Risks of Tribufos Use to Federally Threatened California Red-legged Frog

(*Rana aurora draytonii*)

Pesticide Effects Determination

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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 use of tribufos on agricultural sites. In addition, this assessment evaluates whether these actions can be expected to result in modification of the species' designated critical habitat. This assessment was completed in accordance with the U.S. Fish and Wildlife Service (USFWS) and National Marine Fisheries Service (NMFS) *Endangered Species Consultation Handbook* (USFWS/NMFS, 1998) and procedures outlined in the Agency's Overview Document (USEPA, 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.

Tribufos is an organophosphate chemical used as a defoliant. Currently, tribufos is only registered for use on cotton.

When released to soil, tribufos degrades very slowly under both aerobic and anaerobic conditions with half-lives exceeding 100 days and up to 745 days in sandy loam soil under dark aerobic conditions. Therefore, tribufos is more persistent than is typical for most organophosphate chemicals. The only major degradate of tribufos is 1-butane sulfonic acid. Tribufos is not expected to leach to groundwater or move to surface water through dissolved runoff at high levels, based on an average K_{oc} value of 9,284 mL/g. However, surface water contamination may occur from runoff via adsorption on eroding soil and from spray drift. The potential for volatilization from soil and water is expected to be low. Neither hydrolysis nor photolysis represents important degradation pathways under environmental conditions.

Since CRLFs exist within aquatic and terrestrial habitats, exposure of the CRLF, its prey, and its habitats to tribufos are assessed separately for the two habitats. Tier-II aquatic exposure models are used to estimate high-end exposures of tribufos in aquatic habitats resulting from runoff and spray drift from its use on cotton. The peak model-estimated environmental concentration for tribufos use on cotton in California is 16.3 µg/L. The modeled estimates are supplemented with analysis of available California surface water monitoring data from U. S. Geological Survey's National Water Quality Assessment (NAWQA) program and the California Department of Pesticide Regulation (CDPR). There were no detections of tribufos reported by NAWQA for California surface waters out of a total of 211 collected water samples analyzed for this chemical. Out of 750 water sample results reported by the CDPR surface water database, there were only two tribufos detections (both at 0.01 µg/L). Though these are not targeted studies likely to find peak concentrations, available monitoring data suggest that the peak model-estimated environmental concentration (16.3 µg/L) (modeled for cotton use in California) is protective.

To estimate tribufos exposures to the terrestrial-phase CRLF and its potential prey resulting from uses involving tribufos applications, the T-REX model (version 1.3.1, 2006) is used for foliar applications. The AgDRIFT model (version 2.01) is also used to estimate deposition of tribufos on terrestrial and aquatic habitats from spray drift. The TerrPlant model (version 1.2.2, 2006) is used to estimate tribufos exposures to terrestrial-phase CRLF habitat, including plants inhabiting semi-aquatic and dry areas, resulting from uses involving foliar tribufos applications. The T-HERPS model (version 1.0, 2007) is used to allow for further characterization of dietary exposures of terrestrial-phase CRLFs relative to birds.

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

Typically, organophosphate pesticides have several degradates of toxicological concern, including oxons. However, only one major degradate, 1-butane sulfonic acid, was observed in the available tribufos fate studies. No toxicity data are available on this degradate, but it was determined to be of no toxicological concern in previous risk assessments (*e.g.*, USEPA 2006). Therefore, the degradation product is not considered a major stressor. Additionally, tribufos is itself an oxon. Therefore, only the parent compound is assessed here.

Based on acute exposure, tribufos is characterized as 'highly toxic' to freshwater fish (which are also used as a surrogate for aquatic-phase amphibians) (LC_{50} = 0.245 mg a.i./L); 'very highly toxic' to freshwater invertebrates (EC_{50} = 0.0068 mg a.i./L); 'moderately toxic' to birds (which are used as a surrogate for terrestrial-phase amphibians) (LD_{50} = 151 mg/kg-bw) and mammals (LD_{50} = 192 mg a.i./kg-bw); and 'practically nontoxic to honey bees (LD_{50} >24.17 µg a.i./bee). Like other organophosphates, there is evidence that tribufos inhibits brain, plasma, and erythrocyte cholinesterase (ChE) activity in animals after acute and chronic exposures.

For chronic exposure, the calculated NOAEC value for freshwater fish is 3.5 µg a.i./L based on clinical signs of intoxication, reduced post-hatch survival, and reduced growth. The NOAEC for freshwater invertebrates is 1.56 µg a.i./L based on reduced number of young/adult/day and adult length. For birds, the NOAEC is 280 mg/kg-diet based on reduced egg production and hatchling survival. A mammalian two-generation reproduction study resulted in a NOAEC of 32 mg/kg-diet [1.7 mg/kg/day] based on increased stillborn pups and pup death, decreased F1 and F2 pup body weights, and increased F1 gestation periods. For non-vascular plants, the EC_{50} = 0.148 mg a.i./L and the NOAEC = 0.0585 mg a.i./L. For terrestrial plants (seedling emergence), onion was the most sensitive monocot species tested (NOAEC 0.45 lb a.i./A; EC_{25} >2.0 lb a.i./A) and soybean was the most sensitive dicot species tested (NOAEC of 0.22 lb a.i./A and an EC_{25} of > 2.0 lb a.i./A) based on reduced plant height. In the vegetative vigor study, corn (monocot) and buckwheat (dicot) were the most sensitive species based on reduced dry weight (NOAEC and EC_{25} values for corn were 0.48 lb a.i./A and 1.8 lb a.i./A, respectively; NOAEC and EC_{25} values for buckwheat were 0.48 lb a.i./A and 1.3 lb a.i./A, respectively). Because tribufos affects the abscission zone in plants, plants that do not form abscission zones (such as those used in the vegetative vigor and seedling emergence studies discussed above) are not expected to be as sensitive to tribufos as plants that form abscission zones.

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 tribufos 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, frogs, terrestrial invertebrates, and mammals) or habitat (*i.e.*, aquatic plants and terrestrial upland and riparian vegetation). When RQs for each particular type of effect are below LOCs, the pesticide is determined to have “no effect” on the CRLF. Where RQs exceed LOCs, a potential to cause adverse effects is identified, leading to a conclusion of “may affect.” If a determination is made that use of tribufos 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 tribufos. Additionally, the Agency has determined that there is the potential for modification of CRLF designated critical habitat from the use of the chemical. This is based on the potential for direct effects (to both aquatic and terrestrial-phase CRLF), indirect effects due to potential decreases in aquatic and terrestrial prey items, and the potential for modification of designated critical habitat due to habitat degradation and potential loss of aquatic and terrestrial prey items. A summary of the risk conclusions and effects determinations for the CRLF and its critical habitat is presented in **Tables 1.1** and **1.2**. Further information on the results of the effects determination is included as part of the Risk Description in Section 5.2. Given the LAA determination for the CRLF and potential modification of designated critical habitat, a description of the baseline status and cumulative effects for the CRLF is provided in **ATTACHMENT 2**.

TABLE 1.1 Effects Determination Summary for Tribufos Use and the CRLF.

Assessment Endpoint	Effects Determination [1]	Basis for Determination
Survival, growth, and/or reproduction of CRLF individuals	LAA	**Potential for Direct Effects**
		Aquatic-phase (Eggs, Larvae, and Adults): - RQs for acute and chronic effects for freshwater fish (used as a surrogate for aquatic-phase CRLFs) exceed the endangered species LOC - Many of the CRLF habitat areas are adjacent to and/or overlap with potential tribufos (cotton) use sites.
		Terrestrial-phase (Juveniles and Adults): - Acute dietary-based RQs exceed endangered species LOCs for frogs eating small insectivorous and herbivorous mammals, as well as frogs eating terrestrial invertebrates. - Many of the CRLF habitat areas are adjacent to and/or overlap with potential tribufos (cotton) use sites. - The chance of individual effects (*i.e.*, mortality) for a terrestrial-phase CRLF is as high as ~1 in 1,210.
		Potential for Indirect Effects
		Aquatic prey items, aquatic habitat, cover and/or primary productivity - The Agency's non-endangered species LOCs are exceeded for aquatic invertebrates (acute and chronic), fish (chronic) and aquatic-phase frogs (chronic). - Many of the CRLF habitat areas are adjacent to and/or overlap with potential tribufos (cotton) use sites. - Effects to aquatic invertebrates could extend to 285 km downstream from site of tribufos application. - Therefore, there is a potential for prey item reduction via impacts to all aquatic-phase CRLF prey items.
		Terrestrial prey items, riparian habitat - The Agency's non-endangered species LOCs are exceeded for mammals (15-g and 35-g) and frogs (acute and chronic). - Due to tribufos' mechanism of action, its use could result in effects to non-target plants important to CRLF (*e.g.*, deciduous shrubs and trees) - Many of the CRLF habitat areas are adjacent to and/or overlap with potential tribufos use sites - Therefore, there is a potential for prey item reduction via impacts to terrestrial-phase CRLF prey items.

[1] LAA = 'Likely to Adversely Effect'

TABLE 1.2. Effects Determination Summary for Tribufos Use and CRLF Critical Habitat Impact Analysis.

Assessment Endpoint	Effects Determination	Basis for Determination
Modification of aquatic-phase PCE	Habitat Modification	- There is a potential for effects to terrestrial plants (*e.g.*, deciduous shrubs and trees) based on tribufos' mechanism of action, which could alter aquatic habitats for the CRLF that are important for shelter, foraging, cover, and the normal growth and viability of CRLFs. - There is a potential for prey item reduction via impacts to all aquatic-phase prey items (aquatic invertebrates, fish, and frogs).
Modification of terrestrial-phase PCE		- There is a potential for effects to terrestrial plants (*e.g.*, deciduous shrubs and trees) based on tribufos' mechanism of action, which could alter terrestrial habitats for the CRLF that are important for shelter, foraging, cover, and the normal growth and viability of CRLFs. - There is a potential for prey item reduction via potential reduction in mammalian and amphibian prey items.

Based on the conclusions of this assessment, a formal consultation with the U.S. Fish and Wildlife Service under Section 7 of the Endangered Species Act should be initiated. When evaluating the significance of this risk assessment's direct/indirect and 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 to EPA. 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* (USEPA 1998), the Services' *Endangered Species Consultation Handbook* (USFWS/NMFS 1998) and is consistent with procedures and methodology outlined in the Overview Document (USEPA 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 use of tribufos as a defoliant on cotton. In addition, this assessment evaluates whether the use of tribufos on cotton 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)) 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 (USEPA 2004). Screening level methods include use of standard models such as PRZM-EXAMS, T-REX, TerrPlant, and AgDRIFT, all of which are described at length in the Overview Document. In order to refine our assessment of the potential for direct effects to the frog, T-HERPS was also used to calculate RQs for the CRLF using toxicity data from birds and the ingestion rate of insectivorous iguanids. Use of such information is consistent with the methodology described in the Overview Document (USEPA 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 USEPA 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 tribufos 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 tribufos 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 tribufos 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 tribufos 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 tribufos.

If a determination is made that use of tribufos within the action area associated with the CRLF "may affect" this species or its designated critical habitat, additional information is considered to refine the potential for exposure and for effects to the CRLF and other taxonomic groups upon which these species depend (*e.g.,* aquatic and terrestrial vertebrates and invertebrates, aquatic plants, riparian vegetation, *etc*.). Additional information, including spatial analysis (to determine the geographical proximity of CRLF habitat and tribufos use sites) and further evaluation of the potential impact of tribufos 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 tribufos is expected to directly impact living organisms within the action area (defined in Section 2.7), critical habitat analysis for tribufos 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 tribufos 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

Tribufos, first registered in the U.S. in 1961, is an organophosphate plant growth inhibitor that is currently registered in the U.S. for use on cotton only (as a defoliant). Tribufos is applied via spray to cotton plants prior to harvesting via aerial or ground equipment.

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

Although current registrations of tribufos allow for its use nationwide, this assessment addresses currently registered uses of tribufos 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.

In soil, the only major degradate observed was 1-butane sulfonic acid, identified at about 10% of the initial amount of radioactivity applied at day 272. This degradate was determined to be of no toxicological concern in previous risk assessments (*e.g.*, USEPA 2006a). Therefore, the degradation product is not considered a major stressor, and only the parent compound is assessed here. There are currently no registered multi-a.i. products that contain tribufos in the U.S., and no data on mixtures including tribufos are available.

2.3 Previous Assessments

A Reregistration Eligibility Decision (RED) for tribufos [based on the Interim Registration Eligibility Decision (IRED) signed in 2000] was signed in 2006 (USEPA 2006a). The RED identified acute and chronic risks to birds, mammals, and aquatic invertebrates (both freshwater and estuarine/marine) from the registered use of tribufos on cotton. Acute risks to estuarine/marine fish, but not freshwater fish, were also identified. Neither risks to fish from chronic exposure to tribufos nor risks to plants could be fully evaluated at that time due to a lack of data. After the RED was signed, the following studies were submitted to and reviewed by the Agency:

- Terrestrial field dissipation study (on MS soil) (MRID 458637-01)
- Life-cycle toxicity study with saltwater mysid (MRID 458637-02)
- Early life stage toxicity study with the fathead minnow (MRID 458637-03)
- Tier 2 seedling emergence and vegetative vigor phytotoxicity study with DEF 6 (tribufos end-use product) (MRID 458637-04)
- Vascular aquatic plant toxicity study (MRID 458637-05)
- Acute toxicity study with the fathead minnow (MRID 458637-06)
- Early life stage toxicity study with the sheepshead minnow (MRID 458637-07)

The results from these studies, along with other available data, are considered in this assessment.

2.4 Stressor Source and Distribution

2.4.1 Environmental Fate Properties

Tribufos is a colorless to yellow liquid. Selected chemical properties are summarized below.

Common Name:	Tribufos
Chemical Name:	*S,S,S*-tributyl phosphorotrithioate (see **Fig. 2.1**)
Chemical Family:	Organophosphate
CAS Registry Number:	78-48-8
Empirical Formula:	$C_{12}H_{27}OPS_3$
Molecular Weight:	314.5 g/mole

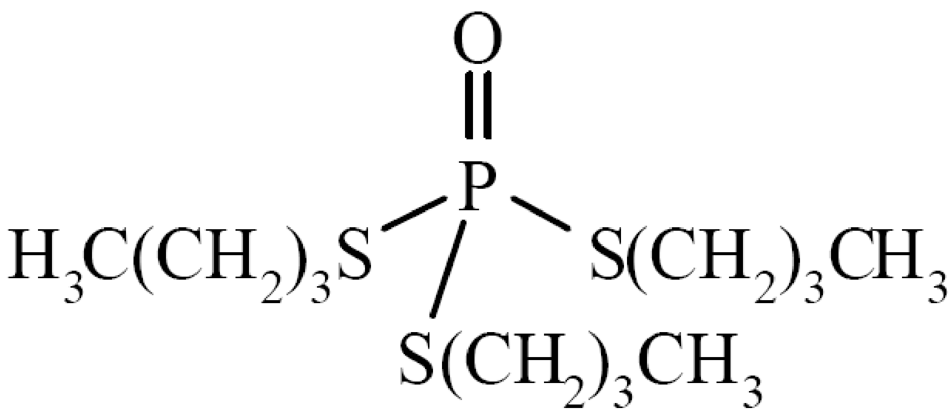


FIGURE 2.1. Tribufos (*S,S,S*-tributyl phosphorotrithioate) Chemical Structure.

In general, tribufos is persistent and immobile. When released to soil, tribufos degrades very slowly under both aerobic and anaerobic conditions with half-lives exceeding 100 days. Tribufos degraded very slowly (745-day half-life) in sandy loam soil incubated aerobically in the dark at 25 ± 1°C for up to 360 days (MRID 420072-04). Therefore, tribufos is more persistent than is typical for most organophosphate chemicals. The only major degradate observed in the aerobic soil metabolism study was 1-butane sulfonic acid, identified at about 10% of the initial amount of radioactivity applied at day 272. Under anaerobic conditions, a half-life of 389 days was observed for tribufos in sandy loam soil (MRID 420072-05). However, because tribufos use sites (cotton fields) are not flooded, the primary route of dissipation appears to be aerobic soil metabolism. Based on laboratory data, there is a potential for tribufos to accumulate in soil with repeated applications.

In a supplemental field dissipation study (MRID 458637-01) the half-life of the chemical in a Mississippi, sandy loam soil was 13.9 days (DT_{90} = 14 – 28 days). The dissipation pattern was biphasic, with an initial rapid decline through approximately 28 days post-treatment, followed by slower dissipation for the duration of the study period. The major route of dissipation of tribufos under terrestrial field conditions was not reported. The patterns of formation and decline of transformation products were not addressed and the transformation products were not identified in the study; therefore, it could not be determined whether the study duration was adequate to capture such information. In addition, only one site representative of the intended tribufos use site (cotton) was studied. The dissipation half-life from this study is not consistent with the laboratory fate data and suggests that there are dissipation pathways that have not been addressed in laboratory fate studies.

Tribufos is moderately soluble, with an aqueous solubility limit of 2.3 mg/L (MRID 41618814). Tribufos is not expected to leach to groundwater, based on K_{oc} values in the range of 4,870 to 12,684 mL/g (average = 9,284 mL/g) (MRID 416188-17). However, surface water contamination may occur from runoff via adsorption to eroding soil and/or spray drift. Due to its relatively high soil/water partitioning, the concentration of tribufos adsorbed to suspended organic carbon and bottom sediment is typically expected to be much greater than that in sediment pore water or the water column. Based on its tendency to bind to soil, ground water contamination as a result of tribufos use is not expected.

The potential for volatilization from soil and water is expected to be low due to tribufos' low vapor pressure (1.7×10^{-6} torr at 25ºC). The Henry's Law Constant for tribufos was calculated to be 9.50E-07 atm-m^3/mol. There was no evidence of tribufos degradation by hydrolysis (MRID 41618814) or photolysis (MRID 41719401) in laboratory studies simulating environmental conditions. The hydrolysis study is classified as supplemental because the kinetics portion and degradate identification portion of the study were run at higher than the solubility limit (5 and 20 mg/L, respectively), and 10.8-30.4% of applied radioactivity adsorbed to the glass vials.

Two bioaccumulation studies on bluegill sunfish (*Lepomis macrochirus*) were submitted for tribufos; one for the in-life portion study of bioaccumulation (MRID 416188-11) and one to supplement that study with metabolite identification (MRID 430804-01). In the in-life study, bluegill sunfish were continuously exposed to a mean concentration of 6.2 µg/L of tribufos for 35 days. After 14 days of depuration, 71-88% of residues were eliminated from fish tissues. Bioconcentration factors (BCF) were determined to be 300X for edible tissue and 1300X for non-edible tissues, with a whole fish bioconcentration factor of 730X. The metabolite identification study found that tribufos was the only compound detected in the edible tissue and water samples. In viscera, tribufos parent was the major extractable residue at 33% of radioactivity. In addition to parent, 47 other metabolites were extracted from the viscera; however only three were identified at radioactivities between 3 - 4%. The BCF of 730X indicates the potential of bioaccumulation of tribufos in potential fish prey items.

Table 2.1 lists the environmental fate properties of tribufos, along with the major and minor degradates detected in the submitted environmental fate and transport studies.

TABLE 2.1 Summary of Tribufos Environmental Fate Properties.

Study	Value (units)	Major Degradates *Minor Degradates*	MRID #	Study Status
Hydrolysis	No evidence of degradation	--	41618814	Supplemental
Direct Aqueous Photolysis	No evidence of degradation (pH 5, 25°C up to 30 days)	--	41719401	Acceptable
Soil Photolysis	No evidence of degradation on a sandy loam soil	--	41618816	Acceptable
Aerobic Soil Metabolism	745 day half-life on sandy loam soil	Only major degradate = 1-butane sulfonic acid (9.9% applied radioactivity on day 272)	42007204	Acceptable
Anaerobic Soil Metabolism	389 day half-life on sandy loam soil	Primary degradate – 1-butane sulfonic acid (max of 3.4% of applied at 61 days – 31 days of anaerobic incubation) and 3.5% at 90 days (60 days of anaerobic incubation)	42007205	Acceptable
Anaerobic Aquatic Metabolism	4 – 6 month half-life (flooded silty clay)	One degradate, 1-butane sulfonic acid, was identified in both floodwater and sediment (max of 29.5 – 30.6% of applied at 6 and 9 months post-treatment) (primarily in floodwater through 2 months and sediment 6 – 12 months)	43325504	Acceptable
$K_{d\text{-}ads}$ / $K_{d\text{-}des}$ (mL/g) $K_{oc\text{-}ads}$ / $K_{oc\text{-}des}$ (mL/g)	Not mobile in sand, sandy loam, silt loam, and clay loam soils Freundlich K_{ads} values = 61 – 106 K_{oc}'s ranged from 4870 – 12684 (average = 9,284)	--	41618817	Acceptable
Terrestrial Field Dissipation	13.9 day half-life (MS soil; sandy loam) DT90 = 14 – 28 days	Not addressed	45863701	Supplemental
Aquatic Field Dissipation	--	--	--	--
Vapor pressure	1.7×10^{-6} torr (at 25ºC)	--	41618803	Acceptable
Henry's Law Constant	9.50E-07 atm-m^3/mol	--	N/A (calculated)	--

2.4.1 Environmental Transport Mechanisms

Due to its relatively high soil/water partitioning, tribufos is not expected to leach to groundwater or move to surface water through dissolved runoff. Although groundwater contamination as a result of tribufos use is not expected, surface water contamination may occur from runoff via adsorption to eroding soil and/or spray drift. Additionally, tribufos is expected to partition from the water column to the sediment based on its K_{oc} value. Based on laboratory data, there is a potential for tribufos to accumulate in soil with repeated applications. Additionally, fish bioconcentration factors (BCFs) in the range of 300 (fillet) and 1,300 (viscera) suggest the possibility of bioaccumulation (MRID 43080401). The potential for volatilization from soil and water is expected to be low.

2.4.2 Mechanism of Action

Tribufos is an organophosphate chemical. When applied to plants and absorbed by its leaves, it injures the cells in the layer immediately beneath the leaf cuticle (via esterase inhibition), causing stress to the plant via stimulation of ethylene production. The increased ethylene concentrations result in the premature abscission of whole green leaves. Due to its mechanism of action, terrestrial plants that form abscission zones (*e.g.*, some woody and deciduous plants) are expected to be more sensitive to tribufos than annual plants. Like other organophosphates, there is evidence that tribufos inhibits brain, plasma, and erythrocyte cholinesterase (ChE) activity in animals after acute and chronic exposures.

2.4.3 Use Characterization

Analysis of labeled use information is the critical first step in evaluating the federal action. The current labels for tribufos represent 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.

The only registration for tribufos in the U.S. is for use on cotton (as a defoliant) to remove leaves prior to harvesting. There are currently four tribufos products registered in the U.S. (one product is a technical grade for use in the manufacture of cotton defoliants and three are end-use products) (see **Table 2.2**). Only the end-use products are assessed here because the technical grade chemical is only labeled for use in producing end use products.

TABLE 2.2. Summary of Tribufos Products Registered in the U.S.

