeled (cotton 2.4 lbs a.i./A) is relatively high (1 in 20.2, with respective lower and upper bounds of 1 in 4 to 1 in 2,060); the lowest application rate modeled (cotton 0.7 lbs a.i./A) yielded a lower probability of individual effect (1 in 29,900, with respective lower and upper bounds of 1 in 26 to 1 in 1.31 x

10^{15}). A probit slope value for the acute honey bee toxicity test was not available; therefore, the effect probability was calculated based on a default slope assumption of 4.5 with upper and lower 95% confidence intervals of 2 and 9 (Urban and Cook, 1986). Given LOC exceedance and high probability of individual effect, especially for small insects, prometryn is likely to indirectly affect the terrestrial-phase CRLF via reduction in terrestrial invertebrate prey items.

No prometryn incidents have been reported involving terrestrial invertebrates.

Given that upper bound RQs for large insects do not exceed Agency's LOC for terrestrial invertebrates and that the probabilities of individual effect are low (on the order of 10^8 for the highest application rate modeled), a "may affect, not likely to adversely affect" determination is given for large insects. However, given that RQs for small insects exceed Agency's LOC for terrestrial invertebrates and that the probabilities of individual effect are high (especially for the highest application rate modeled), a "likely to adversely effect" determination is given for small insects, with uncertainty associated with potential mortality at LC_{50}. In summary, based on available lines of evidence the Agency concludes a "likely to adversely affect" or "LAA" determination for indirect effects to the CRLF, via a reduction in terrestrial invertebrates.

5.2.2.5 Mammals

Life history data for terrestrial-phase CRLFs indicate that large adult frogs consume terrestrial vertebrates, including mice. As previously discussed in Section 5.1.2.2.2, indirect effects to the CRLF via reduction in terrestrial mammal prey items that are exposed to prometryn are expected. Both chronic dose-based and dietary-based RQs greatly exceed the Agency's chronic risk LOC for terrestrial mammals for all uses (Table 5-7). Similarly, the acute dose-based RQ exceeds the Agency's acute risk LOC for cotton (2.4 lbs a.i./A) and celery/fennel/parsley (2.0 lbs a.i./A). In addition, the probability of individual effect is relatively high for the highest application rate modeled (*i.e.*,1 in 1.64 x 10^4, with respective lower and upper bounds of 1 in 23 to 1 in 1.31 x 10^{14}). A probit slope value for the acute rat toxicity test was not available; therefore, the effect probability was calculated based on a default slope assumption of 4.5 with upper and lower 95% confidence intervals of 2 and 9 (Urban and Cook, 1986). Given the LOC exceedances for acute and chronic effects and the relatively high probability of individual effect, prometryn is likely to indirectly affect the CRLF via reduction in terrestrial mammal prey items.

No prometryn incidents have been reported involving mammals.

In summary, based on available lines of evidence the Agency concludes a "likely to adversely affect" or "LAA" determination for indirect effects to the terrestrial-phase CRLF via reduction in small mammals as prey through acute and chronic exposure.

5.2.2.6 Terrestrial-phase Amphibians

Terrestrial-phase adult CRLFs also consume frogs. RQ values representing direct exposures of prometryn to terrestrial-phase CRLFs are used to represent exposures of prometryn to frogs in terrestrial habitats. Based on estimated exposures resulting from ground spray and aerial use of prometryn, acute and chronic risks to frogs are not likely (refer back to section 5.2.1.2 for a more detailed discussion). In light of the lack of risk to listed species LOC exceedances for the avian acute oral endpoint (T-HERPS) and the subacute and chronic dietary endpoints (T-REX), the effects determination for indirect effects to large CRLF adults that feed on other species of frogs as prey, via acute and chronic exposure to prometryn, is “may effect, not likely to adversely affect” or “NLAA”.

5.2.3 Indirect Effects (via Habitat Effects)

5.2.3.1 Aquatic Plants (Non-vascular and Vascular)

Aquatic plants serve several important functions in aquatic ecosystems. Non-vascular aquatic plants are primary producers and provide the autochthonous energy base for aquatic ecosystems. Vascular plants provide structure as attachment sites and refugia for many aquatic invertebrates, fish, and juvenile organisms, such as fish and frogs. In addition, vascular plants also provide primary productivity and oxygen to the aquatic ecosystem. Rooted plants help reduce sediment loading and provide stability to nearshore areas and lower streambanks. In addition, vascular aquatic plants are important as attachment sites for egg masses of CRLFs.

Potential indirect effects to the CRLF based on impacts to habitat and/or primary production were assessed using RQs from freshwater aquatic vascular and non-vascular plant data. As discussed in Section 5.2.2.1, non-vascular plant RQs based on *N. pelliculosa* toxicity to prometryn and EEC estimates from PRZM/EXAMS exceed the Agency’s risk to aquatic plant LOC for all uses. Similarly, vascular plant RQs (Table 5-4) based on the sensitivity of *L. gibba* to prometryn and EEC estimates from PRZM/EXAMS exceed the Agency’s risk to aquatic plant LOC for all uses. Therefore, prometryn is likely to indirectly affect the CRLF via reduction in both non-vascular and vascular aquatic plants.

Not unlike the outcome for the direct effect to the aquatic phase CRLF, comparison of the highest modeled surface water EEC (peak = 377.3 µg/L on parsley fall application) with available NAWQA surface water monitoring data from California indicates that the peak modeled EEC is approximately 607 times higher than the maximum concentration of prometryn (0.621 µg/L) detected in Stanislaus County. The lowest modeled surface water EEC (peak = 49.2 µg/L on fall application on celery/fennel) is 79 times higher than the maximum concentration. LOC exceedences were observed at all modeled EEC levels for both aquatic vascular and non-vascular plants. Therefore, use of modeled EECs is assumed to provide a conservative measure of prometryn exposures for aquatic vascular and non-vascular plants.

