# Risks of Rotenone 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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# 1. 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 FIFRA regulatory actions regarding direct application of rotenone to water in order to kill fish (*i.e*. as a piscicide) for fishery management purposes. 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 (USFWS) and National Marine Fisheries Service (NMFS) *Endangered Species Consultation Handbook* (USFWS/NMFS, 1998 and procedures outlined in the Agency's Overview Document (U.S. EPA, 2004).

The CRLF was listed as a threatened species by USFWS 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 (USFWS, 1996) in California.

Rotenone (CAS 83-79-4) is a selective, non-specific botanical pesticide obtained from extracts of roots, seeds, and leaves of tropical and sub-tropical plants in the genera *Lonchocarpus* and *Derris*. The actual plants from which rotenone is typically derived are not native to California. Rotenone acts as a piscicide by blocking electron transport in cell mitochondria. Rotenone was labeled as a general use insecticide/acaricide in a variety of agricultural and residential settings; however, the technical registrants agreed to cancel all agricultural and residential uses. Therefore, the only remaining use is as a piscicide in fisheries management. Rotenone can be applied to both lentic (ponds, lakes, and reservoirs) and lotic waters (streams and rivers).

Rotenone appears to degrade rapidly in the environment with half-lives on the order of several hours to a several weeks. Abiotic mechanisms, mainly hydrolysis, appear to be the primary routes of dissipation, but biodegradation may contribute to limiting the persistence of rotenone in the environment. The primary degradation product is rotenolone; however, degradation product data under environmental conditions are sparse.

Rotenone's low vapor pressure and Henry's Law constant suggest that volatilization from water surfaces will not be an important environmental fate process, and the potential for inhalation exposure to terrestrial species is considered low. No valid information on soil-water partitioning is available.

Since CRLFs exist within aquatic and terrestrial habitats, exposure of the CRLF, its prey and its habitats to rotenone are generally assessed separately for the two habitats. However, rotenone is not applied to terrestrial habitats and therefore direct effects to terrestrial-phase CRLF is considered low. An assessment of potential risk due to the consumption of contaminated fish and mammalian food items confirm that the risk to the CLRF in terrestrial environments is indeed low. Where streams, rivers and lakes are directly treated with rotenone, aquatic exposure concentrations are assumed to be equal to the specific treatment concentration of rotenone. In this assessment, actual treatment concentrations are used as high-end exposure estimates of rotenone in aquatic habitats resulting from the use of the compound as a piscicide. Therefore, model-generated estimated environmental concentrations are not used in this assessment. Peak environmental concentrations resulting from the piscicidal use represented by the water solubility limit of rotenone, *i.e.*, 200 µg/L were used for acute aquatic assessments. Mean concentrations calculated using zero and first order kinetics were used for chronic estimates. Time periods over risk thresholds were also calculated for various temperature regimes. Since rotenone is typically detoxified with potassium permanganate in flowing water (stream and rivers) and/or is displaced by untreated water, the likelihood of chronic exposure is considered low. Neither rotenone nor its rotenolone degradate is included as an analyte in U. S. Geological Survey's National Water Quality Assessment (NAWQA) program; therefore, monitoring data are not available for historic agricultural or present day uses of the compound.

Since rotenone is applied exclusively to lentic water via boat bailing, perforated hoses, or a Venturi pump attached to the boat outboard motor and is applied to lotic water via drip stations and/or backpack sprayers, the likelihood of exposure to terrestrial animals and plants is considered low. Therefore, modeling of terrestrial exposure using T-REX, T-HERPS and TerrPlant was not conducted in this assessment. While it is possible that terrestrial-phase CRLF may utilize aquatic animals (fish and invertebrates) in their diet, again, the percentage that these prey items make up the diet of terrestrial-phase CRLF is considered low. However, exposure to rotenone residues for piscivorous animals was estimated using allometric equations to explore the potential exposure to rotenone from ingestion of prey containing rotenone residues.

The 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. 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. Because rotenone is applied directly to water, terrestrial habitats are not considered likely to be exposed and, as such, are not likely at risk from the use of rotenone as a piscicide.

Rotenone is classified as highly toxic to fish, aquatic-phase amphibians, and aquatic invertebrates on an acute exposure basis. Chronic exposure to rotenone has been demonstrated to cause decreases in growth and reproduction in freshwater fish and invertebrates, respectively. No data are available to evaluate the toxicity of rotenone to aquatic plants.

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 rotenone use within the action area has the potential to adversely affect the CRLF and its designated critical habitat via direct toxicity or indirectly based on direct effects to its food supply (*i.e*., freshwater invertebrates, algae, fish, and frogs) or habitat (*i.e*., aquatic and semi-aquatic plants). When RQs for a particular type of effect are below LOCs, the pesticide is determined to have "no effect" on the subject species. 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 rotenone use within the action area "may affect" the CRLF and 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 and its critical habitat.

Based on the best available information, the Agency makes a May Affect and Likely to Adversely Affect (LAA) determination for the CRLF from the use of rotenone as a piscicide. Additionally, the Agency has determined that there is the potential for modification of CRLF designated critical habitat from the use of rotenone as a piscicide. RQs for direct effects of rotenone to aquatic-phase CRLFs exceed acute and chronic risk LOCs by factors of 206X and 105X, respectively, and the likelihood of individual mortality of aquatic-phase CRLF is 100% Indirect effects to the CRLF may also occur through the loss of both vertebrate and invertebrate aquatic forage items. Although there are no guideline toxicity study data with which to assess the effects of rotenone to aquatic and/or semi-aquatic plants, the potential for rotenone to cause phytotoxicity is considered low since the chemical is a naturally occurring plant flavinoid, historical use of rotenone on agricultural crops has not resulted in any reported phytotoxicity, and there are no reported ecological incidents involving plants. A summary of the risk conclusions and effects determinations for the CRLF and its critical habitat is presented in **Tables 1** and **2**. Further information on the results of the effects determination is included as part of the Risk Description in Section 5.2.

**Table 1. Effects Determination Summary for Rotenone 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)***:<br><br>Rotenone is intended to kill fish. Since fish represent surrogates for the aquatic-phase CRLF, it is presumed that rotenone will kill aquatic-phase CRLFs. RQ values exceed the acute risk LOC by a factor of 2060X. The likelihood of individual acute mortality is 100%. RQ values exceed the chronic risk LOC by a factor of 83X. |
| | | ***Terrestrial-phase (Juveniles and Adults)***:<br><br>Rotenone is applied directly to water and the likelihood of exposure to terrestrial animals is considered low. |
| | | **Potential for Indirect Effects** |
| | | RQ values exceed the acute risk LOC by a factor of 108X. The likelihood of |

| | | |
|---|---|---|
| | | individual acute mortality is 100%. RQ values for aquatic invertebrates exceed the chronic risk LOC by a factor of 21X<br>Although there are no guideline toxicity study data on the effects of rotenone on plants, rotenone is derived from plants and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants; therefore, the potential risk to plants is considered low. |
| | | ***Terrestrial prey items, riparian habitat***<br>Rotenone is applied directly to water and the likelihood of exposure to terrestrial animals/plants is considered low. Alternative prey items are likely available for terrestrial-phase amphibians even if aquatic vertebrate/invertebrate prey are reduced. |

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

**Table 2. Effects Determination Summary for Rotenone Use and CRLF Critical Habitat Impact Analysis.**

| Assessment Endpoint | Effects Determination [1] | Basis for Determination |
|---|---|---|
| Modification of aquatic-phase PCE | HM[1] | Rotenone is intended to kill fish and RQ values exceed the acute and chronic risk LOCs by factors of 206X and 83X, respectively. The likelihood of acute mortality of both fish and invertebrates in the action area is 100%. As such, there is a high likelihood that the forage base of the aquatic-phase CRLF habitat will be modified. |
| Modification of terrestrial-phase PCE | | Rotenone is applied directly to water and the likelihood of exposure to terrestrial animals/plants is considered low. Therefore, modification of terrestrial-phase habitat is considered low. |

[1] Habitat Modification or No effect (NE)

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 to determine whether there are reasonable and prudent alternatives and/or measures to reduce and/or eliminate potential incidental take.

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. 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. EPA's *Guidance for Ecological Risk Assessment* (U.S. EPA 1998), the Services' *Endangered Species Consultation Handbook* (USFWS/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 (USFWS/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 piscicidal uses of rotenone in fishery management. In addition, this assessment evaluates whether this use pattern is expected to result in modification of 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)) settlement 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 modification 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 typically include use of standard models such as PRZM/EXAMS, T-REX, TerrPlant, AgDRIFT, and AGDISP, all of which are described at length in the Overview Document. Since rotenone use as a piscicide involves direct application of the compound to water and the compound is maintained at a specific concentration and duration of exposure, exposure modeling is not conducted in this evaluation. Rather, maximum treatment concentrations allowed by the label are used to characterize aquatic exposure. Since rotenone is no longer used in terrestrial environments and is now only used as a direct application to water to kill fish, no terrestrial exposure assessment is conducted as exposure is not considered likely. 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 ESA, and the Services' *Endangered Species Consultation Handbook*, the assessment of effects associated with registrations of rotenone is based on an action area. The action area is the area directly or indirectly affected by the federal action, as indicated by the exceedance of the Agency's

Levels of Concern (LOCs). It is acknowledged that the action area for a national-level FIFRA regulatory decision associated with a use of rotenone 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 rotenone 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 rotenone 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 rotenone.

If a determination is made that use of rotenone 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 vertebrates and invertebrates, aquatic plants, riparian semi-aquatic vegetation, *etc*.). Additional information, including spatial analysis (to determine the geographical proximity of CRLF habitat and rotenone use sites) and further evaluation of the potential impact of rotenone on the PCEs is also used to determine whether modification of 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 rotenone is expected to directly impact living organisms within the action area (defined in Section 2.7), critical habitat analysis for rotenone 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 rotenone 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

Rotenone is used to kill fish (*i.e.*, as a piscicide) in fisheries management. Although it was historically also used as an insecticide to control aphids, thrips, suckers, moths, and beetles in organic fruit and vegetable crops and as an insecticide/acaricide to control fire ants, lice/tick control on pets, and warble fly control on livestock, these uses have all been voluntarily cancelled by the technical registrants (USEPA, 2007; **Appendix A**). Existing stocks were to be sold March 11, 2008.

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 rotenone in accordance with the approved product labels for California is "the action" relevant to this ecological risk assessment.

Although current registrations of rotenone allow for use nationwide, this ecological risk assessment and effects determination addresses currently registered uses of rotenone 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.

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. In the case of the product formulations of active ingredients (*i.e.*, a registered 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; USFWS/NMFS, 2004).

Current formulations for rotenone include crystalline preparations (approximately 95% a.i.), emulsified solutions (approximately 50% a.i.), and dusts (approximately 0.75 to 5% a.i.). Rotenone may be used in formulations (*e.g*. Nusyn-Noxfish® Fish Toxicant, Prentox Synpren® Fish Toxicant) with another pesticide, piperonyl butoxide (CAS 51-03-

6) that is intended to act as a synergist although the level of the potential synergistic effects cannot be determined from the current data. Other flavinoids may be present in formulations with rotenone, such as rotenoloids, which occur in plants from which rotenone is obtained. Rotenoloids are also degradation products of rotenone and are similarly structured to the parent compound. Stereo-isomers of the rotenoloids can vary considerably in their biological activity. Formulated end-products of rotenone vary in the extent to which rotenoloids are present as co-extractables and their contribution to the toxicity of rotenone is uncertain.

Rotenone has registered products that contain multiple active ingredients. Analysis of the available open literature and acute oral mammalian $LD_{50}$ data for multiple active ingredient products relative to the single active ingredient is provided in Appendix B. The results of this analysis show that an assessment based on the toxicity of the single active ingredient of rotenone is appropriate.

The major degradate of rotenone is rotenolone which is structurally similar to the parent compound. Since no toxicity data are available for rotenolone, the degradate is presumed to be as toxic as the parent compound.

## 2.3 Previous Assessments

A Reregistration Eligibility Decision (RED) document was prepared for rotenone in 2006 (USEPA, 2006). The results of this screening-level risk assessment indicated that there was a potential for direct acute mortality of freshwater fish and invertebrates from agricultural and nonagricultural uses of rotenone as an insecticide/acaricide and from the use of rotenone as a piscicide in fishery management. Subsequent to the publication of the ecological risk assessment for rotenone, the technical registrants voluntarily cancelled all non-piscicidal uses of rotenone (USEPA, 2007; **Appendix A**). Therefore, the only remaining use of rotenone is as a piscicide.

## 2.4 Stressor Source and Distribution

Rotenone (CAS 83-79-4) is a selective, non-specific botanical pesticide obtained from extracts of roots, seeds, and leaves of various plants that are members of the pea or bean family (*Leguminosae)*, including jewel vine (*Derris spp.*) (EXTOXNET, 1996; Finlayson *et al.*, 2000). The tropical and sub-tropical plants from which rotenone is typically derived are not native to California.

### 2.4.1 Environmental Fate Properties

Rotenone or (6R, 6aS, 12aS)-1,2,6,6a,12,12a-hexahydro-2-isopropenyl-8,9-dimethoxychromenyl[3,4-b]furo(2,3-h)chromen-6-one, is a biochemical related to and derived from isoflavonoid compounds of plants. Rotenone has three chiral centers, and thus has a rather complex stereochemistry. As is usual for plant products, only one of the 8 possible stereoisomers is actually produced by the plants, with the 6 carbon in the Rconfiguration, and the 6a and 12a carbons in the S-configuration. As with many chemicals that have stereoisomers, the environmental fate and biological effects can be influenced by the relative positions of atoms around chiral centers.

The physical and chemical properties that determine the fate and transport of rotenone are detailed in **Table 3**. Rotenone is mobile to moderately mobile in soil and sediment ($K_d$ = 4-426), binding is well-correlated with the specific surface of the soil ($K_{ss}$ = 0.29), and it has a relatively low potential to bioconcentrate in aquatic organisms (BCF < 30). It is not likely to move in the atmosphere due to its low vapor pressure and Henry's Law constant (< $10^{-7}$ L·atm/mol), which limits its volatility. Rotenone degrades fairly rapidly by hydrolysis with a half-life of 12.6 d at pH 5 and 2 d at pH 9. The only degradate identified in the hydrolysis study is rotenolone. Degradation may be faster due to microbial routes; however, data is lacking to describe biologically mediated pathways.

Given the paucity of laboratory fate data for rotenone, the understanding of the fate and transport can be significantly augmented by information from field studies. An aquatic dissipation study was conducted in two experimental ponds located in Wisconsin and maintained at two different temperatures ($5^oC$ and $23–27^oC$). In each experiment, rotenone was applied to achieve a nominal concentration of 0.25 ppm a.i.. It dissipated from the water column with half-lives of 23 hrs in the cold water pond and 10.6 hours in the warm-water pond (MRID 4701520-10). Rotenone degraded from the entire system (water + sediment) with half-lives of approximately 20 days in the cold-water pond and 1.5 days in the warm-water pond. Only the parent was assessed in this study. As would be expected, all the resident fish in the ponds died shortly after the initial application. After 7 days in the cold-water pond and 1 day in the warm-water pond, fat-head minnows (*Pimephales promelas*) were placed in the pond in cages until 9 of 10 survived for 24 hours. This occurred after 4 days in the warm pond and 30 days in the cold-water pond. An important point to note is that the pH of around 8.5 in both ponds should have made hydrolysis of rotenone fairly rapid. This was the case in the warm-water pond, but not so for the cold water pond. This provides at least some evidence that rotenone degradation slows substantially in cool environments.

A great deal of the concentration data related to the application of rotenone to Lake Davis to control northern pike (*Esox lucius*) was collected in 1997 (California Department of Fish and Game, 1999). Lake Davis has a surface area of 4030 acres and a normal capacity of 84,370 acre-feet. It is in the Plumas National Forest in California. One particularly useful aspect of this dataset is that it can be used to estimate the dissipation rate of rotenone from Lake Davis after the initial application, which was made on October 17, 1997. This date was rather late in the season for rotenone application and the water was fairly cold ($9^oC$). Measurements were made at 9 sites on each of 6 time periods extending out to 25 d after the application. The mean concentration of rotenone in the lake on the day of application was 45 $\mu g \cdot L^{-1}$. There was a control sample taken prior to application that showed no rotenone or rotenolone present. Rotenone dissipated from the reservoir with a half-life of 10.3 d. About 55% of the rotenone formed rotenolone. Rotenolone dissipated with a half-life of 5.5 d. Some portion of the dissipation of both rotenone and rotenolone would have been due to discharge from the lake; the volume of discharge was not reported. **Table 3** lists the environmental fate properties of rotenone in the submitted environmental fate and transport studies.

**Table 3. Physical and chemical properties of rotenone.**

| **Parameter** | **Value** | |
|---|---|---|
| Rotenone CAS Number | 83-79-4 | |
| Molecular formula | $C_{23}H_{22}O_6$ | |
| **Property** | **Value** | **Reference** |
| Molecular weight | 394.4 g $mol^{-1}$ | Tomlin, 1994 |
| Melting point | 163–181 °C | Tomlin, 1994 |
| log $K_{ow}$ | 4.10 | Hansch et al., 1995 |
| Water solubility (20 °C) | 0.2 mg $L^{-1}$ | Augustijn-Beckers, 1994 |
| Vapor pressure (25 °C) | $6.9x10^{-10}$ torr | estimated - EPIWIN, 2004 |
| Henry's Law constant | $1.1x10^{-13}$ atm-$m^3$ $mol^{-1}$ | estimated - EPIWIN, 2004 |
| Hydrolysis half-life (25 °C) | 12.6 days<br>3.2 days<br>2.0 days | MRID 000141409 |
| Aqueous photolysis half-life | 191 days[a] (2 m depth, well mixed)<br>21 hours (1 cm surface) | Draper, 2002; Zepp and Cline, 1977 |
| Bioconcentration Factor (BCF) | 10.8 (viscera)<br>27.9 (head)<br>27.6 (carcass) | MRID 455801073 |

[a]Draper (2002) examined the near UV quantum yield and photodegradation kinetics of rotenone in a photoreactor. Using the method of Zepp and Cline (1977), rotenone's fate in the environment can be estimated as a function of molar concentration in water, depth of the water body, and other factors (see Appendix B). A summer time photolysis half-life was estimated for both a well-mixed pond with a depth of 2 m, and a stratified pond in which rotenone remains in the top 1 cm surface layer.

