Risks of Acephate Use to the Federally Listed California Red Legged Frog
(*Rana aurora draytonii*)

Pesticide Effects Determination

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1. Executive Summary

Background

Acephate is an organophosphate insecticide currently registered for use on a variety of field, fruit, and vegetable crops; in food handling establishments; rights-of-ways, fencerows, sewage disposal areas, drainage systems, and on ornamental plants both in greenhouses and outdoors (including lawns, turf, and cut flowers). Acephate use in California entails a variety of application techniques and varies greatly in amounts applied, number of applications, application intervals, and timing of applications. Acephate may move through the environment and be transported away from the site of application by run-off or spray drift. The toxic degradate of acephate, methamidophos, is also considered in this assessment.

Exposure pathways to the CRLF or its critical habitat were considered non-existent for indoor uses that remain indoors which includes use in eating establishments, food processing facilities and other indoor applications. These uses are therefore considered to have “no effect” on the CRLF or its critical habitat. The following summary addresses effects to the CRLF and its critical habitat from the remaining labeled uses.

There were insufficient monitoring data to support an aquatic evaluation based on concentrations found in water samples; specifically there were no targeted monitoring data on acephate for this region. Therefore, it was necessary to estimate exposure based on modeled results.

The mode of action for acephate is similar to other organophosphate insecticides in that the chemical inhibits an enzyme, acetylcholinesterase. This action causes disruption to the central nervous system.

Aquatic Phase

Direct, acute effects to the aquatic phase CRLF are not expected as there are no acute listed LOC exceedences for freshwater fish, the surrogate test species for the aquatic phase CRLF. An acute-to-chronic ratio analysis with other organophosphate insecticides indicated no LOC exceedence for reproductive effects. Indirect effects to the aquatic phase of the frog, due to effects on critical habitat are not expected, since there were no LOC exceedences to aquatic plants, nor effects to water quality. Indirect effects to CRLF, based on food availability are not expected, because the effect on invertebrate food sources is discountable. Thus it was determined that acephate use is not likely to adversely affect the aquatic phase CRLF, or its critical habitat.

Terrestrial Phase

Acephate use is likely to adversely affect the terrestrial phase of the CRLF directly, as determined by acute and chronic LOC exceedences for birds, the surrogate test species for terrestrial phase CRLF. Avian reproductive effects indicate direct chronic fecundity effects to CRLF. Toxic effects on the CRLF prey base are likely to adversely affect the terrestrial phase CRLF as several taxa from the CRLF diet exceed the LOC. Birds, mammals, insects, and small amphibians are all part of the terrestrial CRLF diet. Because multiple components of the diet are expected to be affected, including mammals, birds and insects, an LAA determination was made for indirect effects. An LAA determination for terrestrial critical habitat was concluded based on adverse modification of terrestrial food resources.

Based on LOC exceedences, the overlap of use sites with frog habitat and core areas, and other factors, the following table summarizes the effects determination for the CRLF from methamidophos use.

Assessment Endpoint	Effects determination	Basis for Determination
Aquatic Phase (Eggs, larvae, tadpoles, juveniles, and adults)		
Direct Effects and Critical Habitat Effects		
1. Survival, growth, and reproduction of CRLF	No Effect	All Acute and Chronic RQ are below the listed LOC for surrogate species (rainbow trout)
Indirect Effects		
2. Reduction or modification of aquatic prey base	May Affect, Not Likely to Adversely Affect	Acute LOC is exceeded for aquatic invertebrates, however effect is considered discountable based on low likelihood of individual effect.
3. Reduction or modification of aquatic plant community	No Effect	No LOC Exceedences for any plant species
4. Degradation of riparian vegetation	No Effect	No LOC Exceedences for any plant species. No adverse aquatic critical habitat modification is expected.
Terrestrial Phase (Juveniles and Adults)		
Direct Effects		
5. Survival, growth, and reproduction of CRLF	May Affect, Likely to Adversely Affect	Acute and Chronic LOC exceedences for birds, the surrogate species for direct effects to frogs. Initial Area of Concern overlaps habitat. Use is widespread (nearly all counties). Use is documented in all months. Probability of effect approaches 100% at calculated RQs.
Indirect Effects and Critical Habitat Effects		
6. Reduction or modification of terrestrial prey base	May Affect, Likely to Adversely Affect	Acute and Chronic LOC exceedences for multiple components of CRLF prey base (mammals, birds, and terrestrial invertebrates). LAA to terrestrial phase CRLF and its critical habitat based on acute RQs exceeding 0.5 and chronic RQs over LOC for mammals, insects, birds. Adverse terrestrial critical habitat modification is expected.

Assessment Endpoint	Effects determination	Basis for Determination
7. Degradation of riparian vegetation	No Effect	No plant LOC exceedences.

Effects on Primary Constituent Elements of the Critical Habitat

Aquatic Breeding and Non-breeding Habitat

Adverse effects on the aquatic critical habitat are not expected, as there are is No Effect via aquatic plants, and the effect on invertebrates is discountable.

Upland and Dispersal Habitat

There may be effects on these habitats through reduction in prey base (invertebrates, and small mammals, birds, and amphibians).

There may also be a reduction in shelter for the CRLF (small mammal burrows) due to the effects on mammals.

Action Area

Based on chronic effects to small birds consuming short grass food items, a terrestrial buffer zone of 2,913 feet is needed to delineate the Action Area. This is the distance from the edge of the use site needed to reduce exposure to below the Level of Concern for all taxa considered.

The aquatic Action Area is based on effects to prey items (invertebrates) exposed to the degradate methamidophos. Based on the RQs, terrestrial effects are expected to dominate the Action Area.

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 adverse modification to critical habitat.

2. Problem Formulation

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

2.1 Purpose

The purpose of this endangered species assessment is to evaluate potential direct and indirect effects on individuals of the federally threatened California red-legged frog *(Rana aurora draytonii*) (CRLF) arising from FIFRA regulatory actions regarding use of acephate on labeled use sites. In addition, this assessment evaluates whether these actions can be expected to result in the destruction or adverse modification of the species' critical habitat. Key biological information for the CRLF is included in Section 2.5, and designated critical habitat information for the species is provided in Section 2.6 of this assessment. This ecological risk assessment has been prepared as part of the *Center for Biological Diversity (CBD) vs. EPA et al*. (Case No. 02-1580-JSW(JL)) settlement entered in the Federal District Court for the Northern District of California on October 20, 2006.

In this endangered species assessment, direct and indirect effects to the CRLF and potential adverse modification to its critical habitat are evaluated in accordance with the methods (both screening level and species-specific refinements, when appropriate) described in the Agency's Overview Document (U.S. EPA 2004).

In accordance with the Overview Document, provisions of the ESA, and the Services' *Endangered Species Consultation Handbook,* the assessment of effects associated with registrations of acephate are based on an action area. The action area is considered to be the area directly or indirectly affected by the federal action, as indicated by the exceedance of Agency Levels of Concern (LOCs) used to evaluate direct or indirect effects. It is acknowledged that the action area for a national-level FIFRA regulatory decision associated with a use of acephate 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 for registration of acephate at the use sites described in this document to affect CRLF individuals and/or result in the destruction or adverse modification of designated CRLF critical habitat:

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

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 (Section 2.6).

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 the FIFRA regulatory action regarding acephate as it relates to this species and its designated critical habitat. If, however, direct or indirect effects to individual CRLFs are anticipated and/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 acephate.

If a determination is made that use of acephate within the action area(s) associated with the CRLF "may affect" this species and/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 geographic proximity of the CRLF habitat and175% acephate use sites) and further evaluation of the potential impact of acephate on the PCEs is also used to determine whether destruction or adverse modification to designated critical habitat may occur. Based on the refined information, the Agency uses the best available information to distinguish those actions that "may affect, but are not likely to adversely affect" from those actions that "may affect and are likely to adversely affect" the CRLF and/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 acephate is expected to directly impact living organisms within the action area (defined in Section 2.7), critical habitat analysis for acephate 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 destroy or adversely modify critical habitat are those that alter the PCEs and appreciably diminish the value of the habitat. Evaluation of actions related to use of acephate 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

Acephate (O, S-Dimethyl acetylphosphoramidothioate) is an organophosphate insecticide currently registered for use on a variety of field, fruit, and vegetable crops; in food handling establishments; on ornamental plants both in greenhouses and outdoors (including lawns, turf, and cut flowers); and in and around the home. Acephate was first registered in 1973 for ornamental uses and in 1974 for food uses (agricultural crops). Target pests include: Armyworms, aphids, beetles, bollworms, borers, budworms, cankerworms, crickets, cutworms, fire ants, fleas, grasshoppers, leafhoppers, loopers, mealybugs, mites, moths, roaches, spiders, thrips, wasps, weevils, and whiteflies.

California's Department of Pesticide Regulation maintains a database of all pesticide applications throughout the state and provides this information to the public. According to the Summary of Pesticide Use Reporting Data (2005), the reported pounds of acephate used have decreased by nearly 60%, from approximately 458,000 lbs in 1995 to 194,000 lbs in 2005. The acreage to which acephate was applied has also decreased during that time period, from about 490,000 acres in 1995 to 198,000 acres in 2005. Since California's Department of Pesticide Regulation (CDPR) Pesticide Use Reporting (PUR) data does not account for residential uses, the actual pounds used could be higher.

The end result of the EPA pesticide registration process (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 acephate in accordance with the approved product labels for California is "the action" being assessed.

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

2.2.1. Degradates

The major degradate of acephate in aerobic soil metabolism studies is methamidophos, (up to 23%) which is itself a registered insecticide. Methamidophos was also found in the aqueous photolysis study (maximum formation of 1.6% of the applied amount), the soil photolysis study (5.3 to 8.4% in both irradiated and control), the anaerobic aquatic metabolism study (5% at 7 days), and the aerobic aquatic metabolism study (<1.6%).

Methamidophos is more toxic to vertebrates and invertebrates than is acephate, so its effects will be considered in the aquatic risk assessment. Because terrestrial LOCs are expected to be exceeded based on exposure to the parent acephate alone, methamidophos exposure will not be considered quantitatively for terrestrial wildlife.

FIFRA methamidophos registered uses are also being assessed for the CRLF. Therefore, the effects characterization for methamidophos will be found in detail in the Methamidophos Assessment for CRLF and only summarized within this assessment as needed.

Since there is information on the maximum formation of methamidophos in soil after application of acephate (23% at 32 days in a Fresno loam soil, less than 10% in two other soils), the exposure of aquatic organisms to methamidophos can be quantified in PRZM-EXAMS modeling by assuming that the starting concentration of methamidophos in the soil is 23% of the application rate of acephate. The fate and transport properties of acephate and methamidophos are very similar, thus separate modeling runs are not necessary.

2.2.2. Mixtures

Product Formulations Containing Multiple Active Ingredients

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

Formulated Product Data and Aquatic Exposure

The limitation on the quantitative exposure modeling for formulations is based on the expectation that the varying physical-chemical properties of individual components of pesticide formulations will result in progressively different formulation constituents in environmental media over time. As the proportions of formulation components in environmental media differ from the proportions in the tested formulation, the assumption that environmental residues are toxicologically equivalent to tested formulations cannot be supported beyond the time period immediately following product application. This assumption is especially important in the case of runoff from treated areas to surface waters. In this case, varying fate and transport properties for each formulation component will result in a final proportion of the residues of these components in the receiving surface waters that is significantly different than the proportion of ingredients that are applied and that were tested in an aquatic organism toxicity study using the formulated product. Therefore available formulated product data for products directly applied to aquatic environments or that may drift into aquatic environments were considered, and only for effects resulting from acute exposure to the formulated product (see Overview Document section V.B.1.b.(2) and Services Evaluation memorandum).

Formulated Product Data and Terrestrial Exposure

In situations where available toxicity data indicate that a formulated product may be more toxic to terrestrial wildlife than indicated by active ingredient effects testing, it may be necessary to consider exposure to the formulation. Exposure modeling in these instances is limited to dietary exposure to residues for a time period immediately following pesticide product application.

The limitation on the quantitative exposure modeling for formulations is based on the expectation that the varying physical-chemical properties of individual components of pesticide formulations will result in progressively different formulation constituents in environmental media over time. Because the proportions of formulation components in environmental media differ from the proportions in the tested formulation, the assumption that environmental residues are toxicologically equivalent to tested formulations cannot be supported beyond the time period immediately following product application. Therefore, available formulated product data for terrestrial applications were considered only for effects resulting from acute dietary exposure to the formulated product (see Overview Document section V.B.1.c.(2) and Services Evaluation memorandum).

Acephate has registered products containing multiple active ingredients. These products, their product registration numbers, the active ingredient(s) in the product in addition to acephate, the percentage of each active ingredient in the product, and the available product formulation data are listed in Table 2.1 below. The ECOTOX search strategy for public scientific literature identifies studies addressing multiple active ingredients (see Overview Document and Services Evaluation memorandum). If a multiple active ingredient study was performed on any of the formulated pesticide products in Table 2.1, this assessment considers those studies. In addition to public literature, if the registrant has submitted data on any of the formulated products in Table 2.1, those data as well have been considered and noted.

The below registered products containing multiple active ingredients are in pressurized containers used only for residential and greenhouse uses. Exposure pathways from indoor use to the CRLF are considered unlikely and therefore these uses have “no effect” on the CRLF. A quantitative risk assessment from mixtures to the CRLF was not done due to discountable nature of exposure of the mixtures.

Table 2.1 Data on Acephate Mixtures with Other Active Ingredients

EPA Reg#	Formulated Product	Formulation	Registrant Submitted Studies	Public Scientific Literature Studies*
00023902476	ORTHO SYSTEMIC ROSE & FLORAL SPRAY	Acephate (0.25%) Resmethrin (0.1%) Triforine (0.1%)	none	See Appendix D2 for listing of ECOTOX literature studies.
00023902594	ORTHENEX INSECT & DISEASE CONTROL FORMULA III	Acephate (4%) Fenbutatinoxide (0.75%) Triforine (3.25%)	none	
00023902595	ISOTOX INSECT KILLER FORMULA IV	Acephate (8%) Fenbutatinoxide (0.5%)	none	
00049900441	WHITMIRE TC 136	Acephate (1.5%) Fenpropathrin(1%)	none	

As summarized in Appendix H there are no product LD50 values, with associated 95% Confidence Intervals (CIs) available.

As discussed in U.S. EPA (2000), a quantitative component-based evaluation of mixture toxicity requires data of appropriate quality for each component of a mixture. In this mixture evaluation, an LD50 with associated 95% CI is needed for the formulated product. The same quality of data is also required for each component of the mixture. Given that the formulated products for acephate do not have LD50 data available, it is not possible to undertake a quantitative or qualitative analysis for potential interactive effects. However, because the active ingredients are not expected to have similar mechanisms of action, metabolites, or toxicokinetic behavior, it is reasonable to conclude that an assumption of dose-addition would be inappropriate. Consequently, an assessment based on the toxicity of acephate is the only reasonable approach that employs the available data to address the potential acute risks of the formulated products in Appendix H.

2.2.3. Other use sites not quantitatively assessed

Structural pest control applications, while a significant contribution to the total pounds of acephate reportedly applied each year, are not considered as likely exposure pathways to the CRLF or its critical habitat and are therefore considered to have “no effect” on the CRLF or its critical habitat. Indoor uses for eating establishments, food processing facilities and other indoor applications are not considered further in this assessment as the products are to be used indoors, in small amounts and disposed of according to the label instructions which will not result in exposure to the CRLF or its critical habitat. Therefore these indoor uses are also considered to have “no effect” on the CRLF or its critical habitat.