No prometryn incidents have been reported involving aquatic vascular and non-vascular plants.

In summary, based on all available lines of evidence, the Agency concludes a “likely to adversely affect” or “LAA” determination for indirect effects of prometryn to the CRLF via impacts to habitat and/or primary production through direct effects to non-vascular and vascular aquatic plants.

5.2.3.2 Terrestrial Plants

Terrestrial plants serve several important habitat-related functions for the CRLF. In addition to providing habitat and cover for invertebrate and vertebrate prey items of the CRLF, terrestrial vegetation also provides shelter for the CRLF and cover from predators while foraging. Terrestrial plants also provide energy to the terrestrial ecosystem through primary production. Upland vegetation including grassland and woodlands provides cover during dispersal. Riparian vegetation helps to maintain the integrity of aquatic systems by providing bank and thermal stability, serving as a buffer to filter out sediment, nutrients, and contaminants before they reach the watershed, and serving as an energy source.

Loss, destruction, and alteration of habitat were identified as a threat to the CRLF in the USFWS Recovery Plan (USFWS, 2002). Herbicides can adversely impact habitat in a number of ways. In the most extreme case, herbicides in spray drift and runoff from the site of application have the potential to kill (or reduce growth and/or biomass in) all or a substantial amount of the vegetation, thus removing or impacting structures which define the habitat, and reducing the functions (*e.g.*, cover, food supply for prey base) provided by the vegetation.

Risk to terrestrial plant LOCs are exceeded for monocots in dry and semi-aquatic areas for highest application cotton uses (ground: 2.4 lbs a.i./A) and in dry and semi-aquatic areas as well as spray drift alone for highest application cotton uses (aerial: 2.4 lbs a.i./A; Table 5-7). Dicots are more sensitive as LOC exceedances are estimated in dry and semi-aquatic areas for both application types on cotton (ground and aerial spray at 2.4 lbs a.i./A; Table 5-8). Therefore, based on LOC exceedances observed for both monocots and dicots, prometryn is likely to indirectly affect the CRLF via reduction in terrestrial plants.

Based on the available toxicity data for terrestrial plants, it appears that emerged monocot seedlings are more sensitive to prometryn in the seedling emergence test than monocot plants in the vegetative vigor test. This is demonstrated by the difference in monocot response in the two guideline studies. For example, the oat (a monocot) EC_{25} values for the seedling emergence and vegetative vigor tests are 0.049 lbs a.i./A and 1.4 lbs a.i./A, respectively, representing almost a 26-fold difference in sensitivity (Table 4-5). Dicots show reverse levels of sensitivity in the seedling emergence versus the vegetative vigor toxicity tests. For example, the lettuce (a dicot) EC_{25} values for seedling emergence and vegetative vigor tests are 0.025 lbs a.i./A and 0.01 lbs a.i./A, respectively, representing over a 2-fold difference in sensitivity (Table 4-5). In addition, it is likely the case that the cucumber is a more sensitive dicot tested in the vegetative vigor study. However, the endpoint values obtained for this species are considered invalid and were not used in the assessment. Nevertheless, LOC exceedances were observed at the highest concentration (use on cotton at 2.4 lbs a.i./A) with the next most sensitive dicot species (lettuce) based on the results of the vegetative vigor study.

Riparian vegetation typically consists of three tiers of vegetation, which include a groundcover of grasses and forbs, an understory of shrubs and young trees, and a canopy of mature trees. Frogs spend a considerable amount of time resting and feeding in riparian vegetation; the moisture and cover of the riparian plant community provides good foraging habitat, and may facilitate dispersal in addition to providing pools and backwater aquatic areas for breeding (USFWS, 2002). According to Hayes and Jennings (1988), the CRLF tends to occupy waterbodies with dense riparian vegetation including willows (*Salix* sp.). Upland habitat includes grassland and woodlands, as well as scrub/shrub habitat. While no guideline data are available on the toxicity of woody plants, the available toxicity information from the open literature (Ref. # 41006, Kozlowski & Torrie 1965) indicates prometryn has a greater effect on red pine (*Pinus resinosa* Ait.) seedling survival and dry-weight production than it does on seedling germination. The effect of prometryn (incorporation into soil) on percent germination did not differ greatly from the control at the given time steps for which germination was observed (*i.e.*, 33, 43, and 73 days). The effect of prometryn (incorporation into soil) on survival and dry-weight production was more severe; prometryn killed all seedlings by 110 days after planting at the highest doses (8 and 16 lbs/A) tested and only 14.1% and 10.9% of seedlings survived in the 2 and 4 lbs/A treatments, respectively. Sublethal effects included slight needle curling, chlorosis, and growth inhibition. Prometryn led to significant reductions in total dry-weight production of seedlings relative to controls. In addition to the negative effect on seedling survival and dry-weight production, the study suggests that, soil incorporated prometryn is more toxic than surface-applied prometryn. Therefore, prometryn may potentially have a negative effect on terrestrial woody plant species.