Product Name (EPA Reg. No.)	Registrant	Percent Active Ingredient	Use
DEF® Technical (264-720)	Bayer Crop Science	98	For use in the manufacture of cotton defoliants
DEF® 6 Emulsifiable Defoliant (264-730)	Bayer Crop Science	70.5	Cotton defoliant
Folex® 6 EC (5481-504)	Amvac Chemical Corp.	70.5	Cotton defoliant
DFT® 6 EC Cotton Defoliant (34704-867)	Loveland Products, Inc.	70.5	Cotton defoliant

Tribufos can be used nationally in areas where cotton is grown (primarily the southeastern and southwestern U.S.). The application rates, application methods, and label restrictions are the same for all three of the currently registered tribufos end-use products; however, the application rates are geographically-dependant (see **Table 2.3**). The maximum seasonal application rate (the same for all three end-use products) is 1.875 lb a.i./acre/season in CA and AZ. The maximum application rate for CA will be used in this assessment. The maximum seasonal application rate in other states (*i.e.*, all states except CA and AZ) is 1.125 lb a.i./acre/season.

Because the defoliant must penetrate the leaf surface to work, the performance of tribufos is affected by a variety of factors including the condition of the plant and weather variables during application. Therefore, the registered rates of application vary geographically. Tribufos is most effective when it is applied to cotton leaves with thin leaf cuticles (the waxy layer surrounding the leaf) and is applied at times when light intensity and humidity are high (which result in more open stomata). Higher rates of tribufos are required in the drier and lower humidity climates of CA and AZ because thick leaf cuticles, which are more prevalent in these states, result in lower uptake through the leaves.

In all states, two applications, not to exceed the seasonal maximum application rate, are allowed; however, the application interval is not specified on the current labels. Applications are limited to foliar applications via ground and aerial equipment; applications via irrigation systems are not allowed. According to the labels, higher application rates and/or diesel oil rather than water as the spray carrier is recommended in adverse conditions (*e.g.*, low temperature, especially temperatures below 60^{o}F at night; low humidity) or when plants are stressed.

TABLE 2.3. Summary of Tribufos Application Methods and Rates (for Use as a Cotton Defoliant).

Product	Application method(s)	Application Rate (lbs a.i.)/Acre/Season	Max. No. of Applications/Season	Application Interval	Label Restrictions
DEF® 6 Emulsifiable Defoliant	Ground sprayer Aerial sprayer	All states except CA and AZ: 1.125	2 [the total application rate not to exceed the seasonal maximum rate per acre (a total of 1.125 lb a.i/acre/season in all states except CA and AZ; and a total of 1.875 lb a.i./acre/season in CA and AZ)]	Not specified	Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean high water mark. Do not apply where runoff is likely to occur. Do not apply when weather conditions favor drift from areas treated. Do not apply through any type of irrigation equipment. Use higher rates and/or diesel oil rather than water as the spray carrier in adverse conditions (*e.g.*, low temperature, especially temperatures below 60^{o}F at night; low humidity) or when plants are stressed.
Folex® 6 EC (70.5% a.i.)		CA and AZ: 1.875			
DFT® 6 EC Cotton Defoliant (70.5% a.i.)					

Table 2.4 presents the use and corresponding application rate and methods of application considered in this assessment.

TABLE 2.4. Tribufos Use Assessed for the CRLF

Use (Application Method)	Max. Single Appl. Rate (lb ai/A)	Max. Number of Application per Year
Cotton (foliar ground and aerial)	1.875*	2

*This is also the seasonal maximum application rate.

According to the United States Geological Survey's (USGS) national pesticide usage data (based on information from 1999 to 2004), an average of 2,407,397 lbs of tribufos per year are applied nationally to cotton in the U.S. (see **Fig. 2.2**). Tribufos usage is limited to cotton-growing regions of the U.S. (primarily southwestern and southeastern states).

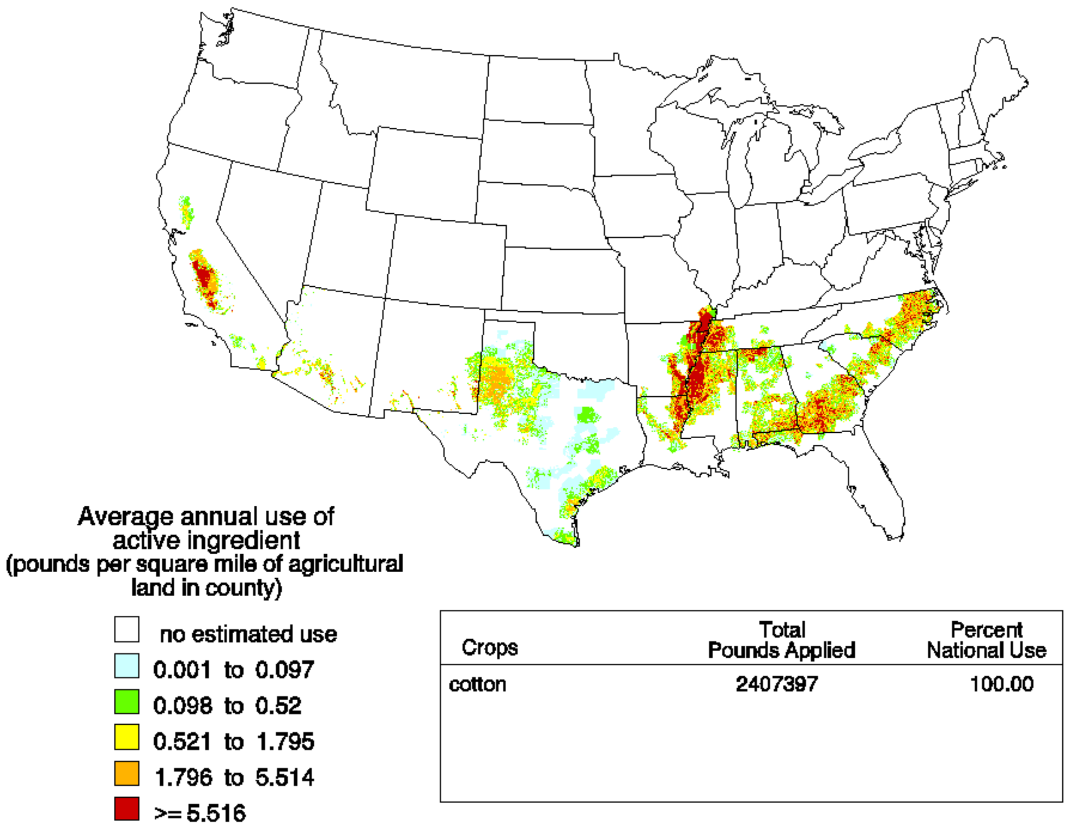


FIGURE 2.2. Estimated Annual Tribufos Usage in the U.S.
(from http://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=02&map=m8009)
[The pesticide use maps available from this site show the average annual pesticide use intensity expressed as average weight (in pounds) of a pesticide applied to each square mile of agricultural land in a county. The area of each map is based on state-level estimates of pesticide use rates for individual crops that were compiled by the CropLife Foundation, Crop Protection Research Institute based on information collected during 1999 through 2004 and on 2002 Census of Agriculture county crop acreage. The maps do not represent a specific year, but rather show typical use patterns over the five year period 1999 through 2004.]

The Agency's Biological and Economic Analysis Division (BEAD) provides an analysis of both national- and county-level usage information (Kaul and Jones 2006) using state-level usage data obtained from USDA-NASS[1], Doane (www.doane.com; the full dataset is not provided due to its proprietary nature) and the California's Department of Pesticide Regulation Pesticide Use Reporting (CDPR PUR) database[2]. CDPR PUR is considered a more comprehensive source of usage data than USDA-NASS or EPA proprietary databases, and thus the usage data reported for tribufos by county in this California-specific assessment were generated using CDPR PUR data. Five years (2002-2006) of usage data were included in this analysis. Data from CDPR PUR were obtained for every pesticide application made on every use site at the section level (approximately one

[1] United States Depart of Agriculture (USDA), National Agricultural Statistics Service (NASS) Chemical Use Reports provide summary pesticide usage statistics for select agricultural use sites by chemical, crop and state. See http://www.usda.gov/nass/pubs/estindx1.htm#agchem.
[2] The California Department of Pesticide Regulation's Pesticide Use Reporting database provides a census of pesticide applications in the state. See http://www.cdpr.ca.gov/docs/pur/purmain.htm.

square mile) of the public land survey system. BEAD summarized these data to the county level by site, pesticide, and unit treated. Calculating county-level usage involved summarizing across all applications made within a section and then across all sections within a county for each use site and for each pesticide. The county level usage data that were calculated include: average annual pounds applied, average annual area treated, and average and maximum application rate across all five years. The units of area treated are also provided where available.

Considering only cotton uses (the only currently registered use of tribufos in the U.S.), the CDPR PUR data indicate that from 2002 to 2006, an average of 222,284 lbs of tribufos were applied to an average of 157,660 acres of cotton per year in CA. This results in an average application rate of 1.41 lb a.i./acre/year (222,284 lbs/157,660 acre). From 2002 to 2006, tribufos was reportedly used in 14 CA counties (listed in descending order for average lb applied/year): Fresno, Kings, Merced, Kern, Riverside, Madera, Imperial, Tulare, Colusa, Yolo, San Bernardino, San Joaquin, Glenn, and Butte (see **Fig. 2.3**). Based on the CA usage data, tribufos use (overall and in each county where the chemical was used) has declined over the past five years. For example, in 2002 a total of 345,355 lbs of tribufos was applied to cotton in CA, whereas a total of 77,133 lbs was applied in 2006 (see **APPENDIX A**). This decrease in tribufos usage is correlated with a decrease in overall cotton acreage in California during the same time period. Based on USDA-NASS data, cotton acreage in California dropped from 690,000 acres in 2002 to 560,000 acres in 2006 (see http://www.nass.usda.gov/Data_and_Statistics/Quick_Stats/).

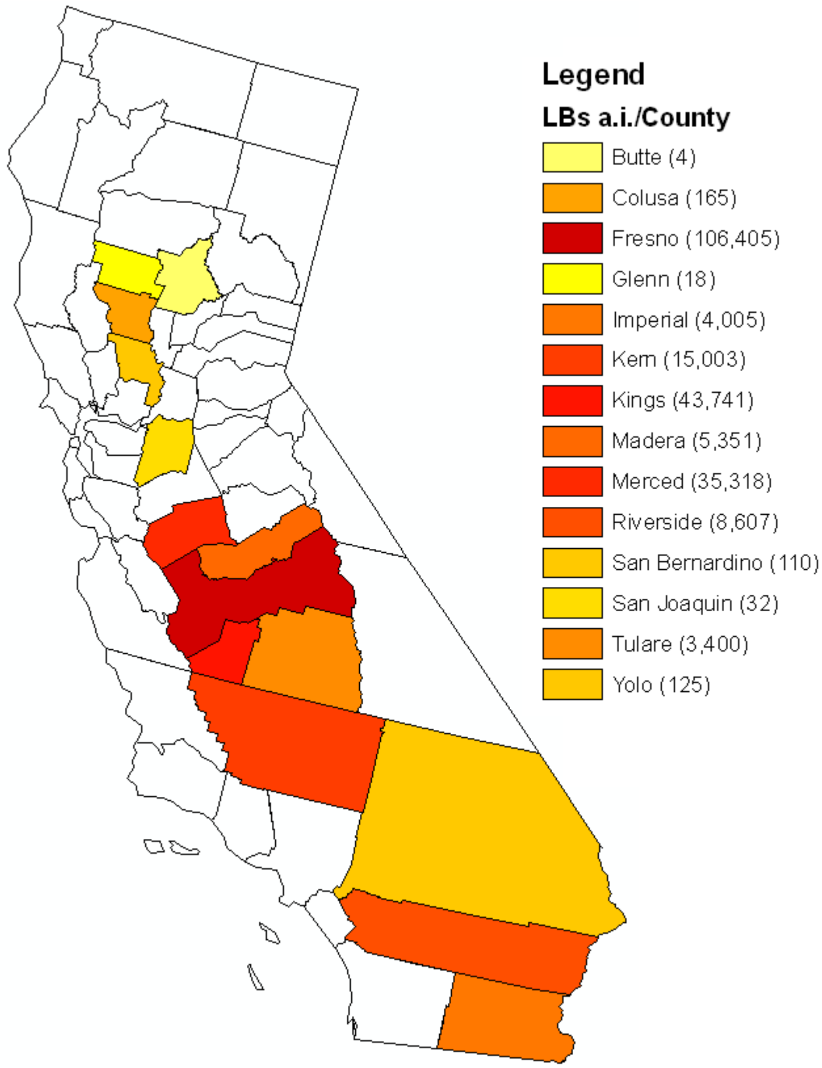


FIGURE 2.3. Average Pounds of Tribufos Applied/Year/CA County from 2002-2006.

Considering each CA county where tribufos was used, the average application rate per county/year from 2002 to 2006 ranged from 0.86 to 1.70 lb a.i./acre. The average 95th% and 99th% application rate and the maximum reported application rate per county/year all ranged from 1.48 to 1.875 lb a.i./acre (some counties reported 99th% and max application rates higher than the registered use rates; however, these values are considered misreports or misuses) (see **Table 2.5**). These data indicate that, in most CA counties, at least some tribufos users are using the chemical at or near maximum registered application rates. The maximum application rate and maximum applications per season for tribufos were consistent throughout the years of analysis of CA PUR data.

TABLE 2.5. Summary of California Department of Pesticide Registration (CDPR) Pesticide Use Reporting (PUR) Data from 2002 to 2005 for the Currently Registered Tribufos Use (Cotton).

Average Pounds Applied/Year (for All Counties)	County	Avg Annual Area Treated (Acres)	Avg App Rate (lb a.i./appl)	Avg 95th% App Rate (lb a.i./appl)	Avg 99th% App Rate (lb a.i./appl)	Avg Max App Rate (lb a.i./appl)
222,284	Butte	4	0.97	0.99	0.99	0.99
	Colusa	102	1.70	1.85	1.85	1.85
	Fresno	65,927	1.61	1.87	4.44*	15.2*
	Glenn	14	1.25	1.48	1.48	1.48
	Imperial	4,639	0.86	1.69	4.23*	4.48*
	Kern	12,839	1.27	1.54	1.85	4.36*
	Kings	32,235	1.39	1.85	1.87	11.98*
	Madera	3,625	1.42	1.85	2.0	8.53*
	Merced	25,768	1.40	1.85	1.89	4.44*
	Riverside	9,533	0.86	1.78	1.85	6.81*
	San Bernardino	79	1.41	1.51	1.51	1.51
	San Joaquin	28	0.92	1.48	1.48	1.48
	Tulare	2,782	1.25	1.66	1.85	1.85
	Yolo	84	1.48	1.48	1.48	1.48
	All counties	157,659	1.27	1.63	1.68	1.52

* These rates are higher than 1.875 lb a.i./acre (the max registered application rate); therefore, they are considered misreports or misuses, and not included in summary calculations.

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.

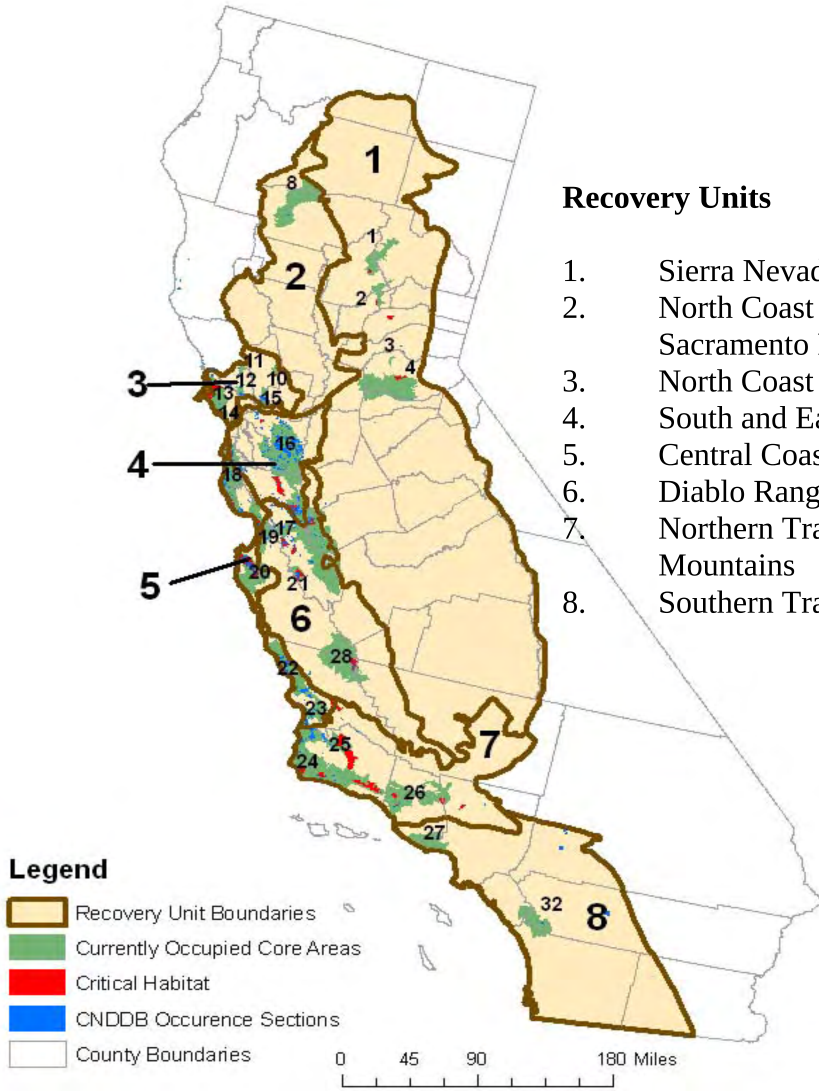
2.5.1 Distribution

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 2.4**). Recovery units, core areas, and other known occurrences of the CRLF from the CNDDB are mapped below and are described in further detail in **APPENDIX B**, 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

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

FIGURE 2.4. Recovery Unit, Core Area, Critical Habitat, and Occurrence Designations for CRLF.

Core Areas

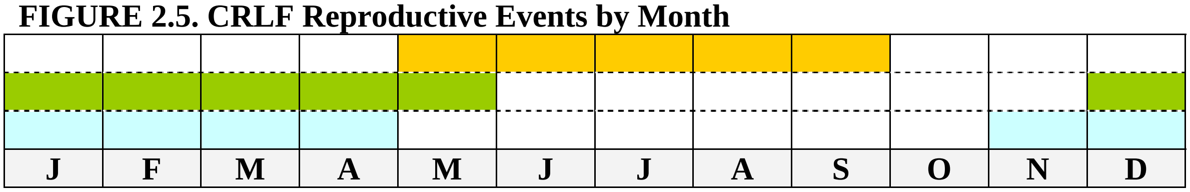
1. Feather River
2. Yuba River- S. Fork Feather River
3. Traverse Creek/ Middle Fork/ American R. Rubicon
4. Cosumnes River
5. South Fork Calaveras River*
6. Tuolumne River*
7. Piney Creek*
8. Cottonwood Creek
9. Putah Creek – Cache Creek*
10. Lake Berryessa Tributaries
11. Upper Sonoma Creek
12. Petaluma 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

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 2.5** depicts CRLF annual reproductive timing.

FIGURE 2.5. 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 2.4.

'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 tribufos 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 tribufos is expected to directly impact living organisms within the action area, critical habitat analysis for tribufos 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 tribufos is likely to encompass considerable portions of the southeastern and southwestern United States based on its use on cotton. However, the scope of this assessment limits consideration of the overall action area to those portions that may be applicable to the protection of the CRLF and its designated critical habitat within the state of California. The Agency's approach to defining the action area under the provisions of the Overview Document (USEPA 2004) considers the results of the risk assessment process to establish boundaries for that action area with the understanding that exposures below the Agency's defined Levels of Concern (LOCs) constitute a no-effect threshold. For the purposes of this assessment, attention is focused on the footprint of the action (*i.e.*, the area where pesticide application occurs), plus all areas where offsite transport (*i.e*., spray drift, runoff, *etc*.) may result in potential exposure within the state of California that exceeds the Agency's LOCs.

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

The definition of action area requires a stepwise approach that begins with an understanding of the federal action. The federal action is defined by the currently labeled use for tribufos. An analysis of available product labels was completed. The analysis indicates that, for tribufos, the only use considered as part of the federal action evaluated in this assessment is the chemical's use on cotton.

Following a determination of the assessed use, an evaluation of the potential "footprint" of tribufos use patterns (*i.e*., the area where pesticide application occurs) is determined. This "footprint" represents the initial area of concern, based on an analysis of available land cover data for the state of California. The initial area of concern is defined as all land cover types and the stream reaches within the land cover areas that represent cotton acreage in California. A map representing all the land cover types that make up the initial area of concern for tribufos is presented in **Figure 2.6**. The National Land Cover Database (NLCD 2001) cultivated crop layer is used to represent sites of potential cotton acreage.

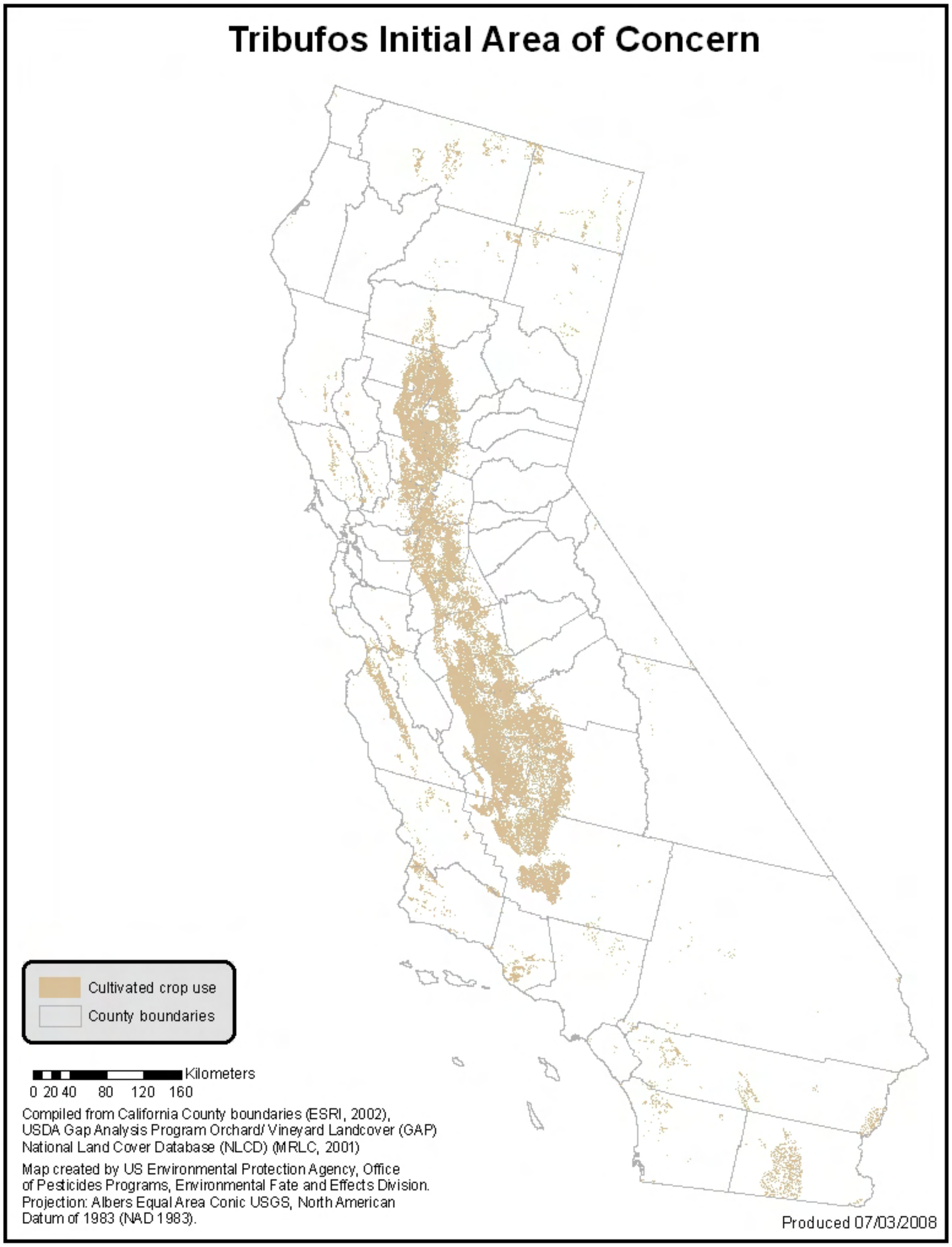


FIGURE 2.6. Initial Area of Concern, or "Footprint" of Potential Use for Tribufos.