### 2.4.1 Environmental Transport Mechanisms

Rotenone is applied directly to water using a boat bailer, perforated hose or via a Venturi pump attached to the boat outboard motor. Direct application of rotenone to surface water may lead to deposition onto nearby or more distant ecosystems. Direct application to surface water is by far the major route of exposure for rotenone. As rotenone is not volatile (vapor pressure: 6.9 x $10^{-10}$ torr, Henry's law constant: 1.1 x $10^{-13}$ atm·$m^3$·$mol^{-1}$) it is not expected to dissipate through the atmosphere. Valid information on the soil-water partitioning of rotenone is not available so its propensity to bind to sediment is not known. In flowing waters, the dominant dissipation mechanism will be transport downstream.

### 2.4.2 Mechanism of Action

Rotenone is a naturally occurring plant flavinoid that acts as a pesticide through uncoupling oxidative phosphorylation within cell mitochondria by blocking electron transport at complex I.

### 2.4.3 Use Characterization

Rotenone has historically been used as a piscicide, insecticide and acaricide. In letters dated March 7, 2006; March 17, 2006; and April 5, 2006; registrants (Prentiss, Foreign Domestic and Tifa, respectively) requested voluntarily cancellation of all uses not related to the piscidal use including: agricultural, livestock, residential, home owner and domestic pet uses. Therefore, the piscicidal uses are the only ones supported for reregistration and the only uses discussed in the Reregistration Eligibility Decision chapter on rotenone (USEPA, 2007; **Appendix A**).

Analysis of labeled use information is the critical first step in evaluating the federal action. The current label for rotenone 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. **Table 3** presents the uses and corresponding application rates and methods of application considered in this assessment.

**Table 4. Rotenone Uses Assessed for the CRLF1**

| Use (boat bailer, perforated hose, Venturi pump) | Max. Single Treat Rate | Max. Number of Application per Year |
|---|---|---|
| Fishery Management/restoration (Lentic Environments) | 250 µg/L | Not specified |
| Fishery Management/restoration (Lotic Environments) | 50 µg/L | Not specified |

Historical uses of rotenone in agriculture are reflected in the California Pesticide Use Reporting (PUR) data provided by the State of California. These data indicate that over the period of 1999 to 2006, rotenone use has steadily declined from 206 lbs in 2000 to 116 lbs/year in 2006. California PUR data are summarized in **Appendix C** and reflect the historical use of rotenone as an insecticide/acaricide. From 1999 to 2006, a total of 1,405 lbs of rotenone was used on agricultural crops. The highest percentages of total use were on tomatoes (13.7%), lettuce (13.4%), spinach (7.4%), and citrus (7.4%). The highest percentage of total reported use of rotenone as an insecticide/acaricide from 1999 – 2006 was in Monterey (28%), Ventura (17%), San Diego (11%) and San Luis Obispo (9.9%) counties. Since the technical registrants agreed to no longer support (voluntarily cancelled) agricultural uses of rotenone, all use sites for which the PUR data indicate past use of rotenone in CA are no longer valid.

Rotenone has been used by fishery managers since the 1950s to kill fish in coves, shorelines, rivers, streams, and some estuarine habitats (Bettoli and Maceina, 1996). Rotenone is typically applied to a surface water body as a piscicide in conjunction with a fisheries management or research program. Use of rotenone as a piscicide is classified for restricted use. Piscicidal users must consult their local state Fish and Game Agency before applying rotenone products (U.S. EPA, 1988). In addition, the following use restrictions are typically recommended, although not required, when rotenone is applied to surface waters: (1) fish killed using rotenone may not be consumed; (2) water treated with rotenone may not be used to irrigate crops; and (3) rotenone-treated water may not be released within one-half mile upstream of a drinking water supply intake or irrigation water intakes. Drinking water restrictions do not apply to municipalities that have an alternative potable water source that may be used until rotenone has dissipated in the treated water supply (Finlayson *et al.*, 2000).

Rotenone has been used extensively as a piscicide throughout the United States. By the mid-1980s, its greatest use was in the management of warm-water fisheries in the Southeast and cool-water fisheries of the Midwest and Mountain states (Gingerich and Rach, 1985). According to a recent survey conducted by the American Fisheries Society (1988–2002; McClay, 2005), rotenone was used for fisheries management in 38 states (including California) and five Canadian provinces. Eleven states and one Canadian province accounted for 89% of rotenone applications to surface waters in North America. Approximately 97% of rotenone applications during this period were made to standing waters (ponds and lakes). Reported uses were grouped into the following five categories, reflecting different fisheries management objectives: (1) obtain a sample to characterize fish populations (34%); (2) manipulate fish populations to maintain desirable fish species for sport fisheries (27%); (3) treat rearing ponds (17%); (4) remove exotic species (10%); and (5) restore threatened and endangered species (7%). Formulated products used in standing waters were roughly equally split between rotenone powder (53%) and liquid (47%) (McClay, 2005).

In a survey conducted by the Fish Management Chemicals Subcommittee of the American Fisheries Society, fishery management agencies reported a total 112,124 kg of rotenone was used nationally over the period 1988 – 2002 (McClay, 2005). Over that time period, California reported using 9,464 kg representing 8.4% of the national total. Average annual use in California over the 14-year period was 636 kg. More recent estimates of the use of rotenone for fishery management purposes were not available.

No data on rotenone uses were located from searches of the National Center for Food and Agricultural Policy (NCFAP, 2005) pesticide database, nor the United States Geological Survey (USGS) National Pesticide Synthesis Project (USGS, 2005) database. Therefore, U.S. maps showing the spatial distribution of annual uses of rotenone at the county level for 1997 or 2002 are not available.

In lotic environments such as streams and rivers, rotenone is applied through drip stations or backpack sprayers where the moving water carries the rotenone downstream. Sprays are made close to the water surface, or in the water, so spray drift onto the shore is minor.

As a result, this potential terrestrial exposure was not assessed. In lentic environments such as lakes, reservoirs and ponds, rotenone is typically pumped through a hose into the propeller wash of an outboard motor (Venturi pump) in order to distribute the chemical uniformly through the water column.

Application rates for direct application to water are determined by estimating the concentration in receiving waters needed to achieve a resource management objective. This concentration in receiving waters is sometimes referred to as a treatment concentration (Finlayson *et al.*, 2000). Therefore, application rates of active ingredient (a.i.) will vary depending on the physical attributes of the water body such as the volume of the receiving water in terms of acre-feet (mean depth of the water in feet multiplied by the surface area in acres), flow rates, and the percentage a.i. in the formulation. Maximum application rates are generally limited to quantities that do not exceed a treatment concentration of 0.25 mg a.i./L for lakes, ponds and reservoirs and 0.05 ppm for streams and rivers with the caveat the slow moving rivers should be treated as if they were a lake. The 0.25 mg/L concentration is recommended for pre-impoundment treatment above a dam. Concentrations up to 0.2 mg/L are recommended for removing carp and bluegill in ponds rich in organic matter. Concentrations as low as 0.005 mg /L are recommended for 'selective treatment'.

Rotenone product labels do not include use information that is typically required for exposure modeling, including minimum application intervals or maximum number of applications per season or year. However, for the fisheries uses, it is unlikely that application would be necessary more than once or, on rare occasions, twice a year. Once the undesirable fish are removed and the water body restocked, it would be unlikely to be treated again for several years. Since a successful application of rotenone to surface water can be expected to result in a complete fish kill, it is assumed that only a single application is typically required in a year to achieve a fisheries management objective.

In streams and rivers, detoxification (or neutralization of rotenone) is sometimes accelerated by a post-treatment application of potassium permanganate ($KMnO_4$) (Engstrom-Heg, 1971, 1972; Lawrence, 1956). Potassium permanganate application rates vary depending on the chemical and physical composition of the water body treated, especially with respect to dissolved electrolytes and organic matter (Engstrom-Heg, 1971, 1972). According to Finlayson et al. (2000), the California Department of Fish and Game (CDFG, 1994) recommends using a ratio between 2:1 and 4:1 ($KMnO_4$: formulated rotenone) for neutralization. For this risk assessment, post-treatment use of permanganate is not considered in the fate and transport modeling; therefore, estimated enviornmental concentrations (EECs) are considered to be protective (*i.e.*, provide high-end estimates) for locations in which a chemical deactivation program is applied. It should be noted, however, that while permanganate may greatly reduce exposure concentrations of rotenone, and permanganate rapidly degrades in the environment, treatment concentrations of potassium permanganate are roughly equivalent to the 96-hour $LC_{50}$ values for permanganate for multiple fish species (see Section 3.4.3), and, as such, would be toxic to the aquatic-phase CRLF. Regardless of whether the compound is deactivated, bioassays are typically conducted and cages containing sentenal species (typically the target fish species) are placed downstream of treated waters to ensure that the chemical does not move outside of the targeted treatment area. Although labels do not specify the maximum number of treatments per year, the logistical constraints of conducting fish renovation efforts typically limit the number of applications that can be undertaken in a year.

## 2.5 Assessed Species

The CRLF was federally listed as a threatened species by USFWS effective June 24, 1996 (USFWS, 1996). It is one of two subspecies of the red-legged frog and is the largest native frog in the western United States (USFWS, 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 1**.

Final critical habitat for the CRLF was designated by USFWS on April 13, 2006 (USFWS 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 (USFWS, 1996). Its range has been reduced by about 70%, and the species currently resides in 22 counties in California (USFWS, 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) (USFWS, 2002).

Populations currently exist along the northern California coast, northern Transverse Ranges (USFWS 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 (USFWS 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) (USFWS 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 1**. Recovery units, core areas, and other known occurrences of the CRLF from the CNDDB are described in further detail in this section, 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 USFWS 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.

*Recovery Units*

Eight recovery units have been established by USFWS for the CRLF. These areas are considered essential to the recovery of the species, and the status of the CRLF "may be considered within the smaller scale of the recovery units, as opposed to the statewide range" (USFWS 2002). Recovery units reflect areas with similar conservation needs and population statuses, and therefore, similar recovery goals. The eight units described for the CRLF are delineated by watershed boundaries defined by US Geological Survey hydrologic units and are limited to the elevational maximum for the species of 1,500 m above sea level. The eight recovery units for the CRLF are listed in **Table 5** and shown in **Figure 2**.

*Core Areas*

USFWS has designated 35 core areas across the eight recovery units to focus their recovery efforts for the CRLF (see **Figure 2**). **Table 5** summarizes the geographical relationship among recovery units, core areas, and designated critical habitat. The core areas, which are distributed throughout portions of the historic and current range of the species, represent areas that allow for long-term viability of existing populations and reestablishment of populations within historic range. These areas were selected because they: 1) contain existing viable populations; or 2) they contribute to the connectivity of other habitat areas (USFWS 2002). Core area protection and enhancement are vital for maintenance and expansion of the CRLF's distribution and population throughout its range.

For purposes of this assessment, designated critical habitat, currently occupied (post-1985) core areas, and additional known occurrences of the CRLF from the CNDDB are considered. Each type of locational information is evaluated within the broader context of recovery units. For example, if no labeled uses of rotenone occur (or if labeled uses occur at predicted exposures less than the Agency's LOCs) within an entire recovery unit, a "no effect" determination would be made for all designated critical habitat, currently occupied core areas, and other known CNDDB occurrences within that recovery unit.

Historically occupied sections of the core areas are not evaluated as part of this assessment because the USFWS Recovery Plan (USFWS 2002) indicates that CRLFs are extirpated from these areas. A summary of currently and historically occupied core areas is provided in **Table 5** (currently occupied core areas are bolded). While core areas are considered essential for recovery of the CRLF, core areas are not federally-designated critical habitat, although designated critical habitat is generally contained within these core recovery areas. It should be noted, however, that several critical habitat units are located outside of the core areas, but within the recovery units. The focus of this assessment is currently occupied core areas, designated critical habitat, and other known CNDDB CRLF occurrences within the recovery units. Federally-designated critical habitat for the CRLF is further explained in Section 2.6.

**Table 5. California Red-legged Frog Recovery Units with Overlapping Core Areas and Designated Critical Habitat**

| Recovery Unit [1] (Figure 2.a) | Core Areas [2,7] (Figure 2.a) | Critical Habitat Units [3] | Currently Occupied (post-1985) [4] | Historically Occupied [4] |
|---|---|---|---|---|
| Sierra Nevada Foothills and Central Valley (1) (eastern boundary is the 1,500m elevation line) | **Cottonwood Creek (partial) (8)** | -- | ✓ | |
| | **Feather River (1)** | BUT-1A-B | ✓ | |
| | **Yuba River-S. Fork Feather River (2)** | YUB-1 | ✓ | |
| | -- | NEV-1[6] | | |
| | **Traverse Creek/Middle Fork American River/Rubicon (3)** | -- | ✓ | |
| | **Consumnes River (4)** | ELD-1 | ✓ | |
| | S. Fork Calaveras River (5) | -- | | ✓ |
| | Tuolumne River (6) | -- | | ✓ |
| | Piney Creek (7) | -- | | ✓ |
| | **East San Francisco Bay (partial)(16)** | -- | ✓ | |
| North Coast Range Foothills and Western Sacramento River Valley (2) | **Cottonwood Creek (8)** | -- | ✓ | |
| | Putah Creek-Cache Creek (9) | -- | | ✓ |
| | **Jameson Canyon – Lower Napa Valley (partial) (15)** | -- | ✓ | |
| | **Belvedere Lagoon (partial) (14)** | -- | ✓ | |
| | **Pt. Reyes Peninsula (partial) (13)** | -- | ✓ | |
| North Coast and North San Francisco Bay (3) | Putah Creek-Cache Creek (partial) (9) | -- | | ✓ |
| | **Lake Berryessa Tributaries (10)** | NAP-1 | ✓ | |
| | **Upper Sonoma Creek (11)** | -- | ✓ | |
| | **Petaluma Creek-Sonoma Creek (12)** | -- | ✓ | |
| | **Pt. Reyes Peninsula (13)** | MRN-1, MRN-2 | ✓ | |
| | **Belvedere Lagoon (14)** | -- | ✓ | |
| | **Jameson Canyon-Lower Napa River (15)** | SO/L | ✓ | |

| | | | | |
|---|---|---|---|---|
| South and East San Francisco Bay (4) | -- | CCS-1A[6] | | |
| | **East San Francisco Bay (partial) (16)** | ALA-1A, ALA-1B, STC-1B | ✓ | |
| | -- | STC-1A[6] | | |
| | **South San Francisco Bay (partial) (18)** | SNM-1A | ✓ | |
| Central Coast (5) | **South San Francisco Bay (partial) (18)** | SNM-1A, SNM-2C, SCZ-1 | ✓ | |
| | **Watsonville Slough- Elkhorn Slough (partial) (19)** | SCZ-2 [5] | ✓ | |
| | **Carmel River-Santa Lucia (20)** | MNT-2 | ✓ | |
| | **Estero Bay (22)** | -- | ✓ | |
| | -- | SLO-8[6] | | |
| | **Arroyo Grande Creek (23)** | -- | ✓ | |
| | **Santa Maria River-Santa Ynez River (24)** | -- | ✓ | |
| Diablo Range and Salinas Valley (6) | **East San Francisco Bay (partial) (16)** | MER-1A-B, STC-1B | ✓ | |
| | -- | SNB-1[6], SNB-2[6] | | |
| | **Santa Clara Valley (17)** | -- | ✓ | |
| | **Watsonville Slough- Elkhorn Slough (partial)(19)** | MNT-1 | ✓ | |
| | **Carmel River-Santa Lucia (partial)(20)** | -- | ✓ | |
| | **Gablan Range (21)** | SNB-3 | ✓ | |
| | **Estrella River (28)** | SLO-1A-B | ✓ | |
| Northern Transverse Ranges and Tehachapi Mountains (7) | -- | SLO-8[6] | | |
| | **Santa Maria River-Santa Ynez River (24)** | STB-4, STB-5, STB-7 | ✓ | |
| | **Sisquoc River (25)** | STB-1, STB-3 | ✓ | |
| | **Ventura River-Santa Clara River (26)** | VEN-1, VEN-2, VEN-3 | ✓ | |
| | -- | LOS-1[6] | | |
| Southern Transverse and Peninsular Ranges (8) | **Santa Monica Bay-Ventura Coastal Streams (27)** | -- | ✓ | |
| | San Gabriel Mountain (29) | -- | | ✓ |
| | Forks of the Mojave (30) | -- | | ✓ |
| | Santa Ana Mountain (31) | -- | | ✓ |
| | **Santa Rosa Plateau (32)** | -- | ✓ | |
| | San Luis Rey (33) | -- | | ✓ |
| | Sweetwater (34) | -- | | ✓ |
| | Laguna Mountain (35) | -- | | ✓ |

Recovery units designated by the USFWS (USFWS 2000, pg 49).
[2] Core areas designated by the USFWS (USFWS 2000, pg 51).
[3] Critical habitat units designated by the USFWS on April 13, 2006 (USFWS 2006, 71 FR 19244-19346).
[4] Currently occupied (post-1985) and historically occupied core areas as designated by the USFWS (USFWS 2002, pg 54).
[5] Critical habitat unit where identified threats specifically included pesticides or agricultural runoff (USFWS 2002).
[6] Critical habitat units that are outside of core areas, but within recovery units.
[7] Currently occupied core areas that are included in this effects determination are bolded.