Six prometryn incidents have been reported for terrestrial plants. An incident on 86 acres of corn lists prometryn as a 'probable' cause of plant damage via carryover, or the presence of residues from an application made in a previous growing season, of a broadcast application. The remaining five incidents are on cotton. Two incidents list prometryn as a 'possible' cause of cotton plant loss on 600 and 1,424 acres via direct treatment. One incident lists prometryn as a 'probable' cause of plant damage on 48 acres of cotton via direct treatment using broadcast application. Two remaining incidents list prometryn as a 'possible' cause of plant damage on 26 and 60 acres of cotton via direct treatment using band application. The latter three cotton incidents utilizing broadcast and band applications were all 'registered uses' of the compound.

In summary, based on all available lines of evidence the Agency concludes a “likely to adversely affect” or “LAA” determination for indirect effects of prometryn to the CRLF via adverse, direct effects to herbaceous terrestrial vegetation, which provides habitat and cover for the CRLF and its prey.

5.2.4 Effects to Designated Critical Habitat

5.2.4.1 Aquatic-Phase PCEs

Three of the four assessment endpoints for the primary constituent elements (PCEs) of designated critical habitat for the aquatic-phase CRLF are related to potential effects to aquatic and/or terrestrial plants:

- Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile and adult CRLFs.
- Alteration in water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs and their food source.
- Reduction and/or modification of aquatic-based food sources for pre-metamorphs (*e.g.*, algae).

Conclusions for potential indirect effects to the CRLF via direct effects to aquatic and terrestrial plants are used to determine whether effects to critical habitat may occur. Based on the results of the effects determinations for aquatic plants (see Sections 5.2.2.1 and 5.2.3.1), critical habitat of the CRLF may be affected via prometryn-related impacts to non-vascular and vascular aquatic plants as food items for tadpoles and habitat for aquatic-phase CRLFs. Prometryn uses that may result in effects to critical habitat via direct effects to non-vascular (Section 5.1.1.2.1) and vascular aquatic plants (Section 5.1.1.3) include applications on celery/fennel (2.0 lbs a.i./A), parsley (2.0 lbs a.i./A), and cotton (2.4 lbs a.i./A). In addition, based on ground spray and aerial applications of prometryn on cotton (Section 5.1.2.3) critical habitat may be affected by a reduction in herbaceous riparian vegetation (Section 5.2.3.2) that provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile and adult aquatic-phase CRLFs. Therefore, there is a potential for habitat effects via impacts to aquatic and terrestrial plants.

The remaining aquatic-phase PCE is “alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.” Other than impacts to algae as food items for tadpoles (discussed above), this PCE is assessed by considering direct and indirect effects to the aquatic-phase CRLF via acute and chronic freshwater fish and invertebrate toxicity endpoints as measures of potential effects. As discussed in Section 5.2.1.1, direct effects to the aquatic-phase CRLF are expected for certain prometryn uses. In addition, prometryn-related effects to freshwater fish as food items are also likely to occur (see Sections 5.2.2.3) even though prometryn-related effects to freshwater invertebrates as food items are not likely to occur (see Section 5.2.2.2). Therefore, prometryn may affect critical habitat by altering chemical characteristics necessary for normal growth and viability of aquatic-phase CRLFs along with potential effects on the availability of some of their non-plant food sources.

5.2.4.2 Terrestrial-Phase PCEs

Two of the four assessment endpoints for the PCEs of designated critical habitat for the terrestrial-phase CRLF are related to potential effects to terrestrial plants:

- Elimination and/or disturbance of upland habitat; ability of habitat to support food source of CRLFs: Upland areas within 200 ft of the edge of the riparian vegetation or drip line surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance.

- Elimination and/or disturbance of dispersal habitat: Upland or riparian dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal.

As discussed above, effects to critical habitat may occur via prometryn-related impacts to sensitive herbaceous terrestrial vegetation (Section 5.2.3.2), which provides habitat, cover, and a means of dispersal for the terrestrial-phase CRLF and its prey, based on ground spray and aerial applications of prometryn at the maximum annual application rate used on cotton.

The third terrestrial-phase PCE is “reduction and/or modification of food sources for terrestrial phase juveniles and adults.” To assess the impact of prometryn on this PCE, acute and chronic toxicity endpoints for terrestrial invertebrates, mammals, and terrestrial-phase frogs are used as measures of effects. Based on the characterization of indirect effects to terrestrial-phase CRLFs via reduction in the prey base (see Section 5.2.2.4 for terrestrial invertebrates, Section 5.2.2.5 for mammals, and 5.2.2.6 for frogs), critical habitat may be affected via a reduction in small insects and mammals as food items.

The fourth terrestrial-phase PCE is based on alteration of chemical characteristics that are necessary for normal growth and viability of juvenile and adult CRLFs and their food source. As discussed in Section 5.2.1.2, direct acute effects to the terrestrial-phase CRLF are possible but not likely and chronic reproductive effects are not expected. As discussed in Sections 5.2.2.4, 5.2.2.5, and 5.2.2.6, indirect effects to the terrestrial-phase CRLF are expected via potential reduction in small insect and mammalian species as food items. Therefore, the ability of prometryn to affect critical habitat by altering chemical characteristics that are necessary for normal growth and viability of terrestrial-phase CRLF directly is not likely. However, prometryn may affect critical habitat by altering chemical characteristics that are necessary for normal growth and viability of small insect and mammalian food sources.

6 Uncertainties

6.1 Exposure Assessment Uncertainties

6.1.1 Maximum Use Scenario

The screening-level risk assessment focuses on characterizing potential ecological risks resulting from a maximum use scenario, which is determined from labeled statements of maximum application rate and number of applications with the shortest time interval between applications. The frequency at which actual uses approach this maximum use scenario may be dependant on pest resistance, timing of applications, cultural practices, and market forces.