Once the initial area of concern is defined, the next step is to define the potential boundaries of the action area by determining the extent of offsite transport via spray drift and runoff where exposure of one or more taxonomic groups to the pesticide exceeds the listed species LOCs.

As previously discussed, the action area is defined by the most sensitive measure of direct and indirect ecological toxic effects including reduction in survival, growth, reproduction, and the entire suite of sublethal effects from valid, peer-reviewed studies.

Due to the lack of a defined no effect concentration (NOEAC) for the most sensitive reported effect to aquatic vascular plants, duckweed (*Lemna gibba*) (NOEAC < 0.0172 mg a.i./L, MRID 458637-05 [see Section 4.1.3]), the spatial extent of the action area (*i.e.*, the boundary where exposures and potential effects are less than the Agency's LOC) for tribufos cannot be determined. Therefore, it is assumed that the action area encompasses the entire state of California, regardless of the spatial extent (*i.e.*, initial area of concern or footprint) of the pesticide use(s).

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."[3] 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, riparian vegetation, and upland and dispersal habitats), the migration pathways of tribufos (*e.g.*, runoff, spray drift, *etc*.), and the routes by which ecological receptors are exposed to tribufos *(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 guideline tests that are performed on a limited number of organisms. Additional ecological effects data from the open literature are also considered. It should be noted that assessment endpoints are limited to direct and indirect effects associated with survival, growth, and fecundity, and do not include the full suite of sublethal effects used to define the action area. According the Overview Document (USEPA 2004), the Agency relies on acute and chronic effects endpoints that are either direct measures of impairment of survival, growth, or fecundity or endpoints for which there is a scientifically robust, peer reviewed relationship that can quantify the impact of the measured effect endpoint on the assessment endpoints of survival, growth, and fecundity.

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

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 tribufos is provided in **Table 2.6**.

TABLE 2.6. Assessment Endpoints and Measures of Ecological Effects.

Assessment Endpoint	Measures of Ecological Effects[4]
Aquatic-Phase CRLF (Eggs, larvae, juveniles, and adults)[1]	
Direct Effects	
1. Survival, growth, and reproduction of CRLF	1a. Bluegill sunfish (*Lepomis macrochirus*) LC_{50} = 0.245 mg a.i./L 1b. Bluegill sunfish (*Lepomis macrochirus*) NOAEC = 0.0035 mg a.i./L [5]
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. Bluegill sunfish (*Lepomis macrochirus*) LC_{50} = 0.245 mg a.i./L 2b. Daphnid (*Daphnia magna*) EC_{50} = 0.0068 mg a.i./L 2c. Algae (*Selenastrum capricornutum*) EC_{50} = 0.148 mg a.i./L 2d. Bluegill sunfish (*Lepomis macrochirus*) NOAEC = 0.0035 mg a.i./L[5] 2e. Daphnid (*Daphnia magna*) NOAEC = 0.0016 mg a.i./L
3. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat, cover, food supply, and/or primary productivity (*i.e.*, aquatic plant community)	3a. Duckweed (*Lemna gibba*) EC_{50} = 1.10 mg a.i./L 3b. Algae (*Selenastrum capricornutum*) EC_{50} = 0.148 mg a.i./L
4. Survival, growth, and reproduction of CRLF individuals via effects to riparian vegetation	4a. Monocot (corn) vegetative vigor EC_{25} = 1.8 lb a.i./acre[3] 4b. Dicot (buckwheat) vegetative vigor EC_{25} = 1.3 lb a.i./acre[3]
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. Bobwhite quail (*Colinus virginianus*)[2] LD_{50} = 151 mg/kg-bw LC_{50} = 1519 mg/kg-diet 5b. Bobwhite quail (*Colinus virginianus*)[2] NOAEC = 280 mg/kg-diet
Indirect Effects and Critical Habitat Effects	
6. Survival, growth, and reproduction of CRLF individuals via effects on terrestrial prey (*i.e.*, terrestrial invertebrates, small mammals , and frogs)	6a. Honey bee (*Apis mellifera*) LD_{50} = >24.17 µg a.i./bee 6b.Bobwhite quail (*Colinus virginianus*)[2] LD_{50} = 151 mg/kg-bw LC_{50} = 1519 mg/kg-diet 6c Rat LD_{50} = 192 mg a.i./kg-bw 6d. No chronic terrestrial invertebrate data available 6e. Bobwhite quail (*Colinus virginianus*)[2] NOAEC = 150 mg/kg-diet 6f. Rat NOAEL = 32 ppm
7. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat (*i.e.*, riparian and upland vegetation)	7a. Monocot (corn) vegetative vigor EC_{25} = 1.8 lb a.i./acre[3] 7b. Dicot (buckwheat) vegetative vigor EC_{25} = 1.3 lb a.i./acre[3]

[1] 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.
[2] Birds are used as surrogates for terrestrial phase amphibians.

[3] Due to its mechanism of action, annual plant species used in the vegetative vigor studies are not expected to be sensitive to tribufos; the results from these studies are used for characterization. No open literature data were available for review.
[4] All registrant-submitted and open literature toxicity data reviewed for this assessment are included in **APPENDICES C** and **D**.
[5] NOAEC is based on acute to chronic ratio using sheepshead minnow endpoints [0.767/0.011= 69.7].

2.8.2 Assessment Endpoints for Designated Critical Habitat

As previously discussed, designated critical habitat is assessed to evaluate actions related to the use of tribufos 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 tribufos 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 tribufos on critical habitat of the CRLF are described in **Table 2.7**. 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 2.7. Summary of Assessment Endpoints and Measures of Ecological Effect for Primary Constituent Elements of Designated Critical Habitat[1]

Assessment Endpoint	Measures of Ecological Effect
Aquatic-Phase CRLF PCEs (Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)	
Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile and adult CRLFs.	a. Algae (*Selenastrum capricornutum*) EC_{50} = 0.148 mg a.i./L b. Monocot (corn) vegetative vigor EC_{25} = 1.8 lb a.i./acre[2] c. Dicot (buckwheat) vegetative vigor EC_{25} = 1.3 lb a.i./acre[2]
Alteration in water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	a. Algae (*Selenastrum capricornutum*) EC_{50} = 0.148 mg a.i./L b. Monocot (corn) vegetative vigor EC_{25} = 1.8 lb a.i./acre[2] c. Dicot (buckwheat) vegetative vigor EC_{25} = 1.3 lb a.i./acre[2]
Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.	a. Bluegill sunfish (*Lepomis macrochirus*) LC_{50} = 0.245 mg a.i./L b. Daphnid (*Daphnia magna*) EC_{50} = 0.0068 mg a.i./L c. Bluegill sunfish (*Lepomis macrochirus*) NOAEC = 0.0035 mg a.i./L[3] d. Daphnid (*Daphnia magna*) NOAEC = 0.0016 mg a.i./L e. Algae (*Selenastrum capricornutum*) EC_{50} = 0.148 mg a.i./L
Reduction and/or modification of aquatic-based food sources for pre-metamorphs (*e.g.*, algae)	a. Algae (*Selenastrum capricornutum*) EC_{50} = 0.148 mg a.i./L
Terrestrial-Phase CRLF PCEs (Upland Habitat and Dispersal Habitat)	
Elimination and/or disturbance of upland habitat; ability of habitat to support food source of CRLFs: Upland areas within 200 ft of the edge of the riparian vegetation or dripline surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance	a. Monocot (corn) vegetative vigor EC_{25} = 1.8 lb a.i./acre[2] b. Dicot (buckwheat) vegetative vigor EC_{25} = 1.3 lb a.i./acre[2] c. Honey bee (*Apis mellifera*) LD_{50} = >24.17 µg a.i./bee d. No chronic terrestrial invertebrate data available e. Bobwhite quail (*Colinus virginianus*): LD_{50} = 151 mg/kg-bw LC_{50} = 1519 mg/kg-diet NOAEC = 280 mg/kg-diet f Rat: LD_{50} = 192 mg a.i./kg-bw NOAEL = 32 ppm
Elimination and/or disturbance of dispersal habitat: Upland or riparian dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal	
Reduction and/or modification of food sources for terrestrial phase juveniles and adults	
Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	

[1] 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] Due to its mechanism of action annual plant species used in the vegetative vigor and seedling emergence studies are not expected to be sensitive to tribufos; the results from these studies are used for characterization. No open literature data were available for review.
[3] The NOAEC is based on acute to chronic ratio using sheepshead minnow endpoints [0.767/0.011= 69.7].

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 (USEPA 1998). For this assessment, the risk is stressor-linked, where the stressor is the release of tribufos to the environment. The following risk hypotheses are presumed for this endangered species assessment:

The labeled use of tribufos 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 tribufos release mechanisms, biological receptor types, and effects endpoints of potential concern. The conceptual models for aquatic and terrestrial phases of the CRLF are shown in **Figures 2.7** and **2.8**, respectively, and the conceptual models for the aquatic and terrestrial PCE components of critical habitat are shown in **Figures 2.9** and **2.10**, respectively. 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.

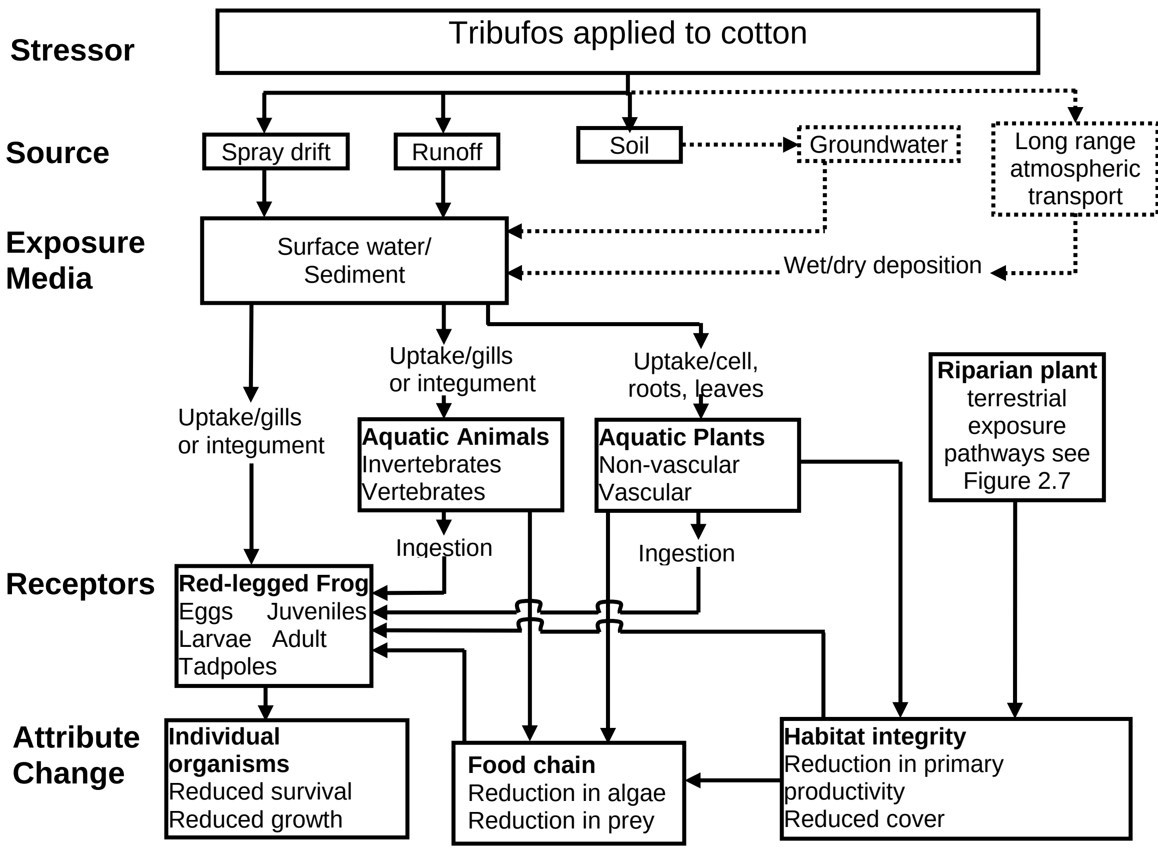


FIGURE 2.7. Conceptual Model for Aquatic-Phase of the CRLF.

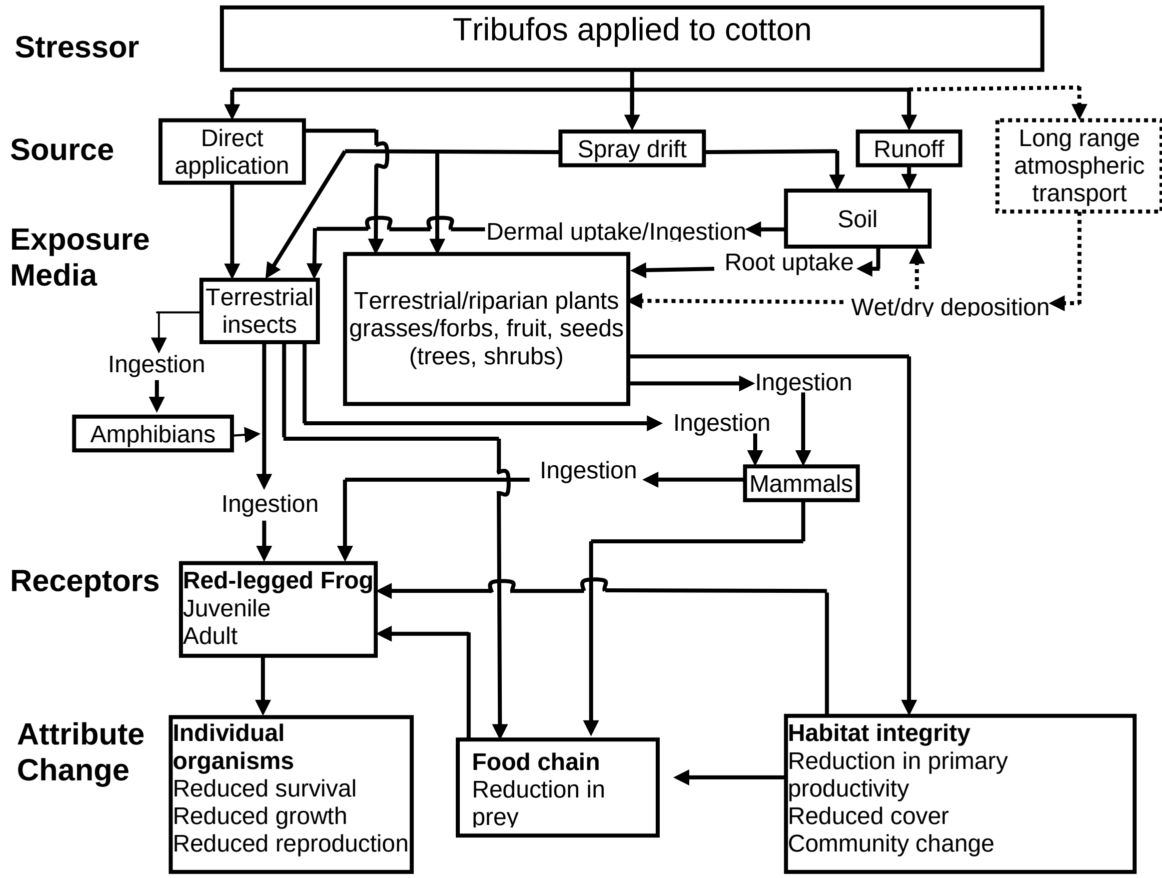


FIGURE 2.8. Conceptual Model for Terrestrial-Phase of the CRLF.

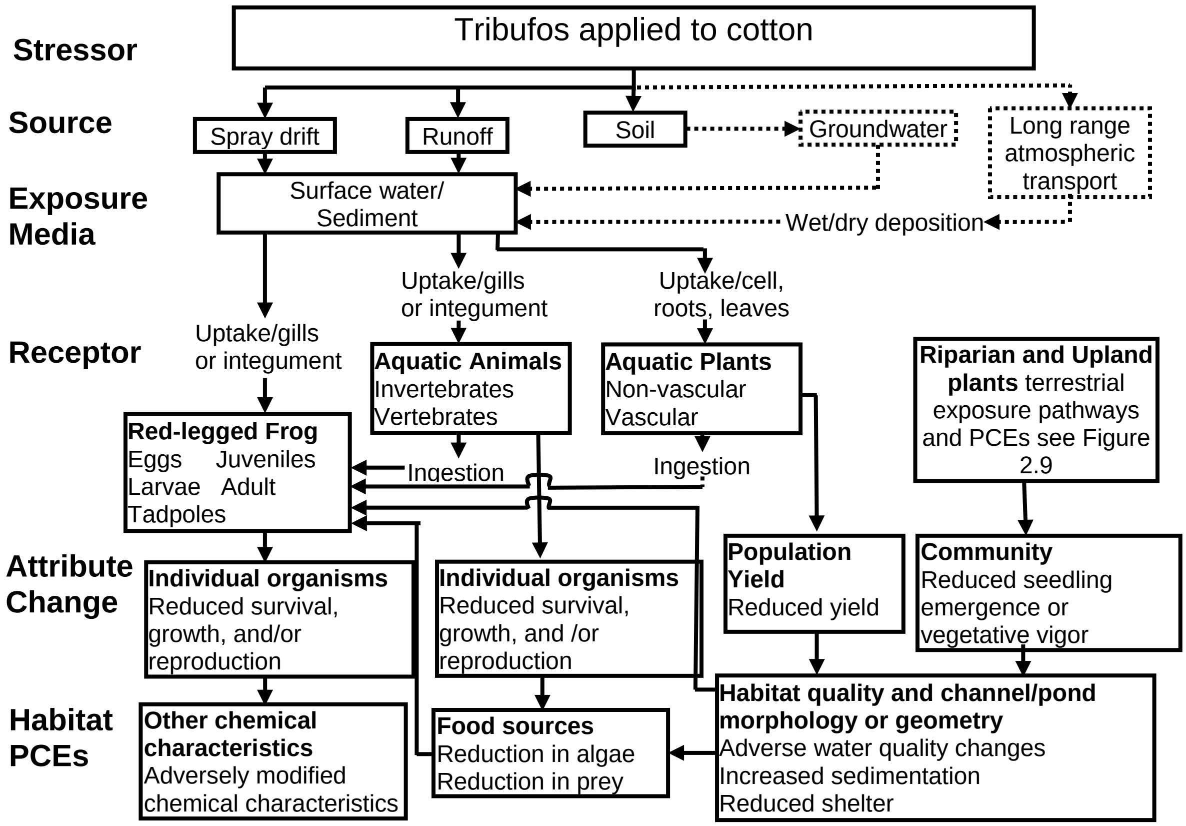


FIGURE 2.9. Conceptual Model for Pesticide Effects on Aquatic Component of CRLF Critical Habitat.

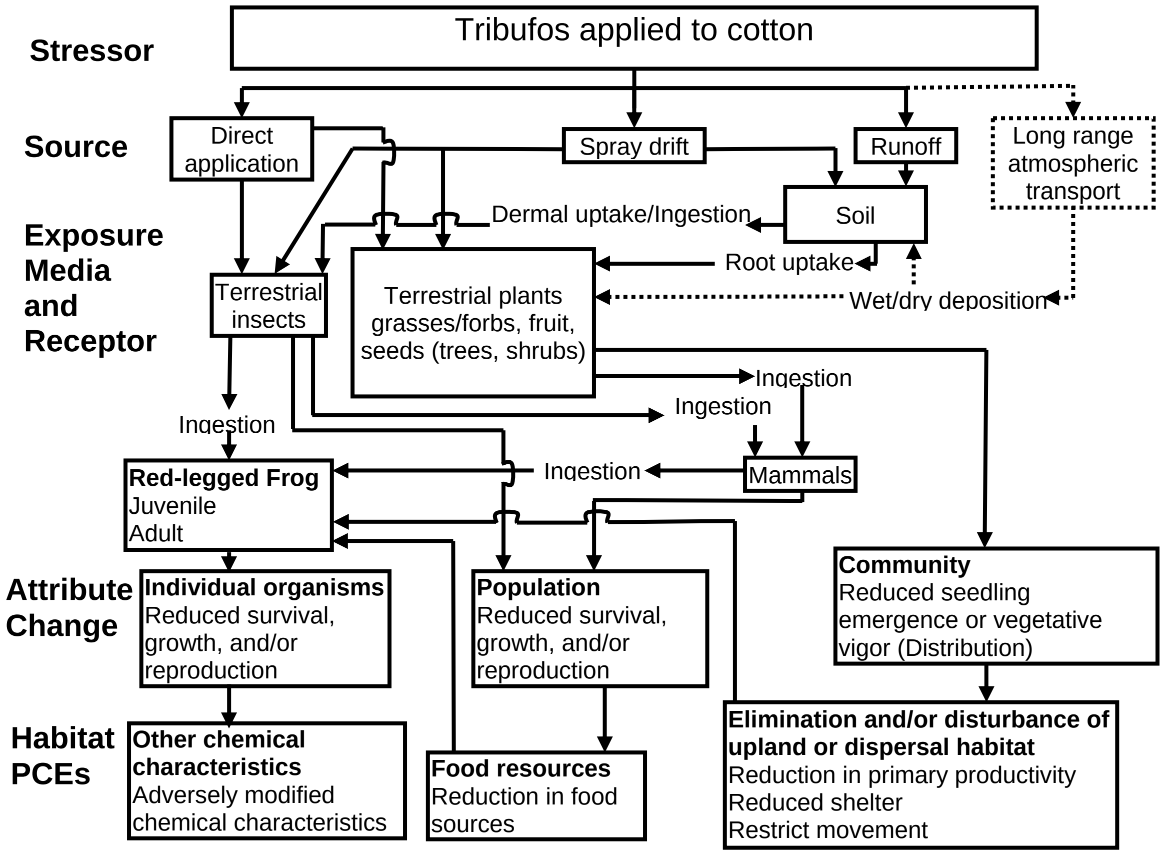


FIGURE 2.10. Conceptual Model for Pesticide Effects on Terrestrial Component of CRLF Critical Habitat.