2.3 Previous Assessments

2.3.1 Acephate Assessments

The Agency published an Interim Reregistration Eligibility Decision for Acephate in September 2001 which identified numerous human health and ecological risks associated with the labeled uses of acephate. Upon completion of the assessment, the Agency decided on a number of label amendments to address the worker, residential, and ecological concerns. Acephate and its degradate methamidophos are highly toxic to honey bees and beneficial predatory insects on an acute contact basis. Acute and chronic risks to birds and chronic risk to mammals were also of concern. The document is available on the web, at: http://www.epa.gov/oppsrrd1/REDs/acephate_ired.pdf. Numerous mitigation requirements (label amendments) resulted from the IRED assessment. However, at this time only those label amendment changes that are reflected by the current labels were assessed as the product user will follow the label rather than the IRED.

Some changes include requiring labeling to protect honeybees and to reduce the potential for spray drift. Also, aerial applications to turf have been deleted as have residential indoor uses also been deleted.

On March 31, 2004 EPA released an assessment of the potential effects of acephate to 26 listed Environmentally Significant Units (ESUs) of Pacific salmon and steelhead. That assessment concluded that acephate would have no effect on the species under consideration. While acephate was noted to have significant toxicity to aquatic invertebrates, as does this assessment, the minimal usage, the size of the watersheds under consideration and the volume of the water bodies serving as habitat to these species taken together, resulted in the determination of no effect to the listed salmon and steelhead.

2.3.2 California Red-legged Frog Assessments

The Agency is currently developing a number of risk assessments for the CLRF, each addressing different pesticide active ingredients. A total of 66 chemicals will be assessed. Metolachlor is among the first group of ten chemicals to be completed. For information regarding the other chemicals in this group[1] please see the relevant document.

[1] Other chemicals assessed in the first group include methamidaphos, methomyl, azinphos-methyl, acephate, imazpyr, aldicarb, metam sodium, diazinon and chloropicrin

2.4 Stressor Source and Distribution

Acephate technical is a colorless to white solid with a melting point of 81-91° C. Acephate is highly soluble in water (79 g/100 ml), acetone (151 g/100 ml), and ethanol (>100 g/100 ml), and is soluble in methanol (57.5 g/100 ml), ethyl acetate (35.0 g/100 ml), benzene (16.0 g/100 ml), and hexane (<0.1g/100 ml) at 25° C. Acephate degrades to another, registered organophosphate insecticide, methamidophos.

Case number: 0042
CAS registry number: 30560-19-1
OPP chemical code: 103301
Empirical formula: $C_4H_{10}NO_3PS$
Molecular weight: 183.16 g/mol
Vapor Pressure: 1.7 x 10^{-6} mm Hg at 24°C
Trade and other names: Orthene®
Technical registrants: Valent U.S.A. Corporation; Micro-Flo Company LLC; United Phosphorous Ltd.; Drexel Chemical Corporation

2.4.1 Environmental Fate Assessment

Aerobic soil metabolism is the main degradation process for acephate. Observed half-lives are less than two days under the nominal or expected use conditions, producing the intermediate degradate methamidophos, which is also an insecticidally active compound. Methamidophos is itself rapidly metabolized by soil microorganisms to carbon dioxide and microbial biomass (half-lives of < 10 days). Acephate is stable against hydrolysis except at high pH (half-life at pH 9 of 18 days) and does not photodegrade. Acephate is not persistent in anaerobic clay sediment: creek water systems in the laboratory, with a half-life of 6.6 days. The major degradates under anaerobic conditions were carbon dioxide and methane, comprising > 60% of the applied acephate after 20 days of anaerobic incubation. No other anaerobic degradates were present at > 10% during the incubation. There are no acceptable data for the aerobic aquatic metabolism of acephate; supplemental information indicates that acephate degrades more rapidly in aquatic systems when sediment is present. Appendix I describes the fate properties of acephate in greater detail.

Acephate is very soluble (80.1-83.5g/100 mL) and very mobile (K_{oc} = 2.7) in the laboratory. Only one K_{oc} value is available, because acephate was adsorbed in only one of the five soils (a clay loam) used in the batch equilibrium studies. When tested in the same soils, methamidophos was determined to be more mobile than acephate; again, only one K_{oc} value is available (K_{oc} = 0.9 in the clay loam soil). Because acephate is not persistent under aerobic conditions, very little acephate is expected to leach to groundwater. If any acephate does reach ground water, it would not be expected to persist, due to its short anaerobic half-life. Volatilization from soil or water is not expected to be a route of dissipation for either acephate or methamidophos.

Field studies conducted in Mississippi (tobacco on silt loam soil), California (bell peppers on silt loam soil), Florida (cauliflower on sand soil) and Iowa (soybeans on loam soil) produced dissipation half-lives of 2 days or less with no detections of parent or the degradate methamidophos below a depth of 50 cm. Laboratory studies showed that bioaccumulation of acephate in bluegill sunfish was insignificant. A maximum bioaccumulation factor of 10x occurred after 14 days' exposure to acephate at 0.007 and 0.7 ppm.

2.4.2 Environmental Transport Assessment

Batch equilibrium studies

Batch equilibrium studies using acephate and methamidophos were conducted using four soils ranging in texture from sand to clay loam. In three of the soils, acephate and methamidophos were not adsorbed in sufficient quantities to permit the calculation of Freundlich adsorption coefficients (Freundlich K_{ads}). For the clay loam soil, the reported adsorption values for parent acephate and its degradate, methamidophos, are listed in the following table:

Soil	pH	CEC (meq/100g)	%clay	%organic matter	Acephate			Methamidophos		
Clay loam	5.8	20.2	32	3.3	K	1/n	r^2	K	1/n	r^2
					0.090	1.06	0.96	0.029	0.64	0.93

Calculated K_{oc} for acephate and methamidophos in this clay loam soil were 2.7 and 0.9, respectively. Because of the minimal adsorption of the chemicals in the adsorption phase of the study, it was not possible to determine desorption values in the soils. Based on the values listed above, it appears that acephate and methamidophos are very mobile in soils.

Volatility

Based on the vapor pressure of acephate (pure active: 1.7×10^{-6} mm Hg/Torr [MRID 40390601]) and its calculated Henry's constant (5.1×10^{-13} atm mole / m^3), it is not expected that acephate will volatilize from either soil or water in significant quantities. Therefore it is not expected that volatilization will be a significant route of dissipation for acephate.

Long Range Transport

Potential transport mechanisms generally include pesticide surface water runoff, spray drift, and secondary drift of volatilized or soil-bound residues leading to deposition onto nearby or more distant ecosystems. The magnitude of pesticide transport via secondary drift depends on the pesticide's ability to be mobilized into air and its eventual removal through wet and dry deposition of gases/particles and photochemical reactions in the atmosphere. A number of studies have documented atmospheric transport and redeposition of pesticides from the Central Valley to the Sierra Nevada mountains

(Fellers et al., 2004, Sparling et al., 2001, LeNoir et al., 1999, and McConnell et al., 1998). Prevailing winds blow across the Central Valley eastward to the Sierra Nevada mountains, transporting airborne industrial and agricultural pollutants into Sierra Nevada ecosystems (Fellers et al., 2004, LeNoir et al., 1999, and McConnell et al., 1998). Therefore, physicochemical properties of the pesticide that describe its potential to enter the air from water or soil (e.g., Henry's Law constant and vapor pressure), pesticide use, modeled estimated concentrations in water and air, and available air monitoring data from the Central Valley and the Sierra Nevadas are considered in evaluating the potential for atmospheric transport of acephate to habitat for the CRLF.

In general, deposition of drifting or volatilized pesticides is expected to be greatest close to the site of application. Computer models of spray drift (AgDRIFT or AGDISP) are used to determine if the exposures to aquatic and terrestrial organisms are below the Agency's Levels of Concern (LOCs). If the limit of exposure that is below the LOC can be determined using AgDRIFT or AGDISP, longer-range transport is not considered in defining the action area. For example, if a buffer zone <1,000 feet (the optimal range for AgDRIFT and AGDISP models) results in terrestrial and aquatic exposures that are below LOCs, no further drift analysis is required. If exposures exceeding LOCs are expected beyond the standard modeling range of AgDRIFT or AGDISP, the Gaussian extension feature of AGDISP may be used. In addition to the use of spray drift models to determine potential off-site transport of pesticides, other factors such as available air monitoring data and the physicochemical properties of the chemical are also considered.

2.4.3 Mechanism of Action

Some of the information for the mode of action below comes from Davies et. al., 1981. Organophosphate insecticides (such as acephate) act upon target pests through a neurotoxic action, which affects the central nervous system. Specifically, the mechanism of action is known to be acetylcholinesterase inhibition. The transmission of nerve impulses across synapses and the junctions between nerve and an organ (gland, muscle, nerve) is accomplished by the release of a chemical agent, acetylcholine. Acetylcholine must be rapidly destroyed or inactivated at or near the site of its release to continue transmission of new impulses. The destruction of acetylcholine at such sites is accomplished by an enzyme, acetylcholinesterase. Acetylcholinesterase is located at the neurosynaptic junctions and breaks the acetylcholine into acetyl and choline fragments. Acetylcholinesterase functions to increase the precision of nerve firing, enabling some nerve cells to fire as rapidly as 1,000 times per second without overlap of the of the neural impulses. Acetylcholinesterase inhibitors prevent the acetylcholinesterase from removing the acetylcholine and thereby causing disruption to the central nervous system. At a high enough concentration of the inhibitors, the muscles may not contract the diaphragm and breathing ceases and death results.

Depending on the organophosphate involved, the dose received, and the duration of exposure; the period for regeneration of the acetylcholinesterase to occur varies among organisms.

2.4.4 Use Characterization

2.4.4.1 Use Profile

Acephate is a an organophosphate insecticide currently registered for use on a variety of field, fruit, and vegetable crops; in food handling establishments; on ornamental plants both in greenhouses and outdoors (including lawns, turf, and cut flowers); and in and around the home. The use profile is based on the current, federally registered uses (Section 3 and 24c for California). There are well over 100 registered labels for acephate, with products ranging from 0.25% to 97.4% ai. Section 3 (nation-wide) and section 24(c) (California) registered uses for acephate are presented in Table 2.2. with the label maximum one time application, maximum annual application rate, and the minimum time between treatments. A complete list of product names and registration numbers is in Appendix G.

Table 2.2. Labeled uses assessed in this document.

Crop/Site (a)	Registration Number	Equipment	Max Application Rate Qty	Max App Rate Unit/Area	Seasonal Max Dose/Crop Cycle	Minimum Interval Between Retreatment (days)
Bean and fruiting vegetable (1)	019713-00400	Aircraft; Ground	1.00	Lb ai /A	2.07 lb	AN; 3
Bermuda grass	CA79013800	Aircraft; Ground	1.00	Lb ai /A	NS	NS
Celery	019713-00400	Aircraft; Ground	1.00	Lb ai /A	2.07 lb	NS; 3
Christmas Tree Plantations	053883-00133	Aircraft; Ground	0.50	Lb ai /A	NS	NS; 28
	066330-00354	Ground	0.50	lb/100 gal/A	NS	NS
	066330-00354	Aircraft	0.50	lb/2 gal	NS	NS
Citrus (non-bearing)	034704-00903	Ground	0.76	Lb ai/ A	NS	AN; 7
	019713-00400	Sprinkler can	0.0012	gal mound	NS	7
	059639-00026	Drencher	0.01	lb ai/mound (4 ft diameter)	NS	NS
Cole Crops (2)	019713-00400	Aircraft; Ground	1.00	Lb ai /A	2.07 lb	AN; 7
Cotton (Unspecified)	019713-00400	Aircraft; Ground	1.00	Lb ai /A	6 lb	AN; 3
	019713-00400	Soil in-furrow treatment	1.13	Lb ai /A	NS	an; 3
	019713-00408	Seed treatment Hopper box; Slurry-type seed treater; Sprayer	0.39 (0.117)	lb cwt (Lb ai/acre)	NS	NS
Cranberry	059639-00026	Aircraft; Ground	1.00	Lb ai /A	0.9975 lb	NS; 7
Drainage Systems	019713-00400	Aircraft; Ground	0.25	Lb ai /A	NS	NS
	070506-00001	Drencher	0.01	lb mound	NS	NS
	070506-00002	Spoon	1.50	Tsp mound	NS	NS
Outdoor Facilities/ Premises (3 and 12)	059639-00026	Aircraft; Ground	0.25	Lb ai /A	NS	AN
	019713-00495	Sprinkler can	0.01	lb mound	NS	NS
	019713-00495	Spoon	1.50	Tsp mound	NS	NS
	070506-00001	Paintbrush	0.08	lb nest	NS	NS
	070506-00001	Sprayer	0.08	lb nest	NS	NS

Crop/Site (a)	Registration Number	Equipment	Max Application Rate Qty	Max App Rate Unit/Area	Seasonal Max Dose/Crop Cycle	Minimum Interval Between Retreatment (days)
	081964-00002	Ground	0.25	lb/3.3 gal	NS	NS
Fencerows/ Hedgerows (4)	066330-00356	Aircraft; Ground; Hydraulic Sprayer	0.25	Lb ai /A	NS	NS
	053883-00133	Spoon	0.002	gal mound	NS	NS
	053883-00133	Sprinkler	0.01	lb mound	NS	NS
	053883-00133	Spoon	1.50	Tsp mound	NS	NS
Golf Course Turf	059639-00087	Ground	0.11	lb 1K sq.ft	NS	NS; 7
	066330-00356 073614-0001	Granule applicator	5.0	Lb ai /A	NS	7
Lettuce	019713-00400	Aircraft; Ground	1.00	Lb ai /A	2.0 lb	AN; 7
Mint/Peppermint/Spearmint	019713-00400	Aircraft; Ground	1.00	Lb ai /A	2.0 lb	AN; 7
Onion (24 C)	CA87007100	Ground	1.00	Lb ai /A	NS	AN
Ornamentals (5)	070506-00001	Paintbrush	0.01	gal in. trunk diam	NS	NS
	059639-00087	Spoon	0.00015	gal pot	NS	NS
	000239-02453	Shaker can	0.00011	gal sq.ft	NS	AN
	070506-00008	Paintbrush	0.0038	gal tree	NS	NS
	000499-00421	Aerosol can	0.50	lb 1K sq.ft	NS	NS
	066330-00358	Sprayer	1.13	tbsp/1.5 gal	NS	7
	000499-00421	Aerosol can	4.00	oz 1.5K sq.ft (L)	NS	NS
	019713-00544	Mist blower; Hydraulic sprayer	1.20	lb/100 gal/A	NS	NS
	000239-02461	Sprayer	0.01	lb/1 gal	NS	7
	000239-02453	Shaker can	0.001	lb sq.ft	NS	30
	081964-00003	Mist blower; Hydraulic sprayer	1.20	Lb ai /A	NS	NS
	059639-00033	Aircraft; Ground; Hydraulic Sprayer	0.45	Lb ai /A	NS	NS
	059639-00028	Hydraulic sprayer	0.75	lb 1K sq.ft	NS	AN
	059639-00087	Granule applicator	0.06	lb 1K sq.ft	NS	NS
	000239-02472	Shaker can	0.00011	gal sq.ft	NS	AN
	059639-00087	Spoon	0.00015	gal pot	NS	NS
	059639-00033	Aircraft; Ground	1.00	Lb ai /A	NS	3
Peanuts	019713-00400	Aircraft; Ground	1.00	Lb ai /A	3.9975 lb	AN; 7
	059639-00033	Hopper box; Planter/seed box	0.20	lb cwt	3.9975 lb	AN; 7
Sewage Disposal Areas	019713-00544	Aircraft; Ground	0.13	Lb ai /A	NS	NS
Soybeans (Unspecified)	059639-00026	Aircraft; Ground	1.00	Lb ai /A	1.5 lb	7