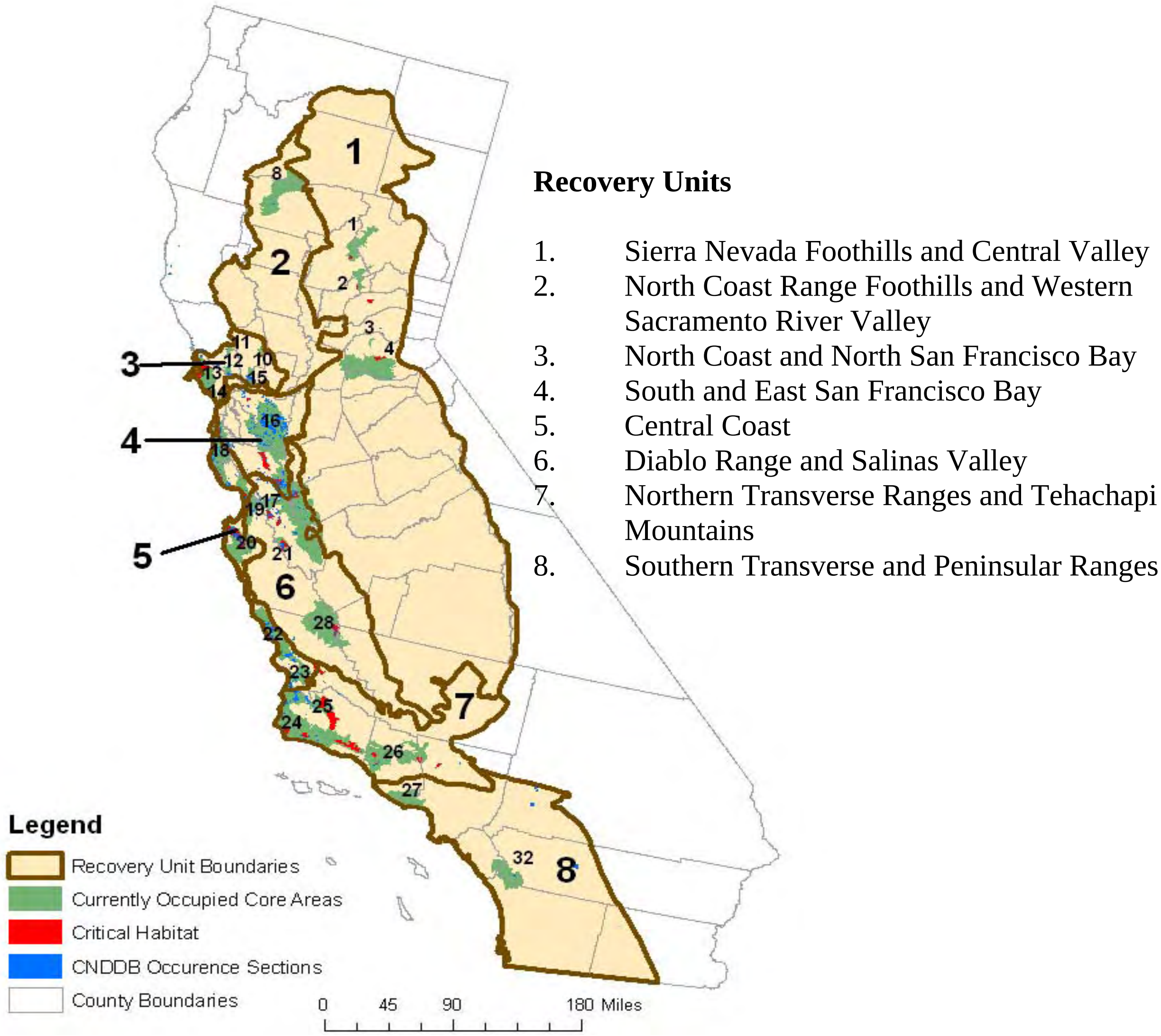


**Figure 1. Recovery Unit, Core Area, Critical Habitat, and Occurrence Designations for CRLF**

## 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

*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.

### 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 (USFWS 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, USFWS 2002); tadpoles have been observed to over-winter (delay metamorphosis until the following year) (Fellers 2005b, USFWS 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 (USFWS 2002). Figure 2 depicts CRLF annual reproductive timing.

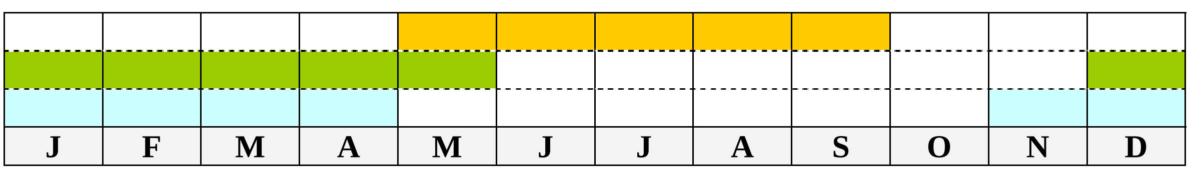


**Figure 2. CRLF Reproductive Events by Month**
Light Blue = **Breeding/Egg Masses**
Green = **Tadpoles (except those that over-winter)**
Orange = **Young Juveniles**
Adults and juveniles can be present all year

### 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 aquatic phase feeding exclusively in water and consuming diatoms, algae, and detritus (USFWS 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 (USFWS 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 (USFWS 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 (USFWS 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://www.fws.gov/endangered/features/rl_frog/rlfrog.html#where).

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 (USFWS 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 (UWFWS 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 USFWS (USFWS 2006; FR 51 19244-19346). A summary of the 34 critical habitat units relative to USFWS-designated recovery units and core areas (previously discussed in Section 2.5.1) is provided in **Table 5.**

'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 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, USFWS 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 FR listing notice in April 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 1** for a full explanation on this special rule.

USFWS has established adverse modification standards for designated critical habitat (USFWS 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 rotenone that may alter the PCEs of the CRLF's critical habitat form the basis of the critical habitat impact analysis. According to USFWS (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) 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.
(3) 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.
(4) Elimination of upland foraging and/or aestivating habitat or dispersal habitat.
(5) Introduction, spread, or augmentation of non-native aquatic species in stream segments or ponds used by the CRLF.
(6) 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 rotenone is expected to directly impact living organisms within the action area, critical habitat analysis for rotenone 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 rotenone is likely to encompass considerable portions of the United States based on fishery management 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. Deriving the geographical extent of this portion of the action area is based on consideration of the types of effects that rotenone may be expected to have on the environment, the exposure levels to rotenone that are associated with those effects, and the best available information concerning the use of rotenone and its fate and transport within the 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 rotenone. An analysis of labeled uses and review of available product labels was completed. For those uses relevant to the CRLF, the analysis indicates that piscicidal use in fishery management of rotenone is the appropriate federal action to be evaluated in this assessment.

Following a determination of the use(s) to be assessed, an evaluation of the potential “footprint” of rotenone’s use pattern is determined. This “footprint” represents the initial area of concern. The initial area of concern is defined as all the lakes, reservoirs, rivers and stream reaches relative to the CRLF. A map representing all the lakes, reservoirs, rivers and stream reaches that make up the initial area of concern for rotenone is presented in **Figures 3** and **4**. Rotenone is applied to water only; therefore, the initial area of concern maps depict the overlay of CRLF habitat and surface water where rotenone could potentially be used.

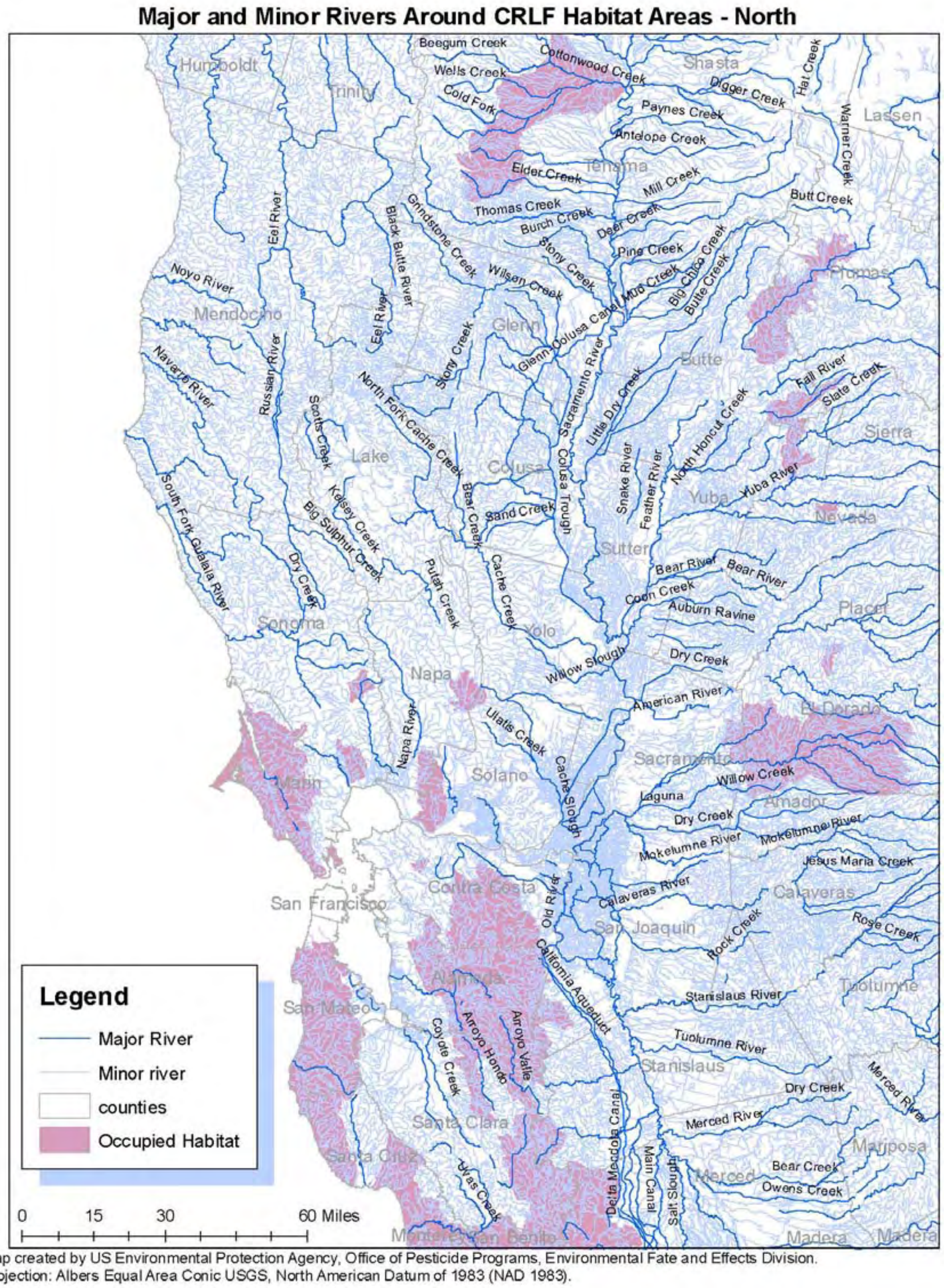


**Figure 3. Initial area of concern, or "footprint" of potential use, for rotenone in the northern range of CRLF occupied habitat**

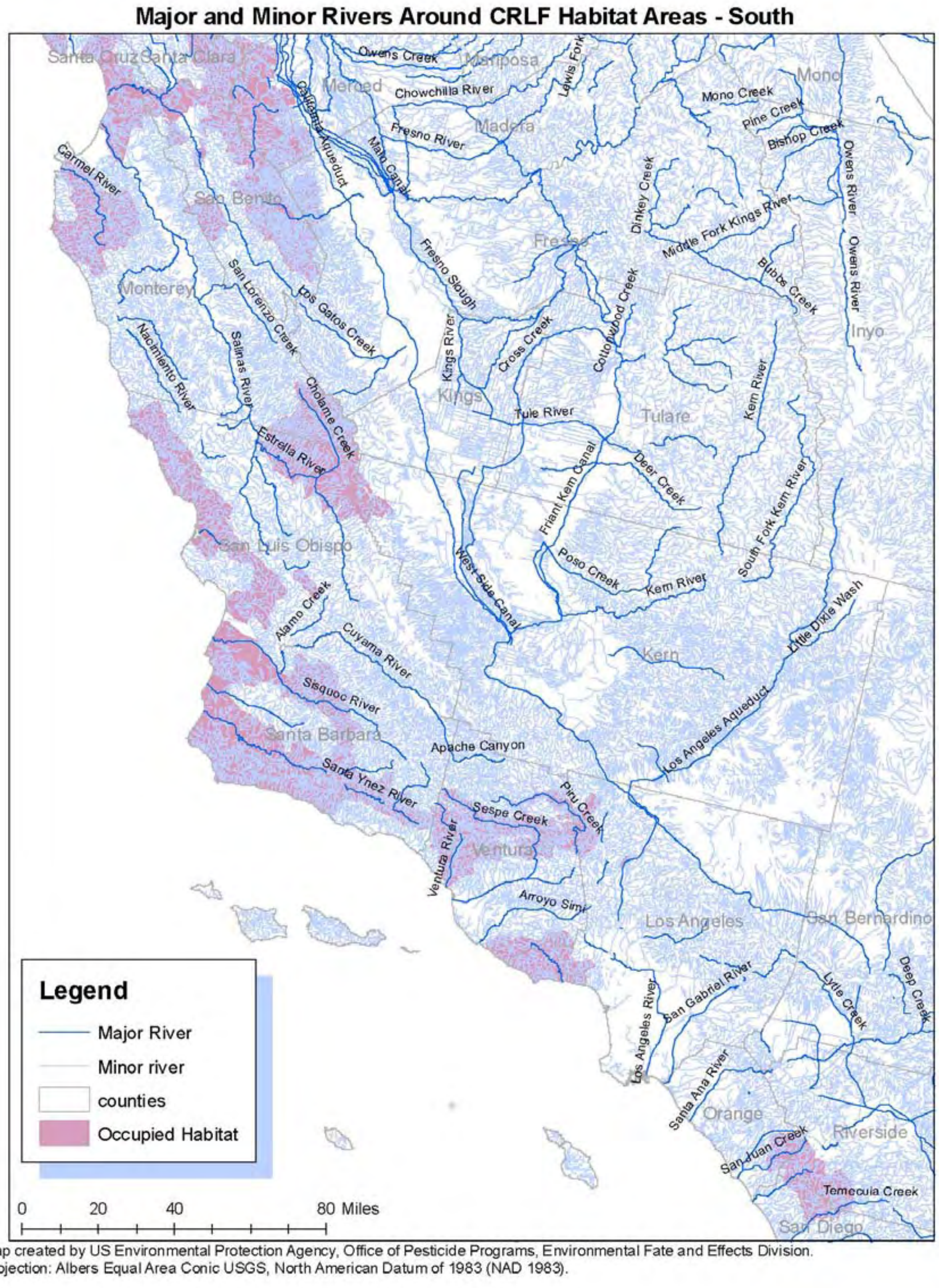


**Figure 4. Initial area of concern, or "footprint" of potential use, for rotenone in the southern range of CRLF occupied habitat**

Once the initial area of concern is defined, the next step is to compare the extent of that area with the results of the screening-level risk assessment. The full extent of the action area is considered to be all water bodies within the state of California that overlay with or are upstream of CRLF habitat. The screening-level risk assessment identifies which taxa, if any, are predicted to be exposed at concentrations above the Agency's Levels of Concern (LOC). The screening-level assessment includes an evaluation of the environmental fate properties of rotenone to determine which routes of transport are likely to have an impact on the CRLF.

Rotenone is not persistent in the environment and its low vapor pressure and Henry's Law constant ($< 10^{-7}$) limit its volatility. When released to water, rotenone generally degrades quickly through abiotic (hydrolytic and photolytic) mechanisms, with half-lives of a few days to several weeks. Since the chemical is added directly to water, its movement is dictated by the extent to which the treated water is confined or rotenone is chemically deactivated through the use of oxidizing agents such as potassium permanganate ($KMnO_4$). Given that potassium permanganate is applied at treatment ratios ranging from 2:1 to 4:1 (potassium permanganate:rotenone), permanganate may be present at exposure concentrations that present a secondary risk to non-target aquatic organisms. However, because of the reactivity of $KMnO_4$, the compound is relatively short-lived particularly in water with high organic carbon levels.

## 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."[1] 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.,* water bodies), the migration pathways of rotenone (*e.g.,* runoff, spray drift, etc.), and the routes by which ecological receptors are exposed to rotenone *(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 modification of its habitat. In addition, potential modification of 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

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

guideline tests that are performed on a limited number of organisms. Additional ecological effects data from the open literature are also considered.

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 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 rotenone is provided in Error! Reference source not found.**6**. As discussed earlier, rotenone is applied directly to water; therefore, the likelihood of exposure to terrestrial organisms is considered low. As such, this assessment does not include a quantitative evaluation of risk to the terrestrial-phase CRLF and/or its habitat.

**Table 6. Assessment Endpoints and Measures of Ecological Effects**

| Assessment Endpoint | Measures of Ecological Effects[2] |
|---|---|
| *Aquatic-Phase CRLF (Eggs, larvae, juveniles, and adults)*[a] | |
| *Direct Effects* | |
| 1. Survival, growth, and reproduction of CRLF | 1a. Rainbow trout acute $LC_{50}$ (guideline) since no suitable amphibian data are available<br>1b. Rainbow trout chronic NOAEC (guideline) |
| *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. *Daphnia magna* $EC_{50}$ (guideline)<br>2b. *Daphnia magna* NOAEC (guideline) |
| 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) | No guideline toxicity study data are available; however, anecdotal data are available for plants. |
| 4. Survival, growth, and reproduction of CRLF individuals via effects to semi-aquatic vegetation | No guideline toxicity study data are available; however, anecdotal data are available for plants. |
| *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. Ring-necked pheasant[b] acute $LD_{50}$ (guideline)<br>5b. No data available for chronic effects |
| *Indirect Effects and Critical Habitat Effects* | |
| 6. Survival, growth, and reproduction of CRLF individuals via effects on terrestrial prey (*i.e.*,terr. invertebrates, small mammals , and frogs) | 6a. Honey bee $LD_{50}$ and rat acute $LD_{50}$ (guideline)[c]<br>6b. Most sensitive terrestrial invertebrate and vertebrate chronic NOAEC (guideline or ECOTOX) |
| 7. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat (*i.e.*, riparian and upland vegetation) | No guideline toxicity study data are available; however, anecdotal data are available for plants. |

[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.