6.1.2 Aquatic Exposure Modeling of Prometryn

The standard ecological water body scenario (EXAMS pond) used to calculate potential aquatic exposure to pesticides is intended to represent conservative estimates, and to avoid underestimations of the actual exposure. The standard scenario consists of application to a 10-hectare field bordering a 1-hectare, 2-meter deep (20,000 m^3) pond with no outlet. Exposure estimates generated using the EXAMS pond are intended to represent a wide variety of vulnerable water bodies that occur at the top of watersheds including prairie pot holes, playa lakes, wetlands, vernal pools, man-made and natural ponds, and intermittent and lower order streams. As a group, there are factors that make these water bodies more or less vulnerable than the EXAMS pond. Static water bodies that have larger ratios of pesticide-treated drainage area to water body volume would be expected to have higher peak EECs than the EXAMS pond. These water bodies will be either smaller in size or have larger drainage areas. Smaller water bodies have limited storage capacity and thus may overflow and carry pesticide in the discharge, whereas the EXAMS pond has no discharge. As watershed size increases beyond 10-hectares, it becomes increasingly unlikely that the entire watershed is planted with a single crop that is all treated simultaneously with the pesticide. Headwater streams can also have peak concentrations higher than the EXAMS pond, but they likely persist for only short periods of time and are then carried and dissipated downstream.

The Agency acknowledges that there are some unique aquatic habitats that are not accurately captured by this modeling scenario and modeling results may, therefore, under- or over-estimate exposure, depending on a number of variables. For example, aquatic-phase CRLFs may inhabit water bodies of different size and depth and/or are located adjacent to larger or smaller drainage areas than the EXAMS pond. The Agency does not currently have sufficient information regarding the hydrology of these aquatic habitats to develop a specific alternate scenario for the CRLF. CRLFs prefer habitat with perennial (present year-round) or near-perennial water and do not frequently inhabit vernal (temporary) pools because conditions in these habitats are generally not suitable (Hayes and Jennings 1988). Therefore, the EXAMS pond is assumed to be representative of exposure to aquatic-phase CRLFs. In addition, the Services agree that the existing EXAMS pond represents the best currently available approach for estimating aquatic exposure to pesticides (U.S. FWS/NMFS 2004).

In general, the linked PRZM/EXAMS model produces estimated aquatic concentrations that are expected to be exceeded once within a ten-year period. The Pesticide Root Zone Model is a process or “simulation” model that calculates what happens to a pesticide in an agricultural field on a day-to-day basis. It considers factors such as rainfall and plant transpiration of water, as well as how and when the pesticide is applied. It has two major components: hydrology and chemical transport. Water movement is simulated by the use of generalized soil parameters, including field capacity, wilting point, and saturation water content. The chemical transport component can simulate pesticide application on the soil or on the plant foliage. Dissolved, adsorbed, and vapor-phase concentrations in the soil are estimated by simultaneously considering the processes of pesticide uptake by plants, surface runoff, erosion, decay, volatilization, foliar wash-off, advection, dispersion, and retardation.

Uncertainties associated with each of these individual components add to the overall uncertainty of the modeled concentrations. Additionally, model inputs from the environmental fate degradation studies are chosen to represent the upper confidence bound on the mean values that are not expected to be exceeded in the environment approximately 90 percent of the time. Mobility input values are chosen to be representative of conditions in the environment. The natural variation in soils adds to the uncertainty of modeled values. Factors such as application date, crop emergence date, and canopy cover can also affect estimated concentrations, adding to the uncertainty of modeled values. Factors within the ambient environment such as soil temperatures, sunlight intensity, antecedent soil moisture, and surface water temperatures can cause actual aquatic concentrations to differ for the modeled values.

Unlike spray drift, tools are currently not available to evaluate the effectiveness of a vegetative setback on runoff and loadings. The effectiveness of vegetative setbacks is highly dependent on the condition of the vegetative strip. For example, a well-established, healthy vegetative setback can be a very effective means of reducing runoff and erosion from agricultural fields. Alternatively, a setback of poor vegetative quality or a setback that is channelized can be ineffective at reducing loadings. Until such time as a quantitative method to estimate the effect of vegetative setbacks on various conditions on pesticide loadings becomes available, the aquatic exposure predictions are likely to overestimate exposure where healthy vegetative setbacks exist and underestimate exposure where poorly developed, channelized, or bare setbacks exist.

In order to account for uncertainties associated with modeling, available monitoring data were compared to PRZM/EXAMS estimates of peak EECs for the different uses. As discussed above, several data values were available from NAWQA for prometryn concentrations measured in surface waters receiving runoff from agricultural areas. The specific use patterns (*e.g.* application rates and timing, crops) associated with the agricultural areas are unknown, however, they are assumed to be representative of potential prometryn use areas. A comparison of NAWQA monitoring data and PRZM/EXAMS modeling indicate the modeling estimates are approximately 600 X greater than the available monitoring data.

PRZM/EXAMS modeling indicates accumulation of prometyrn in the pond due to the high persistence of prometyrn and the static hydrology in the standard pond. This accumulation limits the ability to define reliable 1 in 10 year EECs because of temporal autocorrelation. However, the use of 1-in-10-year EEC provides a conservative estimate of exposure because it represents ~27 years of accumulation.