2.10 Analysis Plan

In order to address the risk hypotheses, 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 tribufos 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 (USEPA 2004), the likelihood of effects to individual organisms from tribufos use is estimated using the probit dose-response slope and either the level of concern (discussed below) or actual calculated risk quotient value.

2.10.1 Measures to Evaluate the Risk Hypothesis and Conceptual Model

2.10.1.1 Measures of Exposure

The environmental fate properties of tribufos along with available monitoring data indicate that runoff and spray drift are the principle potential transport mechanisms of tribufos to the aquatic and terrestrial habitats of the CRLF. In this assessment, transport of tribufos through runoff and spray drift is considered in deriving quantitative estimates of tribufos exposure to CRLF, its prey and its habitats. Tribufos is not expected to volatilize from application sites due to its relatively low vapor pressure.

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

PRZM (v3.12.2, May 2005) and EXAMS (v2.98.4.6, April 2005) are screening simulation models coupled with the input shell pe5.pl (Aug 2007) to generate daily exposures and 1-in-10 year EECs of tribufos that may occur in surface water bodies adjacent to application sites receiving tribufos through runoff and spray drift. PRZM simulates pesticide application, movement and transformation on an agricultural field and the resultant pesticide loadings to a receiving water body via runoff, erosion and spray drift. EXAMS simulates the fate of the pesticide and resulting concentrations in the water body. The standard scenario used for ecological pesticide assessments assumes application to a 10-hectare agricultural field that drains into an adjacent 1-hectare water body, 2-meters deep (20,000 m^3 volume) with no outlet. PRZM/EXAMS was used to estimate screening-level exposure of aquatic organisms to tribufos. The measure of exposure for aquatic species is the 1-in-10 year return peak or rolling mean concentration. The 1-in-10 year peak is used for estimating acute exposures of direct effects to the CRLF, as well as indirect effects to the CRLF through effects to potential prey items, including: algae, aquatic invertebrates, fish and frogs. The 1-in-10-year 60-day mean is used for assessing chronic exposure to the CRLF and fish and frogs serving as prey items; the 1-in-10-year 21-day mean is used for assessing chronic exposure for aquatic invertebrates, which are also potential prey items.

Exposure estimates for the terrestrial-phase CRLF and terrestrial invertebrates and mammals (serving as potential prey) assumed to be in the target area or in an area exposed to spray drift are derived using the T-REX model (version 1.3.1, 12/07/2006) (available at http://www.epa.gov/oppefed1/models/terrestrial/index.htm). This model incorporates the Kenaga nomograph, as modified by Fletcher *et al.* (1994), which is based on a large set of actual field residue data. The upper limit values from the nomograph represented the 95th percentile of residue values from actual field measurements (Hoerger and Kenaga 1972). For modeling purposes, direct exposures of the CRLF to tribufos through contaminated food are estimated using the EECs for the small bird (20 g) which consumes small insects. Dietary-based and dose-based exposures of potential prey (small mammals) are assessed using the small mammal (15 g) which consumes short grass. The small bird (20g) consuming small insects and the small mammal (15g) consuming short grass are used because these categories represent the largest RQs of the size and dietary categories in T-REX that are appropriate surrogates for the CRLF and one of its prey items. Estimated exposures of terrestrial insects to tribufos are bound by using the dietary based EECs for small insects and large insects.

Birds are currently used as surrogates for terrestrial-phase CRLF. However, amphibians are poikilotherms (body temperature varies with environmental temperature) while birds are homeotherms (temperature is regulated, constant, and largely independent of environmental temperatures). Therefore, amphibians tend to have much lower metabolic rates and lower caloric intake requirements than birds or mammals. As a consequence, birds are likely to consume more food than amphibians on a daily dietary intake basis, assuming similar caloric content of the food items. Therefore, the use of an avian food intake allometric equation as a surrogate to amphibians is likely to result in an over-estimation of exposure and risk for reptiles and terrestrial-phase amphibians. Therefore, T-REX (version 1.3.1, 2006) has been refined to the T-HERPS model (version 1.0, 2007), which allows for an estimation of food intake for poikilotherms using the same basic procedure as T-REX to estimate avian food intake.

EECs for terrestrial plants inhabiting dry and wetland areas are derived using TerrPlant (version 1.2.2, 12/26/2006). This model uses estimates of pesticides in runoff and in spray drift to calculate EECs. EECs are based upon solubility, application rate and minimum incorporation depth. But, again, due to its mechanism of action, plants that form abscission zones (*e.g.*, some woody, deciduous plants) are expected to be more sensitive to tribufos than plants that do not form abscission zones. Therefore, the submitted plant studies available for tribufos will only be informative for annual plants, which are not expected to be sensitive to tribufos.

The spray drift model AgDRIFT (version 2.01) is used to assess exposures of terrestrial phase CRLF and its prey to tribufos deposited on terrestrial habitats by spray drift. In addition to the buffered area from the spray drift analysis, the downstream extent of tribufos that exceeds the LOC for the effects determination is also considered.

2.10.1.2 Measures of Effect

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

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

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

It is important to note that the measures of effect for direct and indirect effects to the CRLF and its designated critical habitat are associated with impacts to survival, growth, and fecundity, and do not include the full suite of sublethal effects used to define the action area. According 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 tribufos use, and the likelihood of direct and indirect effects to CRLF in aquatic and terrestrial habitats. The exposure and toxicity effects data are integrated in order to evaluate the risks of adverse ecological effects on non-target species. For the assessment of tribufos 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 LOCs (USEPA, 2004) (see **APPENDIX E**).

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

2.10.2 Data Gaps

Acceptable toxicity data for aquatic- or terrestrial-phase amphibians are not currently available for tribufos. Therefore, toxicity data for surrogate species (*i.e*., fish for aquatic-phase amphibians and birds for terrestrial-phase amphibians) are used to assess the risks of direct effects to the CRLF and indirect effects to the CRLF (via consumption of frogs by terrestrial-phase CRLFs) from the use of tribufos on cotton in California. Additionally, toxicity data from chronic exposure to tribufos are not currently available for the species of freshwater fish that are most acutely sensitive to tribufos (*i.e*., bluegill sunfish), and the freshwater fish chronic toxicity study available for review was not adequate for RQ calculation. Therefore, the chronic effects endpoint used in this assessment for freshwater fish (and, thus, aquatic-phase amphibians) is based on an acute-to-chronic ratio using sheepshead minnow toxicity data (see Section 4.1.1.2 for more discussion). Furthermore, because tribufos could potentially partition to the sediment and no sediment toxicity data are available for review, toxicity to benthic invertebrates may be underestimated in this assessment.

Additionally, although acceptable Tier II terrestrial plant studies are available for tribufos, the plant species studied (*i.e*., annual dicots and monocots) are not expected to be as sensitive to tribufos as other plants important to the CRLF (*e.g*., woody deciduous trees and shrubs) due to its mechanism of action. Therefore, there are uncertainties regarding the toxicity of tribufos to non-target plants that form abscission zones (*e.g*., some woody, deciduous plants). In the absence of data specific for plants that form abscission zones, risks to these terrestrial plants cannot be precluded.

Aerobic aquatic metabolism data for tribufos have not been submitted. Therefore, tribufos is assumed relatively stable (*i.e.*, twice as persistent as in aerobic soils) to this route of degradation in water bodies. Submission of these data may reduce modeled exposure estimates in water bodies.

3. Exposure Assessment

Tribufos is formulated as an emulsifiable concentrate. Applications are limited to foliar applications via ground and aerial equipment; applications via irrigation systems are not allowed. Risks from aerial applications are expected to result in the highest off-target levels of tribufos due to generally higher spray drift levels; however, both aerial and ground applications are modeled.

3.1 Label Application Rates and Intervals

Tribufos labels may be categorized into two types: labels for manufacturing uses (including technical grade tribufos and its formulated products) and end-use products. While technical products, which contain tribufos of high purity, are not used directly in the environment, they are used to make formulated products. The formulated product labels legally limit tribufos's potential use to only those sites that are specified on the labels. The only currently registered use of tribufos within California is for cotton (as a defoliant) (see **Table 3.1**).

TABLE 3.1. Tribufos Use and Application Information for the CRLF Risk Assessment.

Labeled Use	Application Rate	Number of Applications	Application Interval	Application Method
Cotton defoliant	1.875 lbs a.i./acre[1]	2	Not specified	aerial or ground

[1] This is also the maximum application rate allowed per season.

3.2 Aquatic Exposure Assessment

3.2.1 Modeling Approach

Aquatic exposures are quantitatively estimated using the cotton scenario that represents a high exposure site for tribufos use in California. The site represents a 10 hectare field that drains into a 1-hectare pond that is 2 meters deep and has no outlet. Exposure estimates generated using the standard pond are intended to represent a wide variety of vulnerable water bodies that occur at the top of watersheds including prairie pot holes, playa lakes, wetlands, vernal pools, man-made and natural ponds, and intermittent and first-order streams. As a group, there are factors that make these water bodies more or less vulnerable than the standard surrogate pond. Static water bodies that have larger ratios of drainage area to water body volume would be expected to have higher peak EECs than the standard pond. These water bodies will be either shallower or have large drainage areas (or both). Shallow water bodies tend to have limited additional storage capacity, and, thus, tend to overflow and carry pesticide in the discharge whereas the standard pond has no discharge. As watershed size increases beyond 10 hectares, at some point, it becomes unlikely that the entire watershed is planted to a single crop, which is all treated with the pesticide. Headwater streams can also have peak concentrations higher than the standard pond, but they tend to persist for only short periods of time and are then carried downstream.

Crop-specific management practices for cotton were used for modeling, including application rates, number of applications per year, application intervals, and the first application date for each crop. The date of first application was developed based on several sources of information including data provided by BEAD, a summary of individual applications from the CDPR PUR data, rainfall data, and Crop Profiles maintained by the USDA. Based on CDPR PUR data, 98% of tribufos applications to cotton in California from 2001 to 2005 occurred in the months of August through November (see **Fig. 3.1**) when cotton is being defoliated for harvest.

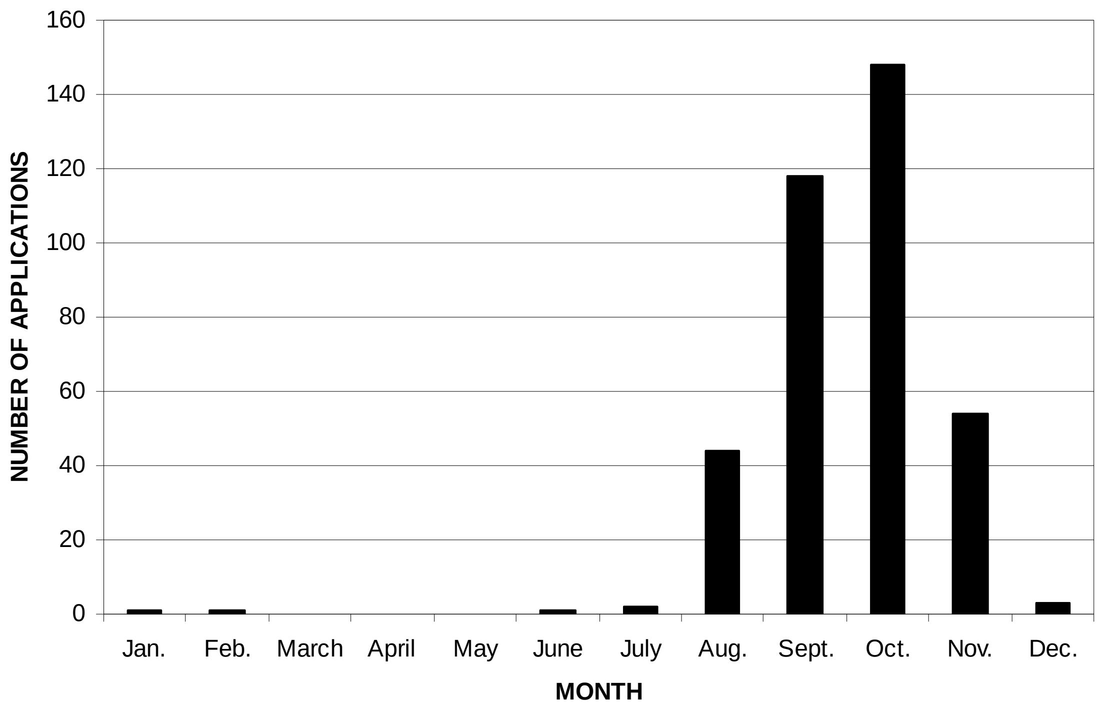


FIGURE 3.1. Tribufos Application Dates by Month in California from CDPR PUR Data (2001 – 2005).

More detail on the crop profiles and the previous assessments may be found at:

http://pestdata.ncsu.edu/cropprofiles/cropprofiles.cfm

3.2.2 Model Inputs

Tribufos is a defoliant used on cotton to aid in mechanical harvesting and is typically applied 7 to 10 days before harvesting in California (http://www.ipmcenters.org/CropProfiles/docs/CAcotton.pdf, prepared in 2002). Tribufos environmental fate data used for generating model parameters are listed in **Table 2.2**. The input parameters for PRZM and EXAMS are in **Tables 3.2 and 3.3**. Although two applications of tribufos are allowed per year in California, the maximum allowed application rate per year is 1.875 lb a.i./acre, whether it is applied in one or two applications. In this assessment, only a single application at the maximum allowable application rate (*i.e.*, 1.875 lb a.i./acre/year) was modeled since it was assumed that this application scenario, as opposed to two applications of 0.938 lb a.i./acre (*i.e.*, 1.875 lb/2 applications), would produce the highest EECs. This is especially true since tribufos is typically applied within a week and a half of harvesting, which would necessitate a relatively short application interval (although an application interval is not specified on the label). An application date of Nov. 11 was selected based on a review of the application dates by month for tribufos use in California (see **Fig. 3.1**) and the rainfall data from the meteorologic data file used in the CA cotton scenario. Selection of the Nov. 11 date captured some of the wettest days during the times when tribufos is typically applied in California and resulted in a conservative estimation of aquatic exposure for the cotton use pattern.

TABLE 3.2. PRZM/EXAMS Crop Input Parameters for Tribufos.

Use Pattern(s)	Scenario	App. Rate in lbs a.i./A (kg a.i./ha)	App. per Year	App. Date	App. Method	CAM Input	IPSCND Input	Application Efficiency	Spray Drift Value
Cotton (defoliant)	CAcotton_WirrigSTD	1.875 (2.10)	1	Nov. 11	Ground	2	1	0.99	0.01
Cotton (defoliant)	CAcotton_WirrigSTD	1.875 (2.10)	1	Nov. 11	Aerial	2	1	0.95	0.05

TABLE 3.3 PRZM/EXAMS Chemical Input Parameters[1] for Tribufos.

Fate Property	Value (unit)	Justification	MRID (or source)
Molecular Weight	314.5 g/mole	Product chemistry data	45863701
Vapor Pressure at 25°C	1.7 x 10^{-6} torr	Study value	41618803
Solubility in Water at 25°C	23 mg/L	Represents 10x the measured water solubility value	
Aerobic Soil Metabolism Half-life[1]	2235 days	Represents 3 times a single total residue half-life.	42007204
Hydrolysis	Stable (at pH values)	Study values	41618814
Aerobic Aquatic Metabolism (water column)[1]	4470 days	2x aerobic soil metabolism	No data
Anaerobic Aquatic Metabolism (benthic)[1]	1167 days	Represents 3 times a single total residue half-life.	42007205
Soil-Water Partitioning Distribution Coefficient (K_d)[1]	76.9 l/kg	Represents the average K_d.	41618817
Photolysis in Water	Stable	Study values	41719401

[1] Inputs determined in accordance with EFED "*Guidance for Chemistry and Management Practice Input Parameters for Use in Modeling the Environmental Fate and Transport of Pesticides*" dated February 28, 2002.

3.2.3 Results

The aquatic EECs for the cotton scenario and application practices for tribufos are listed in **Table 3.4.** The output from PRZM/EXAMS is available in **APPENDIX F.**

TABLE 3.4 Aquatic EECs (µg/L) for Tribufos Use on Cotton in California.

Crop	Application Rate	Application Method	Peak EEC	21-day average EEC	60-day average EEC
CA cotton	1.875 lbs a.i./acre	Aerial	16.34	14.89	13.94
		Ground	11.53	10.62	10.15

3.2.4 Existing Monitoring Data

One step in the process of characterizing EECs is comparing the modeled estimates with available surface water monitoring data. There are limited monitoring data available for tribufos. No surface water monitoring studies that specifically targeted tribufos were available for analysis as part of this assessment. Generally, targeted monitoring data are collected with a sampling programs designed to capture, both spatially and temporally, the maximum use of a particular pesticide. Because none of the available regional monitoring studies were designed specifically for tribufos, they are considered 'non-targeted'. Typically, sampling frequencies employed in monitoring studies are insufficient to document peak exposure values. Because of this and because the data are not spatially nor temporally correlated with pesticide application times/areas, these data are limited in their utility to estimate exposure concentrations for risk assessment purposes. Included in this assessment are data from the USGS NAWQA program (http://water.usgs.gov/nawqa) and data from the California Department of Pesticide Regulation (CDPR).

3.2.4.1 USGS NAWQA Surface Water Data

Surface water monitoring data from the USGS NAWQA program were obtained on June 30, 2008. A total of 211 water samples across various sites throughout California were analyzed for tribufos, including ten sites in five counties (Merced, Sacramento, Riverside, San Joaquin, and Stanislaus) from 2001-2006. Three of the five counties have reported tribufos use from 2002 - 2006 according to CDPR (see Section 2.4.3). There were no positive detections of tribufos reported above the level of detection, which ranged from 0.0044 µg/L to 0.23 µg/L.

3.2.4.2 USGS NAWQA Groundwater Data

Ground water monitoring data from the USGS NAWQA program were obtained on June 30, 2008. A total of 171 water samples at various wells throughout 15 counties in California were analyzed for tribufos between 2001-1006. Of the 15 counties sampled, 11 have reported tribufos use from 2002 - 2006 according to CDPR (see Section 2.4.3). There were no positive detections of tribufos reported above the level of detection, which ranged from 0.0044 µg/L to 0.035 µg/L. As previously discussed, due to its relatively high soil/water partitioning, tribufos is not expected to leach to groundwater.

3.2.4.3 California Department of Pesticide Regulation (CPR) Data

Surface water monitoring data were obtained from the California Department of Pesticide Regulation (CDPR) on June 30, 2008. CDPR maintains a database of monitoring data of pesticides in sampled surface waters, including rivers, creeks, urban streams, agricultural drains, the San Francisco Bay delta region, and storm water runoff from urban areas (http://www.cdpr.ca.gov/docs/emon/surfwtr/surfdes.htm). Samples obtained from sites in the Sacramento Valley and the San Joaquin Valley were analyzed for tribufos. Of the 441 total samples obtained from the San Joaquin Valley from 1991-2005, there were two detections of tribufos found at sites in Stanislaus County in January 1992, both at the level of quantization (LOQ) of 0.01 µg/L. The LOQ for the remaining sites with no tribufos detections ranged from 0.01 – 0.1 µg/L. There were zero detections of tribufos in the Sacramento Valley (309 sample sites) at or above the LOQ (0.05 – 0.1 µg/L).

3.2 Terrestrial Animal Exposure Assessment

T-REX (Version 1.3.1) is used to calculate dietary and dose-based EECs of tribufos for the CRLF and its potential prey (*e.g.*, small mammals and terrestrial insects) inhabiting terrestrial areas. EECs used to represent the CRLF are also used to represent exposure values for frogs serving as potential prey of CRLF adults. T-REX simulates a 1-year time period. For this assessment, spray applications of tribufos are considered, as discussed below.

Although tribufos can be absorbed by plant leaves and is systemic, EECs from T-REX are based solely on residue values, adding uncertainty into our dietary exposure estimates. Additionally, because tribufos is a defoliant and affected leaves could drop and become unpalatable within a few days after exposure, the duration of exposure to contaminated leaves (as a food source) may be limited. Again, this adds uncertainty to our dietary exposure estimates.

Terrestrial EECs for foliar formulations of tribufos were derived for the cotton use using the maximum labeled single application rate (see **Table 3.5**). Given that no data on interception and subsequent dissipation from foliar surfaces are available for tribufos, a default foliar dissipation half-life of 35 days is used based on the work of Willis and McDowell (1987). The output from T-REX is available in **APPENDIX G**.

TABLE 3.5. Input Parameters for Foliar Applications Used to Derive Terrestrial EECs for Tribufos with T-REX.

Use (Application method)	Application rate (lbs ai/A)	Number of Applications	Foliar Dissipation Half-life
Cotton	1.875	1	35 days*

* This is a default value.

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

For modeling purposes, exposures of the CRLF to tribufos through contaminated food are estimated using the EECs for the small bird (20 g) which consumes small insects. Dietary-based and dose-based exposures of potential prey are assessed using the small mammal (15 g) which consumes short grass. Upper-bound Kenaga nomogram values reported by T-REX for these two organism types are used for derivation of EECs for the CRLF and its potential prey (**Table 3.6**). Dietary-based EECs for small and large insects reported by T-REX as well as the resulting adjusted EECs are available in **Table 3.7**. The output from T-REX v. 1.3.1 is available in **APPENDIX G**.

TABLE 3.6. Upper-bound Kenaga Nomogram EECs for Dietary- and Dose-based Exposures of the CRLF and its Prey to Tribufos.

Use	EECs for CRLF		EECs for Prey (small mammals)	
	Dietary-based EEC (ppm)	Dose-based EEC (mg/kg-bw)	Dietary-based EEC (ppm)	Dose-based EEC (mg/kg-bw)
Cotton	253	288	450	429

TABLE 3.7. EECs (ppm) for Indirect Effects to the Terrestrial-Phase CRLF via Effects to Terrestrial Invertebrate Prey Items.

Use	Small Insect	Large Insect
Cotton	253	28

3.3 Terrestrial Plant Exposure Assessment

TerrPlant (Version 1.1.2) is used to calculate EECs for non-target plant species inhabiting dry and semi-aquatic areas. Parameter values for application rate, drift assumption, and incorporation depth are based upon the use and related application method (**Table 3.8**). A runoff value of 0.01 is utilized based on tribufos' solubility, which is classified by TerrPlant as <10 mg/L. For aerial and ground application methods, drift is assumed to be 5% and 1%, respectively. EECs relevant to terrestrial plants consider pesticide concentrations in drift and in runoff. These EECs are listed in **Table 3.8**. An example output from TerrPlant v.1.2.2 is available in **APPENDIX H.**

TABLE 3.8. TerrPlant Inputs and Resulting EECs for Plants Inhabiting Dry and Semi-aquatic Areas Exposed to Tribufos via Runoff and Drift.