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[2] All registrant-submitted and open literature toxicity data reviewed for this assessment are included in Appendix A.

[b] Birds are used as surrogates for terrestrial-phase amphibians.

### 2.8.2 Assessment Endpoints for Designated Critical Habitat

Designated critical habitat is assessed to evaluate actions related to the use of rotenone 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 rotenone effects data are available.

Adverse modification to the critical habitat of the CRLF includes, but is not limited to, the following, as specified by USFWS (2006):

1. Alteration of water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs.
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.
4. Significant alteration of channel/pond morphology or geometry.
5. Elimination of upland foraging and/or aestivating habitat, as well as 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.

Measures of such possible effects by labeled use of rotenone on critical habitat of the CRLF are described in **Table 7**. 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 USFWS (2006).

**Table 7. Summary of Assessment Endpoints and Measures of Ecological Effect for Primary Constituent Elements of Designated Critical Habitat[a]**

<table>
<tr><th>Assessment Endpoint</th><th>Measures of Ecological Effect</th></tr>
<tr><td colspan="2">Aquatic-Phase CRLF PCEs<br>(Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)</td></tr>
<tr><td>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.</td><td>No guideline toxicity study data are available; however, anecdotal data are available for plants.</td></tr>
<tr><td>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.</td><td>No guideline toxicity study data are available; however, anecdotal data are available for plants.</td></tr>
<tr><td>Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.</td><td>a. Rainbow trout acute $LC_{50}$ value and Daphnia magna acute $EC_{50}$ (guideline)<br>b. Rainbow trout and Daphnia magna NOAEC (guideline)</td></tr>
<tr><td>Reduction and/or modification of aquatic-based food sources for pre-metamorphs (e.g., algae)</td><td>No guideline toxicity study data are available; however, anecdotal data are available for plants.</td></tr>
<tr><td colspan="2">Terrestrial-Phase CRLF PCEs<br>(Upland Habitat and Dispersal Habitat)</td></tr>
<tr><td>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</td><td rowspan="4">a. Distribution of $EC_{25}$ values for monocots (seedling emergence, vegetative vigor, or ECOTOX)<br>b. Distribution of $EC_{25}$ values for dicots (seedling emergence, vegetative vigor, or ECOTOX)<br>c. Most sensitive food source acute $EC_{50}$/$LC_{50}$ and NOAEC values for terrestrial vertebrates (mammals) and invertebrates, birds or terrestrial-phase amphibians, and freshwater fish.</td></tr>
<tr><td>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</td></tr>
<tr><td>Reduction and/or modification of food sources for terrestrial phase juveniles and adults</td></tr>
<tr><td>Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.</td></tr>
</table>

[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 rotenone to the environment. The following risk hypotheses are presumed for this endangered species assessment:

The labeled use of rotenone 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 modify 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 modify 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;
- modify 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);
- modify the designated critical habitat of the CRLF by reducing the food supply required for normal growth and viability of juvenile and adult CRLFs;
- modify 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.
- modify 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.
- modify 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 rotenone release mechanisms, biological receptor types, and effects endpoints of potential concern. Since rotenone is applied directly to water and not to terrestrial environments, the likelihood of exposure for terrestrial animals is considered low. Therefore, the conceptual model for aquatic-phase CRLFs is the focus of this assessment and is depicted in **Figure 5;** the conceptual model for the aquatic PCE component of critical habitat is shown in **Figure 6**. 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 modification to designated critical habitat is expected to be negligible.

When used as a piscicide, rotenone is deposited directly into a receiving water (**Figure 1**), which may be a stream, pond, or lake. Once in the water column, non-target aquatic organisms may be exposed to rotenone via three main exposure pathways.

1. **Rotenone can remain soluble in the water column of the receiving water.** Organisms in the water column (aquatic invertebrates, fish, amphibians and aquatic/semi-aquatic plants) can be directly exposed through contact. Organisms can take up soluble rotenone through the gills or integument. If sufficient concentrations exist, individual toxic effects in aquatic invertebrates, fish, aquatic-phase amphibians and/or plants can result, including reductions in populations of species within these taxa. This is expected to be the main route of exposure for rotenone. Terrestrial wildlife could be potentially exposed to rotenone through drinking water.
2. **Rotenone released into water will sorb to sediment and suspended solids.** Receptors such as aquatic invertebrates, fish, and aquatic-phase amphibians can be exposed to sorbed rotenone through incidental ingestion of sediment and/or suspended solids. This can result in changes in populations based on reduced survival, growth and reproduction.

3. **Rotenone can sorb to plant surfaces**. Ingestion of plant materials with sorbed rotenone can result in a similar exposure pathway as described for sediment and suspended solids. Nonvascular or vascular plants can also be exposed to rotenone through direct contact or root uptake. This could result in changes in plant populations due to direct toxicity. Since no plant toxicity data are available for rotenone, this exposure pathway is uncertain.

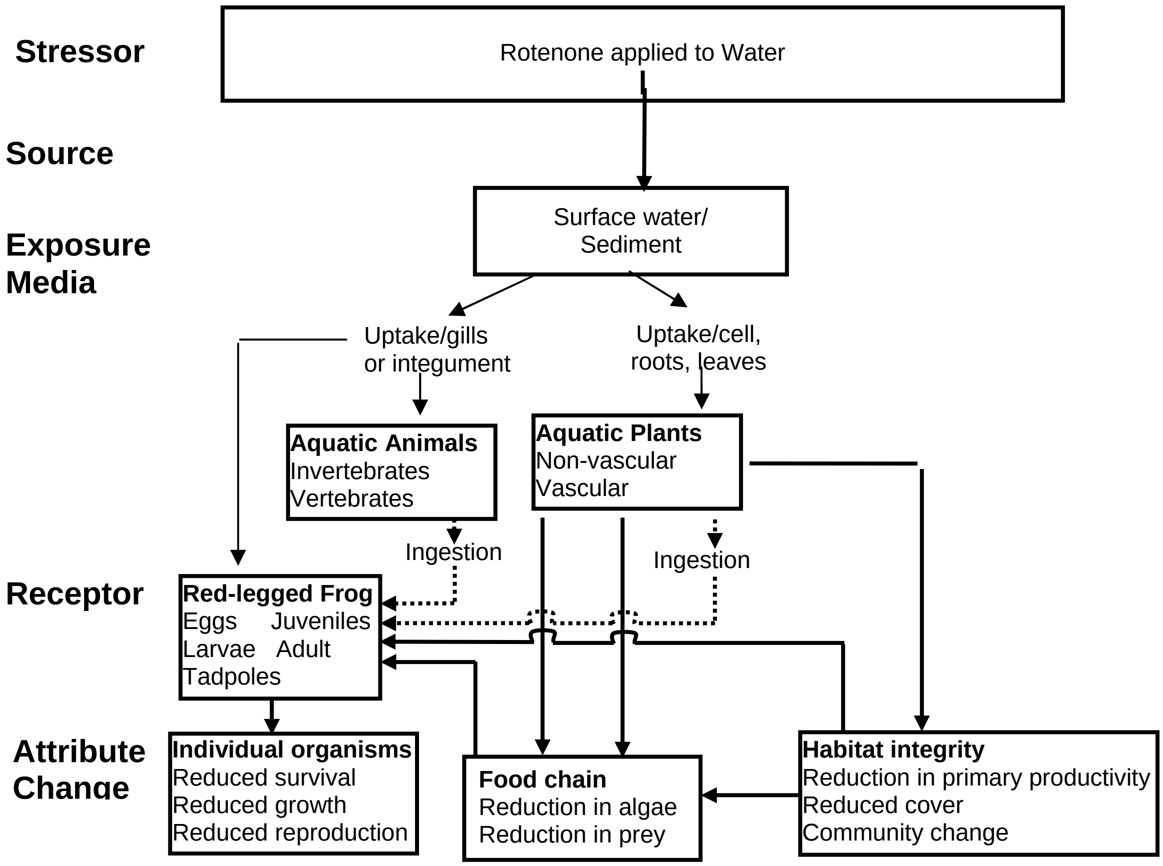


**Figure 5. Conceptual Model for Aquatic-Phase of the CRLF Exposed to Direct Application of Rotenone to Water.**

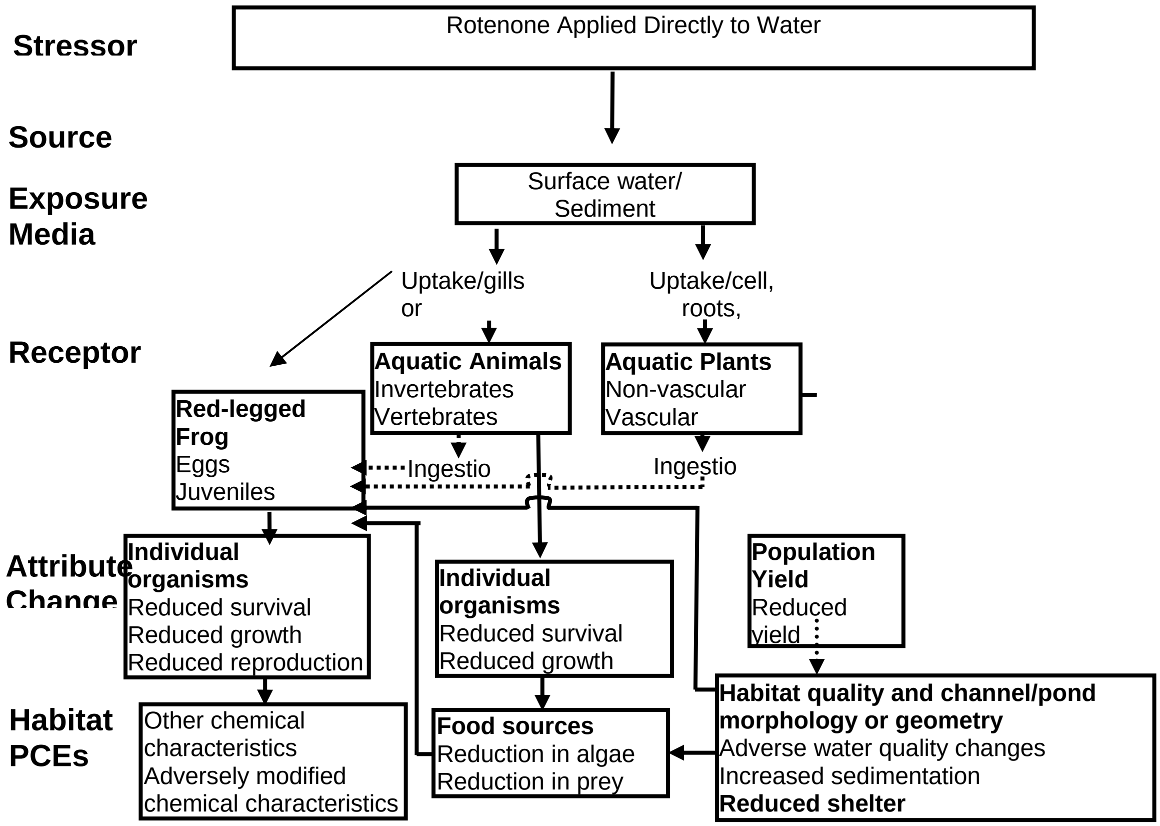


**Figure 6. Conceptual Model for Rotenone Effects on Aquatic Component of CRLF Critical Habitat following Direct Application to Water.**

## 2.10 Analysis Plan

In order to address the risk hypothesis, the potential for direct and indirect effects to the CRLF, its prey, and its habitat is estimated. In the following sections, the use, environmental fate, and ecological effects of rotenone are characterized and integrated to assess the risks. This is accomplished using a risk quotient (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 rotenone 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

Rotenone is applied directly to water in both lentic (lakes and impoundments) and lotic (streams and rivers) environments. The measure of exposure for this assessment is the maximum treatment rate for rotenone in water, i.e., 250 μg active ingredient (a.i.)/L. However, since the solubility limit for rotenone is 200 μg a.i./L, the functional exposure is 200 μg a.i/L. For chronic exposures, the mean concentrations over 21 d (aquatic invertebrates) and 60 d (fish) were calculated using zero-order kinetics ($C = C_0 - k^0 t$) for concentrations from 250 to 200 μg/L and first-order kinetics ($C = C_0 e^{-kt}$) for concentrations below 200 μg/L. In addition the time concentrations were expected to remain over acute LOCs was estimated using similar kinetics for three different temperature regimes. Exposure to piscivorous wildlife is qualitatively assessed by considering the ingestion of food containing rotenone residues.

#### 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. The ECOTOXicology database (ECOTOX; USEPA 2007) 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 USEPA, Office of Research and Development, and the National Health and Environmental Effects Research Laboratory's Mid-Continent Ecology Division.

Typically, the assessment of risk for direct effects to the terrestrial-phase CRLF makes the assumption that toxicity of rotenone 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. Aquatic, semi-aquatic represent habitat of CRLF. As will be discussed later, since rotenone use is limited to direct applications to water, exposure to terrestrial-phase amphibians is considered low.

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.*, $EC_{50}$ 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 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.

#### 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 piscicidal uses of rotenone, and the likelihood of direct and indirect effects to CRLF in aquatic 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 rotenone 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) (USEPA, 2004) (see Appendix D).

For this endangered species assessment, listed species LOCs are used for comparing RQ values for acute and chronic exposures of rotenone directly to the CRLF. If estimated exposures directly to the CRLF of rotenone 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 invertebrates, fish, and frogs), the listed species LOCs are also used. If estimated exposures to CRLF prey of rotenone resulting from the use of rotenone as a piscicide 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 semi-aquatic and aquatic 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 semi-aquatic 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 D**.

### 2.10.2 Data Gaps

No data are available to quantify the following fate characteristics of rotenone: photodegradation on soil (Guideline §161-3), aerobic soil metabolism (Guideline §162-1), and anaerobic soil metabolism (Guideline §162-3). The effects of binding to sediment (Guideline §163-1) cannot be characterized because there is no valid data for adsorption/desorption. However, the lack of these data represents an uncertainty for terrestrial applications of rotenone and does not affect estimates of aquatic exposure used in this assessment where rotenone is applied directly to water. Data for aerobic and anaerobic aquatic metabolism are also lacking. Since there are no valid metabolism data, it is not possible to well characterize the degradates formed by metabolic processes. Degradation half-lives were estimated using aquatic dissipation studies and residue decline data from a monitoring study. Laboratory metabolism data would have substantially improved our understanding of the degradation processes for rotenone.

There are no data available to assess chronic risk to birds (Guideline §71-4). Another source of uncertainty is the lack of data to evaluate the toxicity of rotenone to aquatic (Guideline §123-2) and terrestrial plants (Guideline §123-1). Lack of such data create an uncertainty relative to evaluating the indirect effects of rotenone use to aquatic-phase amphibians and their critical habitat.

## 3. Exposure Assessment

In this assessment acute aquatic exposure is defined as the solubility limit of rotenone in water, *i.e.*, 200 μg a.i./L.

As will be discussed below, rotenone degrades at least moderately rapidly in aquatic environments, thus, it is unlikely that residues would accumulate in water or sediment following successive applications during the year even if successive applications were typically required. Labels generally recommend waiting a period of 2 to 4 weeks prior to restocking after treatment.

The piscicide uses would by design be expected to kill fish at the concentrations at which they are applied. The question then is “how long the concentration stays above the level of concern?” **Table 8** provides some information on the potential duration of exposure at levels where some effects on wildlife may be expected. Dissipation rates from the three available field studies were used to estimate the length of time that the concentration in a water body stays above the levels of concern for fish and for invertebrates (See the following toxicity characterization for the development of these levels of concern). These dissipation rates were from the warm water (25$^{o}$C; $T_{1/2}$=1.5 d) and cold water (5$^{o}$C: $T_{1/2}$ = 20 d) pond aquatic dissipation studies, and derived from monitoring data at Lake Davis (9$^{o}$C: $T_{1/2}$ = 10.3 d), California. They thus cover a range of temperatures found in the environment. These sets of data are described in the Fate and Transport Characterization above. Based on these first-order dissipation rates, and the highest application rates in each type of site, effects might be expected on sensitive species for less than two weeks in warm water environments. However, rotenone can be quite persistent in cold environments where it might persist at levels causing effects for 160 days. In most environments in the lower 48 states, it is unlikely that the water temperature would remain as low as $5^{o}$ C for 160 days; there would sufficient warming of the environment over this period of time to increase the dissipation rate substantially.