Crop/Site (a)	Registration Number	Equipment	Max Application Rate Qty	Max App Rate Unit/Area	Seasonal Max Dose/Crop Cycle	Minimum Interval Between Retreatment (days)
Stone Fruit (6)	059639-00091	Aircraft; Ground	0.97	Lb ai /A	NS	AN
Pome Fruit (7)	059639-00091	Aircraft; Ground	0.97	Lb ai /A	NS	AN
Tree Nut (8)	059639-00091	Aircraft; Ground	0.97	Lb ai /A	NS	AN
Turf (9)	000239-02632	Granule applicator	4.95	Lb ai /A	NS	7
	000239-02632	Hose-end sprayer	6.75	tbsp 1K sq.ft	NS	AN
	000239-02632	Sprayer	0.11	lb 1K sq.ft	NS	14
	059639-00026	Sprinkler can	0.01	lb mound	NS	14
	059639-00026	Sprinkler can	0.15	gal mound	NS	14
	059639-00026	Spoon	1.80	Tsp mound	NS	NS
Uncultivated Land (10)	059639-00026	Aircraft; Ground	0.25	Lb ai /A	NS	NS
	059639-00031	Product container	0.01	lb mound	NS	AN
	059639-00031	Ground	1.80	Tsp mound	NS	NS
	066330-00356	Spoon	0.00	gal mound	NS	NS
	066330-00360	Ground	0.11	lb 1K sq.ft	NS	NS
Vine Crop (11)	059639-00091	Aircraft; Ground	0.97	Lb ai /A	NS	AN

Source: LUIS Report, Updated November 2006.
(a) Similar use sites were combined into one category.
(b) AN = as needed; NS = not specified

1) Bean: Bean- Dried Type; Succulent-Lima; Succulent-Snap; Fruiting Vegetables; Pepper
2) Cole: Brussels Sprouts; Cauliflower
3) Outdoor Facilities/Premises: Commercial/Industrial/Industrial Premises/Equipment (Outdoor Household/Domestic Dwellings Outdoor Premises; Industrial Areas (Outdoor); Meat Processing Plant Premises (Nonfood Contact); Nonagricultural Outdoor Buildings/Structures; Paths/Patios; Paved Areas (Private Roads/Sidewalks); Refuse/Solid Waste Sites (Outdoor)
4) Fencerows/Hedgerows: agricultural rights of way/fencerows/hedgerows; nonagricultural rights of way/fencerows/hedgerows
5) Ornamentals: Crabapple; ornamental and/or shade trees; ornamental ground cover; ornamental herbaceous plants; ornamental nonflowering plants; ornamental woody shrubs and vines
6) Stone Fruit: Apricot; Cherry; Plum; Prune
7) Tree Fruit: Apple; Pear
8) Tree Nut: Almond; Pistachio; Walnuts (English/Black)
9) Turf: Commercial/industrial lawns; ornamental lawns and turf; ornamental sod farm (turf); recreation area lawns; residential lawns
10) Uncultivated Land; agricultural fallow/idleland; agricultural uncultivated areas; nonagricultural uncultivated areas/soils; recreational areas
11) Vine Crop: kiwi fruit; grapes
12) The following uses do not result in exposure of the CRLF or essential habitat because they are solely indoor uses and associated maximum application rates are not included in this table : Commercial Storages/Warehouses Premises; Commercial Transportation Facilities-Nonfeed/Nonfood; Commercial/Industrial/Industrial Premises/Equipment (Indoor);); Eating Establishments; Food Processing Plant Premises (Nonfood Contact); Food Stores/Markets/Supermarkets Premises; Food/Grocery/Marketing/Storage/Distribution Facility Premise; Hospitals/Medical Institutions Premises (Human/Veterinary); Household/Domestic Dwellings; Household/Domestic Dwelling Contents; Household/Domestic Dwellings Indoor Food Handling Areas; Household/Domestic Dwellings Indoor Premises; Poultry Processing Plant Premises (Nonfood Contact); Refuse Solid Waste Containers (Garbage Cans); Refuse/Solid Waste Sites (Indoor);

Acephate was first registered in 1973 for ornamental uses, and in 1974 for food uses (agricultural crops). Use data from 1988 to 1997 indicate that approximately 4 to 5 million pounds of active ingredient (ai) are used domestically each year (USEPA, 2001). Based on California Department of Pesticide Regulation (Cal DPR) Pesticide Use Reporting (PUR) annual reports, annual use of acephate in California ranged from approximately 194,000 to 259,000 pounds a.i. from 2001 through 2005 (Table 2.3)[2].

Table 2.3. Summary of annual acephate use in California from 2001 through 2005.

Year	Pounds a.i. Applied	Number of Applications
2001	240,109	21,098
2002	258,955	20,177
2003	223,749	18,676
2004	201,816	18,624
2005	194,365	16,009

Source: CAL DPR PUR Annual Reports for 2001-2005, viewed April 2007

Food: Acephate is registered for use on beans (green and lima), Brussels sprouts, cauliflower, celery, cottonseed, cranberries, lettuce, peanuts, peppermint, peppers (bell and non-bell), citrus, fruit trees, nut trees, soybeans, and spearmint.

Other Agriculture, Non-food: Acephate is also registered for use on cotton, and as seed treatment on cotton and peanuts (seed for planting), on non-bearing fruit trees, such as ornamental citrus, and on tobacco.

Residential: Acephate is registered for use outdoors around residential buildings, homes, and apartments, for the control of roaches, wasps, fire ants, and crickets, among other pests. It is also registered for outdoor use on home lawns, trees, shrubs and ornamentals.

Public Health: Acephate is registered for use in and around industrial, institutional and commercial buildings, including restaurants, food handling establishments, warehouses, stores, hotels, manufacturing plants, and ships for the control of roaches and fire ants.

Other Nonfood: Acephate is registered for use on sod, golf course turf, field borders, fence rows, roadsides, ditch banks, borrow pits, wasteland, and greenhouse and horticultural nursery floral and foliage plants.

Target pests include: Armyworms, aphids, beetles, bollworms, borers, budworms, cankerworms, crickets, cutworms, fire ants, fleas, grasshoppers, leafhoppers, loopers, mealybugs, mites, moths, roaches, spiders, thrips, wasps, weevils, whiteflies, and others (EPA IRED, 2001).

Formulation types: Wettable Powder, Soluble Powder, Soluble Extruded Pellets, Granular, and Liquid. All forms, except for granular, are mixed with water prior to application and are applied in a liquid form.

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[2] http://www.cdpr.ca.gov/docs/pur/purmain.htm (April 2007)

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

Equipment for agriculture, greenhouse, nursery, and turf uses: Granular acephate can be applied by belly grinder, hand, tractor-drawn spreader, push-type spreader, and shaker can. Liquid acephate (formulated from soluble powders or soluble extruded pellets) may be applied by aircraft, airblast sprayer, backpack sprayer, chemigation, hydraulic sprayers, groundboom spray, handgun, high pressure sprayer, hopper box (seed treatment), low-pressure handwand, slurry (seed treatment), sprinkler can, transplanting in water (tobacco), or by an aerosol generator (greenhouses).

Equipment for residential and public health uses: Residential applications can be made by aerosol can, backpack sprayer, hose-end sprayer, and low-pressure handwand. Residential granular applications can be made by shaker can or by hand. Residential soluble powder applications may be made by sprinkler can or compressed air sprayers.

Method: Acephate may be applied on seed before planting, in-furrow at planting, or as a foliar spray, it may be applied to float beds, plant beds, or as a transplant (tobacco) treatment. For use against fire ants it may be applied directly on their soil mound (drench and dry methods). Acephate is also used indoors as spot, crack and crevice, and bait treatments.

Rates: Rates vary according to method of application and pest. The highest registered maximum one time application rate is 5 lbs ai/A on commercial/residential turf. The highest seasonal application rate is 6 lb ai/A/year (1 lb ai/A at 6 applications per season) for cotton.

2.4.4.2. Use and Useage in California, 2000-2005

The Agency's Biological and Economic Analysis Division (BEAD) provides an analysis of both national- and county-level usage information using state-level usage data obtained from USDA-NASS[3], Doane (www.doane.com; the full dataset is not provided due to its proprietary nature), and the California's Department of Pesticide Regulation Pesticide Use Reporting (CDPR PUR) database[4] . 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 acephate by county in this California-specific assessment were generated using CDPR PUR data. Usage data are averaged together

[3] United States Depart of Agriculture (USDA), National Agricultural Statistics Service (NASS) Chemical Use Reports provide summary pesticide usage statistics for select agricultural use sites by chemical, crop and state. See http://www.usda.gov/nass/pubs/estindx1.htm#agchem.
[4] 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.

over the years 2002 to 2005 to calculate average annual usage statistics by county and crop for acephate, including pounds of active ingredient applied (Table 2.4). California State law requires that every pesticide application be reported to the state and made available to the public.

Table 2.4. Average Annual Pounds of Acephate Applied in California by Counties and Uses. Only the major uses are included. (2002-2005). Information on the remaining uses and counties reported in California can be found in Appendix J

County	Average Annual Pounds Applied	Lettuce	Cotton	Bean	Celery	Non Food Outdoor	Structural Pest Control	Pepper	Landscape Maintenance	Greenhouse	Cauliflower	Mint	Citrus
Average Annual Pounds Applied	**211,133**	**83,897**	**50,215**	**16,409**	**15,438**	**14,329**	**10,979**	**5,866**	**5,038**	**3,961**	**1,662**	**1,370**	**906**
Monterey	**61,958**	50,144	NR	1,280	7,146	310	45	319	707	770	1,188	NR	NR
Fresno	**34,299**	14,427	16,893	1,301	NR	75	169	1,302	2	63	4	NR	28
Kern	**14,189**	129	10,949	996	NR	72	1,031	648	6	1	NR	NR	233
Imperial	**14,021**	5,450	8,287	19	39	50	37	27	NR	NR	40	NR	6
Santa Barbara	**11,014**	6,087	NR	32	1,691	2,414	98	105	33	172	358	NR	NR
Ventura	**10,894**	99	NR	1,153	4,991	1,285	2,738	103	56	204	NR	NR	NR
Kings	**6,616**	226	6,309	13	NR	NR	29	NR	NR	NR	NR	NR	NR
Riverside	**6,190**	76	4,156	36	93	426	337	81	794	58	7	NR	40
Stanislaus	**4,839**	NR	NR	1,982	NR	2,633	60	36	53	51	NR	NR	NR
San Benito	**4,586**	1,984	NR	NR	632	62	6	1,858	4	11	5	NR	NR
San Diego	**4,444**	NR	NR	49	NR	1,858	697	44	749	918	NR	2	NR
San Luis Obispo	**4,314**	3,149	NR	5	714	166	7	60	87	101	25	NR	NR
Merced	**3,692**	NR	2,220	1,405	NR	2	36	NR	0	9	19	NR	NR
Santa Clara	**3,247**	382	NR	7	61	109	430	1,249	713	273	NR	NR	NR
San Joaquin	**3,156**	NR	139	2,011	NR	890	19	NR	6	77	NR	NR	NR
Sutter	**2,798**	NR	11	2,705	NR	69	8	NR	0	NR	NR	NR	NR
Los Angeles	**2,774**	NR	NR	NR	NR	769	1,369	NR	516	86	NR	NR	NR
Santa Cruz	**2,488**	1,731	NR	4	72	164	30	NR	39	442	3	NR	NR
Orange	**2,175**	NR	NR	14	NR	1,322	400	16	271	151	NR	NR	NR

Source: CDPR PUR 2007

The uses considered in this risk assessment represent all currently registered uses according to a review of all current labels. No other uses are relevant to this assessment. Any reported use, such as may be seen in the CDPR PUR database, represent either historic uses that have been canceled, mis-reported uses, or mis-use. Historical uses, mis-reported uses, and misuse are not considered part of the federal action and, therefore, are not considered in this assessment.

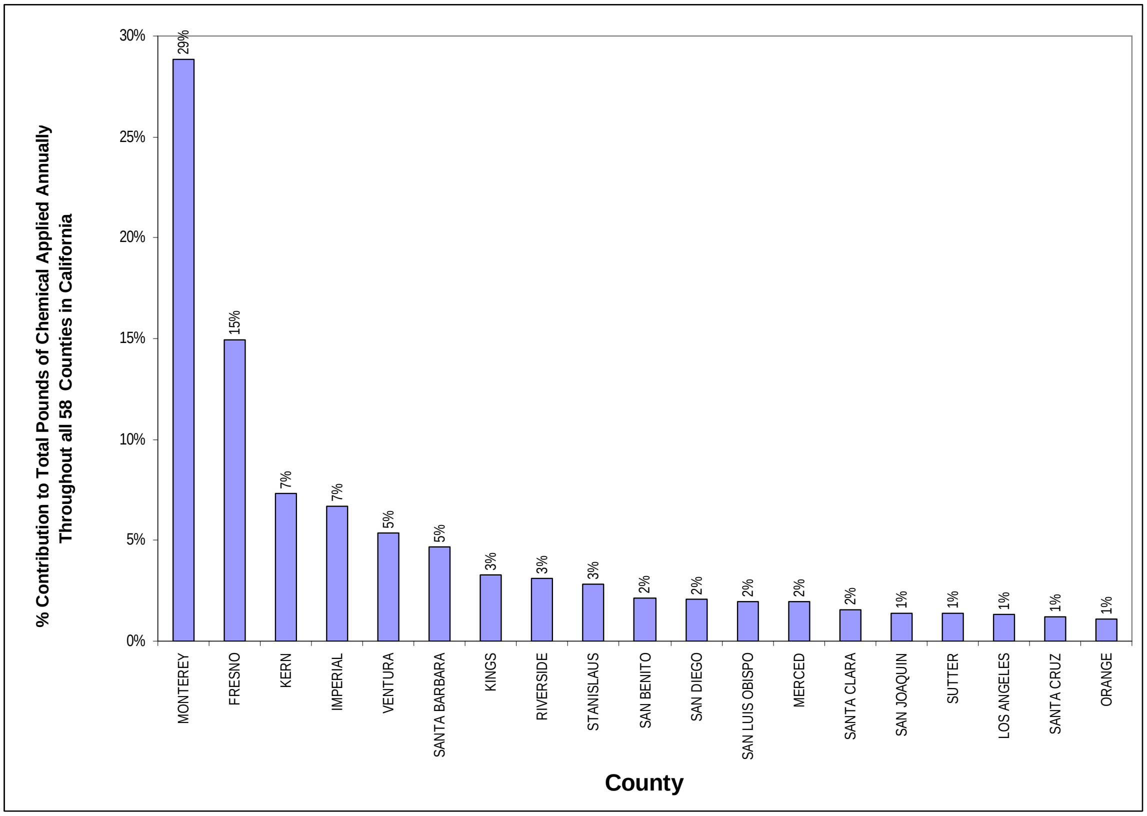


Figure 2.A. Percent Contribution of Each County to the Total Pounds Acephate Applied Annually in California Source: CDPR PUR 2007

Figure 2.A shows useage for the top eight use sites, limited to the top nineteen counties, in terms of pounds applied in 2004. Monterey county is a major use area for acephate. Note that the following counties not included in the figure each contributed less than 1% of the total annual acephate use in California, between 2003-2005: Tulare, San Francisco, Colusa, Madera, Glenn, Solano, San Mateo, Yolo, Contra Costa, Siskiyou, San Bernardino, Sacramento, Sonoma, Butte, Alameda, Modoc, Shasta, Lassen, Marin, Tehama, Placer, Humboldt, Napa, Plumas, Del Norte, Sierra, Tuolumne, Nevada, Mendocino, El Dorado, Mono, Lake, Yuba, Mariposa, Inyo, Amador, Calaveras, Trinity. Figure 2B shows the data for 2003-2005 broken down by month of application, for the top eight uses.