Use	Application rate (lbs a.i./A)	Application method	Drift Value (%)	Spray drift EEC (lbs a.i./A)	Dry area EEC (lbs a.i./A)	Semi-aquatic area EEC (lbs a.i./A)
Cotton	1.875	Foliar - ground	1	0.019	0.038	0.206
Cotton	1.875	Foliar - aerial	5	0.094	0.113	0.281

4. Effects Assessment

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

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

Toxicity endpoints are established based on data generated from guideline studies submitted by the registrant, and from open literature studies that meet the criteria for inclusion into the ECOTOX database maintained by EPA/Office of Research and Development (ORD) (USEPA 2004). Open literature data presented in this assessment were obtained from ECOTOX information obtained on August 2, 2007. 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 for the effects determination 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 tribufos.

A bibliography of the open literature not evaluated for this assessment because it either was rejected by the ECOTOX screen or accepted by ECOTOX but not used (*e.g.*, the endpoint is less sensitive) is included in **APPENDIX I. APPENDIX I** also includes a rationale for rejection of those studies that did not pass the ECOTOX screen and those that were and were not evaluated as part of this endangered species risk assessment. A detailed spreadsheet of the available ECOTOX open literature data, including the full suite of lethal and sublethal endpoints is presented in **APPENDIX J**.

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 used to further refine the characterization of potential ecological effects associated with exposure to tribufos. A summary of the available aquatic and terrestrial ecotoxicity information, use of the probit dose response relationship, and the incident information for tribufos are provided in Sections 4.1 through 4.4, respectively.

As previously stated in Section 2.2, the only major degradate of tribufos, 1-butane sulfonic acid, is not considered a major stressor, and, therefore, no analysis on degradates is included in this assessment.

4.1 Toxicity of tribufos to aquatic organisms

Table 4.1 summarizes the most sensitive aquatic toxicity endpoints for the CRLF, based on an evaluation of both the submitted studies and the open literature, as previously discussed. Toxicity information for each aquatic taxon evaluated in this assessment is summarized in the following sections. All of the endpoints for freshwater fish, freshwater invertebrates, and aquatic plants used for RQ calculations in this assessment come from submitted studies; there were no acceptable studies in the open literature that resulted in more sensitive endpoints for these taxa. Additional toxicity information is provided in **APPENDICES C** and **D.**

TABLE 4.1. Freshwater Aquatic Toxicity Profile for Tribufos.

Assessment Endpoint	Species	Toxicity Value Used in Risk Assessment	Citation MRID # (Author & Date)	Comment
Acute Direct Toxicity to Aquatic-Phase CRLF	Bluegill sunfish (*Lepomis macrochirus*)	96-hr LC_{50} = 245 µg a.i./L (slope = 6.91, 95% CI; 3.11, 10.71)	400980-01	Supplemental – per EFED policy (USEPA 2006b); some deviations from guideline requirements
Chronic Direct Toxicity to Aquatic-Phase CRLF	Bluegill sunfish (*Lepomis macrochirus*)	NOAEC = 3.5 µg a.i./L	N/A	The NOAEC is based on acute to chronic ratio using sheepshead minnow endpoints [0.767/0.011= 69.7 (ACR)]
Indirect Toxicity to Aquatic-Phase CRLF via Acute Toxicity to Freshwater Invertebrates (*i.e*. prey items)	Daphnid (*Daphnia magna*)	48-hr EC_{50} = 6.8 µg a.i./L	400980-01	Supplemental – per EFED policy (USEPA 2006b); raw data were not available for verification
Indirect Toxicity to Aquatic-Phase CRLF via Chronic Toxicity to Freshwater Invertebrates (*i.e*. prey items)	Daphnid (*Daphnia magna*)	NOAEC = 1.56 µg a.i./L LOAEC = 3.23 µg a.i./L	439782-01	Acceptable – the NOAEC is based on reduced number of young/adult/day and reduced adult length
Indirect Toxicity to Aquatic-Phase CRLF via Toxicity to Non-vascular Aquatic Plants	Algae (*Selenastrum capricornutum*)	NOAEC = 58.5 µg a.i./L EC_{50} = 148 µg a.i./L	416188-13	Acceptable – Tier II, 7-day test
Indirect Toxicity to Aquatic-Phase CRLF via Toxicity to Vascular Aquatic Plants	Duckweed (*Lemna gibba*)	NOEAC = <17.2 µg a.i./L EC_{05} = 140 µg a.i./L EC_{50} = 1,100 µg a.i./L	458637-05	Supplemental – analytical verification indicated that the test material was not stable; no definitive NOAEC value; the NOAEC is based on reduced frond count.

Toxicity to aquatic fish and invertebrates is categorized using the system shown in **Table 4.2** (USEPA 2004). Toxicity categories for aquatic plants have not been defined.

TABLE 4.2. 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 tribufos 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 tribufos to the CRLF. Effects to freshwater fish resulting from exposure to tribufos may result in indirect effects to the CRLF via reduction in available food. As discussed in Section 2.5.3, over 50% of the prey mass of the CRLF may consist of vertebrates such as mice, frogs, and fish (Hayes and Tennant 1985).

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

4.1.1.1 Freshwater Fish: Acute Exposure (Mortality) Studies

Tribufos is characterized as ‘highly toxic’ to freshwater fish on an acute exposure basis. The most sensitive freshwater fish species tested was bluegill sunfish (MRID: 400980-01), with an LC_{50} value of 0.245 mg a.i./L (slope = 6.91, with 95% confidence limits of 3.11 and 10.71). Per EFED policy (USEPA 2006b), all data from Mayer and Ellersieck (1986) (MRID: 400980-01) are considered ‘supplemental’ unless, after evaluation of the raw data, it is deemed that the classification can be changed to ‘acceptable’ or ‘invalid’. After review of the raw data, the data for bluegill sunfish were considered ‘supplemental’, scientifically valid, and adequate for RQ calculation. The following study deviations from current guidelines were noted: the acclimation period of 3-days was less than the recommended >14-day acclimation period; the test water pH (8.1) was higher than recommended (7.2-7.6); the following water parameters were not reported: dissolved oxygen, total organic carbon, particulate matter, metals, pesticide, and chlorine; there was only one container used per concentration instead of the recommended three containers per treatment level; and the following information was not provided: lighting during the test and stability of the chemical in the test system. The most sensitive acute LC_{50} values for all freshwater fish species tested are summarized in **Table 4.3**.

TABLE 4.3. Acute Freshwater Fish Toxicity Values for Tribufos.

Freshwater species	Results	Toxicity category	Source or MRID
Bluegill sunfish (*Lepomis macrochirus*)	96-hr LC_{50} = **0.245 mg a.i./L***	Highly toxic	400980-01
Channel catfish (*Ictalurus punctatus*)	96-hr LC_{50} = 0.350 mg a.i./L	Highly toxic	400980-01
Rainbow trout (*Oncorhynchus mykiss*)	96-hr LC_{50} = 0.66 mg a.i./L	Highly toxic	400980-01
Fathead minnow (*Pimephales promelas*)	LC_{50} = 0.92 mg a.i./L	Highly toxic	458637-06

*Most sensitive endpoint used for RQ calculations.

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

Available early life stage toxicity studies are summarized in **Table 4.4**. Only one early life stage freshwater fish study on the fathead minnow is available for tribufos and it is classified as supplemental and not adequate for RQ calculations because a high level of analytical variation (>20%) was observed in the mean results (MRID 458637-03). The NOAEC and LOAEC values from this study were 3.35 and 8.38 µg ai/L, respectively. However, because analytical variation from 28-46% was observed in three of the five treatment levels, and because there was overlap in the measured concentrations from two treatment groups, this endpoint was not used. Therefore, the chronic endpoint used in this assessment was derived using the acute-to-chronic ratio (ACR) for the bluegill sunfish, using the sheepshead minnow (*Cyprinodon variegatus*) endpoints.

Toxicity data for sheepshead minnow, an estuarine/marine species, were used to derive the ACR because they were the only fish species that had adequate toxicity data available for both acute and chronic exposures to tribufos. Additionally, the consistency of the acute toxicity values for saltwater and freshwater species suggests that saltwater species are not considerably more or less sensitive to tribufos than freshwater species. The sheepshead minnow studies resulted in an LC_{50} of 767 µg a.i./L (MRID: 418963-02) and a NOAEC of 11 µg a.i./L (MRID 458637-07) based on clinical signs of intoxication, reduced post-hatch survival, and reduced growth. The LOAEC in the chronic study was 19 µg a.i./L. At this concentration both the post-hatch survival and the total length were reduced by 10% compared to the control. Both studies are classified as acceptable. The calculated NOAEC value for bluegill sunfish is 3.5 µg a.i./L based on the sheepshead minnow-derived ACR (ACR = 69.7). The resulting estimated chronic NOAEC value for the bluegill sunfish (3.5 µg a.i./L) was just slightly higher than the submitted fathead chronic value (3.35 µg a.i./L).

TABLE 4.4. Chronic Freshwater Fish Toxicity Value for Tribufos.

Freshwater species	Results	Source or MRID	Comments
Bluegill sunfish (*Lepomis macrochirus*)	NOAEC = **3.5 µg a.i./L*** (ACR of 69.7 = 245/x)	N/A	Based on an acute to chronic ratio using sheepshead minnow endpoints [767/11= 69.7 (ACR)]

*Most sensitive endpoint used for RQ calculations.

4.1.2 Toxicity to Freshwater Invertebrates

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

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

4.1.2.1 Freshwater Invertebrates: Acute Exposure Studies

All available submitted acute toxicity data for freshwater invertebrates were obtained from Mayer and Ellersieck (1986) (MRID: 400980-01**)** static studies, and are classified by the Agency as supplemental. Per EFED policy (USEPA 2006b), all data from Mayer and Ellersieck (1986) (MRID: 400980-01) are considered 'supplemental' unless, after evaluation of the raw data, it is deemed that the classification can be changed to 'acceptable' or 'invalid'. Mayer and Ellersieck raw data for the aquatic invertebrate studies were not available for review; therefore, all studies were considered 'supplemental'. These data indicate that tribufos is moderately toxic to very highly toxic to freshwater invertebrates on an acute exposure basis. Daphnids (*Daphnia magna*) were the most sensitive species tested, with a 48-hour EC_{50} value of 0.0068 mg a.i./L. The results of the other aquatic invertebrate species tested are summarized in **Table 4.5**. One of the tests resulted in values that exceeded the tribufos solubility limit of 2.3 mg/L (crayfish). Because tribufos could potentially partition to the sediment and no sediment toxicity data are available for review, toxicity to benthic invertebrates may be underestimated by using daphnid data.

TABLE 4.5. Acute Aquatic Invertebrate Toxicity Data for Tribufos.

Freshwater species	Results	Toxicity category	Source or MRID
Daphnid (*Daphnia magna*)*	48-hr EC_{50} = **0.0068** mg a.i./L*	Very highly toxic	400980-01
Scud (*Gammarus pseudolimnaeus*)	96-hr EC_{50} = 0.027 mg a.i./L	Very highly toxic	400980-01
Crayfish (*Orconectes nais*)	96-hr EC_{50} = >5.60 mg a.i./L**	N/A	400980-01
Stonefly (*Pteronarcys californica*)	96-hr EC_{50} = 2.10 mg a.i./L	Moderately toxic	400980-01
Midge (*Chironomus plumosus*)	48-hr EC_{50} = 0.040 mg a.i./L	Very highly toxic	400980-01

* Most sensitive species tested, used for RQ calculation
** Over the solubility limit of 2.3 mg/L

4.1.2.2 Freshwater Invertebrates: Chronic Exposure Studies

There is one acceptable chronic toxicity study available for freshwater invertebrates. In this study, conducted with daphnids, the NOAEC was 1.56 µg a.i./L and the LOAEC was 3.23 µg a.i./L, based on reduced number of young/adult/day (10% reduction compared to the control) and reduced adult length (7% reduction compared to the control) (MRID 439782-01).

4.1.3 Toxicity to Aquatic Plants

Aquatic plant toxicity studies were used as one of the measures of effect to evaluate whether tribufos 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.

A submitted study on aquatic vascular plants (duckweed, *Lemna gibba*) resulted in an EC_{50} of 1.10 mg a.i./L based on reduced frond count (MRID 458637-05). The percent growth inhibition, based on frond count, in the treated culture as compared to the control ranged from 26.2 to 69.8%. The NOEAC for this study could not be determined, because effects were seen at the lowest concentration tested (0.0172 mg a.i./L); however, an EC_{05} value of 0.410 mg a.i./L was estimated based on the raw data. This study was classified as supplemental because analytical verification of test concentrations on Day 14 of the study indicated that the test material was not stable over a 7-day period, and because a definitive NOAEC value was not established.

A Tier II aquatic nonvascular plant toxicity study (MRID 416188-13) was submitted on algae (*Selenastrum capricornutum*). The study resulted in an EC_{50} value of 0.148 mg a.i./L and a NOAEC value of 0.0585 mg a.i./L.

4.2 Toxicity of Tribufos to Terrestrial Organisms

Table 4.6 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. All of the endpoints for birds, mammals, terrestrial invertebrates, and terrestrial plants used for RQ calculations in this assessment come from submitted studies; there were no acceptable studies in the open literature that resulted in more sensitive endpoints for these taxa. Additional toxicity information for terrestrial organisms can be found in **APPENDICES C** and **D**.

TABLE 4.6. Terrestrial Toxicity Profile for Tribufos.

Endpoint	Species	Toxicity Value Used in Risk Assessment	Citation MRID# (Author & Date)	Comment
Acute Direct Toxicity to Terrestrial-Phase CRLF (LD_{50})	Bobwhite quail (*Colinus virginianus*)	LD_{50} = 151 mg/kg-bw (95% C.I. = 128 – 178)	00049258 00120771	Acceptable
Acute Direct Toxicity to Terrestrial-Phase CRLF (LC_{50})	Bobwhite quail (*Colinus virginianus*)	LC_{50} = 1519 mg/kg-diet	416188-04	Acceptable - NOAEC = 556 mg/kg-diet, based on reduced body weight gain
Chronic Direct Toxicity to Terrestrial-Phase CRLF	Bobwhite quail (*Colinus virginianus*)	NOAEC = 280 mg/kg-diet	407571-01	Acceptable - The NOAEC is based on reduced egg production and hatchling survival at 410 mg/kg-diet
Indirect Toxicity to Terrestrial-Phase CRLF (via acute toxicity to mammalian prey items)	Laboratory rat (*Rattus norvegicus*)	LD_{50} = 192 mg/kg-bw	419549-03	Acceptable
Indirect Toxicity to Terrestrial-Phase CRLF (via chronic toxicity to mammalian prey items)	Laboratory rat (*Rattus norvegicus*)	NOAEL = 32 mg/kg-diet (1.7 mg/kg/day) LOAEL = 260 mg/kg-diet (15 mg/kg/day)	420402-01	Acceptable – The NOAEL is based on significant increase in the number of litters with stillborn pups and pup death (including cannibalism) through lactation; decrease in F1 and F2 pup body weights; significant increase in F1 gestation period. These results are from a 2-generation reproduction study.
Indirect Toxicity to Terrestrial-Phase CRLF (via acute toxicity to terrestrial invertebrate prey items)	Honey bee (*Apis mellifera*)	LD_{50} = >24.17 µg a.i./bee	00001999 (Atkins and Anderson 1967)	Supplemental - There was 3% mortality at 24.17 µg a.i./bee
Indirect Toxicity to Terrestrial- and Aquatic-Phase CRLF (via toxicity to terrestrial plants)	<u>Seedling Emergence</u> Monocots	EC_{25} = >2.0 lb a.i./acre	458637-04	Acceptable - Based on onion plant height
	<u>Seedling Emergence</u> Dicots	EC_{25} = >2 lb a.i./acre	458637-04	Acceptable - Based on soybean plant height
	<u>Vegetative Vigor</u> Monocots	EC_{25} = 1.8 lb a.i./acre	458637-04	Acceptable - Based on corn dry weight

Endpoint	Species	Toxicity Value Used in Risk Assessment	Citation MRID# (Author & Date)	Comment
	Vegetative Vigor Dicots	EC_{25} = 1.3 lb a.i./acre	458637-04	Acceptable - Based on buckwheat dry weight

Acute toxicity to terrestrial animals is categorized using the classification system shown in **Table 4.7** (USEPA 2004). Toxicity categories for terrestrial plants have not been defined.

TABLE 4.7. 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 (USEPA 2004). No terrestrial-phase amphibian data are available for tribufos; therefore, acute and chronic avian toxicity data are used to assess the potential direct effects of tribufos to terrestrial-phase CRLFs.

4.2.1.1 Birds: Acute Exposure (Mortality) Studies

Available acute oral and subacute avian dietary studies are summarized in **Table 4.8**. Tribufos is considered slightly to moderately toxic to birds on an acute oral exposure basis, and slightly toxic to practically nontoxic to birds on a dietary exposure basis. The oral acute LD_{50} value for bobwhite quail, the most sensitive species tested, was 151 mg/kg-bw; the corresponding dietary acute LC_{50} value for the bobwhite quail was 1,519 mg/kg-diet (MRIDs 000492-58, 001207-71, and 416188-04). In the acute dietary study (MRID 416188-04), the LOAEC was 1,135 mg/kg-bw based on a reduction in body weight gain (15% reduction when compared to the control).

TABLE 4.8. Toxicity Endpoints Used to Estimate Potential Risk of Direct Effects to Terrestrial-phase CRLFs.

Avian species	Results	Source or MRID	Toxicity Category	Comment
Bobwhite quail (*Colinus virginianus*)	LD_{50} = **151 mg/kg-bw** (95% C.I. = 128 – 178)*	000492-58 001207-71	Moderately toxic	None
Mallard duck (*Anas platyrhynchos*)	LD_{50} = 871 mg/kg-bw	000492-58 001207-71	Slightly toxic	Supplemental (does not fulfill guideline requirements). There were only 8 birds/test group and no food consumption data were provided
Bobwhite quail (*Colinus virginianus*)	LC_{50} = **1519 mg/kg-diet***	416188-04	Slightly toxic	NOAEC = 556 mg/kg-diet; LOAEC = 1135 mg/kg-diet, based on reduced body weight gain
Mallard duck (*Anas platyrhynchos*)	LC_{50} = >5000 mg/kg-diet	416188-05	Practically non-toxic	LOAEC = 313, based on reduced body weight gain; a NOAEC could not be determined because effects were seen at the lowest concentration tested.

*Most sensitive species tested, used for RQ calculations

4.2.1.2 Birds: Chronic Exposure (Growth, Reproduction) Studies

One avian chronic exposure study was submitted to the Agency for review. In a bobwhite quail (*Colinus virginianus*) reproduction toxicity study, NOAEC values of 150 mg/kg-diet and 280 mg/kg-diet were reported, based on reduced eggshell thickness and reduced egg production/hatchling survival, respectively (MRID 407571-01). Because eggshell thickness is not a relevant endpoint for terrestrial-phase CRLFs, the NOAEC value of 280 mg/kg-diet based on reduced egg production and hatchling survival was used in this assessment. The corresponding LOAEC value, based on reduced egg production (71% reduction when compared to the control) and hatchling survival (56% reduction when compared to the control) was 410 mg/kg-diet.

4.2.2 Toxicity to Mammals

Mammalian toxicity data are used to assess potential indirect effects of tribufos to the terrestrial-phase CRLF. Effects to small mammals resulting from exposure to tribufos could also potentially result in indirect effects to 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

Available data indicate that tribufos is moderately toxic to laboratory rats (*Rattus norvegicus*) on an acute oral exposure basis, with an LD_{50} of 192 mg a.i./kg-bw (MRID 419549-03).

4.2.2.2 Mammals: Chronic Exposure (Growth, Reproduction) Studies

A two-generation reproduction study on laboratory rats was conducted to determine the effects of tribufos on mammalian reproduction. Tribufos affected reproductive success (based on increased stillborn pups and pup death, decreased F1 and F2 pup body weights, and increased F1 gestation periods) with a NOAEC of 32 mg/kg-diet [1.7 mg/kg/day] and a LOAEC of 260 mg/kg-diet [13 mg/kg/day] (MRID 420402-01).

Sublethal effects related to cholinesterase (ChE) inhibition have also been noted in a variety of mammals. Plasma and red blood cell (RBC) ChE activity was decreased in rats at 7 mg/kg-diet in a prenatal developmental toxicity study (MRID: 40190601). In a chronic dog study, plasma ChE was decreased at 0.4 mg/kg-diet (MRID: 42007203). Additionally, rabbits showed statistically significant plasma and RBC ChE inhibition at 2 mg/kg-diet in a 21-day toxicity study (MRID: 420072-01).

4.2.3 Toxicity to Terrestrial Invertebrates

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

One study on acute exposure of tribufos to honey bees (*Apis mellifera*) was submitted to the Agency and is considered supplemental (because tribufos was mixed with pyrolite dust in the study). The LD_{50} value was determined to be greater than 24.17 µg a.i./bee (the only concentration tested); there was 3% mortality at this treatment level (MRID 000019-99; Atkins and Anderson 1967). Therefore, tribufos is considered practically nontoxic to bees. Because the LD50 is indiscreet (*i.e.*, it has a ‘greater than’ value), this endpoint could not be used for RQ calculation.

An additional study identified from the open literature (Greenberg *et al*., 2004; ECOTOX Ref.: 92450) includes some information on effects of tribufos on terrestrial invertebrates [specifically boll weevils (*Anthonomus grandis*)]. This study is considered supplemental and not adequate for RQ calculation (*e.g.*, it is a non-guideline study; no controls were used in portions of the study; only one concentration was tested). In the laboratory portion of the study, boll weevils sprayed directly with tribufos at an application rate of 0.42 lb a.i./acre (using the DEF 6 formulation), which is 22% of the maximum allowable labeled rate, had statistically significantly higher mortality than controls 72-hrs post-spray [42.5% (± 2.2) mortality, versus 2.5% (± 5.0), respectively]. Field tests, using the 0.42 lb a.i./acre application rate, also show tribufos exhibiting a toxic effect on boll weevils, with a 36.2% (±21.9) reduction in boll weevil population 72-hrs post-spray (there was no control in this part of the study).

4.2.4 Toxicity to Terrestrial Plants

Terrestrial plant toxicity data are used to evaluate the potential for tribufos 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 of upland and riparian habitat that provides shelter and areas for foraging, predator avoidance and dispersal for juvenile and adult CRLFs.

Plant toxicity data from both registrant-submitted studies and studies in the scientific literature were reviewed for this assessment. Registrant-submitted studies are conducted under conditions and with species defined in EPA toxicity test guidelines. Sub-lethal endpoints such as plant growth, dry weight, and biomass are evaluated for both monocots and dicots, and effects are evaluated at both seedling emergence and vegetative life stages. Guideline studies generally evaluate toxicity to ten crop species. A potential drawback to these studies is that they are conducted on herbaceous crop species only, and extrapolation of effects to other species, such as the woody shrubs and trees and wild herbaceous species, contributes uncertainty to risk conclusions. This is especially relevant for tribufos, which has a mode of action that is expected to have greater impact on plants that form abscission zones (*e.g.*, deciduous trees and shrubs).