**Table 8. Concentrations and Durations of Rotenone Exposure above where Risk Quotients Exceed the Acute Risk Level of Concern for Freshwater Fish and Invertebrates based on Dissipation Rates from Field Studies. LOC for Fish = 0.05*$LC_{50}$ for Rainbow Trout of 0.09 µg/L.**

| Use Sites | Peak EEC | Warm Water Pond ($T_{1/2}$=1.5 d) | Cold Water Pond ($T_{1/2}$=20 d) | Lake Davis ($T_{1/2}$=10.3 d) |
|---|---|---|---|---|
| Lakes/ Reservoirs | 250 µg /L | fish:17.0 d<br>inverts: 10.7 d<br>21 d mean: 26 µg /L<br>60 d mean: 9 µg /L | fish: 227 d<br>inverts: 142 d<br>21 d mean: 173 µg /L<br>60 d mean: 105 µg /L | fish: 117 d<br>inverts: 73.3 d<br>21 d mean: 133 µg /L<br>60 d mean: 61 µg /L |
| Lakes/Reservoirs (lower max rate products) | 200 µg/L | fish: 16.5 d<br>inverts: 10.1 d<br>21 d mean: 21 µg/L<br>60 d mean: 7 µg/L | fish: 220 d<br>inverts: 135 d<br>21 d mean: 142 µg/L<br>60 d mean: 84 µg/L | fish: 113 d<br>inverts: 69.6 d<br>21 d mean: 107 µg/L<br>60 d mean: 49 µg/L |
| Streams/ Rivers | 50 µg /L | fish: 13.5 d<br>inverts: 7.1 d | fish:180 d<br>inverts: 95.1 d | fish: 92.8 d<br>inverts: 49.0 d |

It is a common, but not required, practice to use potassium permanganate to detoxify rotenone in streams. At temperatures above $10^{o}$C, this should occur in 30 minutes if label directions are followed and assuming the detoxification occurs at the rate presumed by the registrant. It is worth reiterating that data supporting the detoxification or degradation of rotenone is not substantial.

It is also worth noting that the maximum label application rate to lakes and reservoirs exceeds the solubility of rotenone in water (200 µg /L at $25^{o}$C). The remaining rotenone above the solubility limit is likely either suspended or in an emulsion. To account for this, the degradation rate of rotenone was estimated using zero order kinetics from 250 to 200 µg /L. Zero order kinetic assumes the degradation rate is constant and does not depend upon the concentration. The zero-order rate constant was estimated by multiplying the first order rate constant by the solubility (200 µg /L). To calculate the time spent over the solubility limit, difference between the application rate and the solubility limit (250 µg /L – 200 µg /L) or 50 µg /L was divided by the zero order rate constant. First order kinetics was then used to estimate the time between the solubility limit and the LOC. These two values were then added together to calculate the total time above the LOC for each temperature regime. Mean 21 d and 60 d concentrations were also estimated using similar methods for each temperature regime and the two higher application rates. Chronic exposure will not occur for rotenone in rivers as transport processes will remove the rotenone downstream before chronic exposure could occur. Therefore chronic EECs were not calculated for the application to streams.

No data are reported in surface or ground waters in California for rotenone and/or its degradate in the USGS National Water Quality Assessment (NAWQA) program since rotenone was not one of the analytes listed.

### 3.2 Terrestrial Exposure Assessment

Rotenone is applied directly to water and not to land; therefore, exposure of terrestrial organisms to rotenone through consumption of terrestrial forage items is not considered likely. In addition, exposure of terrestrial plants in dry and semi-aquatic areas is not considered likely. Therefore, a terrestrial plant exposure assessment was not conducted. The risk characterization does include a brief qualitative description of potential risk posed by potential consumption of rotenone residues by piscivorous animals.

## 4. Effects Assessment

This assessment evaluates the potential for rotenone to directly or indirectly affect the aquatic-phase CRLF or modify its designated aquatic critical habitat. As previously discussed in Section 2.7, assessment endpoints for the CRLF 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 modification of its habitat. In addition, potential modification of critical habitat is 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. Although terrestrial-phase CRLF are not assessed in this document terrestrial-phase effects are normally 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 and aquatic invertebrates and aquatic/semi-aquatic 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 rotenone.

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 aquatic-phase amphibians, freshwater fish, freshwater invertebrates and aquatic plants; however, toxicity data are also provided in this section on birds (surrogate for terrestrial-phase amphibians), mammals, and terrestrial invertebrates for completeness sake.

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 data obtained from the RED (USEPA 2007*a*) as well as ECOTOX (USEAP 2007*b*) information obtained in April 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;
(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 is dependent on whether the information is relevant to the assessment endpoints (*i.e.,* maintenance of 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 rotenone.

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 and/or not appropriate for use in this assessment) are included in **Appendix E. Appendix E** 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 listing of available ECOTOX open literature data, including the full suite of lethal and sublethal endpoints is presented in **Appendix E**.

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 rotenone. A summary of the available aquatic and terrestrial ecotoxicity information, use of the probit dose response relationship, and the incident information for rotenone are provided in Sections 4.1 through 4.4, respectively.

The major degradate of rotenone is rotenolone; however, there are no data available with which to assess the toxicity of rotenolone independent of the parent compound. Rotenolone has a chemical structure similar to that of the parent and for the purposes of this assessment is assumed to be equally toxic to the parent.

A detailed summary of the available ecotoxicity information for all rotenone degradates and formulated products is presented in **Appendix F**.

Rotenone formulations frequently contain other ingredients that may be similarly structured to the active ingredient. Cube root resins and/or "other associated resins" are listed as an active ingredient on a number of labels (*e.g.* Prenitis Cube® Resins, SYPREN-FISH® Toxicant, NOXFISH®, CFT Lugumine®, CHEM-FISH® SYNERGIZED). Several of the formulations (*e.g.* CHEM-FISH® SYNERGIZED, NUSYN – NOXFISH® Toxicant, PRENTOX SYNPREN-FISH® Toxicant) contain piperonyl butoxide (CAS No. 51-03-6). Rotenone can also be formulated with piperonyl sulfoxide (CAS No. 120-62-7). Toxicity data are not available on all of the formulated products of rotenone for all of the surrogate species typically evaluated. However, based on toxicity data collected on both technical grade rotenone (>95% active ingredient) and formulated end-product, the technical grade active ingredient is generally more toxic than formulated end-product [corrected for active ingredient] by at least a factor of two. These data suggest that for the formulated products tested and the toxicity endpoints measured, the inerts do not contribute substantially to the toxicity of the active ingredient. These data also suggest that the similarly structured rotenolones of plant resins (cube root resins) contained in varying amounts in formulated end-products also do not contribute substantially to the toxicity of rotenone. The extent to which the toxicity of untested formulations would be similar to those for which there are data is uncertain.

## 4.1 Toxicity of Rotenone to Aquatic Organisms

**Table 9** 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 F.**

**Table 9. Freshwater Aquatic Toxicity Profile for Rotenone**

| Assessment Endpoint | Species | Toxicity Value Used in Risk Assessment | Citation MRID # (Author & Date) | Probit Slope | Comment |
|---|---|---|---|---|---|
| Acute Direct Toxicity to Aquatic-Phase CRLF | Rainbow Trout (*Oncorhynchus mykiss*) | 96-h $LC_{50}$ = 1.94 µg a.i./L | 439751-02 (Sousa, 1006) | 7.2 | Acceptable |
| Chronic Direct Toxicity to Aquatic-Phase CRLF | Rainbow Trout | NOAEC = 1.01 µg a.i./L (reduced growth) | 400633-02 (Bills *et al.* 1986) | NA | Supplemental |

| | | | | | |
|---|---|---|---|---|---|
| Indirect Toxicity to Aquatic-Phase CRLF via Acute Toxicity to Freshwater Invertebrates (i.e. prey items) | *Daphnia magna* | 48-h $EC_{50}$ = 3.7 µg a.i./L | 400633-03<br><br>(Rach *et al*. 1986) | 4.1 | Supplemental |
| Indirect Toxicity to Aquatic-Phase CRLF via Chronic Toxicity to Freshwater Invertebrates (i.e. prey items) | *Daphnia magna* | NOAEC = 1.25 µg a.i./L<br><br>(reduced number of young) | 400633-03<br><br>(Rach *et al*. 1986) | NA | Supplemental |
| Indirect Toxicity to Aquatic-Phase CRLF via Acute Toxicity to Non-vascular Aquatic Plants | NA | NA | NA | NA | |
| Indirect Toxicity to Aquatic-Phase CRLF via Acute Toxicity to Vascular Aquatic Plants | NA | NA | NA | NA | |

NA = no data available

Toxicity to aquatic fish and invertebrates is categorized using the system shown in **Table 10** (U.S. EPA, 2004). Toxicity categories for aquatic plants have not been defined.

**Table 10. Categories of Acute Toxicity for Aquatic Organisms**

| $LC_{50}$ (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

Given that no acceptable rotenone toxicity data are available for aquatic-phase amphibians; freshwater fish data were used as a surrogate to estimate direct acute and chronic risks to the CRLF. Freshwater fish toxicity data were also used to assess potential indirect effects of rotenone to the CRLF. Effects to freshwater fish resulting from exposure to rotenone may 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, including data from the open literature, is provided below in Sections 4.1.1.1 through 4.1.1.3.

#### 4.1.1.1 Freshwater Fish: Acute Exposure (Mortality) Studies

Rotenone is very highly toxic to the coldwater rainbow trout (96-h $LC_{50}$ = 1.94 µg/L; MRID 439751-02). Acute toxicity testing using technical end-product (5% active ingredient) was also very highly toxic to rainbow trout (96-h $LC_{50}$ = 11.5 µg/L; Acc. No: 121873). Testing of formulated end product (Prenfish®; 5% active ingredient) using wild-caught slimy sculpins (*Cottus cognatus*) of mixed ages, resulted in a 96 $LC_{50}$ of 24 µg/L (Grisak *et al*. 2007).

The range of sensitivity for freshwater fish was evaluated using a genera sensitivity distribution (SD). The method for calculating an SD for a given assessment endpoint is dependent on the number of species and families with results for the given assessment endpoint. The values used to calculate the SD are derived from ECOTOX and registrant-submitted studies and are used for determining distribution only. Not all studies in which values were derived were reviewed. For a species with multiple tests available, the geometric mean $LC_{50}$ value for the specific species was calculated first, and then the mean $LC_{50}$ for the species' genus was calculated. The species' genus log $LC_{50}$ values are assumed to be from a normal distribution and are used to calculate the parameters of this distribution (*i.e.*, mean and SD). **Figure 9** depicts the fish species sensitivity distribution for formulated end-product and indicates that formulated end-products were no more toxic than technical grade rotenone (**Figure 8**). A normal distribution of species mean log $LC_{50}$ values and log SD values was assumed in extrapolating $95^{th}$ percentile $LC_{50}$ values for freshwater fish using technical grade and formulated rotenone (**Appendix G**).

Using this approach, the 95th percentile is 79 and 161 µg/L for technical grade and formulated end-product, respectively. These results indicate that 95% of freshwater fish will have $LC_{50}$ values less than or equal to 79 µg/L for technical grade rotenone versus 161 µg/L for formulated end-product.

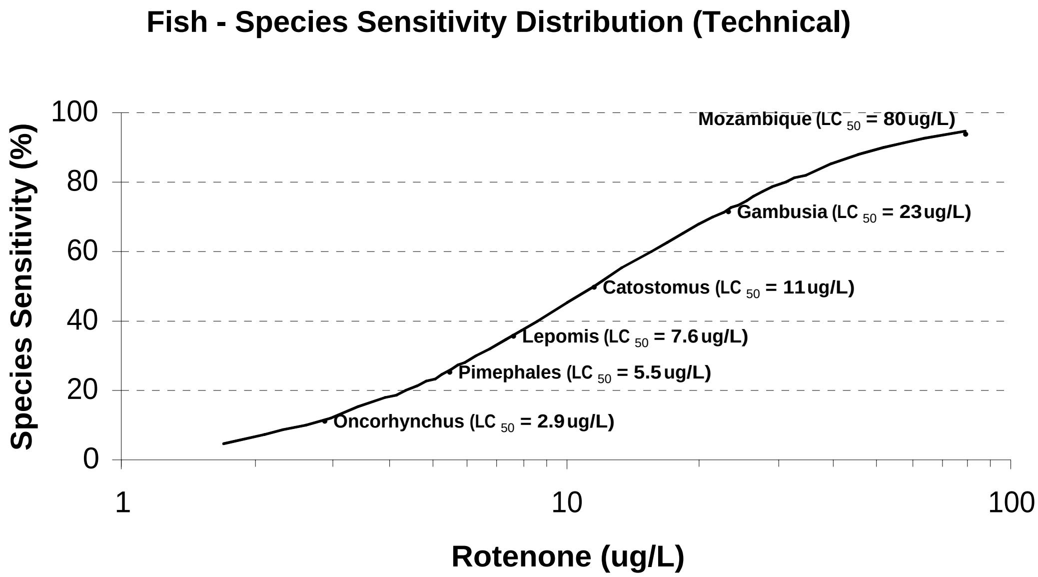


**Figure 7. Freshwater fish genera sensitivity distribution for technical grade rotenone based on the 96-h median lethal concentration.**

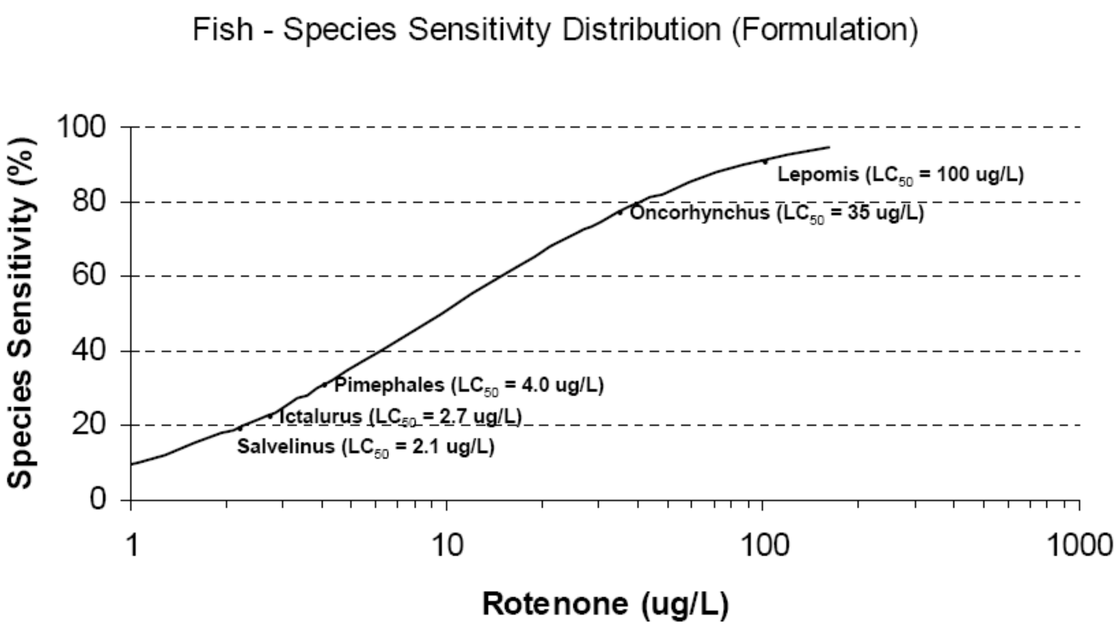


**Figure 8. Freshwater genera species sensitivity distribution for rotenone formulated end-product based on the 96-h median lethal concentration.**

There is considerable variability in the response of fish to rotenone. Both **Figures 8** and **9** show that salmonids are the most sensitive family tested to both technical grade and formulated product. Within the family of salmonids, *Oncorhynchus* is the most sensitive genus tested with technical grade rotenone whereas *Salvelinus* is the most sensitive genus tested with formulated end-product. However, *Oncorhynchus* is one of the least sensitive genera tested with formulated end-product. Additionally, where bluegill sunfish are relatively sensitive to technical grade rotenone ($LC_{50}$=7.6 µg/L), they are considerably less sensitive to the formulated product that was tested ($LC_{50}$=100 µg/L).

Thus, the use of 1.94 µg/L as an acute toxicity endpoint for evaluating the toxicity of rotenone to CRLF is highly conservative and is a more sensitive endpoint than 95% of the species for which data exist.

#### 4.1.1.2 Freshwater Fish: Chronic Exposure (Growth/Reproduction) Studies

A rainbow trout early life-stage test produced a 32-day NOAEC of 1.01 µg/L (MRID 400633-02), based on reduced growth. Length of fish was reduced by roughly 20% at rotenone concentrations of 2.2 µg/L (LOAEC).

#### 4.1.1.3 Freshwater Fish: Sublethal Effects and Additional Open Literature Information

None of the data reported in ECOTOX contained more sensitive acute toxicity endpoints than what is reported in **Table 9** for rainbow trout.

Sublethal effects were studied in adult zebrafish (*Danio rerio*) where the fish were exposed to nominal rotenone concentrations of 2 µg/L for 4 weeks; no locomotor effects were observed (Bretaud *et al.* 2004). In the same study, larval zebrafish exposed to nominal rotenone concentrations of 5 and 10 µg/L (dissolved in dimethylsulfoxide) for 4 days (24 hr post-fertilization to 5 days post-fertilization) did not display any changes in locomotor activity. Larvae exposed to up to 30 µg/L rotenone performed similarly to controls; however, at 50 µg/L, larvae showed what was described as a "degenerating phenotype" and died within 4 days of exposure. The authors attributed the lack of neurotoxic effects to the blood-brain barrier, *i.e.*, limiting access of rotenone to central nervous system neurons, or rapid metabolism of rotenone in the periphery. These data suggest that in non-mammalian species and under slightly more realistic exposure conditions, rotenone did not result in neuron degeneration.

#### 4.1.1.4 Aquatic-phase Amphibian: Acute and Chronic Studies

No acceptable toxicity data were located on the effects of rotenone or its rotenolone degradate on aquatic-phase amphibians.

### 4.1.2 Toxicity to Freshwater Invertebrates

Freshwater aquatic invertebrate toxicity data were used to assess potential indirect effects of rotenone to the CRLF. Effects to freshwater invertebrates resulting from exposure to rotenone may 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, including data published in the open literature, is provided below in Sections 4.1.2.1 through 4.1.2.3.

#### 4.1.2.1 Freshwater Invertebrates: Acute Exposure Studies

Rotenone is very highly toxic to freshwater invertebrates (*Daphnia magna* 48-h $EC_{50}$ = 3.7 µg/L; MRID 400633-03) on an acute exposure basis.