6.1.3 Potential Groundwater Contributions to Surface Water Chemical Concentrations

Although the potential impact of discharging groundwater on CRLF populations is not explicitly delineated, it should be noted that groundwater could provide a source of pesticide to surface water bodies – especially low-order streams, headwaters, and groundwater-fed pools. This is particularly likely if the chemical is persistent and mobile. Soluble chemicals that are primarily subject to photolytic degradation will be very likely to persist in groundwater, and can be transportable over long distances. Similarly, many chemicals degrade slowly under anaerobic conditions (common in aquifers) and are thus more persistent in groundwater. Much of this groundwater will eventually be discharged to the surface – often supporting stream flow in the absence of rainfall. Continuously flowing low-order streams in particular are sustained by groundwater discharge, which can constitute 100% of stream flow during baseflow (no runoff) conditions. Thus, it is important to keep in mind that pesticides in groundwater may have a major (detrimental) impact on surface water quality, and on CRLF habitats.

SciGrow may be used to determine likely ‘high-end’ groundwater vulnerability, with the assumption (based upon persistence in sub- and anoxic conditions, and mobility) that much of the compound entering the groundwater will be transported some distance and eventually discharged into surface water. Although concentrations in a receiving water body resulting from groundwater discharge cannot be explicitly quantified, it should be assumed that significant attenuation and retardation of the chemical will have occurred prior to discharge. Nevertheless, groundwater could still be a significant consistent source of chronic background concentrations in surface water, and may also add to surface runoff during storm events (as a result of enhanced groundwater discharge typically characterized by the ‘tailing limb’ of a storm hydrograph).

6.1.4 Action Area Uncertainties

An example of an important simplifying assumption that may require future refinement is the assumption of uniform runoff characteristics throughout a landscape. It is well documented that runoff characteristics are highly non-uniform and anisotropic, and become increasingly so as the area under consideration becomes larger. The assumption made for estimating the aquatic action area (based on predicted in-stream dilution) was that the entire landscape exhibited runoff properties identical to those commonly found in agricultural lands in this region. However, considering the vastly different runoff characteristics of: a) undeveloped (especially forested) areas, which exhibit the least amount of surface runoff but the greatest amount of groundwater recharge; b) suburban/residential areas, which are dominated by the relationship between impermeable surfaces (roads, lots) and grassed/other areas (lawns) plus local drainage management; c) urban areas, that are dominated by managed storm drainage and impermeable surfaces; and d) agricultural areas dominated by Hortonian and focused runoff (especially with row crops), a refined assessment should incorporate these differences for modeled stream flow generation. As the zone around the immediate (application) target area expands, there will be greater variability in the landscape; in the context of a risk assessment, the runoff potential that is assumed for the expanding area will be a crucial variable (since dilution at the outflow point is determined by the size of the expanding area). Thus, it important to know at least some approximate estimate of types of land use within that region. Runoff from forested areas ranges from 45 – 2,700% less than from agricultural areas; in most studies, runoff was 2.5 to 7 times higher in agricultural areas (Okisaka *et al.* 1997; Karvonen *et al.* 1999; McDonald *et al.* 2002; Phuong and van Dam 2002). Differences in runoff potential between urban/suburban areas and agricultural areas are generally less than between agricultural and forested areas. In terms of likely runoff potential (other variables – such as topography and rainfall – being equal), the relationship is generally as follows (going from lowest to highest runoff potential):

Three-tiered forest < agroforestry < suburban < row-crop agriculture < urban.

There are, however, other uncertainties that should serve to counteract the effects of the aforementioned issue. For example, the dilution model considers that 100% of the agricultural area has the chemical applied, which is almost certainly a gross over-estimation. Thus, there will be assumed chemical contributions from agricultural areas that will actually be contributing only runoff water (dilutant); so some contributions to total contaminant load will really serve to lessen rather than increase aquatic concentrations. In light of these (and other) confounding factors, Agency believes that this model gives us the best available estimates under current circumstances.

6.1.5 Usage Uncertainties

County-level usage data were obtained from California’s Department of Pesticide Regulation Pesticide Use Reporting (CDPR PUR) database. Eight years of data (1999 – 2006) were included in this analysis because statistical methodology for identifying outliers, in terms of area treated and pounds applied, was provided by CDPR for these years only. No methodology for removing outliers was provided by CDPR for 2001 and earlier pesticide data; therefore, this information was not included in the analysis because it may misrepresent actual usage patterns. CDPR PUR documentation indicates that errors in the data may include the following: a misplaced decimal; incorrect measures, area treated, or units; and reports of diluted pesticide concentrations. In addition, it is possible that the data may contain reports for pesticide uses that have been cancelled.

The CPDR PUR data does not include home owner applied pesticides; therefore, residential uses are not likely to be reported. As with all pesticide usage data, there may be instances of misuse and misreporting. The Agency made use of the most current, verifiable information; in cases where there were discrepancies, the most conservative information was used.

6.1.6 Terrestrial Exposure Modeling of Prometryn

The Agency relies on the work of Fletcher *et al.* (1994) for setting the assumed pesticide residues in wildlife dietary items. These residue assumptions are believed to reflect a realistic upper-bound residue estimate, although the degree to which this assumption reflects a specific percentile estimate is difficult to quantify. It is important to note that the field measurement efforts used to develop the Fletcher estimates of exposure involve highly varied sampling techniques. It is entirely possible that much of these data reflect residues averaged over entire above ground plants in the case of grass and forage sampling.