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

The results of the Tier II seedling emergence and vegetative vigor toxicity studies on non-target terrestrial plants are summarized below in **Table 4.9.** All tests were conducted using the tribufos formulated product DEF 6® (70.8% a.i.).

TABLE 4.9. Non-target Terrestrial Plant Seedling Emergence and Vegetative Vigor Toxicity (Tier II) Data.

Crop	Type of Study Species	NOAEC (lb ai/A)	EC_{25} (lb ai/A)	Most sensitive parameter	Slope
Seedling Emergence (MRID 458637-04)					
Monocots	corn	2.0	>2.0	none	N/A
	onion*	0.45	>2.0	plant height	0.354
	ryegrass	2.0	>2.0	none	N/A
	wheat	2.0	>2.0	none	N/A
Dicots	buckwheat	2.0	>2.0	none	N/A
	cucumber	2.0	>2.0	none	N/A
	soybean*	0.22	>2.0	plant height	0.308
	sunflower	2.0	>2.0	none	N/A
	tomato	2.0	>2.0	none	N/A
	turnip	0.94	>2.0	plant height	2.16
Vegetative Vigor (MRID 458637-04)					
Monocots	corn*	0.48	1.8	dry weight	1.39
	onion	0.47	>1.9	dry weight	0.988
	ryegrass	2.1	>2.1	none	N/A
	wheat	2.1	>2.1	dry weight	0.056
Dicots	buckwheat*	0.48	1.3	dry weight	4.08
	cucumber	0.23	>1.9	dry weight	0.209
	soybean	0.99	2.1	dry weight	1.19
	sunflower	1.9	>1.9	none	N/A
	tomato	0.47	1.6	dry weight	1.97
	turnip	0.23	>1.9	dry weight	0.275

* Indicates the most sensitive monocot/dicot.

The submitted Tier II seedling emergence study (MRID 45867-04) indicated that onion was the most sensitive monocot species tested (NOAEC 0.45 lb a.i./A; EC_{25} >2.0 lb a.i./A) based on reduced plant height. Soybean was the most sensitive dicot species tested, also based on reduced plant height, with a NOAEC of 0.22 lb a.i./A and an EC_{25} of > 2.0 lb a.i./A. All other species (monocots and dicots) had NOAEC and EC_{25} values at 2.0 lb a.i./A and > 2.0 lb a.i./A (the highest concentration tested) with the exception of turnip, a dicot species with a NOAEC of 0.94 lb a.i./A.

The Tier II vegetative vigor study (MRID 45867-04) indicated that corn (monocot) and buckwheat (dicot) were the most sensitive species based on reduced dry weight. The NOAEC and EC_{25} values for corn were 0.48 lb a.i./A and 1.8 lb a.i./A, respectively. The NOAEC and EC_{25} values for buckwheat were 0.48 lb a.i./A and 1.3 lb a.i./A, respectively. All other species tested showed that dry weight was the most sensitive endpoint for all species, though ryegrass (monocot) and sunflower (dicot) did not exhibit effects at any test concentration. The endpoint values from this study are summarized in **Table 4.9.**

Because tribufos affects the abscission zone in plants, plants that do not form abscission zones (such as those used in the vegetative vigor and seedling emergence studies discussed above) are not expected to be as sensitive to tribufos as plants that form abscission zones. One study identified from the open literature (Greenberg *et al*., 2004; ECOTOX Ref.: 92450) does describe effects (defoliation) to cotton from a tribufos application at a rate of 0.42 lb a.i./acre (using the DEF 6 formulation). At this application rate (which is 22% of the maximum allowable labeled rate), an average of 80.2% (± SD of 6.1) of the leaves per plant (N = 9) were dropped 7 days post-application, whereas in the untreated control plot, 3.3% (± SD of 1.6) of the leaves per plant (N = 10) were dropped in the same time period.

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 (USEPA, 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 tribufos 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. This analysis is presented in Section 5.2.

4.4 Incident Database Review

A review of the EIIS database for ecological incidents involving tribufos was completed on June 18, 2008. There is only one incident in the EIIS database associated with tribufos (I016036-024). This incident involved damage to 53 acres of lettuce in Riverside, California, in 2004. The damage was described as ‘uniform throughout’ the field, although the specific type of damage to the lettuce was not specified in the incident report. Damage was attributed to spray drift of tribufos and diuron that had been legally applied by air to a cotton field adjacent to the damaged lettuce field. The damaged lettuce tested positive for tribufos (50 ppb). No other residue information was provided in the report. Due to limitations with data in the EIIS, a low number or lack of reported incidents in the database cannot be used as evidence that additional incidents have not occurred.

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 tribufos 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 E**). For acute exposures to the CRLF and its animal prey in aquatic habitats, as well as terrestrial invertebrates, the LOC is 0.05. For acute exposures to the CRLF and mammals, the LOC is 0.1. The LOC 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 1-in-10 year EECs based on the label-recommended tribufos usage scenario for cotton summarized in **Table 3.4** and the appropriate aquatic toxicity endpoint from **Table 4.1**. Risks to the terrestrial-phase CRLF and its prey (*e.g.*, terrestrial insects, small mammals and terrestrial-phase frogs) are estimated based on exposures resulting from applications of tribufos (**Tables 3.6** through **3.7**) and the appropriate toxicity endpoint from **Table 4.6**. Exposures are also derived for terrestrial plants, as discussed in Section 3.3 and summarized in **Table 3.8**, based on the highest application rates of tribufos use within the action area.

5.1.1 Exposures in the Aquatic Habitat

5.1.1.1 Direct Effects to Aquatic-Phase CRLF

Potential direct acute effects to the aquatic-phase CRLF are based on the modeled peak EEC in the standard pond for aerial and ground application methods, and the lowest acute toxicity value for freshwater fish. For aerial applications, the acute RQ of 0.07 exceeds the Agency's acute endangered species LOC of 0.05 for aquatic organisms. In order to assess direct chronic risks to the aquatic-phase CRLF, 60-day EECs and the lowest chronic toxicity value for freshwater fish are used. The resulting chronic RQ of 3.98 exceeds the Agency's chronic LOC for freshwater fish. For ground applications, the acute RQ of 0.05 equals the Agency's acute endangered species LOC for aquatic organisms. The resulting chronic RQ of 2.90, using the ground application EEC, exceeds the Agency's chronic LOC for freshwater fish. (See **Table 5.1**).

TABLE 5.1. Summary of Acute and Chronic RQs for Freshwater Fish.

Use/Application Rate & Method	Species	Peak EEC (µg/L)	60-day EEC (µg/L)	Acute RQ*	Chronic RQ*
Cotton/1.875 lb a.i./acre (Aerial)	Bluegill sunfish (*Lepomis macrochirus*)	16.30	13.94	**0.07**	**3.98**
Cotton/1.875 lb a.i./acre (Ground)	Bluegill sunfish (*Lepomis macrochirus*)	11.53	10.15	**0.05**	**2.90**
* = LOC exceedances (acute listed species RQ $\geq$ 0.05; chronic RQ $\geq$ 1.0) are bolded. Acute RQ = use-specific peak EEC /bluegill sunfish LC_{50} = 245 µg a.i./L. Chronic RQ = use-specific 60-day EEC /ACR-derived NOAEC = 3.5 µg a.i./L.					

Based on acute and chronic RQs that exceed the Agency's LOCs, tribufos has the potential to directly affect the aquatic-phase CRLF, using freshwater fish data as a surrogate. These RQs are further described as they relate to the effects determination in Section 5.2. Additionally, the bioconcentration factor (BCF) of 730X indicates the potential of bioaccumulation of tribufos in potential fish or frog prey items. Given the aerial application aquatic EEC of 16.34 ppb, fish tissue residues could be greater than 11,900 µg/kg (>11.9 ppm) (730 BCF x 16.3 ppb = 11,899 ppb). Therefore, due to the potential for bioaccumulation, CRLFs could be also exposed to tribufos via the ingestion of contaminated aquatic prey items.

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 tribufos to the aquatic-phase CRLF (tadpoles) via reduction in non-vascular aquatic plants in its diet are based on peak EECs from the standard pond and the lowest toxicity value (EC_{50}) for aquatic non-vascular plants. The resulting RQ of 0.11 is below the Agency's LOC for aquatic plants (see **Table 5.2**).

TABLE 5.2. Summary of RQs Used to Estimate Indirect Effects to the CRLF via Effects to Non-Vascular Aquatic Plants (diet of CRLF in tadpole life stage and habitat of aquatic-phase CRLF).

Use	Application rate (lb ai/A) and type	Peak EEC (µg/L)	Indirect effects RQ* (food and habitat)
Cotton	1.875 (foliar)	16.34	0.11

* RQ = use-specific peak EEC/non-vascular aquatic plant EC_{50} (148 µg a.i./L).

Based on these results, tribufos is not expected to indirectly affect the CRLF via reduction in non-vascular aquatic plants as diet of tadpoles or as habitat for aquatic-phase adults and juveniles.

Aquatic Invertebrates

A summary of the acute and chronic RQ values for exposure to aquatic invertebrates (as prey items of aquatic-phase CRLFs) is provided in **Table 5.3**. Indirect effects to the aquatic-phase CRLF via effects to prey (invertebrates) in aquatic habitats are based on peak EECs in the standard pond and the lowest acute toxicity value for freshwater invertebrates. For aerial application methods, the resulting acute RQ of 2.40 exceeds the Agency's endangered species LOC of 0.05 for aquatic invertebrates. Based on the 21-day EEC and the lowest chronic toxicity value for freshwater invertebrates, the resulting chronic RQ of 9.54 exceeds the Agency's LOC of 1 for aquatic organisms. For ground application methods, the resulting acute RQ of 1.70 exceeds the Agency's endangered species LOC for aquatic invertebrates, and the resulting chronic RQ of 6.81 also exceeds the Agency's LOC. Based on LOC exceedances for both acute and chronic RQs (aerial and ground applications), tribufos has the potential to indirectly affect the CRLF via reduction in freshwater invertebrate prey items. These RQs are further described as they related to the effects determination in Section 5.2.

TABLE 5.3. Summary of Acute and Chronic RQs for Aquatic Invertebrates.

Use/Application Rate & Method	Species	Peak EEC (µg/L)	21-day EEC (µg/L)	Acute RQ*	Chronic RQ*
Cotton/1.875 lb a.i./acre (Aerial)	Daphnid (*Daphnia magna*)	16.34	14.89	**2.40**	**9.54**
Cotton/1.875 lb a.i./acre (Ground)	Daphnid (*Daphnia magna*)	11.53	10.62	**1.70**	**6.81**

* = LOC exceedances (acute listed species RQ $\geq$ 0.05; chronic RQ $\geq$ 1.0) are bolded. Acute RQ = use-specific peak EEC /daphnid EC_{50} = 6.8 µg a.i./L. Chronic RQ = use-specific 21-day EEC /daphnid NOAEC = 1.56 µg a.i./L.

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 5.1**) are used to assess potential indirect effects to the CRLF based on a reduction in freshwater fish and frogs as food items. Additionally, the bioconcentration factor (BCF) of 730X indicates the potential of bioaccumulation of tribufos in potential fish prey items. Given the aerial application aquatic EEC of 16.34 ppb, fish tissue residues could be greater than 11,900 µg/kg (>11.9 ppm) (730 BCF x 16.3 ppb = 11,899 ppb).

Based on chronic RQs for freshwater fish that meet or exceed the Agency's LOCs, and the potential for bioaccumulation in freshwater fish prey items, tribufos has the potential to indirectly affect the CRLF via reduction in freshwater fish and frogs as food items.

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

Indirect effects to the CRLF via direct toxicity to aquatic plants are estimated using 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, were used to derive RQs. The resulting RQ for vascular plants of 0.02 does not exceed the Agency's LOC for plants (see **Table 5.4**). Therefore, based on the lack of effects to vascular aquatic plants, tribufos has no effect on the CRLF via reduction in habitat or primary productivity as they relate to aquatic plants.

TABLE 5.4. Summary of RQs Used to Estimate Indirect Effects to the CRLF via Effects to Vascular Aquatic Plants (habitat of aquatic-phase CRLF)[a]

Use	Application rate (lb ai/A) and type	Peak EEC (µg/L)	Indirect effects RQ* (food and habitat)
Cotton	1.875 (foliar)	16.34	0.02

[a] RQs used to estimate indirect effects to the CRLF via toxicity to non-vascular aquatic plants are summarized in Table 5.2.
* RQ = use-specific peak EEC /duckweed EC_{50} = 1,100 µg a.i./L.

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 direct effects to terrestrial-phase CRLFs are based on foliar applications of tribufos.

Potential direct acute effects to the terrestrial-phase CRLF are derived by considering dose- and dietary-based EECs modeled in T-REX for a small bird (20 g) consuming small invertebrates (**Table 3.6**) and acute oral and subacute dietary toxicity endpoints for avian species. Resulting acute dietary- and dose-based RQs (0.17 and 2.65, respectively) exceed the Agency's acute endangered species LOC of 0.1 for birds. Additionally, the dose-based RQ also exceeds the Agency's non-endangered species acute risk LOC of 0.5 (see **Table 5.5**).

Potential direct chronic effects of tribufos to the terrestrial-phase CRLF are derived by considering dietary-based exposures modeled in T-REX for a small bird (20g) consuming small invertebrates. Chronic effects are estimated using the lowest available toxicity data for birds that is relevant for the terrestrial-phase CRLF. EECs are divided by toxicity values to estimate chronic dietary-based RQs. The chronic dietary-based RQ (1.61) also exceeds the Agency's chronic LOC (see **Table 5.5**).

TABLE 5.5. Summary of Acute and Chronic RQs* Used to Estimate Direct Effects to the Terrestrial-phase CRLF.

Use (Application Rate)	Dietary-based Acute RQ[1]	Dose-based Acute RQ[1]	Dietary-based Chronic RQ[1]
Cotton (1.875 lb ai/A)	**0.17**	**2.65**	**1.61**

* = LOC exceedances (acute endangered species RQ $\geq$0.1; chronic RQ $\geq$ 1) are bolded.
[1] Based on bobwhite quail LC_{50} = 1,519 mg a.i./kg-diet, LD_{50} =151 mg a.i./kg-bw, and NOAEC = 280 mg a.i./kg-diet.

Based on acute and chronic RQs that exceed the Agency's LOCs, tribufos has the potential to directly affect the terrestrial-phase of the CRLF. These RQs are further described as they relate to the effects determination in Section 5.2.

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

Terrestrial Invertebrates

In order to assess the risks of tribufos to terrestrial invertebrates, which are considered prey of CRLF in terrestrial habitats, the honey bee is used as a surrogate for terrestrial invertebrates. The toxicity value for terrestrial invertebrates is calculated by multiplying the lowest available acute contact LD_{50} of >24.2 µg a.i./bee by 1 bee/0.128g, which is based on the weight of an adult honey bee. EECs (µg a.i./g of bee) calculated by T-REX for small and large insects (Table 3.6) are divided by the calculated toxicity value for terrestrial invertebrates, which is >189 ppm (*i.e.*, µg a.i./g). Resulting RQs are <1.35 and <0.15 for small and large insects, respectively. Since the toxicity data for honeybees are based on a single concentration that resulted in 3% mortality, a discreet LD_{50} for terrestrial insects could not be determined. Although it is unclear whether the non-definitive "less than" RQ values exceed the LOC of 0.05, potential risks to terrestrial invertebrates cannot be precluded.

Additional information from the open literature (Greenberg *et al*., 2004; ECOTOX Ref.: 92450) indicates mortality to individuals and population effects in boll weevils exposed to tribufos at an application rate of 0.42 lb a.i./acre (using the DEF 6 formulation), which is 22% of the maximum allowable labeled rate. Therefore, although neither study could be used for RQ calculation, based on the effects demonstrated in boll weevil study and the indiscreet LD_{50} for honey bees, tribufos has the potential to indirectly affect the CRLF via reduction in terrestrial invertebrate prey items (see **Table 5.6**).

TABLE 5.6. Summary of RQs Used to Estimate Indirect Effects to the Terrestrial-phase CRLF via Direct Effects on Terrestrial Invertebrates as Dietary Food Items.

Use (Application Rate)	Small Insect RQ*	Large Insect RQ*
Cotton (1.875 lb ai/A)	**<1.34**	**<0.15**

* = LOC exceedances (RQ ≥ 0.05) are bolded. Because a definitive endpoint was not established for terrestrial invertebrates (*i.e.*, the value is greater than the highest test concentration), the RQ represents an upper bound value.

Mammals

Potential indirect effects to large terrestrial-phase CRLFs associated with reduction in small mammals as food items are derived for dietary-based and dose-based exposures modeled in T-REX for a small mammal (15 g) consuming short grass. Effects from acute and chronic exposures are estimated using the most sensitive mammalian toxicity data. EECs are divided by the toxicity value to estimate acute and chronic dose-based RQs as well as chronic dietary-based RQs. The resulting acute dose-based RQ of 1.02 (see **Table 5.7**), and dose- and dietary-based chronic RQs of 122 and 14.1, respectively, exceed the Agency's LOCs. Based on acute and chronic LOC exceedances, tribufos has the potential to indirectly affect the CRLF via reduction in small mammal prey items. These RQs are further described as they relate to the effects determination in Section 5.2.

TABLE 5.7. Summary of Acute and Chronic RQs* Used to Estimate Indirect Effects to the Terrestrial-phase CRLF via Direct Effects on Small Mammals as Dietary Food Items.

Use (Application Rate)	Acute RQ	Chronic RQ	
	Dose-based Acute RQ[1]	Dose-based Chronic RQ[2]	Dietary-based Chronic RQ[3]
Cotton (1.875 lb a.i./A)	**1.02**	**122**	**14.1**

* = LOC exceedances (acute risk RQ ≥ 0.5 and chronic RQ ≥ 1) are bolded.
[1] Based on dose-based EEC and tribufos rat acute oral LD_{50} = 192 mg/kg-bw.
[2] Based on dose-based EEC and tribufos rat NOAEL = 1.6 mg/kg-bw.
[3] Based on dietary-based EEC and tribufos rat NOAEC = 32 mg/kg-diet.

Frogs

An additional prey item of the adult terrestrial-phase CRLF is other species of frogs. In order to assess risks to these organisms, dietary-based and dose-based exposures modeled in T-REX for a small bird (20 g) consuming small invertebrates are used. The results for the potential for indirect effects to frogs are the same for those described above for direct effects to terrestrial-phase frogs [see Section 5.1.2.1 and associated table (**Table 5.5**) for details]. Based on acute and chronic RQs that exceed the Agency's LOCs, tribufos has the potential to indirectly affect the CRLF via reduction in frogs as prey items.

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

Potential indirect effects to the CRLF resulting from direct effects on riparian and upland vegetation are assessed using RQs from terrestrial plant seedling emergence and vegetative vigor EC_{25} data as a screen. Based on the available toxicity data, no RQs for terrestrial plants exceed the Agency's LOC (see **Table 5.8**). Example output from TerrPlant v.1.2.2 is provided in **APPENDIX H.** Because the available toxicity studies were conducted on plant species that do not form abscission zones, data from these studies may underestimate the potential toxicity of tribufos to non-target plants that do form abscission zones (*e.g.*, some woody, deciduous plants). Therefore, based on the mode of action of tribufos as a known defoliant, the associated plant incident discussed in Section 4.4, the lack of available toxicity data for terrestrial plants with abscission zones, tribufos has the potential to indirectly affect the CRLF via reduction in terrestrial plants (*e.g.*, deciduous shrubs and trees) that comprise part of its habitat.

TABLE 5.8. RQs for Monocots and Dicots Inhabiting Dry and Semi-Aquatic Areas Exposed to Tribufos via Runoff and Drift*.

Use	Application rate (lbs a.i./A)	Application method	Drift Value (%)	Spray drift RQ	Dry area RQ	Semi-aquatic area RQ
Cotton	1.875	Foliar – ground	1	<0.1	<0.1	0.10
		Foliar – aerial	5	<0.1	<0.1	0.14

* The RQs are the same for both monocots and dicots.

5.1.3 Primary Constituent Elements of Designated Critical Habitat

For tribufos use on cotton, the assessment endpoints for designated critical habitat PCEs involve a reduction and/or modification of habitat and/or food sources necessary for normal growth and viability of aquatic- and terrestrial-phase CRLFs. Because these endpoints are also being assessed relative to the potential for direct and indirect effects to aquatic- and terrestrial-phase CRLF, the effects determinations for indirect effects from the potential loss of food items and habitat modification are used as the basis of the effects determination for potential modification to designated critical habitat. Potential effects to taxa that are related to potential impacts to critical habitat (*i.e.*, a reduction and/or modification of habitat and/or food sources necessary for normal growth and viability of aquatic- and terrestrial-phase CRLFs) are described in Section 5.1 (see above).

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 for the use of tribufos on cotton in California. However, if direct or indirect effect LOCs are exceeded and/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 tribufos. A preliminary effects determination of 'may effect' is made for the CRLF and it's designated critical habitat based on LOC exceedances for freshwater fish (acute and chronic), aquatic invertebrates (acute and chronic), birds (acute and chronic), mammals (acute and chronic), and potential effects to terrestrial plants that form abscission zones. Furthermore, risks to terrestrial invertebrates cannot be precluded because a discreet toxicity endpoint is not available. A summary of the risk estimation results are provided in **Table 5.9** for direct and indirect effects to the CRLF and in **Table 5.10** for the PCEs of designated critical habitat for the CRLF.

TABLE 5.9. Risk Estimation Summary for Tribufos - Direct and Indirect Effects to CRLF.

Assessment Endpoint	LOC Exceedances (Y/N)	Description of Results of Risk Estimation
Aquatic Phase (eggs, larvae, tadpoles, juveniles, and adults)		
Direct Effects Survival, growth, and reproduction of CRLF individuals via direct effects on aquatic phases	Y	Based on the freshwater fish acute and chronic RQs that exceed the Agency's LOCs, tribufos has the potential to directly affect the aquatic-phase of the CRLF.
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects to food supply (*i.e.*, freshwater invertebrates, non-vascular plants)	Y	Based on acute and/or chronic RQs that exceed the Agency's LOCs, tribufos has the potential to indirectly affect the aquatic-phase of the CRLF via effects to freshwater invertebrates, fish, and frogs (as a food supply).
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects on habitat, cover, and/or primary productivity (*i.e.*, aquatic plant community)	N	The freshwater aquatic plant RQs were calculated based on the most sensitive EC_{50} value rather than the NOAEC value, because there are no obligate relationships between the CRLF and any aquatic plant species. RQs for vascular and non-vascular aquatic plants are less than the Agency's LOC; therefore, tribufos is not expected to indirectly affect the CRLF via effects to aquatic plants.
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects to riparian vegetation, required to maintain acceptable water quality and habitat in ponds and streams comprising the species' current range.	N (however, effects to some terrestrial plants cannot be precluded)	Although the RQs based on the results of the submitted terrestrial plant studies do not exceed Agency LOCs, toxicity data are not available for terrestrial plants with abscission zones that are expected to be sensitive to tribufos. Given that tribufos is a known defoliant, it is associated with one plant incident, and it has a mechanism of action that could result in effects to plants important to CRLF habitat (*e.g.*, deciduous scrubs and trees), tribufos has the potential to indirectly affect the CRLF via reduction in terrestrial plants.
Terrestrial Phase (Juveniles and adults)		
Direct Effects Survival, growth, and reproduction of CRLF individuals via direct effects on terrestrial phase adults and juveniles	Y	Avian acute and chronic RQs exceed the Agency's LOCs; therefore, tribufos has the potential to directly affect the terrestrial-phase of the CRLF.
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects on prey (*i.e.*, terrestrial invertebrates, small	Y	Acute and chronic LOCs are exceeded for mammals and birds. Additionally, an LOC exceedance for terrestrial invertebrates cannot be precluded for tribufos. Therefore, there is a potential for tribufos to indirectly affect the terrestrial-phase CRLF via a reduction in prey items.