#### 4.1.2.2 Freshwater Invertebrates: Chronic Exposure Studies

In a 21-day full life-cycle study with *Daphnia magna*, the NOAEC was 1.25 µg/L (MRID 400633-03), based on roughly a 50% reduction in number of young produced.

#### 4.1.2.3 Freshwater Invertebrates: Open Literature Data

No more sensitive data on rotenone effects on freshwater invertebrates were found in the open literature.

### 4.1.3 Toxicity to Aquatic Plants

Effects to aquatic plants are used to evaluate whether rotenone 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.

No guideline aquatic plant toxicity study data were submitted and no useable data were located in the open literature for rotenone. However, rotenone is derived from plants and has in the past been used extensively on terrestrial plants as an insecticide/acraracide without report of any phytotoxicity. Rotenone has also been frequently used as a pisicide without report of any effects on aquatic plants. Additionally, there are no reports of adverse incidents involving either terrestrial and/or aquatic plants in the Ecological Incident Information System.

### 4.1.4 Freshwater Field/Mesocosm Studies

Benthic macroinvertebrate monitoring data collected by the California Department of Game and Fish (**Appendix H**) indicate on Silver King Creek (Alpine County, CA) and

Silver Creek (Mono County, CA), at the applications rates used, *i.e*., 12.5 µg a.i./L and 25 µg a.i./L, respectively, benthic macroinvertebrates were not completely eliminated from the treatment area. While there is a considerable amount of variability in the data, a qualitative assessment suggests that overall abundance may be temporarily affected (within 1 week) by rotenone treatment, although invertebrate numbers appear to return to pretreatment levels within a year. Less clear though are the potential effects of repeated rotenone treatments on macroinvertebrate diversity. Both studies indicate rotenone treatments affected the total number of stonefly (*Plecoptera spp*.) taxa as well as the total number of stoneflies. It is possible that rotenone treatments may select for more tolerant species; the ability of an aquatic system to recover from such effects would depend on the extent to which immigration from non-impacted sites can take place.

## 4.2 Toxicity of Rotenone to Terrestrial Organisms

Although risk to terrestrial-phase CRLF and their habitat is consider low since rotenone is no longer applied to terrestrial habitats, data on the toxicity of rotenone is included below. **Table 11** summarizes the most sensitive terrestrial toxicity endpoints for the CRLF, based on an evaluation of both the submitted studies and the open literature. A brief summary of submitted and open literature data considered relevant to this ecological risk assessment for the CRLF is presented below.

**Table 11. Terrestrial Toxicity Profile for Rotenone.**

| Endpoint | Species | Toxicity Value Used in Risk Assessment | Citation MRID# (Author & Date) |
|---|---|---|---|
| Acute Direct Toxicity to Terrestrial-Phase CRLF ($LD_{50}$) | Ring-necked pheasant (*Phasianus colchicus*) | $LD_{50}$ = 1680 mg/kg | MRID 143250 (Tucker 1968) |
| Acute Direct Toxicity to Terrestrial-Phase CRLF ($LC_{50}$) | Ring-necked pheasant | $LC_{50}$ = 1608 mg/kg feed | Acc. No. 248788 (Hill *et al*. 1975) |
| Chronic Direct Toxicity to Terrestrial-Phase CRLF | ND | ND | ND |
| Indirect Toxicity to Terrestrial-Phase CRLF (via acute toxicity to mammalian prey items) | Laboratory rat (*Rattus norvegicus*) | $LD_{50}$ = 39.5 mg/kg | Accession No. 00141408 (Kehoe and MacKenzie 1983) |
| Indirect Toxicity to Terrestrial-Phase CRLF (via chronic toxicity to mammalian prey items) | Laboratory rat | NOAEL = 7.5 mg/kg diet (0.5 mg/kg ; decreased body weight) | |
| Indirect Toxicity to Terrestrial-Phase CRLF (via acute toxicity to terrestrial invertebrate prey items) | Honey bee (*Apis mellifera*) | $LD_{50\ (contact)}$ > 60 µg a.i./bee | MRID 05001991 (Stevenson, J. H. 1978) |
| Indirect Toxicity to Terrestrial- and Aquatic-Phase CRLF (via toxicity to terrestrial plants) | Seedling Emergence Monocots | ND | ND |
| | Seedling Emergence Dicots | ND | ND |
| | Vegetative Vigor Monocots | ND | ND |
| | Vegetative Vigor Dicots | ND | ND |

ND=no data.

Acute toxicity to terrestrial animals is categorized using the classification system shown in **Table 12** (U.S. EPA, 2004). Toxicity categories for terrestrial plants have not been defined.

**Table 12. 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 rotenone; therefore, acute and chronic avian toxicity data are used to assess the potential direct effects of rotenone to terrestrial-phase CRLFs.

#### 4.2.1.1 Birds: Acute Exposure (Mortality) Studies

Avian acute oral toxicity data are available for the mallard duck and ring-necked pheasant. In these studies only female birds were tested. The $LD_{50}$s for the mallard duck and ring-necked pheasant, based on formulated product (32.4% a.i. rotenone), were 2200 mg/kg and 1680 mg/kg, respectively (**Table 11**; MRID 143250). Regurgitation occurred at concentrations above 1500 mg/kg. Based on these data, rotenone is classified as slightly toxic to birds and the taxa for which they serve as surrogates (reptiles and terrestrial phase amphibians) on an acute oral exposure basis.

Subacute dietary toxicity studies on formulated product (34.5% rotenone) have been conducted using ring-necked pheasants (*Phasianus colchicus*), Japanese quail (*Coturnix japonica*), and mallard ducks (*Anas platyrhynchos*). Toxicity ($LD_{50}$) values for 5-day subacute dietary toxicity studies in the three species are 1608, 1882, and 2600 ppm, respectively (**Table 11**; ACC No. 248788). Based on the most sensitive species tested, *i.e.*, ring-necked pheasants, rotenone is classified as slightly toxic to birds on a subacute dietary exposure basis.

#### 4.2.1.2 Birds: Chronic Exposure (Growth, Reproduction) Studies

No chronic toxicity data were available to assess the chronic effects of rotenone on birds.

#### 4.2.1.3 Terrestrial-phase Amphibian Acute and Chronic Studies

No acute or chronic toxicity data are available for the direct effects of rotenone or rotenolone on terrestrial-phase amphibians.

### 4.2.2 Toxicity to Mammals

Mammalian toxicity data are used to assess potential indirect effects of rotenone to the terrestrial-phase CRLF. Effects to small mammals resulting from exposure to rotenone may 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).

#### 4.2.2.1 Mammals: Acute Exposure (Mortality) Studies

Rotenone was more toxic to female rats ($LD_{50}$ = 39.5 mg/kg) compared to male rats ($LD_{50}$ = 102 mg/kg), based on an acute oral exposure (MRID 00145496)

#### 4.2.2.2 Mammals: Chronic Exposure (Growth, Reproduction) Studies

In a two-generation rat reproductive study, adult and offspring toxicity was indicated by decreased body weight (MRID 00141408). An NOAEC of 7.5 mg/kg (0.5 and 0.6 mg/kg/day for male and female, respectively) was determined based on decreased F1 and F2 pup body weight and body weight gain. The offspring toxicity LOAEL for rotenone in male and female rats was 35.7 ppm (2.4 and 3.0 mg/kg/day for male and females, respectively), based on decreased body weight (10 - 50%) and body weight gain (20 - 60%) in both generations (MRID 00141408).

As discussed in the ecological risk assessment in support of the reregistration eligibility decision on rotenone (USEPA 2003), continuous jugular vein infusion of rotenone at 2 to 3 mg/kg/day dissolved in dimethylsulfoxide for more than 5 weeks produced behavioral, biochemical and neuropathological effects that resemble Parkinson's disease in rats (Betarbet *et al.* 2000). An analogous route and level of exposure is not considered likely from the use of rotenone in fishery management.

### 4.2.3 Toxicity to Terrestrial Invertebrates

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

#### 4.2.3.1 Terrestrial Invertebrates: Acute Exposure (Mortality) Studies

Acute contact and oral toxicity studies in honey bees (*A. mellifera*) using technical grade rotenone (95% a.i) yielded $LD_{50}$ values of greater than 60 µg a.i./bee (MRID 05001991) and greater than 30 µg a.i./bee (MRID 05001991), respectively. Based on these results, rotenone is classified as practically non-toxic to honey bees on an acute contact and oral exposure basis.

#### 4.2.3.2 Terrestrial Invertebrates: Open Literature Studies

No additional information was available from open literature for quantitative purposes.

### 4.2.4 Toxicity to Terrestrial Plants

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

No toxicity studies were submitted by the registrant to evaluate the toxicity of rotenone to terrestrial plants. However, as noted previously, up until recently the chemical has been used as an insecticide/acaricide on a large number of agricultural crops without any reported phytotoxicity. On this basis, no effects of rotenone on terrestrial plants are expected.

## 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 rotenone 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 OPP Ecological Incident Information System (EIIS) database for ecological incidents involving rotenone was completed on April 1, 2008.

A total of seven rotenone-related field incidents have been reported in the OPP Ecological Incident Information System (EIIS) database; however, none were reported as being associated with the registered use of rotenone. The majority (6/7) of the reported incidents were a result of direct applications to water. The one incident reported for a residential use of rotenone (I005754-014) involved eleven humming birds being killed due to a cotton bail treated with Black Flag®. Although the incident report claims that the active ingredient of Black Flag® is rotenone, information available to this reviewer suggests that the active ingredient of Black Flag® is actually a synthetic pyrethroid and not rotenone.

There is one additional incident report that is associated with mortality of terrestrial animals in New York (I009311-001) in which a swan and a duck were killed following the treatment of a pond with synergized rotenone containing piperonyl butoxide. Two Canadian geese and one mute swan were found dead several days after the treatment; however, it was uncertain whether the mortalities could be clearly associated with the rotenone treatment.

Of the incidents associated with the piscicidal use of rotenone, the receiving waters included ponds, lakes and rivers. Of the two pond incidents reported on ponds, one occurred in South Carolina (B0000-500-56) where 300 sunfish and tadpoles were reported killed. Insufficient details were reported to understand the circumstances surrounding this incident; however, rotenone is believed to have been intentionally added to the pond for the purposes of removing its resident fish.

The second incident involving ponds (I000636-028) occurred in Missouri and resulted in thousands of fish being killed. A bait pond had been treated with rotenone to remove carp and the treated water from the pond was released into an adjoining creek. Dead fish were observed for 2.4 miles downstream.

The one incident associated with a river (I000402-001) occurred in Illinois and was an intentional misuse of the rotenone, which resulted in mortality of 11,206 fish of different species. The majority (90%) of the fish killed were minnows.

The one reported incident associated with a lake (I006514-001), where aquatic animals were affected, occurred on Lake Davis, California (this incident was due to the same treatment described in the fate assessment). Lake Davis was intentionally treated to control Northern pike and resulted in "thousands" of fish being killed and is further described in **Appendix H**. Although the EIIS does not state whether the field incident was from a registered use of the pesticide, it appears from the information available that the fish mortality was from the registered use of rotenone as a piscicide and was intentional. Additional information on this incident, was obtained from the California Department of Game and Fish and from the California Environmental Protection Agency and is discussed in greater detail in the RED (USEPA 2007a). According to a report by the California Department of Game and Fish, Lake Davis was intentionally treated over a two-day period by the California Department of Game and Fish following well defined standard operating procedures. Although potassium permanganate was used to detoxify rotenone and prevent its movement downstream, an insufficient amount of permanganate was used and fish were killed outside of the intended treatment area. The lower amount of permanganate used to detoxify the rotenone was, however, an unapproved deviation from the protocol (personal communication: Brian Finlayson, Pesticide Investigation Unit, California Department of Fish and Game, 2005).

Finally, hundreds of fish were reported killed in South Carolina (B0000300-025) following terrestrial (pesticidal) applications of rotenone; however, there are so few details concerning this report that it is difficult to understand which, if any, uses of rotenone could be implicated.

## 5. 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 rotenone 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 D**). For acute exposures to the CRLF and its animal prey in aquatic habitats, the LOC is 0.05. The LOC for chronic exposures to CRLF and its prey, as well as acute exposures to plants is 1.0.

Risk to the aquatic-phase CRLF is estimated by calculating the ratio of exposure to toxicity using the maximum treatment concentration based on the labeled rotenone application rate as a piscicide summarized in **Table 4** and the appropriate aquatic toxicity endpoint from **Table 9.**

### 5.1.1 Exposures in the Aquatic Habitat

#### 5.1.1.1 Direct Effects to Aquatic-Phase CRLF

At a maximum treatment rate of 250 µg a.i./L, acute estimated environmental concentrations of rotenone are expected to be equivalent to rotenone's solubility limit of 200 µg/L. At this exposure concentration, the RQ value ($RQ=EEC/LC_{50}$) for fish is 103 (200/1.94) and exceeds the acute risk level of concern for direct effects ($RQ \geq 0.5$) by a factor of 206X (**Table 13**). When used as a piscicide at the maximum application rate, rotenone is considered likely to cause acute mortality in aquatic-phase CRLF in the treatment area. Durations of exposure above the LOC were estimated based on the field studies and available monitoring data. In warm waters ($25^{o}C$), rotenone is expected to persist at concentrations where risk quotients would be above the acute risk LOC (based on the most sensitive species) for 17 days. However, in cold water ($5^{o}C$) it may persist for as long as 227 days above the acute risk LOC (**Table 8**). As noted above, temperatures would be unlikely to stay as low as $5^{o}C$ for 227 days except perhaps in Alaska or at high altitudes. Even at application rates that have been recommended for streams, *i.e.*, 50 µg a.i./L, the acute RQ value for fish and aquatic-phase CRLF (RQ=26) exceeds the acute risk level of concern by a factor of 52X. Application rates would have to be less than 1 µg a.i./L not to exceed the acute risk LOC for freshwater fish and aquatic-phase amphibians, and treatment rates would have to be less than 0.1 µg a.i./L not to exceed the acute risk to endangered species LOC ($RQ \geq 0.05$).

**Table 13. Summary of Direct Effect RQs for the Aquatic-phase CRLF.**

| Direct Effects to CRLF[a] | Surrogate Species | Toxicity Value (µg/L) | EEC (µg/L)[b] | RQ | Probability of Individual Effect | LOC Exceedance and Risk Interpretation |
|---|---|---|---|---|---|---|
| Acute Direct Toxicity | Rainbow trout | $LC_{50}$ = 1.94 | Peak: 200 | 103 | 1 in 1[c] | Yes[d] |
| Chronic Direct Toxicity | | NOAEC = 1.01 | 60-day: 9 | 8.9 | Not calculated for chronic endpoints | Yes[e] |

[a] RQs associated with acute and chronic 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.
[b] The highest EEC based on the maximum treatment rate of rotenone in lentic environments.
[c] A probit slope value for the acute rainbow trout toxicity test is 7.2.
[d] RQ > acute endangered species LOC of 0.05.
[e] RQ > chronic LOC of 1.0.

At the maximum application rate where initial concentrations are equivalent to the solubility limit and with a degradation half-life of 1.5 days (warm water), the 60-day average estimated environmental concentration is 9 µg/L. The chronic risk quotient (RQ=EEC/NOAEC) for fish at this concentration is 8.9 (9/1.01) (**Table 13**). With a half-life of 20 days (cold water pond), the 60-day average water concentrations is 105 µg a.i./L, and the chronic RQ value for fish is 105. Chronic risk was not calculated for streams as downstream movement of the treated water and deactivation with potassium permanganate would preclude exposure for durations sufficient to cause chronic effects. In order not to exceed the chronic risk LOC, the maximum application would have to be less than or equal to 1 µg a.i./L.

Given that both acute and chronic RQ values exceed the acute and chronic risk LOCs and the likelihood of individual mortality is 100% from acute exposure, the preliminary determination for direct effects on the aquatic-phase CRLF is a "may affect."

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

<u>Non-vascular Aquatic Plants</u>

Indirect effects of rotenone to the aquatic-phase CRLF (tadpoles) via reduction in non-vascular aquatic plants in its diet cannot be quantified because no guideline aquatic plant toxicity study data are available for either rotenone or its degradate rotenolone. However, there are anecdotal data suggesting that rotenone is not toxic to plants. First, rotenone is derived from a relatively common group of plants, the legumes. Additionally, rotenone has been used for many years as an insecticide/acaricide on terrestrial plants without any report of phytotoxicity, and rotenone has been frequently used in aquatic habitats without any report of phytotoxicity. Additionally, there are no reports of adverse effects on plants in the Ecological Incident Information System. Since there have been no reports of phytotoxicity following relatively frequent use of rotenone on plants in both the terrestrial and aquatic environments, the determination is "no effect".

<u>Aquatic Invertebrates</u>

Indirect acute effects to the aquatic-phase CRLF via effects to prey (invertebrates) in aquatic habitats are based on peak EECs and the lowest acute toxicity value for freshwater invertebrates. For chronic risks, 21-day EECs and the lowest chronic toxicity value for invertebrates 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 14**.

Similar to what was discussed above, at a maximum treatment rate of 250 µg a.i./L, the acute estimated environmental concentration of rotenone is equivalent to rotenone's solubility limit of 200 µg/L. At this exposure concentration, the RQ value ($RQ=EEC/EC_{50}$) for invertebrates is 54 (200/3.7) (**Table 14**), and it exceeds the acute risk level of concern ($RQ \geq 0.5$) by a factor of 108X. When used as a piscicide at the maximum application rate, rotenone is likely to cause acute mortality of aquatic invertebrate species in the treatment area. In warm waters (25°C), rotenone is expected to persist at concentrations where risk quotients would be above the acute risk LOC (based on the most sensitive species) for less than 11 days. However, in cold water (5°C) it may persist for as long as 142 days above the acute risk LOC (**Table 14**). As noted above, temperatures would be unlikely to stay as low as 5°C for 142 days except at high altitudes. At the maximum application rate for streams, *i.e.*, 50 µg a.i./L, the acute RQ value for invertebrates (RQ=14) exceeds the acute risk level of concern.