It was assumed that ingestion of food items in the field occurs at rates commensurate with those in the laboratory. Although the screening assessment process adjusts dry-weight estimates of food intake to reflect the increased mass in fresh-weight wildlife food intake estimates, it does not allow for gross energy differences. Direct comparison of a laboratory dietary concentration- based effects threshold to a fresh-weight pesticide residue estimate would result in an underestimation of field exposure by food consumption by a factor of 1.25 – 2.5 for most food items.

Differences in assimilative efficiency between laboratory and wild diets suggest that current screening assessment methods do not account for a potentially important aspect of food requirements. Depending upon species and dietary matrix, bird assimilation of wild diet energy ranges from 23 – 80%, and mammal's assimilation ranges from 41 – 85% (U.S. EPA 1993). If it is assumed that laboratory chow is formulated to maximize assimilative efficiency (*e.g.*, a value of 85%), a potential for underestimation of exposure may exist by assuming that consumption of food in the wild is comparable with consumption during laboratory testing. In the screening process, exposure may be underestimated because metabolic rates are not related to food consumption.

For the terrestrial exposure analysis of this risk assessment, a generic bird or mammal was assumed to occupy either the treated field or adjacent areas receiving a treatment rate on the field. Actual habitat requirements of any particular terrestrial species were not considered, and it was assumed that species occupy, exclusively and permanently, the modeled treatment area. Spray drift model predictions suggest that this assumption leads to an overestimation of exposure to species that do not occupy the treated field exclusively and permanently.

Given that no data on interception and subsequent dissipation from foliar surfaces is available for prometryn, a default foliar dissipation half-life of 35 days was used based on the work of Willis and McDowell (1987). In addition, the highest application rate (2.4

lbs a.i./A) on cotton at pre-plant incorporation (Reg No. 100-620, 9779-297, 10163-94, 34704-692, 66222-15) was used in T-REX to calculate the most conservative estimates of the risk quotients. Dill, parsley, celery/fennel, and even other cotton uses all have application rates below this value. Therefore, the risk quotient calculations are representative of the upper bound estimates of risk to the CRLF. It should be noted, however, that the CRLF may potentially be exposed to prometryn via other routes such as through the skin or via ingestion of drinking water contaminated with prometryn. However, there are no approved methods or models available to the Agency for characterizing these routes of exposure. The T-REX model is assumed to provide a reasonable representation of exposure and risk, given the best available information and associated uncertainties that may lead to an overestimation and an underestimation of risk.

Guideline studies generally evaluate toxicity to ten agricultural crop species. A drawback to these tests is that they are conducted on herbaceous crop species only, and extrapolation of effects to other species, such as the woody shrubs and trees and wild herbaceous species, contributes uncertainty to risk conclusions.

Commercial crop species have been selectively bred, and may be more or less resistant to particular stressors than wild herbs and forbs. The direction of this uncertainty for specific plants and stressors, including prometryn, is largely unknown. Homogenous test plant seed lots also lack the genetic variation that occurs in natural populations, so the range of effects seen from tests is likely to be smaller than would be expected from wild populations.

6.1.7 Spray Drift Modeling

Although there may be multiple prometryn applications at a single site, it is unlikely that the same organism would be exposed to the maximum amount of spray drift from every application made. In order for an organism to receive the maximum concentration of prometryn from multiple applications, each application of prometryn would have to occur under identical atmospheric conditions (*e.g.*, same wind speed and – for plants – same wind direction) and (if it is an animal) the animal being exposed would have to be present directly downwind at the same distance after each application. Although there may be sites where the dominant wind direction is fairly consistent (at least during the relatively quiescent conditions that are most favorable for aerial spray applications), it is nevertheless highly unlikely that plants in any specific area would receive the maximum amount of spray drift repeatedly. It appears that in most areas (based upon available meteorological data) wind direction is temporally very changeable, even within the same day. Additionally, other factors, including variations in topography, cover, and meteorological conditions over the transport distance are not accounted for by the AgDRIFT/AGDISP model (*i.e.*, it models spray drift from aerial and ground applications in a flat area with little to no ground cover and a steady, constant wind speed and direction). Therefore, in most cases, the drift estimates from AgDRIFT/AGDISP may overestimate exposure even from single applications, especially as the distance increases from the site of application, since the model does not account for potential obstructions (*e.g.*, large hills, berms, buildings, trees, *etc*.). Furthermore, conservative assumptions are often made regarding the droplet size distributions being modeled (‘ASAE Very Fine to Fine’ for orchard uses and ‘ASAE Very Fine’ for agricultural uses), the application method (*e.g.*, aerial), release heights and wind speeds. Alterations in any of these inputs would change the area of potential effect.

6.2 Effects Assessment Uncertainties

6.2.1 Age Class and Sensitivity of Effects Thresholds

It is generally recognized that test organism age may have a significant impact on the observed sensitivity to a toxicant. The acute toxicity data for fish are collected on juvenile fish between 0.1 and 5 grams. Aquatic invertebrate acute testing is performed on recommended immature age classes (*e.g.*, first instar for daphnids, second instar for amphipods, stoneflies, mayflies, and third instar for midges).

Testing of juveniles may overestimate toxicity at older age classes for pesticide active ingredients that act directly without metabolic transformation because younger age classes may not have the enzymatic systems associated with detoxifying xenobiotics. In so far as the available toxicity data may provide ranges of sensitivity information with respect to age class, this assessment uses the most sensitive life-stage information as measures of effect for surrogate aquatic animals, and is therefore, considered as protective of the CRLF.