Assessment Endpoint	LOC Exceedances (Y/N)	Description of Results of Risk Estimation
terrestrial mammals and terrestrial phase amphibians)		
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects on habitat (*i.e.*, riparian vegetation)	N (however, effects to some terrestrial plants cannot be precluded)	Although the RQs based on the results of the submitted terrestrial plant studies do not exceed Agency LOCs, toxicity data are not available for terrestrial plants with abscission zones that are expected to be sensitive to tribufos. Given that tribufos is a known defoliant, it is associated with one plant incident, and it has a mechanism of action that could result in effects to plants important to CRLF habitat (*e.g*., deciduous scrubs and trees), tribufos has the potential to indirectly affect the CRLF via reduction in terrestrial plants.

TABLE 5.10. Risk Estimation Summary for Tribufos – PCEs of Designated Critical Habitat for the CRLF.

Assessment Endpoint	LOC Exceedances (Y/N)	Description of Results of Risk Estimation
Aquatic Phase PCEs (Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)		
Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile and adult CRLFs.	N (however, effects to some terrestrial plants cannot be precluded)	The RQs based on the results of the submitted terrestrial plant studies do not exceed Agency LOCs. However, tribufos is a known defoliant, it is associated with one plant incident, and it has a mechanism of action that could result in effects to plants important in maintaining aquatic critical habitat for the CRLF. Tribufos is not likely to modify CRLF critical habitat via effects to aquatic plants.
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.	N (however, effects to some terrestrial plants cannot be precluded)	The RQs based on the results of the submitted terrestrial plant studies do not exceed Agency LOCs. However, tribufos is a known defoliant, it is associated with one plant incident, and it has a mechanism of action that could result in effects to plants important in maintaining aquatic critical habitat for the CRLF. Tribufos is not likely to modify CRLF critical habitat via effects to aquatic plants.
Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.	Y	Based on LOC exceedances, there is a potential for habitat modification via direct impacts to aquatic-phase CRLFs and effects to freshwater invertebrates, fish, and frogs as food items.

Assessment Endpoint	LOC Exceedances (Y/N)	Description of Results of Risk Estimation
Reduction and/or modification of aquatic-based food sources for pre-metamorphs (*e.g.*, algae)	N	Because the RQ was below the Agency's LOC for aquatic non-vascular plants, tribufos is not likely to modify the CRLF critical habitat via effects to algae used as food for metamorphs.
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	N (however, effects to some terrestrial plants cannot be precluded)	RQs based on the results of the submitted terrestrial plant studies do not exceed Agency LOCs. However, tribufos is a known defoliant, it is associated with one plant incident, and it has a mechanism of action that could result in effects to plants important to CRLF critical habitat.
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	N (however, effects to some terrestrial plants cannot be precluded)	RQs based on the results of the submitted terrestrial plant studies do not exceed Agency LOCs. However, tribufos is a known defoliant, it is associated with one plant incident, and it has a mechanism of action that could result in effects to plants important to CRLF critical habitat.
Reduction and/or modification of food sources for terrestrial phase juveniles and adults	Y	Acute LOCs are exceeded for mammals and acute and chronic LOCs are exceeded for birds. Additionally, an LOC exceedance for endangered terrestrial invertebrates cannot be precluded for tribufos.
Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	Y	Acute LOCs are exceeded for mammals and acute and chronic LOCs are exceeded for birds. Additionally, an LOC exceedance for endangered terrestrial invertebrates cannot be precluded for tribufos.

Based on the results of the risk estimation, the following risk hypotheses (as discussed in Section 2.9.1.) are not rejected:

The labeled use of tribufos 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 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.

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

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

- <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 receive runoff and spray drift containing tribufos.

There are very little monitoring data for tribufos to compare with modeling results and because these data come from non-targeted studies, they are of limited value for analysis. Additionally, there were no aquatic incidents reported for tribufos. Therefore, modeled results are used for assessing risks to aquatic species in this assessment.

As noted previously, all analyses were performed on the only registered use of tribufos, as a cotton defoliant. Based on surrogate freshwater fish toxicity data for tribufos, there is potential for tribufos to both directly and indirectly affect the CRLF. Both the acute and chronic RQs (0.07 and 3.98, respectively) for the most sensitive species tested, bluegill sunfish, exceeded the freshwater fish LOCs, indicating there is potential for direct effects via both acute and chronic toxicity to the CRLF from tribufos use, when considering aerial application methods. The estimated environmental concentration value from modeling ground application methods results reduces these values slightly. The resulting acute freshwater fish RQ of 0.05 equals the endangered species LOC, while the chronic RQ value (2.90) remains above the LOC. This indicates that ground applications of tribufos also have the potential to directly affect the CRLF via both acute and chronic toxicity.

Only one study was submitted that evaluated chronic effects of tribufos on freshwater fish (fathead minnow). Since this species was not the most acutely sensitive species tested, and because the study was not adequate for RQ calculation, an acute-to-chronic ratio was calculated for bluegill sunfish (the most acutely sensitive species), using values for the sheepshead minnow (which had both acute and chronic values submitted). It should be noted that the sheepshead species is an estuarine/marine species, not a freshwater species; however, the consistency of the acute toxicity values for saltwater and freshwater species does not suggest that saltwater species are considerably more or less sensitive to tribufos than freshwater species. The resulting estimated chronic NOAEC value for the bluegill sunfish was similar to the submitted fathead chronic value.

The probit dose-response slope was used to calculate the chance of an individual event corresponding to the listed species acute LOC, since no acute RQs exceeded the Agency's LOC. The analysis uses the EFED spreadsheet IEC (version 1.1.xls). Using the acute endangered species LOC of 0.05, and a slope derived from the acute bluegill sunfish study (MRID: 400980-01) (slope = 6.91, with 95% confidence limits of 3.11 and 10.71), the chance of an individual mortality for the aquatic-phase CRLF is ~ 1 in 8.10E+18 (**Table 5.11**).

TABLE 5.11. Chance of Individual Acute Effects to Freshwater Fish Using the Probit Slope Response Relationship.

LOC OR USE SITE SCENARIO (RQ)	RQ	PROBIT SLOPE	CHANCE OF AN INDIVIDUAL EFFECT
Cotton (ground)	0.05	Slope = 6.91	~ 1 in 8.10E+18
		Lower Bound = 3.11	~ 1 in 38,400
		Upper Bound = 10.71	~ 1 in 5.08E+43
Cotton (aerial)	0.07	Slope = 6.91	~ 1 in 1.37E+15
		Lower Bound = 3.11	~ 1 in 6.09E+03
		Upper Bound = 10.71	~ 1 in 5.20E+34

Because the RQs for acute and chronic effects from both aerial and ground applications exceed (or are equal to) the listed species LOCs for fish, some potential cotton use sites in CA overlap with CRLF range (see **Fig. 5.1**), and usage data show that the maximum single application rate for tribufos is being used in California, there is potential for direct adverse effects to the aquatic-phase CRLF found immediately adjacent to application sites from the use of tribufos.

5.2.1.2 Terrestrial-Phase CRLF

For 20 g birds that eat small insects, which are used as a surrogate for terrestrial-phase CRLF, the acute dose-based RQ (2.65), the acute dietary-based RQ (0.17) and the chronic dietary-based RQ (1.61) exceed the Agency's LOCs at the maximum application rate for tribufos use on cotton in California. Therefore, direct adverse effects to terrestrial-phase CRLFs may occur from tribufos use on cotton.

In order to refine the assessment of potential direct effects to the terrestrial-phase CRLF, T-HERPS was used to calculate RQs for the CRLF using toxicity data from birds and the ingestion rate of insectivorous iguanids (see **APPENDIX K** for more details). The acute RQs from the dose-based analysis are exceeded for 37 g and 238 g frogs that eat small herbivorous mammals (RQs = 1.86 and 0.29, respectively) and 37 g frogs that eat small insectivorous mammals (RQ = 0.12) (see **Table 5.12**). Additionally, the acute dietary-based RQ exceeded the Agency's endangered species LOC for frogs eating small insectivorous and herbivorous mammals. The acute dietary-based and chronic RQs for frogs consuming small herbivorous mammals also exceeded the Agency's LOCs, indicating that terrestrial-phase CRLF that eat small mammals are at potential risk from acute and chronic exposure to tribufos from its labeled use on cotton. CRLF that eat small insects are also at potential risk from acute exposure to tribufos.

TABLE 5.12. Upper Bound Kenaga Acute and Chronic Terrestrial Herpetofauna RQs (from T-HERPS) (1 Application, 1.875 lb a.i./Acre, 35-Day Foliar Half-life).

Size Class (grams)	Small Insects	Large Insects	Small Herbivore Mammals	Small Insectivore Mammal	Small Amphibians
	RQ	RQ	RQ	RQ	RQ
Acute Dose-Based					
1.4	0.07	0.01	N/A	N/A	N/A
37	0.06	0.01	**1.86**	**0.12**	<0.01
238	0.04	<0.01	**0.29**	0.02	<0.01
Acute Dietary-Based					
	0.17	0.02	**0.20**	0.01	0.01
CHRONIC RQs					
	0.90	0.10	**1.06**	0.07	0.03

Bolded numbers exceed the Agency's LOCs.

Using the acute endangered species LOC of 0.1 and a slope derived from the acute oral bobwhite quail study (MRID: 00049258) (slope = 7.9 with 95% confidence limits of 4.3 and 11.5), the chance of an individual mortality for terrestrial-phase CRLF is ~ 1 in 7.17E+14 (see **Table 5.13**). For 37 g frogs that eat small herbivorous mammals the chance of individual effects goes up to 100% (see **Table 5.13**).

A slope for the bobwhite quail sub-acute dietary study could not be determined; therefore, a default slope of 4.5 (with upper and lower bounds of 2 and 9, respectively) was used to calculate the chance of individual effects based on dietary toxicity. Using the acute endangered species LOC of 0.1 and the default slope, the chance of an individual mortality for terrestrial-phase CRLF is ~ 1 in 294,000 (see **Table 5.13**). The chance for individual effects for a frog eating small insects, using dietary-based data, is ~1 in 3,740. For frogs that eat herbivorous mammals, the chance of individual effects is ~1 in 1,210.

TABLE 5.13. Chance of Individual Effects for Terrestrial-Phase CRLF from Tribufos Use (1 Application, 1.875 lb a.i./Acre, 35-Day Foliar Half-life).

Size Class (grams)	Slope	LOC	Chance of Ind. Effects	Small Insect (RQ)	Chance of Ind. Effects	Herb. Mam. (RQ)	Chance of Ind. Effects	Insect-eating Mam. (RQ)	Chance of Ind. Effects
Dose-Based									
N/A	7.9	0.1	~1 in 7.17E+14	N/A	N/A	**1.86**	~1 in 1.02	**0.12**	~1 in 5.75E+12
	4.3		~1 in 1.17E+05				~1 in 1.14		~1 in 2.66E+04
	11.5		~1 in 1.52E+30				~1 in 1		~1 in 5.99E+25
Dietary-Based									
N/A	4.5	0.1	~1 in 2.94E+05	**0.17**	~1 in 3,740	**0.20**	~1 in 1,210	N/A	N/A
	2		~1 in 44		~1 in 16.2		~1 in 12.3		
	9		~1 in 8.86E+18		~1 in 4.62E+11		~1 in 6.33E+09		

N/A = Non-applicable (*i.e.*, the chance of individual effects is less likely than for the chance of individual effects at the level of the LOC.

To explore the reduction in application rate that would be required to reduce RQs so that they no longer exceeded Agency LOCs, various single application rates were modeled in T-HERPS. Single application rates ≤1.75 lbs a.i./A result in RQs below the Agency's chronic risk LOC and single application rates ≤0.09 lbs a.i./A result in RQs below the Agency's acute endangered species LOC. The tribufos usage data for California provided by BEAD indicate that the *maximum* single application rate for all counties in California that reported tribufos use between 1999 and 2006 was >0.09 lb a.i./acre (range = 0.99 to 1.875 lb a.i./acre, excluding applications rates higher than allowed on the label). Additionally, all of the counties report an *average* single application rate ≥ 0.09 lb a.i./acre (range = 0.86 to 1.7 lb a.i./acre). This indicates that there is a potential risk of direct acute effects and chronic effects on terrestrial-phase CRLF from tribufos use on cotton in California.

These potential risks for direct effects to terrestrial-phase CRLF are associated with food items contaminated with tribufos on the site of application during application. However, off-site exposure to tribufos may occur via spray drift. AgDRIFT (Tier I, aerial, ASAE Very Fine to Fine droplet size distribution) and the smallest ratio of LOC/RQ (acute) from T-HERPS [a frog that eats small herbivorous mammals (LOC = 0.1)/(RQ = 1.86) = 0.05] are used to determine what the fraction of the maximum single application rate (1 application at 1.875 lb a.i./acre) needs to be applied for the RQ to be below the LOC. Based on this analysis, spray drift up to 741 ft from the field of application could contaminate a small herbivorous mammal to a level high enough to affect a CRLF that ingests it.

Based on this analysis, a terrestrial-phase CRLF frog may be directly adversely affected through ingestion of contaminated forage items on the site of application during application from tribufos use at the maximum labeled use rate for California. Additionally, terrestrial-phase frogs foraging on items potentially contaminated up to 741 ft from the site of application could be directly impacted by tribufos use. A map of potential tribufos use sites in California [based on agricultural landcover from the National Land Cover Dataset (MRLC 2001)] and known CRLF habitat and designated critical habitat shows that potential tribufos use sites and frog habitat overlap (or are in close proximity to each other) in some areas of California (see **Fig. 5.1**). Because use sites for tribufos potentially overlap with the CRLF's range and designated critical habitat, and the usage data show that the maximum single application rate for tribufos is being used in California, the Agency concludes that there is a potential for direct effects to terrestrial-phase CRLF from tribufos use.

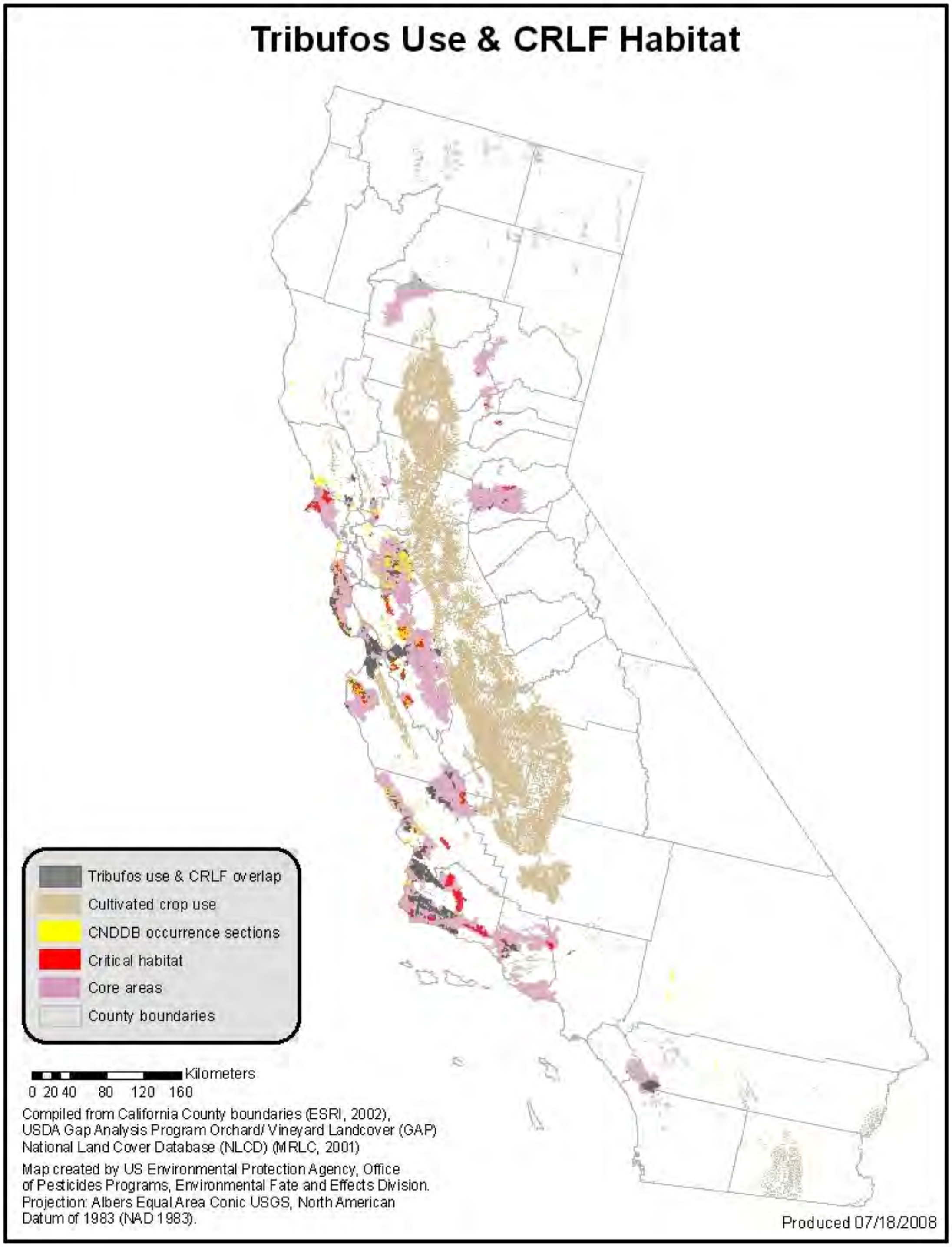


FIGURE 5.1. Overlap of Potential Tribufos Use Sites with CRLF Range and Designated Critical Habitat.

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. No effects to aquatic plants are expected from the use of tribufos (RQs < 1); therefore, only aquatic invertebrates, fish and frogs will be characterized for potential indirect effects to the aquatic-phase CRLF.

5.2.2.2 Aquatic Invertebrates

The potential for tribufos 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.

The acute endangered species and chronic LOCs (0.05, and 1) are exceeded for aquatic invertebrates, for both aerial (RQs: acute = 2.40; chronic = 9.54) and ground (RQs: acute = 1.70; chronic = 6.81) applications. Additionally, the acute and chronic RQs for aquatic invertebrates also exceeded the Agency's non-listed LOCs (0.5 and 1, respectively), indicating the potential for tribufos to indirectly affect the CRLF via reduction in prey items. Tribufos can partition to the sediment; however, no sediment toxicity data are available for review. Therefore, toxicity to benthic invertebrates may be under- or over-estimated using the daphnid data.

The probit dose-response slope was used to calculate the chance of an individual event corresponding to the listed species acute LOC and RQ. Because no raw data were available for analysis, the default slope assumption of 4.5 was used (Urban and Cook 1986). The results of the individual acute effects to freshwater invertebrates for the RQ and listed species LOC using IEC (version 1.1.xls) analysis are presented in **Table 5.14**. Using the acute endangered species LOC of 0.05, and the default slope of 4.5, (95% confidence limits of 2 and 9), the chance of an individual mortality for the aquatic invertebrates is ~ 1 in 4.18E+08. Using the daphnid acute RQ of 2.40 for aerial applications, and the default slope of 4.5 (95% confidence limits of 2 and 9), the chance of an individual mortality for the aquatic invertebrates is ~ 1 in 1.05. For ground applications, again using the default slope of 4.5 (95% confidence limits of 2 and 9), the chance of an individual mortality for the aquatic invertebrates is ~ 1 in 1.18. Therefore, the probability of an individual effect approaches 100%, for both ground and aerial applications.

TABLE 5.14. Chance of Individual Acute Effects to Freshwater Invertebrates Using the Probit Slope Response Relationship.

LOC OR USE SITE SCENARIO (RQ)	LOC OR RQ	PROBIT SLOPE		CHANCE OF AN INDIVIDUAL EFFECT
Acute Endangered Species LOC	**0.05**	Slope	4.5	~ 1 in 4.18E+08
		Upper Bound	2	~ 1 in 216
		Lower Bound	9	~ 1 in 1.75E+31
Cotton (aerial)	**2.40**	Slope	4.5	~ 1 in 1.05
		Upper Bound	2	~ 1 in 1.29
		Lower Bound	9	~ 1 in 1.00
Cotton (ground)	**1.70**	Slope	4.5	~ 1 in 1.18
		Upper Bound	2	~ 1 in 1.48
		Lower Bound	9	~ 1 in 1.02

Based on the downstream dilution analysis (described in detail in **APPENDIX L**), exposure values that would result in aquatic invertebrate RQs that exceed the acute risk LOC could extend up to 177 stream miles from a site of tribufos application. Therefore, based on the exposure analysis, non-listed LOC exceedances, the chance of individual effects, and the downstream dilution analysis, CRLFs within 285 km of use sites may be indirectly affected by tribufos use due to a loss of potential aquatic invertebrate prey items.

5.2.2.3 Fish and Aquatic-phase Frogs

The potential for direct effects to listed fish and aquatic-phase frogs is discussed above in Section 5.2.1.1. Because fish and frogs are also considered potential prey items for the aquatic-phase CRLF, indirect effects via potential prey item reduction are also considered here. For freshwater fish, also used as a surrogate for aquatic-phase amphibians, the maximum single application rate for tribufos results in acute and chronic RQs for freshwater fish of 0.07 and 3.98, respectively. The freshwater fish acute RQ does not exceed the non-listed species acute risk LOC of 0.5 and the probit dose analysis suggests that there is a chance of individual effects of ~ 1 in 1.37E+15. Therefore, the impacts to non-listed freshwater fish and aquatic-phase amphibians from acute exposure to tribufos are not expected to reach levels high enough to affect them as a food supply for a generalist species (*i.e.*, CRLF).

The effects seen in the chronic sheepshead minnow study (*i.e.*, 10% reduction in length and post-hatch survival at 19 µg a.i./L), however, are large enough to potentially impact populations of sensitive species. An ACR using the sheepshead minnow LOAEC and LC_{50} and the bluegill LC_{50} results in a potential LOAEC of 6.1 µg a.i./L for the bluegill sunfish (767/19 = 40.4; 245/40.4 = 6.1). This is below the 60-day peak EEC (13.94 µg a.i./L) for aerial applications of tribufos and below the 60-day peak EEC (10.15 µg a.i./L) for ground applications. However, for chronic exposure, the potential for indirect effects to the CRLF from loss of fish/aquatic amphibian prey cannot be precluded, and is assumed.