**Table 14. 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) .**

| Direct Effects to CRLF[a] | Surrogate Species | Toxicity Value (µg/L) | EEC (µg/L)[b] | RQ | Probability of Individual Effect | LOC Exceedance and Risk Interpretation |
|---|---|---|---|---|---|---|
| Acute Direct Toxicity | Water flea | $EC_{50}$ = 3.7 | Peak: 200 | 54 | 1 in 1[c] | Yes[d] |
| Chronic Direct Toxicity | | NOAEC = 1.25 | 21-day: 26 | 21 | Not calculated for chronic endpoints | Yes[e] |

[a] RQs associated with acute and chronic 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.
[b] The highest EEC based on the maximum treatment rate of rotenone in lentic environments.
[c] A probit slope value for the acute *Daphnia magna* toxicity test is 4.1.
[d] RQ > acute endangered species LOC of 0.05 and the acute risk to non-listed species LOC of 0.5
[e] RQ > chronic LOC of 1.0.

At the maximum application rate where initial concentrations are equivalent to the solubility limit and with a degradation half-life of 1.5 days (warm water), the 21-day average estimated environmental concentration is 26 µg a.i./L. The chronic risk quotients (RQ=EEC/NOAEC) for freshwater invertebrates at this concentration is 21 (26/1.25) (**Table 14**). With a half-life of 20 days (cold water pond), the 21-day average water concentration is 173 µg a.i./L, and the chronic RQ value for invertebrates is 138. As noted above, chronic risk estimates are not being calculated for streams as chronic exposure will not occur in these environments. With chronic RQ values ranging from 21 to 138 for freshwater invertebrates, the chronic risk LOC (RQ≥ 1) is exceeded. In order not to exceed the chronic risk LOC, the maximum application would have to be less than or equal to 1 µg a.i./L.

Given that both acute and chronic RQ values exceed the acute and chronic risk LOCs and the likelihood of individual mortality is 100% from acute exposure, the preliminary determination for indirect effects on the aquatic-phase CRLF through loss of forage is a "may affect."

Fish and Frogs

Fish and frogs also represent potential prey items of adult aquatic-phase CRLFs. RQs associated with acute and chronic direct toxicity to the CRLF (**Table 14**) are used to assess potential indirect effects to the CRLF based on a reduction in freshwater fish and frogs as food items. Based on RQ values exceeding both acute and chronic risk LOCs the preliminary determination is "may affect."

#### 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 typically estimated using the most sensitive non-vascular and vascular plant toxicity endpoints. Because there are no obligate relationships between the CRLF and any aquatic plant species, the most sensitive $EC_{50}$ values, rather than NOAEC values, are typically used to derive RQs. As noted previously, no guideline toxicity study data are available for either rotenone or its degradate rotenolone. However, there are anecdotal data suggesting that rotenone is not toxic to plants. First, rotenone is derived from a relatively common group of plants, the legumes. Rotenone has also been used for many years as an insecticide/acaricide on terrestrial plants without any report of phytotoxicity, and rotenone has been frequently used in aquatic habitats without any report of phytotoxicity. Additionally, there are no reports of adverse effects on plants in the Ecological Incident Information System. Although RQ values for non-vascular aquatic plants could not be calculated, anecdotal data indicate that plants are not affected; therefore, the preliminary determination for indirect effects to the CRLF via reduction in habitat and/or primary productivity is "no effect."

### 5.1.2 Exposures in the Terrestrial Habitat

#### 5.1.2.1 Direct Effects to Terrestrial-phase CRLF

As previously discussed in Section 3.3, potential rotenone exposure to terrestrial-phase CRLFs is considered low since it is applied exclusively to water and does not involve terrestrial applications.

Since the piscicidal use of rotenone is a direct application to water, there is little likelihood that terrestrial forage items for birds will contain rotenone residues from this use. While it is possible that some piscivorous animals may feed opportunistically on dead or dying fish located along the surface of treated waters, most protocols for the use of rotenone as a piscicide recommend that dead fish be collected and buried, rendering the fish less available for consumption. However, whole body residues in fish killed with rotenone ranged from 0.22 µg/g in yellow perch (*Perca flavescens*) to 1.08 µg/g in common carp (*Cyprinus carpio*) (Jarvinen and Ankley 1998). For a 68 g yellow perch, this represents a total of 15 µg of rotenone per fish and for an 88 g carp, it represents 95 µg/fish. The avian subacute dietary $LC_{50}$ value for Japanese quail (*Coturnix japonica*) is 1882 mg/kg diet (Hill et al., 1975) and is the closest surrogate species for an adult terrestrial-phase CRLF. The animal would have to minimally consume 125,000 perch or 19,800 small carp. Thus, it is not likely that many piscivorous animals could consume the number of fish that would be required to equate to the subacute dietary toxicity of rotenone.

Additionally, in some circumstances backpack applicators may apply rotenone to the margins of treated water bodies to assure that potential shoreline refuges are eliminated (personal communication: Dr. Brian Finlayson, Chair, Fish Management Chemicals Subcommittee of the American Fisheries Society. April 2006). While this represents a potential exposure pathway for birds that may be foraging along the shoreline, the likelihood of effects on birds is considered low. Therefore, the determination for direct effects of rotenone to terrestrial-phase CRLF is "no effect".

#### 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

Since rotenone is no longer applied to terrestrial environments the likelihood of exposure to terrestrial invertebrates is low.

##### 5.1.2.2.2 Mammals

The use of rotenone to achieve fishery management purposes, where the compound is applied directly to water, is not likely to represent a means of exposure to wild mammals relative to consumption of rotenone residues on terrestrial forage items. Additionally, formulated end-products of the piscicide are roughly three times less toxic to mammals based on an acute exposure basis. Finally, fishery managers make an effort to collect most of the fish killed during treatment and these fish are then buried. Based on the off-chance, however, that different-sized mammals could forage on dead or dying fish, it is possible to estimate the potential amount of rotenone in their diet. Using daily food intake as estimated by Nagy (1987), a 15 g mammal will consume about 3.2 g of food based on the allometric equation:

$$F=0.621*BW^{0.564}/(1-W) \qquad \textbf{(1)}$$

where F is the food intake in grams of fresh weight, BW is the body mass of the organism in grams, and W is the mass fraction of water in the food. For this assessment W is assumed to be 0.1. Using data previously cited from the common carp with a body weight of 88 grams, a small mammal would only consume 3.6% (3.2 ÷ 88) of the total carp body mass. According to the data for common carp, total body residues of rotenone in carp amounted to 1.08 µg/g. A 15-g mammal consuming 3.2 grams represents an equivalent dose of 3.5 µg of rotenone; this value is well below the median lethal dose of rotenone (39.5 mg/kg*0.015 kg=0.59 mg) for similarly sized mammals. Extrapolations from one-sized mammal to another size need to consider differences the scaling of toxicity for difference in body weight. The $LD_{50}$ can be adjusted for body weight based on the formula recommended by Mineau *et al.* 1996:

$$\text{Adj. } LD_{50} = LD_{50}(TW/AW)^{(0.25)} \qquad \textbf{(2)}$$

where the adjusted $LD_{50}$ is the median 50% lethal dose for the species being assessed, $LD_{50}$ is the median lethal dose in the test organism, AW is the body weight of the assessed organism and TW is the body weight for the test organism. Since we are assessing a small mammal, 15 g is a suitable value for AW. The test organism is a rat weighing about 350 g. With a test $LD_{50}$ of 39.5 mg/kg, the adjusted $LD_{50}$ is 87 mg/kg. Using the daily intake equation, a 15-g mammal will consume about 3.2 g of food. If the animal fed exclusively on carp killed by rotenone, the equivalent dose would be 3.2 g *1.08 µg/g or 3.5 µg of rotenone. Once again, this value is below the estimated median lethal equivalent concentration adjusted for body weight (87 mg/kg * 0.015 kg=1.31 mg). Therefore, although fish are typically collected and buried to the extent possible following a rotenone treatment, even if fish were available for consumption by mammals scavenging along the shoreline for dead or dying fish, it is not likely that the mammals would be able to consume sufficient quantities of rotenone to result in acute toxicity.

As mentioned previously, rotenone may be applied to shorelines where mammals could be potentially foraging. The likelihood of effects to mammals is considered low since it is likely that only a very small percentage of the shoreline is treated for the piscicidal use. Additionally, the qualitative assessment of the number of prey items a mammal would have to consume to produce and effect indicated a low likelihood of effects. Therefore, potential risk to mammals from this use is considered low.

#### 5.1.2.2.3 Frogs

Since rotenone is applied directly to water and terrestrial exposure is not considered likely, potential effects of the piscicidal use of rotenone on terrestrial-phase frogs that may serve as prey for terrestrial-phase CRLF is considered low. To the extent though that terrestrial-phase CRLF are dependent on aquatic-phase amphibians which are likely to be subject to both acute and chronic effects from the use of rotenone as a piscicide, the preliminary effects determination for indirect effects on terrestrial-phase CRLF through reduction in prey is "may effect".

#### 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 typically assessed using RQs from terrestrial plant seedling emergence and vegetative vigor $EC_{25}$ data as a screen. However, there are no guideline toxicity study data with which to evaluate potential effects of rotenone on terrestrial plants. Up until recently, rotenone has been applied to a wide variety of agricultural crops with no reported phytotoxicity, and there are no reported incidents involving rotenone and effects on plants. Furthermore, rotenone is now only applied directly to water and not to terrestrial environments. Therefore, the likelihood of adverse effects on terrestrial plants is considered low and the preliminary determination for indirect effects to CRLF through reductions in terrestrial plant community is “no effect”.

### 5.1.3 Primary Constituent Elements of Designated Critical Habitat

For rotenone use, the assessment endpoints for designated critical habitat PCEs involve a reduction and/or modification of food sources necessary for normal growth and viability of aquatic-phase CRLFs, and/or a reduction and/or modification of food sources for terrestrial-phase juveniles and adults. Because these endpoints are also being assessed relative to the potential for indirect effects to aquatic- and terrestrial-phase CRLF, the effects determinations for indirect effects from the potential loss of food items are used as the basis of the effects determination for potential modification to designated critical habitat.

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

Three of the four assessment endpoints for the aquatic-phase primary constituent elements (PCEs) of designated critical habitat for the 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).

The preliminary effects determination for the first two aquatic-phase PCEs of designated habitat related to potential effects on aquatic and/or terrestrial plants is “no effect”, based on the risk estimation for plants. To assess the impact of rotenone on the third PCE, acute and chronic freshwater fish and invertebrate toxicity endpoints are used as measures of effects. RQs for these endpoints were calculated in Sections 5.1.1.1 and 5.1.1.2. Based on both acute and chronic effects on aquatic vertebrates and invertebrates that may serve as forage for aquatic-phase amphibians the preliminary effect determination is “may affect”.

The remaining aquatic-phase PCE is “alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.” Rotenone use is not expected to affect the chemical characteristics of the water since primary productivity is not considered likely to be affected. Therefore, the determination for the fourth PCE is “no effect”.

#### 5.1.3.2 Terrestrial-Phase (Upland Habitat and Dispersal Habitat)

Two of the four assessment endpoints for the terrestrial-phase PCEs of designated critical habitat for the 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 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 preliminary effects determination for terrestrial-phase PCEs of designated habitat related to potential effects on terrestrial plants is “no effect”, based on the risk estimation provided in Section 5.1.2.3.

The third terrestrial-phase PCE is “reduction and/or modification of food sources for terrestrial phase juveniles and adults.” To assess the impact of rotenone on this PCE, acute and chronic toxicity endpoints for birds, mammals, and terrestrial invertebrates are used as measures of effects. Since rotenone is applied directly to water, exposure to either terrestrial animals or plants is not considered likely. However, to the extent that the terrestrial-phase CRLF is dependent on aquatic-phase amphibians/aquatic invertebrates as prey and these forage items are likely to be affected through both acute and chronic effects of rotenone applications to water, indirect effects on the terrestrial-phase CRLF forage base may occur. Therefore, the preliminary effects determination is “may affect”.

The fourth terrestrial-phase PCE is based on alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source. Direct acute and chronic RQs for terrestrial-phase CRLFs are presented in Section 5.1.2. Since rotenone is applied directly to water, exposure to either terrestrial animals or plants is not considered likely; therefore, the preliminary determination for the fourth terrestrial-phase PCE is “no effect”.

## 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.

If the RQs presented in the Risk Estimation (Section 5.1) show no direct or indirect effects for the CRLF, and no modification to PCEs of the CRLF's designated critical habitat, a "no effect" determination is made, based on rotenone's use as a piscicide within the action area. However, if direct or indirect effect LOCs are exceeded or effects may modify the PCEs of the CRLF's critical habitat, the Agency concludes a preliminary "may affect" determination for the FIFRA regulatory action regarding rotenone. A summary of the results of the risk estimation is provided in **Table 15** for direct and indirect effects to the CRLF and in **Table 16** for the PCEs of designated critical habitat for the CRLF.

**Table 15. Risk Estimation Summary for rotenone - Direct and Indirect Effects to CRLF .**

| Assessment Endpoint | LOC Exceedance (Y/N) | Basis For Preliminary Determination |
|---|---|---|
| ***Aquatic-Phase (eggs, larvae, tadpoles, juveniles, and adults)*** | | |
| Survival, growth, and reproduction of CRLF individuals via direct effects on aquatic phases | Yes | Acute and chronic RQ values for direct effects to the aquatic-phase CRLF exceed the acute and chronic risk LOCs by factors of 206X and 105X, respectively. Likelihood of individual mortality from acute exposure is 100%. |
| Survival, growth, and reproduction of CRLF individuals via effects to food supply (*i.e.*, freshwater invertebrates, non-vascular plants) | Yes | Acute and chronic RQ values for indirect effects to the aquatic-phase CRLF through acute and chronic effects to aquatic invertebrates exceed the acute and chronic risk LOCs by factors of 108X and 21X, respectively Likelihood of individual mortality from acute exposure is 100%. |
| Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat, cover, and/or primary productivity (*i.e.*, aquatic plant community) | No | Although there are no guideline toxicity study data on the effects of rotenone on plants, rotenone is derived from plants and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants; therefore, the potential risk to plants is considered low. |
| 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. | No | Although there are no guideline toxicity study data on the effects of rotenone on plants, rotenone is derived from plants and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants; therefore, the potential risk to plants is considered low. |

| | | |
|---|---|---|
| ***Terrestrial-Phase (Juveniles and adults)*** | | |
| Survival, growth, and reproduction of CRLF individuals via direct effects on terrestrial-phase adults and juveniles | No | Rotenone is applied directly to water and the likelihood of exposure to terrestrial animals is considered low. RQ values were not calculated based on the lack of exposure. |
| Survival, growth, and reproduction of CRLF individuals via effects on prey (*i.e.*, terrestrial invertebrates, small terrestrial mammals and terrestrial-phase amphibians) | Yes | Rotenone is applied directly to water and the likelihood of exposure to terrestrial animals is considered low. However, to the extent that the terrestrial-phase CRLF is dependent on aquatic-phase amphibians/aquatic invertebrates as prey and these forage items are likely to be affected through both acute and chronic effects of rotenone, indirect effects on the terrestrial-phase CRLF forage base may occur. |
| Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat (*i.e.*, riparian vegetation) | No | Rotenone is applied directly to water and the likelihood of exposure to terrestrial animals is considered low. Although there are no guideline toxicity study data on the effects of rotenone on plants, rotenone is derived from plants and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants; therefore, the potential risk to plants is considered low. |

**Table 16. Preliminary Effects Determination Summary for Rotenone – PCEs of Designated Critical Habitat for the CRLF.**

| Assessment Endpoint | LOC Exceedance (Y/N) | Basis For Preliminary Determination |
|---|---|---|
| ***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 avoidance, and aquatic dispersal for juvenile and adult CRLFs. | No | Although there are no guideline toxicity study data on the effects of rotenone on plants, rotenone is derived from plants and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants; therefore, the potential risk to plants is considered low. |
| 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. | No | Although there are no guideline toxicity study data on the effects of rotenone on plants, rotenone is derived from plants and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants; therefore, the potential risk to plants is considered low. |

| Assessment Endpoint | LOC Exceedance (Y/N) | Basis For Preliminary Determination |
|---|---|---|
| Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source. | Yes | Acute and chronic RQ values for direct effects to the aquatic-phase CRLF exceed the acute and chronic risk LOCs by factors of 206X and 105X, respectively. Likelihood of individual mortality from acute exposure is 100%. Forage items for the aquatic-phase CRLF are likely to be subject to both acute mortality and chronic effects. |
| Reduction and/or modification of aquatic-based food sources for pre-metamorphs (*e.g.*, algae) | No | Although there are no guideline toxicity study data on the effects of rotenone on plants, rotenone is derived from plants and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants; therefore, the potential risk to plants is considered low. |
| ***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 | No | Rotenone is applied directly to water and as such, exposure of terrestrial habitats is not considered likely. Although there are no guideline toxicity study data on the effects of rotenone on plants, rotenone is derived from plants and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants; therefore, the potential risk to plants is considered low. |
| 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 | No | Rotenone is applied directly to water and as such, exposure of terrestrial habitats is not considered likely. Although there are no guideline toxicity study data on the effects of rotenone on plants, rotenone is derived from plants and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants; therefore, the potential risk to plants is considered low. |
| Reduction and/or modification of food sources for terrestrial-phase juveniles and adults | Yes | Rotenone is applied directly to water and as such, exposure of terrestrial habitats is not considered likely. However, to the extent that the terrestrial-phase CRLF is dependent on aquatic-phase amphibians/aquatic invertebrates as prey and these forage items are likely to be affected through both acute and chronic effects of rotenone, indirect effects on the terrestrial-phase CRLF forage base may occur. |
| Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source. | No | Rotenone is applied directly to water and as such, exposure of terrestrial habitats is not considered likely. Although there are no guideline toxicity study data on the effects of rotenone on plants, rotenone is derived from plants and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants; therefore, the potential risk to plants is considered low. |

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.