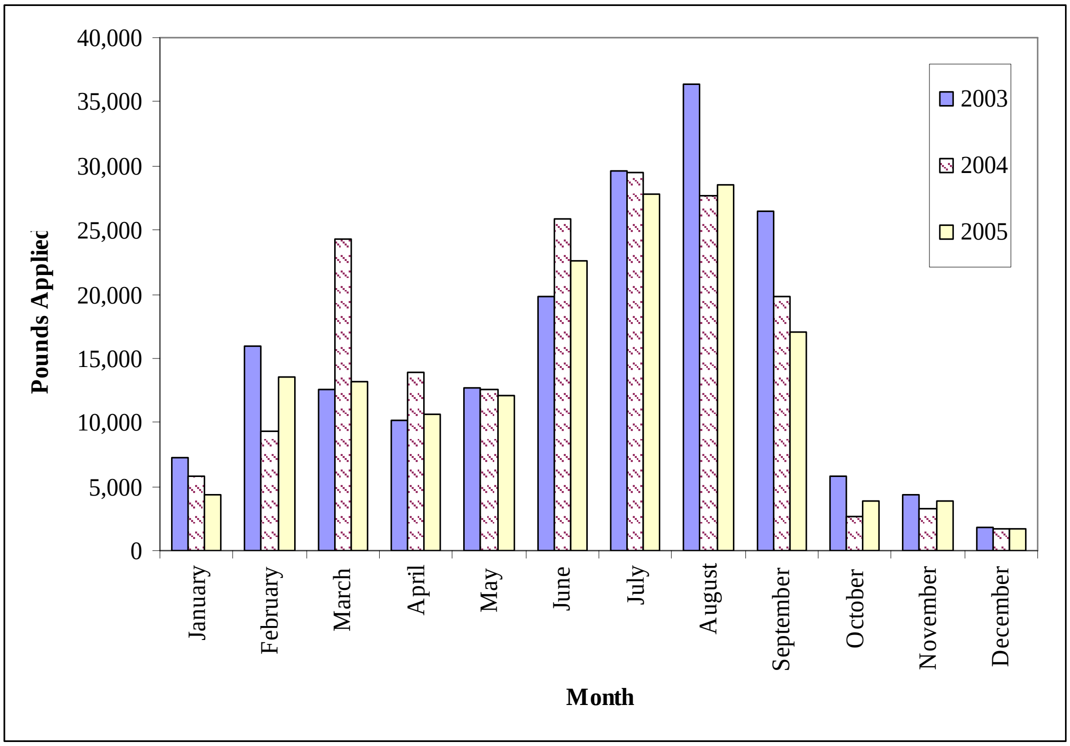


Figure 2.B. Pounds of A.I. applied per month to the following sites in 2003, 2004, 2005: Bean, Celery, Citrus, Cotton, Landscape Maintenance, Lettuce, Pepper, Pistachio. Source: CDPR PUR 2007

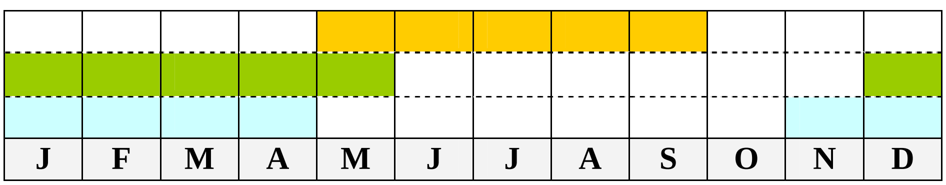


Figure 2.C – CRLF Reproductive Events by Month
Light Blue = **Breeding/Egg Masses**
Green = **Tadpoles (except those that over-winter)**
Orange = **Young Juveniles**
Adults and juveniles can be present all year

Above, Figure 2.C represents the CRLF reproductive cycle over time, and is presented in parallel to Figure 2.B, depicting the timing of acephate application to illustrate the temporal co-occurrence of reproductive events with acephate usage. The months when there are egg masses and tadpoles present (November to May) correspond to high usage on lettuce, celery and cotton. While this does not account for spatial differences between the location of the frog habitat and timing of reproductive events, it does show that there is general overlap between the timing of acephate applications and CRLF reproduction.

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 elevation 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.D). Recovery units, core areas, and other known occurrences of the CRLF from the CNDDB are described in further detail in this section, and designated critical habitat is addressed in Section 2.6. Recovery units are large areas defined at the watershed level that have similar conservation needs and management strategies. The recovery unit is primarily an administrative designation, and land area within the recovery unit boundary is not exclusively CRLF habitat. Core areas are smaller areas within the recovery units that comprise portions of the species' historic and current range and have been determined by USFWS to be important in the preservation of the species. Designated critical habitat is generally contained within the core areas, although a number of critical habitat units are outside the boundaries of core areas, but within the boundaries of the recovery units. Additional information on CRLF occurrences from the CNDDB is used to cover the current range of the species not included in core areas and/or designated critical habitat, but within the recovery units.

Recovery Units

Eight recovery units have been established by USFWS for the CRLF. These areas are considered essential to the recovery of the species, and the status of the CRLF "may be considered within the smaller scale of the recovery units, as opposed to the statewide range" (USFWS 2002). Recovery units reflect areas with similar conservation needs and population status, and therefore, similar recovery goals. The eight units described for the CRLF are delineated by watershed boundaries defined by US Geological Survey hydrologic units and are limited to the elevational maximum for the species of 1,500 m above sea level. The eight recovery units for the CRLF are listed in Table 2.5 and shown in Figure 2.D.

Core Areas

USFWS has designated 35 core areas across the eight recovery units to focus their recovery efforts for the CRLF (see Figure 2.D). Table 2.5 summarizes the geographical relationship among recovery units, core areas, and designated critical habitat. The core areas, which are distributed throughout portions of the historic and current range of the species, represent areas that allow for long-term viability of existing populations and reestablishment of populations within historic range. These areas were selected because they: 1) contain existing viable populations; or 2) they contribute to the connectivity of other habitat areas (USFWS 2002). Core area protection and enhancement are vital for maintenance and expansion of the CRLF's distribution and population throughout its range.

For purposes of this assessment, designated critical habitat, currently occupied (post-1985) core areas, and additional known occurrences of the CRLF from the CNDDB are considered. Each type of locational information is evaluated within the broader context of recovery units. For example, if no labeled uses of acephate occur (or if labeled uses occur at predicted exposures less than the Agency's LOCs) within an entire recovery unit, a "no effect" determination would be made for all designated critical habitat, currently occupied core areas, and other known CNDDB occurrences within that recovery unit. Historically occupied sections of the core areas are not evaluated as part of this assessment because the USFWS Recovery Plan (USFWS 2002) indicates that CRLFs are extirpated from these areas. A summary of currently and historically occupied core areas is provided in Table 2.5 (currently occupied core areas are bolded). While core areas are considered essential for recovery of the CRLF, core areas are not federally-designated critical habitat, although designated critical habitat is generally contained within these core recovery areas. It should be noted, however, that several critical habitat units are located outside of the core areas, but within the recovery units. The focus of this assessment is currently occupied core areas, designated critical habitat, and other known CNDDB CRLF occurrences within the recovery units. Federally-designated critical habitat for the CRLF is further explained in Section 2.6.

Table 2.5. California Red-legged Frog Recovery Units with Overlapping Core Areas and Designated Critical Habitat

Recovery Unit [1] (Figure 2.a)	**Core Areas [2,7] (Figure 2.a)**	**Critical Habitat Units [3]**	**Currently Occupied (post-1985) [4]**	**Historically Occupied [4]**
Sierra Nevada Foothills and Central Valley (1) (eastern boundary is the 1,500m elevation line)	**Cottonwood Creek (partial) (8)**	--	✓	
	Feather River (1)	BUT-1A-B	✓	
	Yuba River-S. Fork Feather River (2)	YUB-1	✓	
	--	NEV-1[6]		
	Traverse Creek/Middle Fork American River/Rubicon (3)	--	✓	
	Consumnes River (4)	ELD-1	✓	
	S. Fork Calaveras River (5)	--		✓
	Tuolumne River (6)	--		✓
	Piney Creek (7)	--		✓
	East San Francisco Bay (partial)(16)	--	✓	
North Coast Range Foothills and Western Sacramento River Valley (2)	**Cottonwood Creek (8)**	--	✓	
	Putah Creek-Cache Creek (9)	--		✓
	Jameson Canyon – Lower Napa Valley (partial) (15)	--	✓	
	Belvedere Lagoon (partial) (14)	--	✓	
	Pt. Reyes Peninsula (partial) (13)	--	✓	
North Coast and North San Francisco Bay (3)	Putah Creek-Cache Creek (partial) (9)	--		✓
	Lake Berryessa Tributaries (10)	NAP-1	✓	
	Upper Sonoma Creek (11)	--	✓	
	Petaluma Creek-Sonoma Creek (12)	--	✓	
	Pt. Reyes Peninsula (13)	MRN-1, MRN-2	✓	
	Belvedere Lagoon (14)	--	✓	
	Jameson Canyon-Lower Napa River (15)	SOL-1	✓	
South and East San Francisco Bay (4)	--	CCS-1A[6]		
	East San Francisco Bay (partial) (16)	ALA-1A, ALA-1B, STC-1B	✓	
	--	STC-1A[6]		
	South San Francisco Bay (partial) (18)	SNM-1A	✓	
Central Coast (5)	**South San Francisco Bay (partial) (18)**	SNM-1A, SNM-2C, SCZ-1	✓	

Table 2.5. California Red-legged Frog Recovery Units with Overlapping Core Areas and Designated Critical Habitat

Recovery Unit [1] (Figure 2.a)	Core Areas [2,7] (Figure 2.a)	Critical Habitat Units [3]	Currently Occupied (post-1985) [4]	Historically Occupied [4]
	Watsonville Slough- Elkhorn Slough (partial) (19)	SCZ-2 [5]	✓	
	Carmel River-Santa Lucia (20)	MNT-2	✓	
	Estero Bay (22)	--	✓	
	--	SLO-8[6]		
	Arroyo Grande Creek (23)	--	✓	
	Santa Maria River-Santa Ynez River (24)	--	✓	
Diablo Range and Salinas Valley (6)	**East San Francisco Bay (partial) (16)**	MER-1A-B, STC-1B	✓	
	--	SNB-1[6], SNB-2[6]		
	Santa Clara Valley (17)	--	✓	
	Watsonville Slough- Elkhorn Slough (partial)(19)	MNT-1	✓	
	Carmel River-Santa Lucia (partial)(20)	--	✓	
	Gablan Range (21)	SNB-3	✓	
	Estrella River (28)	SLO-1A-B	✓	
Northern Transverse Ranges and Tehachapi Mountains (7)	--	SLO-8[6]		
	Santa Maria River-Santa Ynez River (24)	STB-4, STB-5, STB-7	✓	
	Sisquoc River (25)	STB-1, STB-3	✓	
	Ventura River-Santa Clara River (26)	VEN-1, VEN-2, VEN-3	✓	
	--	LOS-1[6]		
Southern Transverse and Peninsular Ranges (8)	**Santa Monica Bay-Ventura Coastal Streams (27)**	--	✓	
	San Gabriel Mountain (29)	--		✓
	Forks of the Mojave (30)	--		✓
	Santa Ana Mountain (31)	--		✓
	Santa Rosa Plateau (32)	--	✓	
	San Luis Rey (33)	--		✓
	Sweetwater (34)	--		✓
	Laguna Mountain (35)	--		✓

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

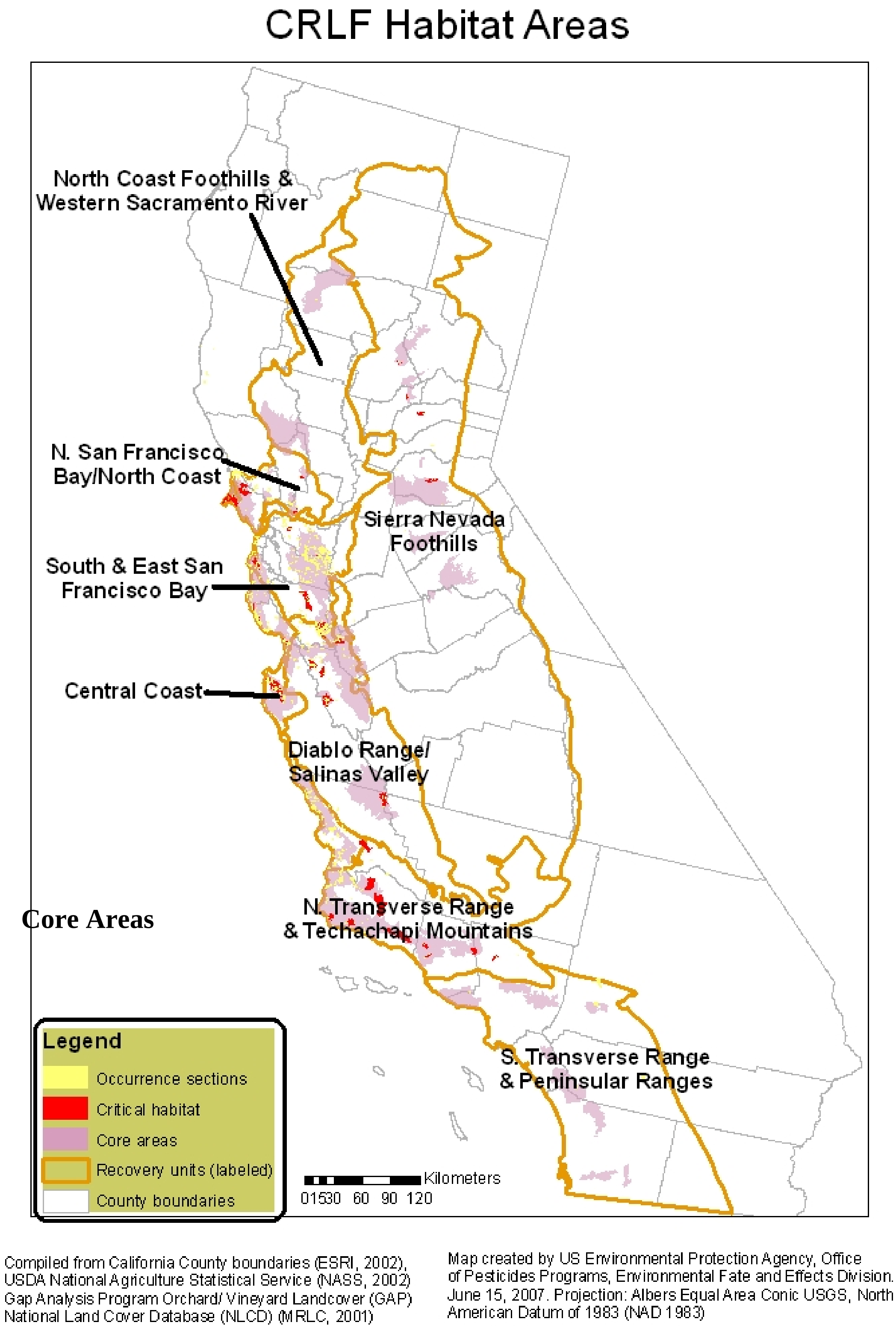


Figure 2.D Recovery Unit and Core Area Designations for CRLF

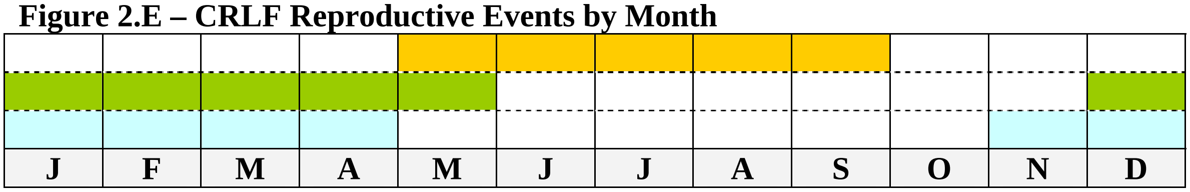
Other Known Occurrences from the CNDBB

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

2.5.2 Reproduction

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

Figure 2.E – 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, shading water of moderate depth is a habitat feature that appears 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 (USFWS 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.D.