5.2.2.4 Terrestrial Invertebrates

When the terrestrial-phase CRLF reaches juvenile and adult stages, its diet is mainly composed of terrestrial invertebrates. An acute contact LD_{50} for terrestrial invertebrates could not be determined based on available data. For honey bees, a contact concentration of 24.2 µg a.i./bee (equivalent to 188 ppm) resulted in 3% mortality of exposed adults. Only one concentration was used in this study; therefore, a definitive LD_{50} value and response slope could not be determined. Using an LD_{50} of >24.2 µg a.i./bee results in RQs less than 1.35 and 0.15 for small and large insects, respectively; however, it is not clear if the actual RQs are above or below the interim LOC of 0.05 for endangered terrestrial invertebrates.

The chance of individual effects for terrestrial invertebrates using the IECv1.1.xls spreadsheet, the endangered species LOC of 0.05, and default slope of 4.5 (upper and lower bound = 2 and 9) is ~1 in 4.18E+08 (with upper and lower bounds of ~1 in 216, and ~1 in 1.75E+31).

As stated above, in the submitted honey bee study, a concentration of 189 ppm resulted in 3% mortality. Based on T-REX, a tribufos application of 1.875 lb a.i./acre results in EEC values of 28 and 253 ppm for large and small insects, respectively. Therefore, the concentration on the site of application at the maximum allowable application rate is not expected to reach levels high enough to cause 3% mortality in large insects. For small insects, the concentration on the site of application is expected to be 1.35 times the concentration that would result in 3% mortality. AgDRIFT (Tier III aerial application, very fine to fine ASAE droplet size distribution) was used to model the fraction of applied pesticide that is predicted to be 1 ft off the field. The resulting fraction of applied pesticide is 49% (*i.e.*, 0.92 lb a.i./A). Inputting an application rate of 0.92 lb a.i./A into T-REX results in EECs of 124 ppm for small insects, which is below the concentration that resulted in 3% mortality in adult bees. Therefore, the concentration of tribufos off the site of application is not expected to reach levels high enough to cause 3% mortality in small insects based on the available honey bee data.

Based on an available boll weevil study (Greenberg *et al*., 2004; ECOTOX Ref.: 92450) significant impacts [42.5% mortality (laboratory study) and a 36.2% population decrease (field study)] were seen 72-hrs post-application at 22% of the maximum allowed single application rate. This indicates that boll weevils are likely more sensitive to tribufos than honey bees and that effects on or near the site of application could significantly impact sensitive terrestrial invertebrate species. This study is considered supplemental, however, and not adequate for RQ calculation; therefore, its results are discussed qualitatively.

Therefore, the Agency concludes that there is a potential for indirect effects to the CRLF at or near the site of application based on a loss of terrestrial prey items from the use of tribufos on cotton.

5.2.2.5 Mammals

Life history data for terrestrial-phase CRLFs indicate that large adult frogs consume terrestrial vertebrates, including mice. The acute dose-based RQ (1.02) for a small mammal (15 g) that eats short grass resulted in an RQ that exceeds the Agency's acute risk LOC of 0.5. The dose- and dietary-based chronic RQs (122 and 14.1, respectively) also exceed the Agency's chronic risk LOC of 1. To explore the potential for effects across a variety of potential mammalian prey for the CRLF, the RQs for 15 g and 35 g mammals that have different dietary categories were calculated using T-REX.

Acute dose-based (for 15 g mammals) and chronic dietary-based RQs exceed the Agency's acute and chronic risk LOCs for the short grass, tall grass, and broadleaf plants/small insects dietary categories (see **Table 5.15**). The acute dose-based RQs for 35 g mammals exceed (or are near) the Agency's acute risk LOC for the short grass and broadleaf plants/small insects dietary categories. All of the chronic dose-based RQs for both 15 g and 35 g mammals (range 1.5 – 122) exceed the Agency's chronic risk LOC (the RQs for 15 g and 35 g mammals that eat short grass exceed the LOC by more than 100 X). The effects seen in the chronic rat studies at concentrations of ≥260 mg/kg-diet [13 mg/kg/day] (MRID 420402-01) (*i.e.*, increased stillborn pups and pup death, decreased F1 and F2 pup body weights, and increased F1 gestation periods) have the potential to impact mammals at the population level. Based on T-REX, EECs on dietary food items on the field of tribufos application are expected to exceed the 260 mg/kg-diet concentration. Therefore, the impacts of tribufos use to mammals potentially extend beyond 15 g mammals that eat short grass. Additionally, cholinesterase inhibition has been seen in a variety of mammals after exposure to tribufos (MRIDs: 40190601, 42007203, and 42007201)

TABLE 5.15. Mammalian RQ Values for Acute and Chronic Exposure to Tribufos from the Maximum Single Application Rate (1.875 lb a.i./Acre).

DIETARY CATEGORY	BODY SIZE	ACUTE RQ (Dose-Based)	Chronic RQ (Dose-Based)	Chronic RQ (Dietary-Based)
Short Grass	**15 g**	**1.02**	**122**	**14**
	35 g	**0.87**	**104**	
Tall Grass	**15 g**	**0.47**	**56**	**6.5**
	35 g	0.40	**48**	
Broadleaf Plants/Small Insects	**15 g**	**0.57**	**69**	**7.9**
	35 g	**0.49**	**59**	
Fruits/Pods/Seeds/Large Insects	**15 g**	0.06	**7.6**	0.88
	35 g	0.05	**6.5**	
Granivore	**15 g**	0.01	**1.7**	N/A
	35 g	0.08	**1.5**	

Bolded numbers indicate RQs that are near or exceed the Agency's acute or chronic risk LOC for mammals

The chance of individual effects for mammals using the IECv1.1.xls spreadsheet and rat acute toxicity data [default slope = 4.5 (upper and lower bound = 2 and 9)] ranges from 50% to ~ 1 in 8.86E+18 depending on the size and dietary category (see **Table 5.16**).

TABLE 5.16. Chance of Individual Effects for Small Mammals from Maximum Seasonal Application Rates for Tribufos Use in California.

DIETARY CATEGORY	BODY SIZE	ACUTE RQ	SLOPE	CHANCE OF INDIVUAL EFFECTS
Acute Endangered Species LOC (0.1)	N/A	N/A	4.5	~ 1 in 294,000
			2	~ 1 in 44
			9	~ 1 in 8.86E+18
Short Grass	**15 g**	**1.02**	4.5	~ 1 in 1.94
			2	~ 1 in 1.97
			9	~ 1 in 1.88
	35 g	**0.87**	4.5	~ 1 in 2.55
			2	~ 1 in 2.21
			9	~ 1 in 3.41
Tall Grass	**15 g**	**0.47**	4.5	~ 1 in 14.3
			2	~ 1 in 3.91
			9	~ 1 in 632
	35 g	0.40	4.5	~ 1 in 27.3
			2	~ 1 in 4.69
			9	~ 1 in 5,850
Broadleaf Plants/Small Insects	**15 g**	**0.57**	4.5	~ 1 in 7.35
			2	~ 1 in 3.2
			9	~ 1 in 71.4
	35 g	**0.49**	4.5	~ 1 in 12.2
			2	~ 1 in 3.73
			9	~ 1 in 377
Fruits/Pods/Seeds/Large Insects	**15 g**	0.06	4.5	~ 1 in 52,200,000
			2	~ 1 in 138
			9	~ 1 in 5.04E+27
	35 g	0.05	4.5	~ 1 in 4.18E+08
			2	~ 1 in 216
			9	~ 1 in 1.75E+31
Granivore	**15 g**	0.01	4.5	~ 1 in 8.86E+18
			2	~ 1 in 31,600
			9	~ 1 in 1.03E+72
	35 g	0.08	4.5	~ 1 in 2,510,000
			2	~ 1 in 70.8
			9	~ 1 in 3.64E+22

Based on T-REX, the single application rate would need to drop to 0.06 lb a.i./acre before all of the acute dose-based RQs (for 15 and 35 g mammals) would no longer exceed the Agency's acute risk to non-listed species LOCs. The application rate would need to be reduced to 0.015 lb a.i./acre before all of the chronic RQs would no longer exceed the chronic LOC. The usage data indicate that tribufos (single application) is being used in California at rates above 0.9 lb a.i./acre.

Because use sites for tribufos potentially overlap with the CRLF's range and designated critical habitat (see **Fig. 5.1**), and the usage data show that the maximum single application rate for tribufos is being used in California, the Agency concludes that there is a potential for indirect effects to terrestrial-phase CRLF due to loss of mammalian prey items from tribufos use in CA.

5.2.2.6 Terrestrial-phase Amphibians

Terrestrial-phase adult CRLFs also consume frogs. RQ values representing direct exposures of tribufos to terrestrial-phase CRLFs are used to represent exposures of tribufos to frogs in terrestrial habitats. For frogs, although the acute dietary-based RQs for frogs that eat small insects and small herbivorous mammals (RQs = 0.17 and 0.2, respectively) exceed the listed species LOC, none of the acute RQs exceed the acute risk LOC (see Section 6.1.2). The chronic risk LOC, however, is exceeded for frogs that eat herbivorous mammals (RQ = 1.06). Therefore, frogs that eat herbivorous mammals might be affected by tribufos, thus, impacting those frogs as a potential food source for CRLF. The Agency concludes that there is a slight (but not discountable) potential for indirect effects to the CRLF based on a loss of amphibian prey items from the use of tribufos on cotton.

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 primary energy base for aquatic ecosystems. Vascular plants provide energy and structure 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 nearshore areas and lower streambanks. In addition, vascular aquatic plants are important as attachment sites for egg masses of CRLFs.

Potential indirect effects to the CRLF based on impacts to habitat and/or primary production were assessed using RQs from freshwater aquatic vascular and non-vascular plant data. No effects are anticipated to aquatic vascular and non-vascular plants from use of tribufos because associated RQ values are less than the Agency's LOC.

5.2.3.2 Terrestrial Plants

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

Based on the available registrant-submitted toxicity data, RQs for terrestrial plants do not exceed the Agency's LOC (see **Table 5.8**). Because the studies from which these data were drawn were conducted on plant species that do not form abscission zones, these data are expected to underestimate the potential toxicity of tribufos to non-target plants that do form abscission zones (*e.g.*, some woody, deciduous plants).

Only one study (adequate for use in risk assessment) for tribufos and a plant that forms abscission zones was found in the open literature (Greenberg *et al*., 2004; ECOTOX Ref.: 92450). This study was conducted on the target species (*i.e*., cotton); therefore, it is unclear how the results might relate to other plant species. In the Greenberg *et al*. (2004) study, an average of approximately 80% of the leaves per plant were dropped 1-week post-application at an application rate 22% of the maximum allowable labeled rate (*i.e*., 0.42 lb a.i./acre, using the DEF 6 formulation).

One terrestrial plant incident in the EIIS database is associated with tribufos (I016036-024). This incident, which damaged 53 acres of lettuce in Riverside, California, also involved the herbicide diuron. Although the damaged lettuce tested positive for tribufos (50 ppb) and no other residues were identified, the fact that an herbicide was also involved in the incident makes it difficult to determine what specific effects, if any, were due to tribufos alone, diuron alone, and/or both chemicals together.

However, based on the fact that tribufos is a known defoliant, has a mechanism of action that could result in effects to plants important to CRLF habitat (*e.g*., deciduous shrubs and trees), and has use sites that potentially overlap CRLF range, tribufos has the potential to indirectly affect the CRLF via reduction in terrestrial plants.

5.2.4. Indirect Effects (via Loss of Prey Base/Habitat) - Conclusion

The data taken together indicate that tribufos has the potential to indirectly affect both the aquatic-phase and terrestrial-phase CRLF via reduction in prey base. Aquatic invertebrates are sensitive to tribufos, and are vulnerable to mortality in areas adjacent to tribufos use sites (and up to 285 km downstream from tribufos use sites). Adverse effects are possible to small mammals, frogs, and fish from tribufos use. The potential effects to CRLF (both in scale and type) from the loss of aquatic and terrestrial prey items would depend on a variety of factors, including (but not limited to): the ability of the prey community to recover either through reproduction or re-colonization of an affected area, and the sensitivity of specific prey to tribufos. Exploring the specific effects these factors might have on the CRLF is beyond the scope of this assessment. However, since tribufos has the potential to affect a wide range of the aquatic-phase CRLF food items (*i.e*., aquatic invertebrates, fish, and frogs), it is reasonable to assume that the ability of a CRLF to substitute less-favored foods in its diet when choice foods are not available is diminished in affected aquatic areas, and that there would be variable rates of community recovery depending on the sensitivity of the prey species affected.

In the terrestrial environment, CRLFs also face a potential reduction in mammalian, terrestrial invertebrate, and amphibian prey availability and selection. Additionally, because tribufos is a known defoliant, terrestrial habitat impacts cannot be discounted. Given its mechanism of action and its use on sites that potentially overlap with CRLF range, tribufos has the potential to affect terrestrial CRLF habitat, which is important in maintaining shelter, cover from predators, and also provide habitat for prey items important to the CRLF.

5.2.5 Modification to Designated Critical Habitat

5.2.5.1 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).

Conclusions for potential indirect effects to the CRLF via direct effects to aquatic and terrestrial plants are used to determine whether modification to critical habitat may occur. As discussed above for aquatic plants (Sections 5.2.2.1 and 5.2.3.1) and terrestrial plants (Section 5.2.3.2), the use of tribufos is not expected to adversely impact aquatic plants; however, effects to some terrestrial plants important to the CRLF are expected from tribufos use in California.

The remaining aquatic-phase PCE is "alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source." Other than impacts to algae as food items for tadpoles (discussed above), this PCE is assessed by considering direct and indirect effects to the aquatic-phase CRLF via acute and chronic freshwater fish and invertebrate toxicity endpoints as measures of effects. Based on our analyses discussed above, there is a potential for habitat modification via impacts to aquatic-phase CRLFs (Sections 5.2.1.1) and effects to freshwater invertebrates and fish as food items (Sections 5.2.2.2 and 5.2.2.3) from tribufos use in California.

5.2.5.2 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.

As already discussed, adverse effects to some terrestrial plants important to the CRLF are expected from tribufos use in California (Section 5.2.3.2).

The third terrestrial-phase PCE is "reduction and/or modification of food sources for terrestrial phase juveniles and adults." To assess the impact of tribufos on this PCE, acute and chronic toxicity endpoints for terrestrial invertebrates, mammals, and terrestrial-phase frogs are used as measures of effects. Based on the potential for a reduction in mammalian, terrestrial invertebrate, and amphibious prey items (Sections 5.2.2.5 and 5.2.2.6), the Agency concludes there is a potential for habitat modification via indirect effects to terrestrial-phase CRLFs via reduction in prey base.

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. Based on the preceding discussions, the Agency concludes there is a potential for habitat modification via direct (Section 5.2.1.2) and indirect effects (Sections 5.2.2.5 and 5.2.2.6) to terrestrial-phase CRLFs.

6. Uncertainties

6.1 Exposure Assessment Uncertainties

6.1.1 Maximum Use Scenario

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

6.1.2 Aquatic Exposure Modeling of Tribufos

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

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

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

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

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

As discussed above, a few detections were reported in CDPR for tribufos concentrations measured in surface waters receiving runoff from agricultural areas. The specific use patterns (*e.g*. application rates and timing, crops) associated with the agricultural areas are unknown; however, they are assumed to be representative of potential tribufos use areas. Because the monitoring data were not from targeted studies, they were not used for characterization of exposure in this assessment.

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

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

6.1.3 Uncertainties Associated with Calculating Areas of Effect

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

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

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

6.1.4 Usage Uncertainties

County-level usage data were obtained from California's Department of Pesticide Regulation Pesticide Use Reporting (CDPR PUR) database. Six years of data (1999 –

2005) 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. CDPR PUR documentation indicates that errors in the data may include the following: a misplaced decimal; incorrect measures, area treated, or units; and reports of diluted pesticide concentrations. As with all pesticide usage data, there may be instances of misuse and misreporting. The Agency made use of the most current, verifiable information; in cases where there were discrepancies, the most conservative information was used.

6.1.5 Terrestrial Exposure Modeling of Tribufos

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

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

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

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

6.1.6 Spray Drift Modeling

It is unlikely that the same organism would be exposed to the maximum amount of spray drift from every application made. In order for an organism to receive the maximum concentration of tribufos from multiple applications, each application of tribufos would have to occur under identical atmospheric conditions (*e.g.*, same wind speed and same wind direction) and (if it is an animal) the animal being exposed would have to be located in the same location (which receives the maximum amount of spray drift) after each application. Additionally, other factors, including variations in topography, cover, and meteorological conditions over the transport distance are not accounted for by the AgDRIFT model (*i.e*., it models spray drift from aerial and ground applications in a flat area with little to no ground cover and a steady, constant wind speed and direction). Therefore, in most cases, the drift estimates from AgDRIFT/ may overestimate exposure, especially as the distance increases from the site of application, since the model does not account for potential obstructions (*e.g*., large hills, berms, buildings, trees, *etc*.). Furthermore, conservative assumptions are made regarding the droplet size distributions being modeled (‘ASAE Very Fine to Fine’ for agricultural uses), the application method (*i.e*., aerial), release heights and wind speeds. Alterations in any of these inputs would decrease the area of potential effect.

6.2 Effects Assessment Uncertainties

Toxicity data from chronic exposure to tribufos are not currently available for the species of freshwater fish that are most acutely sensitive to tribufos (*i.e*., bluegill sunfish). Therefore, the chronic effects endpoint used in this assessment for freshwater fish (and, thus, aquatic-phase amphibians) is based on an acute-to-chronic ratio using toxicity data from sheepshead minnows. Because the ACR was determined by using endpoints from an estuarine/marine species, chronic risks to freshwater fish may be over- or under-estimated, depending on the relative sensitivity of those species. Additionally, tribufos is expected to partition from the water column to the sediment, however, toxicity data from benthic organisms are not currently available. The Agency’s LOCs are intentionally set low, and conservative estimates are made in the screening level risk assessment to account for these types of uncertainties.

Although acceptable Tier II terrestrial plant studies are available for tribufos, the plant species studied (*i.e*., annual dicots and monocots) are not expected to be as sensitive to tribufos as other plants important to the CRLF (*e.g*., woody deciduous trees and shribs) due to its mechanism of action. Therefore, there are uncertainties regarding the toxicity of tribufos to non-target plants that form abscission zones (*e.g*., some woody, deciduous plants). In the absence of data for non-target plants that form abscission zones, risks were presumed.

Additionally, based on its bioconcentration factors (BCF) (*i.e*., 300X for edible tissue and 1300X for non-edible tissues, with a whole fish bioconcentration factor of 730X), tribufos has the potential to bioaccumulate in fish prey items. Therefore, exposure values for organisms that eat contaminated fish may be underestimated (especially for chronic exposures).

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

Acceptable toxicity data for aquatic- or terrestrial-phase amphibians are not currently available for tribufos. Therefore, toxicity data for surrogate species (*i.e.*, fish for aquatic-phase amphibians and birds for terrestrial-phase amphibians) are used to assess the risks of direct effects to CRLF from the use of tribufos on cotton in California. 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.

6.2.3 Sublethal Effects

When assessing acute risk, the screening risk assessment relies on the acute mortality endpoint as well as a suite of sublethal responses to the pesticide, as determined by the testing of species response to chronic exposure conditions and subsequent chronic risk assessment. Consideration of additional sublethal data in the effects determination is exercised on a case-by-case basis and only after careful consideration of the nature of the sublethal effect measured and the extent and quality of available data to support establishing a plausible relationship between the measure of effect (sublethal endpoint) and the assessment endpoints.

6.2.4 Location of Wildlife Species

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

7. Risk Conclusions

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

Based on the best available information, the Agency makes a Likely to Adversely Affect determination for the CRLF from the use of tribufos on cotton. Additionally, the Agency has determined that there is the potential for modification of CRLF designated critical habitat from the use of the chemical. This is based on the potential for direct effects (to both aquatic and terrestrial-phase CRLF), indirect effects due to potential decreases in aquatic and terrestrial prey items, and the potential for modification of designated critical habitat due to habitat degradation and potential loss of aquatic and terrestrial prey items. 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 7.1** and **7.2**. Baseline status and cumulative effects for the CRLF can be found in **ATTACHMENT 2**.

TABLE 7.1 Effects Determination Summary for Tribufos Use and the CRLF.

Assessment Endpoint	Effects Determination [1]	Basis for Determination
Survival, growth, and/or reproduction of CRLF individuals	LAA	**Potential for Direct Effects**
		Aquatic-phase (Eggs, Larvae, and Adults): - RQs for acute and chronic effects for freshwater fish (used as a surrogate for aquatic phase CRLFs) exceed the endangered species LOC - Many of the CRLF habitat areas are adjacent to and/or overlap with potential tribufos (cotton) use sites.
		Terrestrial-phase (Juveniles and Adults): - Acute dietary-based RQs exceeded endangered species LOCs for frogs eating small insectivorous and herbivorous mammals, as well as frogs eating terrestrial invertebrates. - Many of the CRLF habitat areas are adjacent to and/or overlap with potential tribufos (cotton) use sites. - The chance of individual effects (*i.e.*, mortality) for a terrestrial-phase CRLF is as high as ~1 in 1,210.
		Potential for Indirect Effects
		Aquatic prey items, aquatic habitat, cover and/or primary productivity - The Agency's non-endangered species LOCs are exceeded for invertebrates (acute and chronic), fish (chronic) and aquatic-phase frogs (chronic). - Many of the CRLF habitat areas are adjacent to and/or overlap with potential tribufos (cotton) use sites. - Effects to aquatic invertebrates could extend to 285 km downstream from site of tribufos application. - Therefore, there is a potential for prey item reduction via impacts to all aquatic-phase CRLF prey items.
		Terrestrial prey items, riparian habitat - The Agency's non-endangered species LOCs are exceeded for mammals (15-g and 35-g) and frogs (chronic). - Due to tribufos' mechanism of action, the use of tribufos could result in effects to non-target plants important to CRLF (*e.g.*, deciduous shrubs and trees) - Many of the CRLF habitat areas are adjacent to and/or overlap with potential tribufos use sites - Therefore, there is a potential for prey item reduction via impacts to terrestrial-phase CRLF prey items.

[1] LAA = 'Likely to Adversely Effect'

TABLE 7.2. Effects Determination Summary for Tribufos Use and CRLF Critical Habitat Impact Analysis.

Assessment Endpoint	Effects Determination	Basis for Determination
Modification of aquatic-phase PCE	Habitat Modification	- There is a potential for effects to terrestrial plants (*e.g.*, deciduous shrubs and trees) based on tribufos' mechanism of action, which could alter aquatic habitats for the CRLF that are important for shelter, foraging, cover, and the normal growth and viability of CRLFs. - There is a potential for prey item reduction via impacts to all aquatic-phase prey items (aquatic invertebrates, fish, and frogs).
Modification of terrestrial-phase PCE		- There is a potential for effects to terrestrial plants (*e.g.*, deciduous shrubs and trees) based on tribufos' mechanism of action, which could alter terrestrial habitats for the CRLF that are important for shelter, foraging, cover, and the normal growth and viability of CRLFs. - There is a potential for prey item reduction via potential reduction in mammalian and amphibious prey items.

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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