6.2.2 Use of Surrogate Species Effects Data

Guideline toxicity tests or open literature studies on prometryn are not available for frogs or any other aquatic-phase amphibian; therefore, freshwater fish are used as surrogate species for aquatic-phase amphibians. Therefore, endpoints based on freshwater fish ecotoxicity data are assumed to be protective of potential direct effects to aquatic-phase amphibians including the CRLF, and extrapolation of the risk conclusions from the most sensitive tested species to the aquatic-phase CRLF is likely to overestimate the potential risks to those species. Efforts are made to select the organisms most likely to be affected by the type of compound and usage pattern; however, there is an inherent uncertainty in extrapolating across phyla. In addition, the Agency’s LOCs are intentionally set very low, and conservative estimates are made in the screening level risk assessment to account for these uncertainties.

6.2.3 Sublethal Effects

When assessing acute risk, the screening risk assessment relies on the acute mortality endpoint as well as a suite of sublethal responses to the pesticide, as determined by the testing of species response to chronic exposure conditions and subsequent chronic risk assessment. Consideration of additional sublethal data in the effects determination is exercised on a case-by-case basis and only after careful consideration of the nature of the sublethal effect measured and the extent and quality of available data to support establishing a plausible relationship between the measure of effect (sublethal endpoint) and the assessment endpoints. To the extent to which sublethal effects are not considered in this assessment, the potential direct and indirect effects of prometryn on CRLF may be underestimated.

6.2.4 Location of Wildlife Species

For the terrestrial exposure analysis of this risk assessment, a generic bird or mammal was assumed to occupy either the treated field or adjacent areas receiving a treatment rate on the field. Actual habitat requirements of any particular terrestrial species were not considered, and it was assumed that species occupy, exclusively and permanently, the modeled treatment area. Spray drift model predictions suggest that this assumption leads to an overestimation of exposure to species that do not occupy the treated field exclusively and permanently.

7 Risk Conclusions

In fulfilling its obligations under Section 7(a)(2) of the Endangered Species Act, the information presented in this endangered species risk assessment represents the best data currently available to assess the potential risks of prometryn to the CRLF and its designated critical habitat.

Based on the best available information, the Agency makes a "Likely to Adversely Affect" determination for the CRLF from the use of prometryn. The Agency has determined that there is a potential for effects to CRLF designated critical habitat from the use of the chemical. A description of the baseline status and cumulative effects for the CRLF is provided in **Attachment 2**.

The LAA effects determination applies to those areas where it is expected that the pesticide's use will directly or indirectly affect the CRLF or its designated critical habitat. To determine this area, the footprint of prometryn's use pattern is identified, using land cover data that correspond to prometryn's use pattern. The spatial extent of the LAA effects determination also includes areas beyond the initial area of concern that may be impacted by runoff and/or spray drift. The identified direct and indirect effects and modification to critical habitat are anticipated to occur only for those currently occupied core habitat areas, CNDDB occurrence sections, and designated critical habitat for the CRLF that overlap with the initial area of concern plus 997 ft for aerial spray applications (and 190 ft for ground spray applications) from its boundary (refer to analysis in Section 5.1.4). It is assumed that non-flowing waterbodies (or potential CRLF habitat) are included within this area.

In addition to the spray drift buffer, the results of the downstream dilution extent analysis result in a distance of 285 km which represents the maximum continuous distance of downstream dilution from the edge of the initial area of concern (refer to analysis in Section 5.1.4). If any of these streams reaches flow into CRLF habitat, there is potential to affect either the CRLF or modify its habitat. These lotic aquatic habitats within the CRLF core areas and critical habitats potentially contain concentrations of prometryn sufficient to result in LAA determination or effects to critical habitat.

Appendix D provides maps of the initial area of concern, along with CRLF habitat areas, including currently occupied core areas, CNDDB occurrence sections, and designated critical habitat. It is expected that any additional areas of CRLF habitat that are located 997 ft for aerial spray applications (and 190 ft for ground spray applications) (to account for offsite migration via spray drift) and 285 km of stream distance (to account for downstream dilution) outside the initial area of concern may also be impacted and are part of the full spatial extent of the LAA/effects to critical habitat determinations.

A summary of the risk conclusions and effects determinations for the CRLF and its critical habitat, given the uncertainties discussed in Section 6, is presented in **Error! Reference source not found.** and **Error! Reference source not found.**.

Table 7-1. Effects Determination Summary for Prometryn Use and the CRLF

Assessment Endpoint	Effects Determination [1]	Basis for Determination
Survival, growth, and/or reproduction of CRLF individuals	LAA[1]	**Potential for Direct Effects** ***Aquatic-phase (Eggs, Larvae, and Adults)***: Acute risk to freshwater fish LOCs are exceeded for all parsley uses (Spring, Fall: 2 lbs a.i./A) and most cotton uses (Fall, pre-emergence ground spray and pre-emergence aerial spray: 2.4 lbs a.i./A). No chronic risk LOCs are exceeded based on the most sensitive toxicity data for freshwater fish across any of the evaluated uses (Table 5-1). ***Terrestrial-phase (Juveniles and Adults)***: Prometryn is practically non-toxic to birds on an acute oral and sub-acute dietary exposure basis and as such, the studies did not provide definitive toxicity endpoints. No mortality was observed in the acute oral study and only a single mortality was observed in the sub-acute dietary toxicity study and the mortality was not considered treatment-related. There are no chronic risk LOC exceedances for birds consuming small insects at the maximum annual application rate for prometryn (2.4 lbs a.i./A to cotton) in T-REX using dietary-based RQs. **Potential for Indirect Effects** ***Aquatic prey items, aquatic habitat, cover and/or primary productivity*** Risk to aquatic plant LOC exceedances are observed for non-vascular and vascular aquatic plants for all uses. For a complete list see Table 5-2, 5-4. Acute risk LOC exceedances are not observed for freshwater invertebrates. The same finding applies here for fish and frog prey items as in the direct effects (aquatic-phase CRLF) component above. ***Terrestrial prey items, riparian habitat*** Risk to terrestrial invertebrate LOC exceedances are observed for small insects at the maximum annual application rate for cotton (2.4 lbs a.i./A). Small terrestrial mammals are also potentially affected by prometryn as acute and chronic risk LOC exceedances are observed for small mammals. The same description applies here for frog prey items as in the direct effects (terrestrial-phase CRLF) component above. Risk to terrestrial plant LOCs are exceeded for monocots in dry and semi-aquatic areas for cotton uses (ground: 2.4 lbs a.i./A) and in dry and semi-aquatic areas as well as spray drift alone for cotton uses (aerial: 2.4 lbs a.i./A). Dicots are more sensitive as LOC exceedances are estimated in dry and semi-aquatic areas for both application types on cotton (ground and aerial spray at 2.4 lbs a.i./A).
[1] No effect (NE); May affect, but not likely to adversely affect (NLAA); May affect, likely to adversely affect (LAA)		