'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.

Please note that a more complete 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 acephate 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 may 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 acephate is expected to directly impact living organisms within the action area, critical habitat analysis for acephate 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.

2.6.1. Special Rule Exemption for Routine Ranching Activities

As part of the critical habitat designation, the Service promulgated a special rule exemption regarding routine ranching activities where there is no Federal nexus from take prohibitions under Section 9 of the ESA. (USFWS 2006, 71 FR 19285-19290). The Service's reasoning behind this exemption is that managed livestock activities, especially the creation of stock ponds, provide habitat for the CRLF. Maintenance of these areas as rangelands, rather than conversion to other uses should ranching prove to be economically infeasible is, overall, of net benefit to the species.

Several of the specific activities exempted include situations where pesticides may be used in accordance with labeled instructions. In this risk assessment, the Agency has assessed the risk associated with these practices using the standard assessment methodologies. Specific exemptions, and the reasoning behind each of the exemptions is provided below. The rule provides recommended best management practices, but does not require adherence to these practices by the landowner.

1. Stock Pond Management and Maintenance
    a. Chemical control of aquatic vegetation. These applications are allowed primarily because the Service felt "it is unlikely that vegetation control would be needed during the breeding period, as the primary time for explosive vegetation control is during the warm summer months." The Service recommends chemical control measures be used only "outside of the general breeding season (November through April) and juvenile stage (April through September) of the CRLF." Mechanical means are the preferred method of control.
    b. Pesticide applications for mosquito control. These applications are allowed because of concerns associated with human and livestock health. Alternative mosquito control methods, primarily introduction of nonnative fish species, are deemed potentially more detrimental to the CRLF than chemical or bacterial larvicides. The Service believes "it unlikely that [mosquito] control would be necessary during much of the CRLF breeding season," and that a combination of management methods, such as manipulation of water levels, and/or use of a bacterial larvicide will prevent or minimize incidental take.

2. Rodent Control. The Service notes "we believe the use of rodenticides present a low risk to CRLF conservation." In large part, this is due to the fact that "it is

unknown the extent to which small mammal burrows are essential for the conservation of CRLF."

a. Toxicant-treated grains. No data were available to evaluate the potential effects of these compounds (primarily anti-coagulants) on the CRLF. Grain is not a typical food item for the frog, but individuals may be indirectly exposed by consuming invertebrates which have ingested treated grain. There is a possibility of dermal contact, especially when the grain is placed in the burrows. Placing treated grain into the burrows is not prohibited, but should this method of rodent control be used, the Service recommends bait-station or broadcast application methods to reduce the probability of exposure.
b. Burrow fumigants. Use of burrow fumigants is not prohibited, but the Service recommends "not using burrow fumigants within 0.7 mi (1.2 km) in any direction from a water body" suitable as CRLF habitat.

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 acephate is likely to encompass considerable portions of the United States based on the large array of agricultural uses and non-agricultural uses. However, the scope of this assessment limits consideration of the overall action area to those portions that may be applicable to the protection of the CRLF and its designated critical habitat within the state of California. Deriving the geographical extent of this portion of the action area is the product of consideration of the types of effects that acephate may be expected to have on the environment, the exposure levels to acephate that are associated with those effects, and the best available information concerning the use of acephate and its fate and transport within the state of California.

The definition of action area requires a stepwise approach that begins with an understanding of the federal action. The federal action is defined by the currently labeled uses for acephate. An analysis of labeled uses and review of available product labels was completed. This analysis indicates that, for acephate, the following uses are considered as part of the federal action evaluated in this assessment:

All outdoor uses that result in spray drift or run-off exposure are included in the initial area of concern. Indoor uses are not considered part of the Action Area since exposure of the CRLF is unlikely.

After a determination of which uses will be assessed, an evaluation of the potential "footprint" of the use pattern should be determined. This "footprint" represents the initial area of concern and is based on available land cover data for labeled outdoor uses. Local land cover data available for the state of California were analyzed to refine the understanding of potential acephate use. The initial area of concern is defined as all land cover types that represent the labeled uses described above. A map representing all the land cover types that make up the initial area of concern is presented in Figure 2.F.

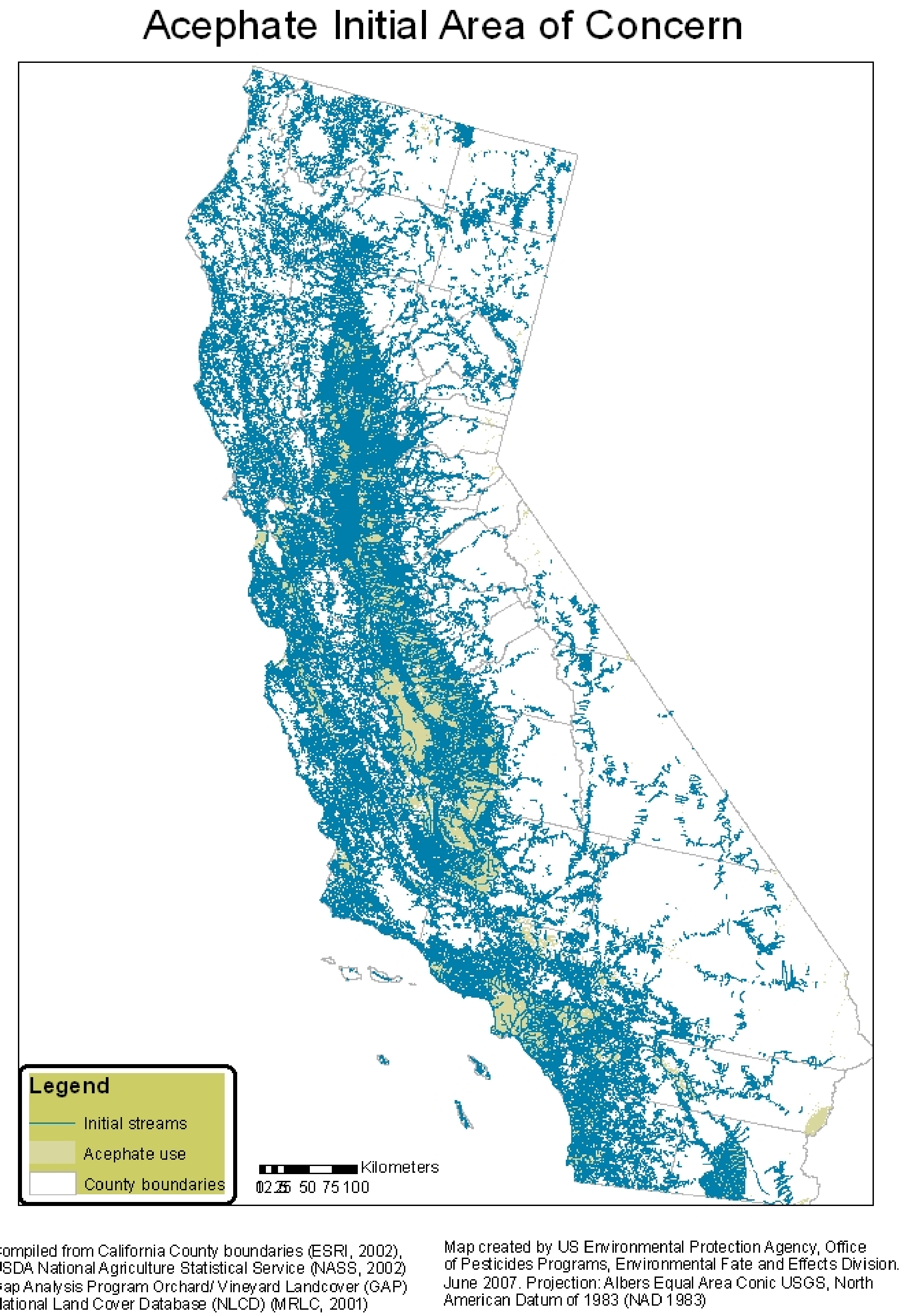


Figure 2.F. Initial Area of Concern

Once the initial area of concern is defined, the next step is to compare the extent of that area with the results of the screening level risk assessment. The screening level risk assessment will define which taxa, if any, are predicted to be exposed at concentrations above the Agency's Levels of Concern (LOC). The screening level assessment includes an evaluation of the environmental fate properties of acephate to determine which routes of transport are likely to have an impact on the CRLF.

LOC exceedances are used to describe how far effects may be seen from the initial area of concern. Factors considered include: spray drift, downstream run-off, atmospheric transport, etc. This information is incorporated into GIS and a map of the action area is created.

Based on the environmental fate assessment, the dominant routes of exposure to acephate and its degradate methamidophos are believed to be run-off and spray drift. Transport through groundwater is not considered to be a route of exposure due to the low persistence of both compounds. Volatilization and long-range transport are not considered to be a route of exposure due to the low vapor pressure and Henry's Law constant of both compounds. Based on its low log Kow (-0.85) bioaccumulation is not expected to be a concern.

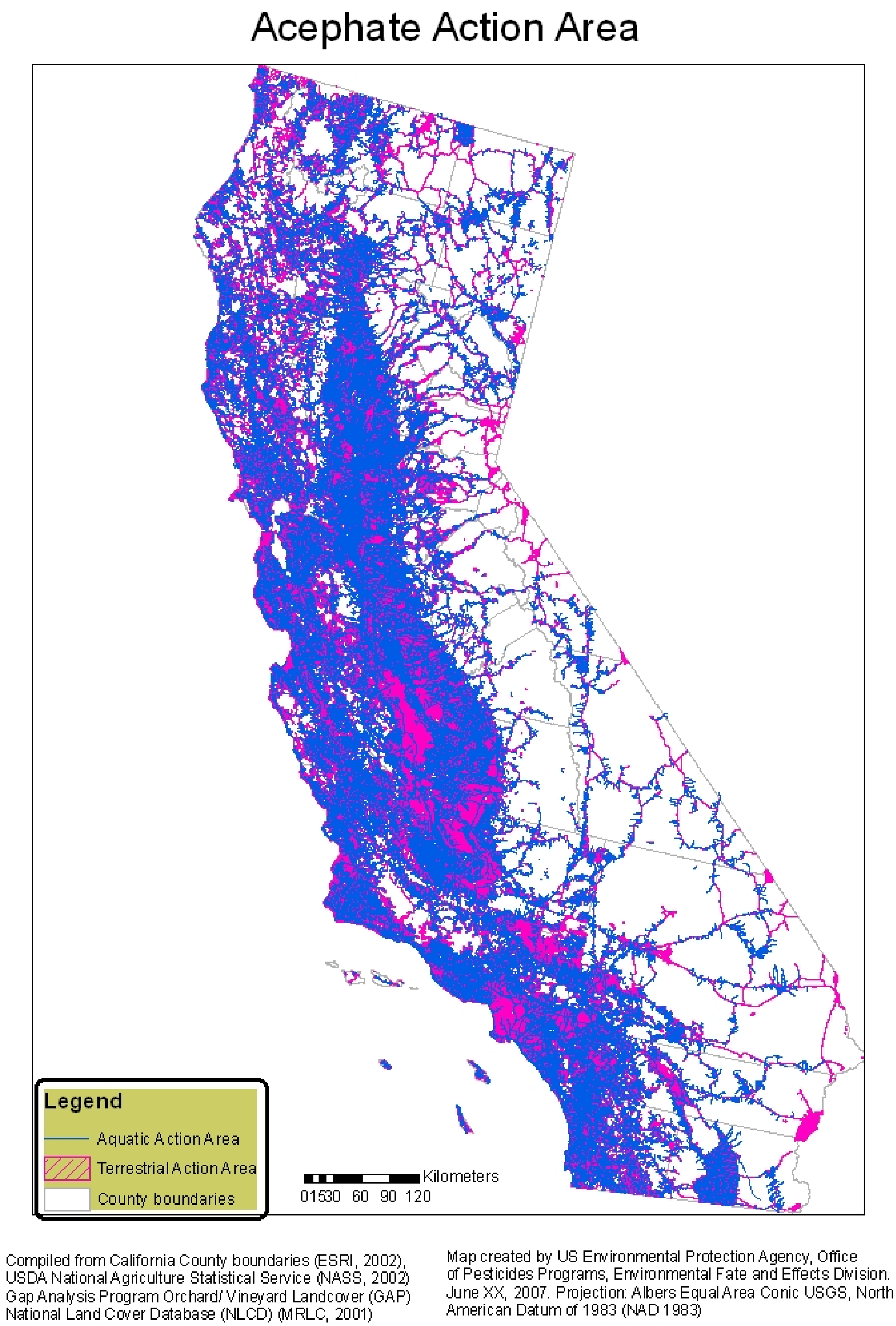


Figure 2.G Acephate Action Area

Subsequent to defining the action area, an evaluation of usage information was conducted to determine area where use of acephate may impact the CRLF. This analysis is used to characterize where predicted exposures are most likely to occur but does not preclude use in other portions of the action area. A more detailed review of the county-level use information was also completed. These data suggest that, as presented in Figures 2A, exposure is likely to be greatest in the counties of highest reported usage. Most of the acephate usage in California is concentrated in a few counties.

Action Area Calculation

The Action Area for acephate will be dominated by its effects on terrestrial species, due to the much higher RQ values in the terrestrial analysis. The highest risk quotient for any animal was for birds (avian) chronic risk based on dietary analysis (RQ = 167).

The dose (lb/acre) that results in an RQ below the chronic level of concern is 0.006 lb ai/A (1 lb/acre÷167). No adjustment is needed for LOC, since it is 1 for chronic risk.

The AgDISP model with the far-field Gaussian extension was used to calculate the spray drift buffer needed to reduce exposures to below 0.006 lb/acre. The following inputs were used; all other inputs were default values. This analysis indicates that the required spray drift buffer needed to define the Action Area for terrestrial effects is 2,913 feet (about 0.55 mile).

Table 2.6. AgDISP Input Parameters

Input parameter	Value
Release height	15 feet
Wind Speed	15 mph
Drop Size Distribution	ASAE Very fine to Fine
Spray volume rate	5 gallons per acre
Non-volatile fraction	0.032 (1.33 lb product in 5 gal = 42 lb water)
Active Fraction	0.024 (nonvol frac x % a.i. = 75%)
Canopy	None
Specific gravity (Carrier)	1
Initial Average Deposition	0.006 lb/acre

2.8 Assessment Endpoints and Measures of Ecological Effect

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

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 acephate is provided in Table 2.7.