Since rotenone is applied directly to aquatic habitat, and there are currently no restrictions upon where rotenone could be applied to the waters of California, these determinations apply to designated critical habitats for the CRLF.

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:

- <u>Significance of Effect</u>: 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.
- <u>Likelihood of the Effect Occurring</u>: Discountable effects are those that are extremely unlikely to occur.
- <u>Adverse Nature of Effect</u>: 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 considers life stages of the frog that are obligatory aquatic organisms, including eggs and larvae. It also considers submerged terrestrial-phase juveniles and adults, which spend a portion of their time in water bodies that may treated with rotenone.

Acute RQ values exceed the acute risk LOC for direct acute mortality of aquatic-phase amphibians. The likelihood of direct individual mortality is 100% given that at maximum treatment concentrations (200 µg/L), the $LC_{50}$ for the most sensitive fish species tested (rainbow trout $LC_{50}$=1.94 µg/L) is exceeded by a factor of 103X. Although the $LC_{50}$ value is for a 96-hr exposure, there is a strong likelihood that at the maximum treatment concentration, acute mortality will ensue shortly after application. Additionally, calculation of the likelihood of individual effects demonstrates that at the acute RQ value of 206, a 100% chance of mortality will occur. At the acute risk to listed species LOC of 0.05, the likelihood of individual effects in 1 in 2.7 x $10^{20}$ and would be considered insignificant; however, given that the RQ is several orders of magnitude higher than the acute risk to listed species LOC, the likelihood of individual effects is considerably higher. Also, rotenone is intended to kill fish and there are incident reports demonstrating fish kills in areas where rotenone moved outside of targeted treatment area, the available data indicates that aquatic-phase CRLFs would be subject to both acute and chronic effects from the use of rotenone as a piscicide. Therefore, the determination is for a may affect and "Likely to Adversely Affect (LAA)".

#### 5.2.1.2 Terrestrial-Phase CRLF

Since rotenone is applied directly to water, the likelihood of exposure to terrestrial animals is considered low. Therefore, the determination for direct effects to terrestrial-phase CRLF is for "no effect".

### 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. Although, no aquatic plant guideline toxicity study data are available for either rotenone or its degradate rotenolone, rotenone is derived from plants of the Leguminosae family and there are no reports of adverse effects on plants. Additionally, up until recently, rotenone was used as an insecticide/acaricide on roughly 96 agricultural crops with no apparent phytotoxicity. Therefore, the determination for indirect effects to the aquatic-phase CRLF via reduction in habitat/primary productivity is "no effect".

#### 5.2.2.2 Aquatic Invertebrates

The potential for rotenone 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.

RQ values for freshwater invertebrates exceed the acute and chronic risk LOCs by factors of 108X and 21X, respectively. Additionally, the likelihood of individual mortality from acute exposure is 100% at the highest RQ value calculated. However at the acute risk to listed species LOC, the likelihood of individual effects, *i.e.*, 1 out of 2.69 x $10^{20}$, is considered insignificant. Given the likelihood of individual acute mortality at the maximum treatment concentrations, there is a likelihood of indirect effects on the aquatic-phase CRLF through reductions in aquatic invertebrate forage items and the determination is may affect and "Likely to Adversely Affect (LAA)".

#### 5.2.2.3 Fish and Aquatic-phase Frogs

As described in Section 5.2.1.1, direct applications of rotenone to water as a piscicide are intended to kill fish and by extension, aquatic-phase amphibians. RQ values exceed acute and chronic risk LOCs by factors of 206X and 105X, respectively, at the maximum treatment concentration allowed by the label. The likelihood of individual mortality at a maximum treatment concentration of 200 µg/L is 100% and there are incident data indicating that in areas where rotenone has extended beyond targeted treatment areas, large numbers of fish have been killed. Therefore, the determination for indirect effects on aquatic-phase CRLF through reductions in fish and other species of aquatic-phase frogs that may serve as forage is may affect and "Likely to Adversely Affect (LAA)".

#### 5.2.2.4 Terrestrial Invertebrates

Since rotenone is applied directly to water, the likelihood of exposure to terrestrial areas is considered low. As such, the likelihood of risk to terrestrial invertebrates is considered low and the determination for indirect effects to terrestrial-phase CRLF through reductions in terrestrial invertebrate forage items is "no effect".

#### 5.2.2.5 Mammals

Since rotenone is applied directly to water, the likelihood of exposure to terrestrial areas is considered low. As such, the likelihood of risk to terrestrial vertebrates is considered low. Additionally, a qualitative assessment of the risk to piscivorous mammals from consumption of rotenone residues in prey indicates a low likelihood of effects. Therefore, the determination for indirect effects to terrestrial-phase CRLF through reductions in terrestrial vertebrate forage items is "no effect".

#### 5.2.2.6 Terrestrial-phase Amphibians

Since rotenone is applied directly to water, the likelihood of exposure to terrestrial areas is considered low. While it is possible that terrestrial-phase amphibians could consume fish and invertebrates immobilized by rotenone through direct application to water, it is not considered likely that these potential forage items would be attractive to terrestrial-phase frogs given that the fish and invertebrates would be dead. Available forage items would be reduced through the loss of the aquatic vertebrates/invertebrates; however, terrestrial invertebrates would not be impacted and thus could remain a reasonable alternative forage base for terrestrial-phase amphibians. Therefore, the determination for effects on terrestrial-phase CRLF is may affect but "Not Likely to Adversely Affect".

### 5.2.3 Indirect Effects (via Habitat Effects)

#### 5.2.3.1 Aquatic Plants (Vascular and Non-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, rather than energy, to the system, as attachment sites for many aquatic invertebrates, and refugia for juvenile organisms, such as fish and frogs. Emergent plants help reduce sediment loading and provide stability to near shore areas and lower stream banks. In addition, vascular aquatic plants are important as attachment sites for egg masses of CRLFs. Although no aquatic plant guideline toxicity study data are available for either rotenone or its degradate rotenolone, rotenone is derived from plants and there are no reported incidents involving plants. Additionally, up until recently, rotenone was used as an insecticide/miticide on roughly 96 agricultural crops with no apparent phytotoxicity. Therefore, the determination for indirect effects to the aquatic-phase CRLF via reduction in habitat/primary productivity is a "no effect".

#### 5.2.3.2 Terrestrial Plants

Since rotenone is applied directly to water, exposure to terrestrial plants is considered negligible. Although there are no guideline toxicity study data on the effects of rotenone on plants, rotenone is derived from plants and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants; therefore, the potential risk to plants is considered low. Therefore, since exposure is negligible and effects are not considered likely, risk is likewise negligible and the determination is for a "no effect".

### 5.2.4 Modification to Designated Critical Habitat

#### 5.2.4.1 Aquatic-Phase PCEs

Three of the four assessment endpoints for the aquatic-phase primary constituent elements (PCEs) of designated critical habitat for the 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).

The effects determinations for indirect effects to the CRLF via direct effects to aquatic and terrestrial plants are used to determine whether modification to critical habitat may occur. In spite of the lack of toxicity data on either aquatic or terrestrial plants, rotenone is derived from plants and has been used on a wide range of agricultural plants as an insecticide/acaricide with no apparent sign of phytotoxicity. Rotenone use as a piscicide is not expected to affect either of the first two PCE. The third 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 effects. Fish, aquatic-phase amphibians and aquatic invertebrates are considered likely to be subject to both acute and chronic effects from the use of rotenone as a piscicide. Therefore, these taxa are likely to be reduced in rotenone-treated waters and as such the PCE would have been modified. The determination for the third PCE is for "Habitat Modification (HM)".

The remaining aquatic-phase PCE is "alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source." Since rotenone is not likely to affect plants nor is it likely to affect the chemical characteristics of the habitat, the determination is for "no effect".

#### 5.2.4.2 Terrestrial-Phase PCEs

Two of the four assessment endpoints for the terrestrial-phase PCEs of designated critical habitat for the 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.

Rotenone is no longer applied to the terrestrial environment. Rather, it is now applied exclusively to aquatic environments. Additionally, since rotenone is derived from plants and there are no reported incidents involving terrestrial plants, the determination is for "no effect (NE)".

The third terrestrial-phase PCE is "reduction and/or modification of food sources for terrestrial phase juveniles and adults." To assess the impact of rotenone on this PCE, acute and chronic toxicity endpoints for terrestrial invertebrates, mammals, and terrestrial-phase frogs are used as measures of effects. Although the preliminary determination was for a may affect since the forage base for terrestrial amphibians (serving as prey to terrestrial-phase CRLF) could be reduced through the loss of aquatic forage items, alternative terrestrial prey items are considered sufficiently available. Therefore, the determination is for "no effect (NE)".

The fourth terrestrial-phase PCE is based on alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source. Since rotenone is not applied to terrestrial environment, the likelihood of exposure is considered low; therefore the determination is for “no effect”.

# 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 treatment rates, which is determined from labeled statements of maximum treatment rate. The frequency at which actual uses approach this maximum treatment rate may be dependant on fishery management objectives, timing of applications, cultural practices, and market forces.

### 6.1.2 Aquatic Exposure Modeling of Rotenone

Aquatic exposure estimates are based on the maximum treatment concentration for rotenone in lentic environments. Lower treatment concentrations are likely used; however, the use of lower rates is dependent on environmental conditions and management objectives.

### 6.1.3 Usage Uncertainties

County-level usage data were obtained from California’s Department of Pesticide Regulation Pesticide Use Reporting (CDPR PUR) database. Four 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. However, these data only include the historic agricultural and non-agricultural uses of rotenone, but do not include the use of the chemical as a piscicide.

### 6.1.4 Deactivation Uncertainties

Although there is anecdotal information regarding the efficacy of rotenone deactivation with potassium permanganate, there is uncertainty regarding the spatial and temporal aspects of the deactivation process. Bioassays are typically employed to demonstrate that rotenone has been deactivated and has not moved beyond the designated treatment area; however, it is possible that the bioassay species may not be representative of the most sensitive species and that the effects of rotenone could extend further than anticipated. Additionally, at this time, there are no data to indicate what products are formed through the deactivation process.

## 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 and open literature data on rotenone 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 sub-lethal data in the assessment 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 rotenone 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.

### 6.2.5 Lack of Aquatic Plant Toxicity Data

As mentioned previously, there is uncertainty regarding the potential toxicity of rotenone to aquatic plants. However, rotenone is derived from plants and, up until recently, the compound was used extensively on plants as an insecticide/acaricide with no reported adverse effects on plants. To the extent that rotenone is toxic to plants, this assessment is not conservative.

## 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 rotenone to the CRLF and its designated critical habitat.

Based on the best available information, the Agency makes a Likely to Adversely Affect (LAA) determination for the CRLF from the use of rotenone. Additionally, the Agency has determined that there is the potential for modification of CRLF designated critical habitat (HM) from the use of the chemical. RQs for direct effects of rotenone to CRLF exceed acute and chronic risk LOCs by factors of 206X and 105X, respectively, and the likelihood of individual mortality of aquatic-phase CRLF is 100%. Indirect effects to the CRLF may also occur through the loss of both aquatic vertebrate and invertebrate forage items. Although there are no guideline toxicity study data with which to assess the effects of rotenone to aquatic and/or terrestrial plants, the potential for rotenone to cause phytotoxicity is considered low since the chemical is naturally occurring plant flavinoid, historical use of rotenone on agricultural crops has not resulted in any reported phytotoxicity, and there are no reported ecological incidents involving plants.

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 **Tables 17** and **18**.

**Table 17. Effects Determination Summary for Direct and Indirect Effects of Rotenone on the CRLF.**

| Assessment Endpoint | Effects Determination[1] | Basis for Determination |
|---|---|---|
| ***Aquatic-Phase CRLF (Eggs, Larvae, and Adults)*** | | |
| Direct Effects:<br>Survival, growth, and reproduction of CRLF individuals via direct effects on aquatic phases | LAA | Rotenone is intended to kill fish and RQ values exceed the acute risk LOC by a factor of 206X. The likelihood of individual acute mortality is 100%. RQ values for fish exceed the chronic risk LOC by a factor of 105X. |
| Indirect Effects:<br>Survival, growth, and reproduction of CRLF individuals via effects to food supply (*i.e.*, freshwater invertebrates, non-vascular plants, fish, and frogs) | Freshwater invertebrates: LAA | RQ values exceed the acute risk LOC by a factor of 108X. The likelihood of individual acute mortality is 100%. RQ values for aquatic invertebrates exceed the chronic risk LOC by a factor of 21X |
| | Non-vascular aquatic plants: NE | Rotenone is a naturally occurring plant flavinoid and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants. |
| | Fish and frogs: LAA | Rotenone is intended to kill fish and RQ values exceed the acute risk LOC by a factor of 206X. The likelihood of individual acute mortality is 100%. RQ values for fish exceed the chronic risk LOC by a factor of 105X. |
| Indirect Effects:<br>Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat, cover, and/or primary productivity (*i.e.*, aquatic plant community) | Non-vascular aquatic plants: NE | Although there are no guideline toxicity study data on the effects of rotenone on plants, rotenone is derived from plants and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants; therefore, the potential risk to plants is considered low. |
| | Vascular aquatic plants: NE | Although there are no guideline toxicity study data on the effects of rotenone on plants, rotenone is derived from plants and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants; therefore, the potential risk to plants is considered low. |
| Indirect Effects:<br>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. | NE | Although there are no guideline toxicity study data on the effects of rotenone on plants, rotenone is derived from plants and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants; therefore, the potential risk to plants is considered low. Additionally, rotenone is applied directly to water and exposure to terrestrial plants is not considered likely. |
| ***Terrestrial-Phase CRLF (Juveniles and adults)*** | | |
| Direct Effects:<br>Survival, growth, and reproduction of CRLF individuals via direct effects on terrestrial phase adults and juveniles | NE | Rotenone is applied directly to water and the likelihood of exposure to terrestrial animals is considered low. |
| Indirect Effects:<br>Survival, growth, and reproduction of CRLF individuals via effects on prey (*i.e.*, terrestrial invertebrates, small terrestrial | Terrestrial invertebrates: NE | Rotenone is applied directly to water and the likelihood of exposure to terrestrial animals is considered low. |
| | Mammals: NE | Rotenone is applied directly to water and the likelihood of exposure to terrestrial animals is considered low. |

| | | |
|---|---|---|
| vertebrates, including mammals and terrestrial phase amphibians) | | Qaulitative assessment of potential risks to mammals indicates that any potential exposure through consumption of residues in aquatic prey items would not result in effects. |
| | Frogs: NLAA | Rotenone is applied directly to water and the likelihood of exposure to terrestrial animals is considered low. Although the forage base for terrestrial amphibans (serving as prey to terrestrial-phase CRLF) could be reduced through the loss of aquatic forage items, alternative terrestrial prey items are considered sufficiently available. |
| Indirect Effects:<br>Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat (*i.e.*, riparian vegetation) | NE | Although there are no guideline toxicity study data on the effects of rotenone on plants, rotenone is derived from plants and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants; therefore, the potential risk to plants is considered low. Additionally, rotenone is applied directly to water and exposure to terrestrial plants is not considered likely. |
| [1] NE = no effect; NLAA = may affect, but not likely to adversely affect; LAA = likely to adversely affect | | |

**Table 18. Effects Determination Summary for the Critical Habitat Impact Analysis.**

| Assessment Endpoint | Effects Determination[1] | Basis for Determination |
|---|---|---|
| ***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. | NE | Although there are no guideline toxicity study data on the effects of rotenone on plants, rotenone is derived from plants and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants; therefore, the potential risk to plants is considered low. |
| 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.[3] | NE | Although there are no guideline toxicity study data on the effects of rotenone on plants, rotenone is derived from plants and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants; therefore, the potential risk to plants is considered low. |
| Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source. | HM | Rotenone is intended to kill fish and RQ values exceed the acute risk LOC by a factor of 206X. The likelihood of individual acute mortality is 100%. RQ values for fish exceed the chronic risk LOC by a factor of 83X; therefore, habitat modification is likely given substantial effects to aquatic forage base (availability of food). |
| Reduction and/or modification of aquatic-based food | NE | Although there are no guideline toxicity study data |

[3] 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.

| | | |
|---|---|---|
| sources for pre-metamorphs (*e.g.*, algae) | | on the effects of rotenone on plants, rotenone is derived from plants and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants; therefore, the potential risk to plants is considered low. |
| ***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 | NE | Although there are no guideline toxicity study data on the effects of rotenone on plants, rotenone is derived from plants and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants; therefore, the potential risk to plants is considered low. |
| 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 | NE | |
| Reduction and/or modification of food sources for terrestrial-phase juveniles and adults | NE | Although there are no guideline toxicity study data on the effects of rotenone on plants, rotenone is derived from plants and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants; therefore, the potential risk to plants is considered low. Additionally, rotenone is applied directly to water and exposure to terrestrial plants is not considered likely. Although the forage base for terrestrial amphibians (serving as prey to terrestrial-phase CRLF) could be reduced through the loss of aquatic forage items, alternative terrestrial prey items are considered sufficiently available. |
| Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source. | NE | Although there are no guideline toxicity study data on the effects of rotenone on plants, rotenone is derived from plants and there are no reported phytotoxic effects associated with the historical use of the compound as an insecticide/acaricide on plants; therefore, the potential risk to plants is considered low. Additionally, rotenone is applied directly to water and exposure to terrestrial plants is not considered likely. |
| 1 NE = No effect; HM = Habitat modification | | |

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 to seek concurrence with the LAA determinations and to determine whether there are reasonable and prudent alternatives and/or measures to reduce and/or eliminate potential incidental take.

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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