Table 7-2. Effects Determination Summary for Prometryn Use and CRLF Critical Habitat Impact Analysis

Assessment Endpoint	Effects Determination	Basis for Determination
Modification of aquatic-phase PCE	Habitat Effects	Risk to terrestrial plant LOCs are exceeded for monocots in dry and semi-aquatic areas for cotton uses (ground: 2.4 lbs a.i./A) and in dry and semi-aquatic areas as well as spray drift alone for cotton uses (aerial: 2.4 lbs a.i./A). Dicots are more sensitive as LOC exceedances are estimated in dry and semi-aquatic areas for both application types on cotton (ground and aerial spray at 2.4 lbs a.i./A). Risk to aquatic plant LOC exceedances are observed for non-vascular and vascular plants for all uses. Acute risk to freshwater fish LOCs are exceeded for all parsley uses (Spring, Fall: 2 lbs a.i./A) and most cotton uses (Fall, pre-emergence ground spray and pre-emergence aerial spray: 2.4 lbs a.i./A). No chronic risk LOCs are exceeded based on the most sensitive toxicity data for freshwater fish and for any evaluated use. Acute and chronic risk LOC exceedances are not observed for freshwater invertebrates.
Modification of terrestrial-phase PCE		Risk to terrestrial plant LOCs are exceeded for monocots in dry and semi-aquatic areas for cotton uses (ground: 2.4 lbs a.i./A) and in dry and semi-aquatic areas as well as spray drift alone for cotton uses (aerial: 2.4 lbs a.i./A). Dicots are more sensitive as LOC exceedances are estimated in dry and semi-aquatic areas for both application types on cotton (ground and aerial spray at 2.4 lbs a.i./A). No definitive acute RQs could be derived because the acute avian effects data shows no mortality at the highest test concentrations. Chronic risk LOC exceedances are not observed for birds consuming small insects when considering a cotton application (2.4 lbs a.i./A) in T-REX using dietary based RQs. This direct effects description applies for frog prey items as an indirect effect on the CRLF. Risk to terrestrial invertebrate LOC exceedances are observed for small insects at the maximum annual application rate for cotton (2.4 lbs a.i./A). Small terrestrial mammals are also affected by prometryn as acute and chronic risk LOC exceedances are observed for small mammals.

Based on the conclusions of this assessment, a formal consultation with the U. S. Fish and Wildlife Service under Section 7 of the Endangered Species Act should be initiated. When evaluating the significance of this risk assessment's direct/indirect and adverse habitat modification effects determinations, it is important to note that pesticide exposures and predicted risks to the species and its resources (i.e., food and habitat) are not expected to be uniform across the action area. In fact, given the assumptions of drift and downstream transport (i.e., attenuation with distance), pesticide exposure and associated risks to the species and its resources are expected to decrease with increasing distance away from the treated field or site of application. Evaluation of the implication of this non-uniform distribution of risk to the species would require information and assessment techniques that are not currently available. Examples of such information and methodology required for this type of analysis would include the following:

- Enhanced information on the density and distribution of CRLF life stages within specific recovery units and/or designated critical habitat within the action area. This information would allow for quantitative extrapolation of the present risk assessment's predictions of individual effects to the proportion of the population extant within geographical areas where those effects are predicted. Furthermore, such population information would allow for a more comprehensive evaluation of the significance of potential resource impairment to individuals of the species.
- Quantitative information on prey base requirements for individual aquatic- and terrestrial-phase frogs. While existing information provides a preliminary picture of the types of food sources utilized by the frog, it does not establish minimal requirements to sustain healthy individuals at varying life stages. Such information could be used to establish biologically relevant thresholds of effects on the prey base, and ultimately establish geographical limits to those effects. This information could be used together with the density data discussed above to characterize the likelihood of adverse effects to individuals.
- Information on population responses of prey base organisms to the pesticide. Currently, methodologies are limited to predicting exposures and likely levels of direct mortality, growth or reproductive impairment immediately following exposure to the pesticide. The degree to which repeated exposure events and the inherent demographic characteristics of the prey population play into the extent to which prey resources may recover is not predictable. An enhanced understanding of long-term prey responses to pesticide exposure would allow for a more refined determination of the magnitude and duration of resource impairment, and together with the information described above, a more complete prediction of effects to individual frogs and potential modification to critical habitat.

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