Table 2.7 Summary of Assessment Endpoints and Measures of Ecological Effects for Direct and Indirect Effects of acephate on the California Red-legged Frog

Assessment Endpoint	Measures of Ecological Effects[6]	Toxicity Endpoint (see effects table for endpoint selection, Section 4)
Aquatic Phase (eggs, larvae, tadpoles, juveniles, and adults)[a]		
1. Survival, growth, and reproduction of CRLF individuals via direct effects on aquatic phases	1a. Most sensitive fish acute LC_{50} 1b. Most sensitive fish chronic NOAEC 1c. Most sensitive fish early-life stage NOAEC	1a. Rainbow trout acute 96-hr LC_{50} 832 ppm ai 1b. none available 1c. Rainbow trout 0.215 ppm ai

[5] From U.S. EPA (1992). *Framework for Ecological Risk Assessment.* EPA/630/R-92/001.
[6] All registrant-submitted and open literature toxicity data reviewed for this assessment are included in Appendix A.

Table 2.7 Summary of Assessment Endpoints and Measures of Ecological Effects for Direct and Indirect Effects of acephate on the California Red-legged Frog		
Assessment Endpoint	**Measures of Ecological Effects[6]**	**Toxicity Endpoint (see effects table for endpoint selection, Section 4)**
		(Acute-Chronic-Ratio)
2. Survival, growth, and reproduction of CRLF individuals via effects to food supply (*i.e.*, freshwater invertebrates, non-vascular plants)	2a. Most sensitive fish (1), aquatic invertebrate *(2)*, and aquatic plant (3-) EC_{50} or LC_{50} (guideline) 2b. Most sensitive aquatic invertebrate (1-*)* and fish (2) chronic NOAEC (guideline or ECOTOX)	2a1. Rainbow trout acute 96-hr LC_{50} 832 ppm ai 2a2. -*Daphnia magna* acute 48-hr EC_{50} = 1.1 ppm ai 2a3. Skeletonema costatum algae 5-day EC_{50} >50 ppm ai 2b1. *Daphnia magna* NOAEC = 0.015 ppm ai 2b2. none available
3. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat, cover, and/or primary productivity (*i.e.*, aquatic plant community)	3a. Vascular plant EC_{50} (duckweed guideline test or ECOTOX vascular plant) 3b. Non-vascular plant EC_{50} ()	3a. none available 3b. Skeletonema costatum algae 5-day EC_{50} >50 ppm ai
4. 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.	4a. Distribution of EC_{25} values for monocots (seedling emergence, vegetative vigor, or ECOTOX) 4b. Distribution of EC_{25} values for dicots (seedling emergence, vegetative vigor, or ECOTOX)[7]	4a. EC25 and NOEC values are greater than 4.5 lb/acre 4b. EC25 and NOEC values are greater than 4.5 lb/acre
Terrestrial Phase (Juveniles and adults)		
5. Survival, growth, and reproduction of CRLF individuals via direct effects on terrestrial phase adults and juveniles	5a. Most sensitive bird[b] () or terrestrial-phase amphibian acute LC_{50} or LD_{50} (guideline) 5b. Most sensitive bird[b] () or terrestrial-phase amphibian chronic NOAEC (guideline or ECOTOX)	5a. Dark eyed junko acute oral LD_{50} = 106 mg ai/kg-bw 5b. Mallard duck Reproductive study NOEL = 5 ppm ai
6. Survival, growth, and reproduction of CRLF individuals via effects on prey (*i.e.*, terrestrial invertebrates, small terrestrial vertebrates, including mammals and terrestrial phase amphibians)	6a. Most sensitive terrestrial invertebrate (1-) and vertebrate (2-) acute EC_{50} or LC_{50} (guideline or ECOTOX)[c] 6b. Most sensitive terrestrial invertebrate(1) and vertebrate(2-) chronic NOAEC (guideline or ECOTOX)	6a1. Honey bee acute contact LD_{50} = 1.20 ug ai/bee 6a2. Rat Acute oral LD_{50} = 866 mg ai/kg bw 6b1. None available 6b2. Rat 3- generation reproductive study NOAEL = 50 mg ai/kg bw-day diet[4]
7. Survival, growth, and reproduction of CRLF individuals via indirect	7a. Distribution of EC_{25} for monocots (seedling emergence, vegetative vigor, or ECOTOX	7a. All monocot EC25 and NOEC values are greater than 4.5 lb/acre

[7] The available information indicates that the California red-legged frog does not have any obligate relationships.

Table 2.7 Summary of Assessment Endpoints and Measures of Ecological Effects for Direct and Indirect Effects of acephate on the California Red-legged Frog		
Assessment Endpoint	**Measures of Ecological Effects**[6]	**Toxicity Endpoint (see effects table for endpoint selection, Section 4)**
effects on habitat (*i.e.*, riparian vegetation)	7b. Distribution of EC_{25} for dicots (seedling emergence, vegetative vigor, or ECOTOX)[5]	7b. All dicot EC25 and NOEC values are greater than 4.5 lb/acre
[a] Adult frogs are no longer in the "aquatic phase" of the amphibian life cycle; however, submerged adult frogs are considered "aquatic" for the purposes of this assessment because exposure pathways in the water are considerably different that exposure pathways on land. [b] Birds are used as surrogates for terrestrial phase amphibians. [c] Although the most sensitive toxicity value is initially used to evaluate potential indirect effects, sensitivity distribution is used (if sufficient data are available) to evaluate the potential impact to food items of the CRLF.		

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 acephate 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 destroy or adversely modify critical habitat are those that alter the PCEs and may 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 acephate effects data are available.

Assessment endpoints and measures of ecological effect selected to characterize potential modification to designated critical habitat associated with exposure to acephate are provided in Table 2.7. Adverse modification to the critical habitat of the CRLF includes the following, as specified by USFWS (2006) and previously discussed in Section 2.6:

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 acephate 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 2.8. Summary of Assessment Endpoints and Measures of Ecological Effect for Primary Constituent Elements of Designated Critical Habitat		
Assessment Endpoint	**Measures of Ecological Effect**[8]	
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.	a. Most sensitive aquatic plant EC_{50} (guideline or ECOTOX) b. Distribution of EC_{25} values for terrestrial monocots (seedling emergence, vegetative vigor, or ECOTOX) c. Distribution of EC_{25} values for terrestrial dicots (seedling emergence, vegetative vigor, or ECOTOX)	a. Skeletonema costatum algae 5-day EC_{50} >50 ppm ai b. All monocot EC25 and NOEC values are greater than 4.5 lb/acre c. All dicot EC25 and NOEC values are greater than 4.5 lb/acre
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.[9]	a. Most sensitive EC_{50} values for aquatic plants (guideline or ECOTOX) b. Distribution of EC_{25} values for terrestrial monocots (seedling emergence or vegetative vigor, or ECOTOX) c. Distribution of EC_{25} values for terrestrial dicots (seedling emergence, vegetative vigor, or ECOTOX)	a. Skeletonema costatum algae 5-day EC_{50} >50 ppm ai b. All monocot EC25 and NOEC values are greater than 4.5 lb/acre c. All dicot EC25 and NOEC values are greater than 4.5 lb/acre
Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.	a. Most sensitive EC_{50} or LC_{50} values for fish or aquatic-phase amphibians and aquatic invertebrates (guideline or ECOTOX) b. Most sensitive NOAEC values for fish or aquatic-phase amphibians and aquatic invertebrates (guideline or ECOTOX)	a. Skeletonema costatum algae 5-day EC_{50} >50 ppm ai b. All monocot EC25 and NOEC values are greater than 4.5 lb/acre c. All dicot EC25 and NOEC values are greater than 4.5 lb/acre
Reduction and/or modification of aquatic-based food sources for pre-metamorphs (*e.g.*, algae)	a. Most sensitive aquatic plant EC_{50} (guideline or ECOTOX)	a. Skeletonema costatum algae 5-day EC_{50} >50 ppm ai
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	a. Distribution of EC_{25} values for monocots (seedling emergence, vegetative vigor, or ECOTOX)	a. Skeletonema costatum algae 5-day EC_{50} >50 ppm ai b. All monocot EC25 and

[8] All toxicity data reviewed for this assessment are included in Appendix A.
[9] 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.

habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance	b. Distribution of EC_{25} values for dicots (seedling emergence, vegetative vigor, or ECOTOX) c. Most sensitive food source acute EC_{50}/LC_{50} and NOAEC values for terrestrial vertebrates (mammals) and invertebrates, birds or terrestrial-phase amphibians, and freshwater fish.	NOEC values are greater than 4.5 lb/acre c. All dicot EC25 and NOEC values are greater than 4.5 lb/acre
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.		

2.9 Conceptual Model

2.9.1 Risk Hypotheses

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

- Labeled uses of acephate within the action area may directly affect the CRLF by causing mortality or by adversely affecting growth or fecundity;
- Labeled uses of acephate within the action area may indirectly affect the CRLF by reducing or changing the composition of food supply;
- Labeled uses of acephate within the action area may indirectly affect the CRLF and/or adversely 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;
- Labeled uses of acephate within the action area may indirectly affect the CRLF and/or adversely 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;

Based on the results of the submitted terrestrial plant toxicity tests, it appears that seedlings and emerged plants may not be sensitive to acephate.

There are two Tier II multiple dose phytotoxicity tests for acephate (seedling emergence and vegetative vigor) that were submitted by the registrant. The EC_{25} is greater than 4.5 lb ai/A and the NOEC is 4.5 lb ai/A. A typical application rate for acephate is 1.0 lb/A.

Based on the results of the submitted terrestrial plant toxicity tests, it appears that seedlings and emerged plants are not sensitive to acephate and therefore acepahte *No Effect* on the CRLF based on these endpoints.

- Labeled uses of acephate within the action area may adversely 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);
- Labeled uses of acephate within the action area may adversely modify the designated critical habitat of the CRLF by reducing the food supply required for normal growth and viability of juvenile and adult CRLFs;
- Labeled uses of acephate within the action area may adversely 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.
- Labeled uses of acephate within the action area may adversely 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.
- Labeled uses of acephate within the action area may adversely 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 stressor (acephate and methamidophos), 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.H and 2.I, and the conceptual models for the aquatic and terrestrial PCE components of critical habitat are shown in Figures 2.J and 2.K. Exposure routes shown in dashed lines are not quantitatively considered because the resulting exposures are expected to be so low as not to cause adverse effects to the CRLF.

Long-range atmospheric transport is not expected due to the non-volatility and non-persistent nature of acephate. Likewise, groundwater transport is considered unlikely due to the non-persistence of acephate, even when its mobility in soil is considered. The operative routes of exposure will be spray drift at the time of application, and run-off due to precipitation within a few days of application.

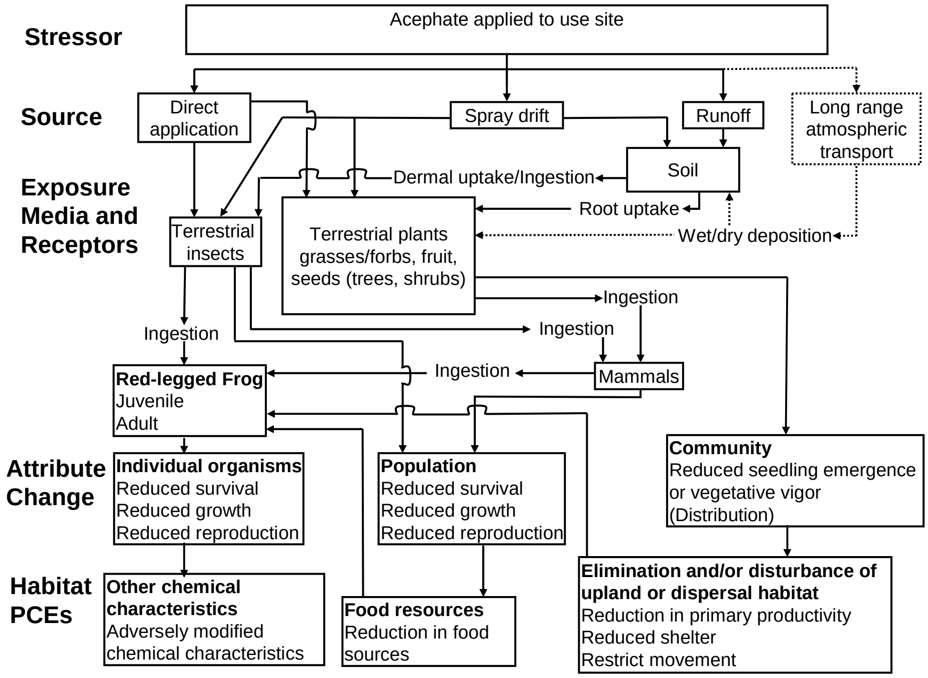


Figure 2.H. Conceptual Diagram for Terrestrial Phase Effects on CRLF

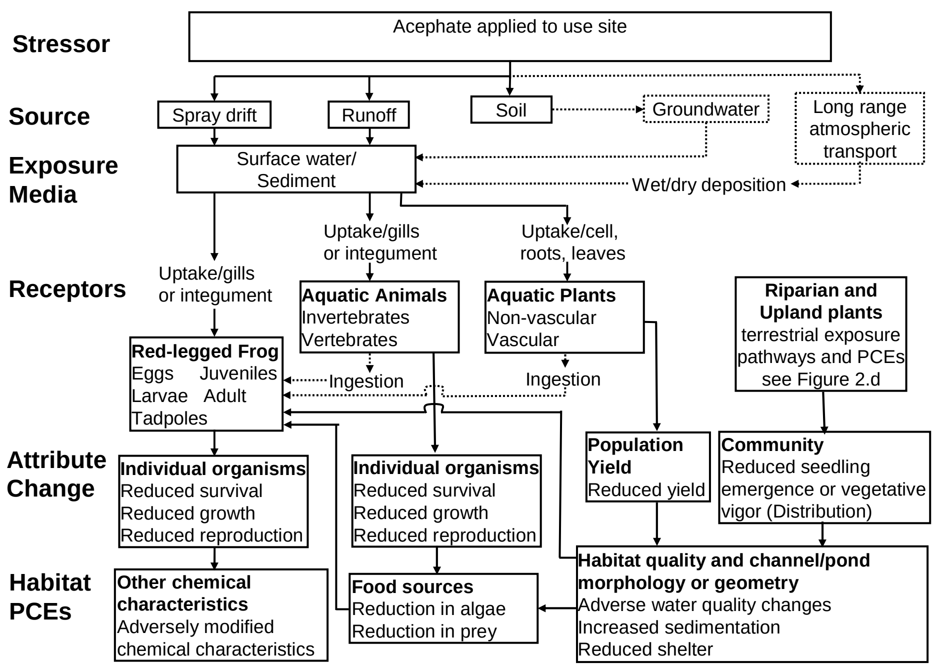


Figure 2.I . Conceptual Diagram for Effects on Aquatic Phase CRLF

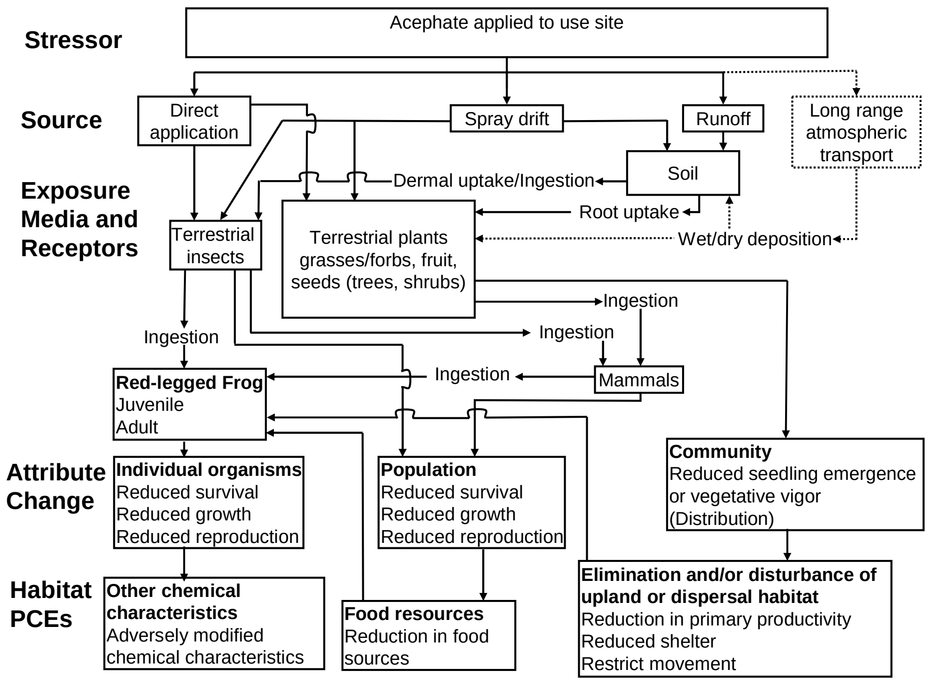


Figure 2.J . Conceptual Diagram for Effects Terrestrial Critical Habitat

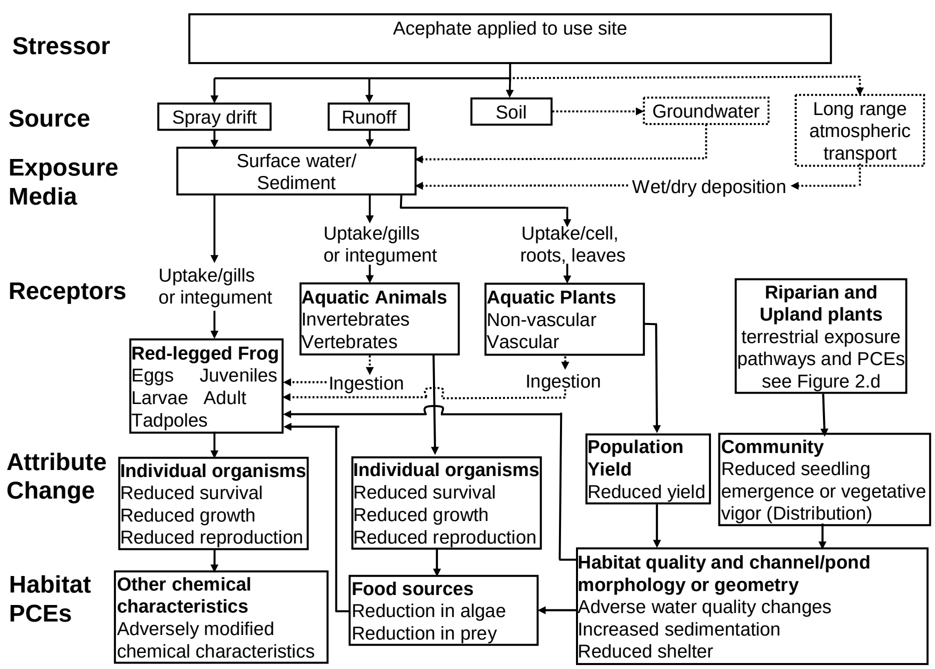


Figure 2.K . Conceptual Diagram for Effects on Aquatic Critical Habitat

2.10 Analysis Plan

Analysis of risks to the California Red-Legged Frog (both direct and indirect) and to its critical habitat will be assessed consistent with the Overview Document (USEPA, 2004) and Agency guidance for ecological risk assessments (USEPA 1998).

There are a number of labeled uses for acephate for indoor applications. Indoor uses include: Commercial Storages/Warehouses Premises; Commercial Transportation Facilities-Nonfeed/Nonfood; Commercial/Industrial/Industrial Premises/Equipment (Indoor); Eating Establishments; Food Processing Plant Premises (Nonfood Contact); Food Stores/Markets/Supermarkets Premises; Food/Grocery/Marketing/Storage/ Distribution Facility Premise; Hospitals/Medical Institutions Premises (Human/Veterinary); Household/Domestic Dwellings; Household/Domestic Dwelling Contents; Household/Domestic Dwellings Indoor Food Handling Areas; Household/Domestic Dwellings Indoor Premises; Poultry Processing Plant Premises (Nonfood Contact); Refuse Solid Waste Containers (Garbage Cans); Refuse/Solid Waste Sites (Indoor).

These applications have been considered. There is no exposure pathway from indoor applications to the CRLF or its habitat and therefore, indoor applications are determined to have **No Effect** on the CRLF.

2.10.1 Exposure Analysis

Direct effects to the aquatic phase CRLF will be assessed by comparing modeled surface water exposure concentrations of acephate and its degradate methamidophos to acute and chronic (early life stage hatching success and growth) effect concentrations for aquatic phase amphibians (surrogate freshwater fish) from laboratory studies (see the Effects Analysis section below). Effects to aquatic dietary food resources (aquatic invertebrates, algae) of the aquatic phase CRLF or effects to aquatic habitat that support the CRLF will also be assessed by comparing modeled surface water exposure concentrations of total acephate residues to laboratory established effect levels appropriate for the taxa.

A Tier 1 analysis (GENEEC 2.0) will be done first, since the toxicity endpoints for acephate are all above 1 ppm, and it is not anticipated that any LOCs will be exceeded, with the possible exception of invertebrates. As a refinement step, surface water concentrations of acephate and methamidophos will be quantified using the Tier 2 model, PRZM-EXAMS, if any LOCs are exceeded at the Tier 1 level. The definitive Tier 2 assessment of methamidophos is given in the methamidophos CRLF assessment document.

For the screening assessment, the standard EXAMS water body of 2 meters maximum depth, and 20,000 cubic meters volume, will be used. Since acephate is applied by numerous application methods, the model accounts for loading of acephate into the surface water via spray drift, run-off and erosion (Figure 2.J). Agricultural scenarios appropriate for labeled acephate uses will be used to account for local soils, weather and growing practices which impact the magnitude and frequency of acephate loading to the surface water. Maximum labeled application rates, with maximum number of applications and shortest intervals, will be used to help define (1) the Action Area within California for the Federal Action and (2) for evaluating effects to the CRLF.

Methamidophos is a major degradate of acephate. Methamidophos is also a registered organophosphate insecticide, and is registered for use in the U.S. on cotton, potatoes, and tomatoes and alfalfa grown for seed. Risks from acephate use, which includes its major degradate methamidophos, are assessed in this effects determination. The Agency is evaluating the risks to the CRLF posed by registered methamidophos uses separately.

Concentrations of acephate estimated by PRZM-EXAMS represent acephate loading in water bodies adjacent to any treated field and assume that the concentration applies to any water body within the treated area.

Risks to the terrestrial phase CRLF will be assessed by comparing modeled exposure to effect concentrations from laboratory studies. Exposure in the terrestrial phase will be quantified using the TREX model, which automates the calculation of dietary exposure according to the Hoerger-Kenaga nomogram, as modified by Fletcher (1994). The nomogram tabulates the 90th and 50th percentile exposure expected on various classes of food items, and scales the exposure (in dietary terms) to the size and daily food intake of several size classes of birds and mammals. Birds are also used as surrogates to represent reptiles and terrestrial-phase amphibians. A foliar decay half-life of 8.2 days, the maximum for acephate found in Willis and McDowell (1987) will be substituted for the default 35-day value. Effects from methamidophos are not considered quantitatively as LOC is expected to be exceeded for parent acephate.

2.10.2 Effects Analysis

As previously discussed in Section 2.8.1 and 2.8.2, assessment endpoints for the frog include direct toxic effects on survival, reproduction, and growth of the species itself, as well as indirect effects, such as reduction of the prey base and/or modification of CRLF habitat. Direct effects to the CRLF are based on toxicity information for freshwater fish and birds, which are generally used as a surrogate for aquatic and terrestrial phase amphibians, respectively. The open literature will be screened also for available frog toxicity data. Indirect effects to the CRLF are assessed by looking at available toxicity information relative to the frog's prey items and habitat requirements (freshwater invertebrates, freshwater vertebrates, aquatic plants, terrestrial invertebrates, terrestrial vertebrates, and terrestrial plants). Both guideline and open literature toxicity data will be identified and evaluated for use in determining RQ values.

Acephate's toxicity dataset is incomplete; chronic fish studies are lacking. Other organophosphates will be screened for available chronic fish data that can be used to derive acute to chronic ratio (ACR) for acephate.

Toxicity studies for acephate degradates (where available) will be discussed for exposure to the aquatic phase of the CRLF and incorporated into this risk assessment.

2.10.3 Action Area Analysis

The Action Area for the federal action is the geographic extent of exceedence of Listed species Levels of Concern (LOC) for any taxon or effect (plant or animal, acute or chronic, direct or indirect) resulting from the maximum label-allowed use of acephate. To define the extent of the Action Area, the following exposure assessment tools will be used: PRZM-EXAMS, TREX, AgDrift, and ArcGIS, a geographic information system (GIS) program. Other tools may be used as required if these are inadequate to define the maximum extent of the Action Area.

To determine the downstream extent of the Action area for any aquatic effects, acephate residues are also estimated for downstream from the treated areas by assuming dilution with stream water (derived from land area) from unaffected sources propagating downstream, until a point is reached beyond which there are no relevant LOC exceedances. Once the distribution of predicted stream water concentrations is obtained, it is further processed using a model that calculates expected dilution in the stream according to contributing land area. As the land area surrounding the field on which acephate is applied is enlarged, it encompasses a progressively greater drainage area; in effect, a progressively larger 'sub-watershed' is created, with a concomitant increase in dilution at the drainage point. This drainage point moves down-gradient along the stream channel as the sub-watershed is expanded. At a certain point the predicted stream concentrations will fall below the LOC. The area below this point is then assumed not to be at risk, with the upstream areas (up to the initial application area) assumed to present the potential for (direct and indirect) impact on the RLF. Additional acephate inputs within the same watershed will cause the area bounded by (that is, within) the LOC to increase, extending the length of stream that is likely to be impacted.

In order to determine the extent of the action area downstream from the initial area of concern, the Agency will need to complete the screening level risk assessment. Once all aquatic risk quotients (RQs) are calculated, the Agency determines which RQ to level of concern (LOC) ratio is greatest for all aquatic organisms (plant and animal). For example, if both fish and aquatic plants have the same RQ of 1, the fish RQ to LOC ratio (1/0.05) would be greater than for plants (1/1). Therefore, the Agency would identify all stream reaches downstream from the initial area of concern where the PCA (percent cropped area) for the land uses identified for acephate are greater than 1/20, or 5%. All streams identified as draining upstream catchments greater than 5% of the landclass of concern, will be considered part of the action area.

3. Exposure Assessment

3.1 Label Application Rates and Intervals

The registered uses of acephate in California include cotton, lettuce, citrus, celery, peppers, beans, mint, Bermuda grass for seed, landscape maintenance, pistachio, structural pest control, greenhouses, plants in containers, transplants, flowers, and others (see Table 2.2).

The application rates, intervals, and frequency are summarized in Table 3.1.

Table 3.1. Label Use Rates for Acephate in California

Use	Application Rate (lb ai/acre)	Number of applications allowed	Application Interval	Application Type
Beans & Lima beans – dry and succulent forms	1 lb a.i.	2	7 days	air or ground
Celery	1 lb a.i.	2	3 days	Air or ground
Cole Crops – Brussels sprouts and cauliflower	1 lb a.i.	2	not specified	
Cotton	1 lb a.i.	6	3, 5, 7 days or as necessary	Air, ground, in-furrow
Head lettuce – crisphead type	1 lb a.i.	2	Not specified	Air or ground
Mint – peppermint and spearmint	1 lb a.i.	2	10 days	Air or ground
Non-bearing Citrus	0.75 lb a.i.	Not specified	7 days or as necessary	Ground or soil drench
Non-crop areas – field borders, fencerows, roadsides, ditchbanks, borrowpits	0.25 lb a.i.	Not specified	Not specified	Ground or air
Non-crop area – wastelands	0.125 lb a.i.	1	Not applicable	Ground or air
Peanuts	1 lb a.i.	4	Not specified	Air or ground
Peppers – bell	1 lb a.i.	2	As necessary	Air or ground
Bermuda grass for seed	1 lb a.i.	Not specified	Not specified	Air or ground
Nursery stock, non-bearing deciduous fruit trees, nut trees, and vines in nursery fields or non-baring orchards	1 lb a.i.	Not specified	As needed	Air or ground

The largest number of applications with the shortest interval between applications will be used whenever the label does not specify the number of applications or application interval.

3.2 Aquatic Exposure Assessment

As discussed in section 2.5, the CRLF occupies a variety of shallow, static and flowing aquatic habitats in the aquatic phase of its life cycle (egg to tadpole). The current range of the CRLF is represented by the core areas, critical habitat and occurrence sections in Figure 2.D.

3.2.1. Conceptual Model of Exposure

Aquatic exposure of the CRLF within the action area is estimated with the PRZM-EXAMS model (EPA, 2004). Screening-level exposures (estimated environmental concentrations, EEC) are produced using the standard farm pond of 20,000 cubic meters volume. Watersheds where acephate is used are assumed to have 100% cropped area. The downstream extent of streams with exposures above the Level of Concern (LOC) is estimated (using GIS methods) by expanding the watershed considered until uncontaminated stream flow dilutes the initial pond concentration to below the LOC.

Standard assumptions of 1% spray drift for ground application and 5% drift for aerial application are used. If the pond concentration from PRZM-EXAMS exceeds the LOC, a spray drift buffer is calculated (using AgDrift model) that will reduce the pond concentration to below the LOC. If a spray drift buffer cannot be used to reduce the pond concentration to below the LOC, then a separate spray drift buffer (neglecting run-off) is calculated with AgDrift to ensure that pond concentrations are below the LOC (see section 2.10.3 above).

3.2.2 Existing Monitoring Data

There is very little useful water monitoring data for acephate, due to its non-persistent nature. There were no data for acephate or methamidophos in the California surface water database or in the USGS NAWQA surface water monitoring program. The assessment will therefore be based on modeled concentrations as described in section 3.2.1.

3.2.3 Modeling Approach

Use sites and the PRZM scenarios used to represent them are given in Table 3.4.
Risk quotients (RQs) were initially based on EECs derived using the Pesticide Root Zone Model/Exposure Analysis Modeling System (PRZM/EXAMS) standard ecological pond scenario according to the methodology specified in the Overview Document (U.S. EPA, 2004). Where LOCs for direct/indirect effects and/or adverse habitat modification are exceeded based on the modeled EEC using the static water body (i.e., “may affect”), refined modeling may be used to differentiate “may affect, but not likely to adversely

affect" from "may affect and likely to adversely affect" determinations for the CRLF and its designated critical habitat.

The general conceptual model of exposure for this assessment is that the highest exposures are expected to occur in the headwater streams adjacent to agricultural fields. Many of the streams and rivers within the action area defined for this assessment are in close proximity to agricultural use sites.

Twenty-six California scenarios were developed for the CRLF assessment. Each scenario is intended to represent a high-end exposure setting for a particular crop. Each scenario location is selected based on various factors including crop acreage, runoff and erosion potential, climate, and agronomic practices. Once a location is selected, a scenario is developed using locally specific soil, climatic, and agronomic data. Each PRZM scenario is assigned a specific climatic weather station providing 30 years of daily weather values.

Specific California PRZM scenarios were chosen for this assessment, including citrus, lettuce, row crop (representing beans, celery, and peppers), cotton, turf (representing bermudagrass for seed and landscape maintenance), almond (representing nut trees), fruit (representing various fruit trees) and cole crops (broccoli, cauliflower). Non-crop areas were not modeled because the application rates are lower than the agricultural uses, and agricultural scenarios are believed to represent non-agricultural exposures adequately. Structural pest control was not modeled due to lack of an appropriate PRZM scenario, and the low likelihood of exposure. All scenarios were used within the standard framework of PRZM/EXAMS modeling using the standard graphical user interface (GUI) shell, PE4v01.pl.

3.2.3.1 Model Inputs

A Tier 1 assessment (GENEEC 2.0) was run because the toxicity endpoints for acephate are in the part-per-million range, and it was not deemed likely that any LOCs would be exceeded. Tier 2 analysis (PRZM-EXAMS) was run if any LOCs were exceeded at the Tier 1 level.

The estimated water concentrations from surface water sources were calculated using Tier 2 PRZM (Pesticide Root Zone Model) and EXAMS (Exposure Analysis Modeling System). PRZM is used to simulate pesticide transport as a result of runoff and erosion from a standardized watershed, and EXAMS estimates environmental fate and transport of pesticides in surface waters. The linkage program shell (PE4v01.pl) that incorporates the site-specific scenarios was used to run these models.

The PRZM/EXAMS model was used to calculate concentrations using the standard ecological water body scenario in EXAMS. Weather and agricultural practices were simulated over 30 years so that the 1 in 10 year exceedance probability at the site was estimated for the standard ecological water body.

Models to estimate the effect of setbacks on load reduction for runoff are not currently available. It is well documented that vegetated setbacks can result in a substantial reduction in pesticide load to surface water (USDA, NRCS, 2000). Therefore, the aquatic EECs presented in this assessment are likely to over-estimate exposure in areas with well-vegetated setbacks. While the extent of load reduction cannot be accurately predicted through each relevant stream reach in the action area, data from USDA (USDA, 2000) suggest reductions could range from 11 to 100%.

The date of first application was set at March 1, because most uses for which there are data (PUR) show use in California in most months of the year, and March corresponds to both a rainy part of the year (thereby capturing higher run-off values), and the reproductive season of the frog.

The appropriate PRZM input parameters were selected from the environmental fate data submitted by the registrant and in accordance with US EPA-OPP EFED water model parameter selection guidelines, Guidance for Selecting Input Parameters in Modeling the Environmental Fate and Transport of Pesticides, Version 2.3, February 28, 2002. Exposures for the toxic degradate methamidophos were calculated by applying two correction factors: 0.77 for the molecular weight difference, and 0.23 for the maximum amount of methamidophos formed in an acephate soil metabolism study (MRID 0014991). Thus, methamidophos exposures are (0.77)*(0.23) = 0.18 times the acephate exposures.

Table 3.2. Summary of PRZM/EZAMS Environmental Fate Data Used for Aquatic Exposure Inputs for Acephate CRLF Assessment

Fate Property	Value	MRID (or source)	Comments
Molecular Weight	183.16	calculated from structure	none
Henry's constant	5.1 E-13 atm-m3/mole	Acephate IRED	Calculated from vapor pressure and solubility
Vapor Pressure	1.7 E-6 torr	Acephate IRED	MRID 40390601; 40645901
Solubility in Water	801000 mg/L	Acephate IRED	MRID 40390601
Photolysis in Water	Stable	Acephate IRED	MRID 41081603
Aerobic Soil Metabolism Half-lives	2.3 days	Acephate IRED	MRID 00014991 3 times single value of 14 hours, as per Input Parameter Guidance
Hydrolysis	163 days	Acephate IRED	MRID 41081603
Aerobic Aquatic Metabolism (water column)	4.6 days	Acephate IRED	2 time soil input value as per Input Parameter Guidance
Anaerobic Aquatic Metabolism (benthic)	4.6 days	Acephate IRED	2 time soil input value as per Input Parameter Guidance
Kd	0.09	Acephate IRED	MRID 40504811

Fate Property	Value	MRID (or source)	Comments
Application Efficiency	95 % for aerial 99 % for ground	Default value[c]	as per Input Parameter Guidance
Spray Drift Fraction[b]	5 % for aerial 1 % for ground	Default value	as per Input Parameter Guidance for ecological assessments
Application method (CAM)	2	Foliar spray	none
Incorporation depth	0 cm	Foliar spray	none

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.

3.2.4. Aquatic EEC Results

The tables (3.3, 3.4) below present the results of the GENEEC and PRZM-EXAMS modeling. Details of the results are found in Appendix B.

Table 3.3. GENEEC Modeled Exposure to Acephate for Maximum Use Rate (Cotton)

Crop/Use	Peak, ppb	21-day average, ppb	60-day average, ppb
Cotton, aerial, 6 applications of 1 lb/acre spaced at 3 days	85.4	43.6	18.4

Table 3.4. PRZM-EXAMS Modeled Exposures to Acephate

PRZM Scenario (Crop/Use)	Air or Ground	Application Rate (lb/acre)	Number of Applications @ interval (day)	Peak EEC (ppb)	21-day avg EEC (ppb)	60-day avg (ppb)
Citrus	G	0.5	1	0.55	0.33	0.20
Cotton	A	1	6 @ 3	19.2	12.8	6.7
	G	1	6 @ 3	13.4	7.5	3.5
Lettuce	A	1	2 @ 7	16.7	10.7	5.0
	G	1	2 @ 7	15.0	9.0	4.1
Row Crop (beans, celery, peppers)	A	1	2 @ 3	9.7	5.3	2.4
	G	1	2 @ 3	5.9	3.3	1.5
Turf (Bermudagrass for seed)	A	1	1	4.5	2.7	1.3
	G	1	1	2.5	1.6	0.76
Almond (pistachio)	A	1	1	18.1	13.6	7.5
	G	1	1	12.9	8.1	4.0
Fruit trees	A	1	1	14.0	9.7	5.0
	G	1	1	7.3	4.6	2.1
Cole crops	A	1	2 @ 7	13.4	9.0	4.4
	G	1	2 @ 7	11.3	7.1	3.4

3.3. Terrestrial Exposure Assessment

As discussed in section 2.5, adult CRLF occupy a variety of terrestrial dispersal habitats. The current range of the CRLF is represented by the core areas, critical habitat and occurrence sections in Figure 2.D.

3.3.1 Conceptual Model of Exposure

Terrestrial exposure of the CRLF on agricultural fields within the Action Area is estimated with the TREX model, which automates exposure analysis according to the Hoerger-Kenaga nomogram. Exposure to animals off the field is estimated with the AgDrift and AgDISP models.

Selection of Foliar Half-life Value for T-REX

Willis and McDowell (1987) was consulted for data on acephate persistence on foliage to replace the default value of 35 days. The default value was not believed to be reasonable for a non-persistent pesticide like acephate. Table III (p. 35) of this reference gives eight values for acephate, five of which are for dislodgeable residues (range 0.7 to 8.2 days), and three of which are for total residues (range 2.8 to 3.5 days). Normally, total residue values would be used for acephate, since it has a low Koc and is taken up through the roots (i.e., acts systemically). This rule is applied because it is believed that residues will be higher and more persistent if the pesticide is taken up into the plant, rather than just being on the surface of the foliage (which is measured by dislodgeable residue). Of the eight values, only one was measured on a crop in California (lemons), and it was measured as dislodgeable residue. This value was also the longest, and therefore most conservative of the values, at 8.2 days. The next longest value was 3.5 days (total residue) on citrus in Florida. Since the crops are similar (lemon and citrus) and the dislodgeable value exceeds the total value, contrary to what is expected, the value of 8.2 days was selected as the input to T-REX.

3.3.2. Modeling Approach

On-field exposure of the CRLF and its prey was estimated with TREX, using both maximum label rates of 1 lb/acre, 6 applications spaced at 3 day intervals (cotton). A low-rate scenario (0.25 lb/acre) was also done to bound possible risks. The decay rate used on foliage and other food items was 8.2 days (Willis & McDowell, 1987, p. 35), which was measured for acephate on lemons in California. Direct risk to the CRLF was bounded using 20-gram and 100-gram avian weight classes, since the weight of young adult frogs falls in this range. The CRLF was assumed to consume the broadleaf plant/small insect food category, since the bulk of its diet is invertebrates, and the small insect food category provides a higher dose. In addition, large CRLF also consumes other frogs and mice.

The T-HERPS model was used to characterize risk to the CRLF, by applying food intake rates and prey items appropriate to frogs, in place of the bird food intake rates assumed in T-REX.

Indirect risks to the CRLF through effects on its prey base were estimated in two ways. First, indirect effects via larger prey (Pacific tree frog and California mouse) were estimated conservatively using the 20-gram weight class for the Pacific tree frog and the

15-gram weight class for the mouse. The short-grass food category was used since it provides the highest dose and is eaten by the mouse.

Indirect effects via smaller prey (terrestrial invertebrates) were estimated using the LD50 data for the honey bee, and an assumed body weight of 0.128 grams. The dose was calculated as the large insect EEC in ppm (avian, dose-based, 20-gram animal), divided by the body weight of the bee. The LD50 (ppm) was calculated as the LD50 (micrograms per bee) divided by the body weight. The RQ was then the dose divided by the LD50 (ppm).

To define the Action Area, the dose (in lb/acre) needed to bring all RQs below their respective LOC (0.1 for acute, non-endangered birds and mammals, and 1.0 for chronic) was calculated by dividing the LOC by the RQ, and multiplying the result by the single application rate (1 lb/acre):

Dose below LOC (lb/acre) = (LOC/RQ)*(application rate, lb/acre).

The AgDrift or AgDISP model was then used to calculate the buffer distance needed to reduce the dose to below the LOC. If the result was beyond the range of these models, then the Gaussian extension to AgDISP was used.

3.3.3. Model Inputs

TREX and T-HERPS model inputs included application rate (1 lb/acre) number of applications (6), application interval (3 days), and foliar decay rate (8.2 days).

3.3.4 Results

See Appendix F and Appendix F1 for T-REX and T-Herps details of EEC calculations. Summaries are given here.

3.3.4.1 EECs for Direct Effects to CRLF

Tables 3.5 to 3.6. present the results of the TREX analysis. The EECs in Table 3.5 are based on the maximum exposure (cotton scenario) of 6 applications of 1 lb a.i./acre, spaced at 3 day intervals. The EECs in Table 3.6. are based on the lowest labeled application rate of 0.13 lb a.i./acre (for sewage disposal areas) and an assumption of one application.

Table 3.5. Summary of EEC for Direct Effects to CRLF (Maximum exposure)

Frog size, g	EEC, ppm	
	Small insects	Large Insects
20	536.5	59.6
100	306	34

Table 3.6. Summary of EEC Direct Effects to CRLF (lowest exposure)

Frog size, g	EEC, ppm	
	Small insects	Large Insects
20	20	2.2
100	11.4	1.3

3.3.4.2. EECs for Indirect Effects to CRLF

The EECs in Table 3.7. are based on the maximum exposure (cotton scenario) of 6 applications of 1 lb a.i./acre, spaced at 3 day intervals, and those in Table 3.8. on the low exposure scenario of 0.13 lb a.i./acre. The lowest weight classes (15-20 g) and highest residue (short grass) categories were used to provide a protective assessment of exposure.

Table 3.7. Summary of EEC for Indirect Effects on CRLF (Maximum exposure)

Avian, 20 gram body weight	
Food Item Category	Dose-Based EEC, mg/kg-bw
Short Grass	954
Tall Grass	437
Broadleaf Plants/small Insects	536
Fruits/pods/seeds/large insects	59.6
Mammal, 15-gram Body Weight	
Food Item Category	Dose-Based EEC, mg/kg-bw
Short Grass	798
Tall Grass	366
Broadleaf Plants/small Insects	449
Fruits/pods/seeds/large insects	49.9

Table 3.8. Summary of EEC for Indirect Effects on CRLF (lowest exposure)

Food Item Category	Dose-Based EEC, mg/kg-bw
Avian, 20-gram body weight	
Short Grass	35.5
Tall Grass	16.3
Broadleaf Plants/small Insects	20
Fruits/pods/seeds/large insects	2.2
Mammal, 15-gram Body Weight	
Food Item Category	Dose-Based EEC, mg/kg-bw
Short Grass	29.75
Tall Grass	13.6
Broadleaf Plants/small Insects	16.7
Fruits/pods/seeds/large insects	1.9

4. Effects Assessment

This assessment evaluates the potential for acephate to adversely affect the California Red-Legged Frog (CRLF). As previously discussed in Section 2.7, selected assessment endpoints for the CRLF include assessment of direct toxic effects on the survival, reproduction, and growth of the frog itself, as well as indirect effects, such as reduction of the prey base and/or modification of its habitat (Tables 2.7 and 2.8). Taxa selected as measurement endpoints include freshwater fish as a prey item and also as a surrogate for aquatic phase of CRLF, if no amphibian toxicity data are available; freshwater aquatic invertebrates (prey item); birds as surrogates for terrestrial phase of CRLF and other amphibians (prey item); small mammals (prey item); terrestrial invertebrates (prey item); aquatic plants, and terrestrial plants (Tables 2.7 and 2.8). Toxicity data for freshwater fish and birds are used as surrogate data for aquatic-phase and terrestrial-phase amphibians (U.S. EPA, 2004).

Information on the toxicity of acephate to selected taxa is characterized based on registrant-submitted studies and a comprehensive review of the open literature on acephate. Values used for each measurement endpoint identified in Tables 2.7 and 2.8 are selected from this data. Currently, no FIFRA data requirements exist for aquatic-phase or terrestrial-phase frogs and are therefore not part of typical registrant submitted data packages. However, some aquatic-phase frog survival data for acephate are available from open literature (Table 4.1), these data were reviewed for use in the risk determination. A summary of the available ecotoxicity information; the selected individual, population, and community-level endpoints for characterizing risks; and interpretation of the LOC, in terms of the probability of an individual effect based on probit dose response relationship are provided in Sections 4.1 through 4.3, respectively. In addition, toxicity data on acephate's relevant degradate methamidophos, are discussed briefly and cross-referenced to the methamidophos effects determination document for the CRLF (USEPA, 2007).

4.1 Evaluation of Aquatic and Terrestrial Ecotoxicity Studies

Toxicity measurement endpoints are selected from data from guideline studies submitted by the registrant, and from open literature studies that meet the criteria for inclusion into the ECOTOX database maintained by EPA/Office of Research and Development (ORD) (U.S. EPA, 2004). Open literature data presented in this assessment were obtained from a search of the ECOTOX database (12/29/2006). Table 4.1 summarizes the most sensitive results for each measurement endpoint, based on an evaluation of both the submitted studies and the open literature, as previously discussed. A brief summary of submitted and open literature data considered relevant to this ecological risk assessment is presented below. Additional information is provided in Appendix A.

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 further evaluated for use in the assessment 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, matching measurement endpoints listed in Tables 2.7 and 2.8, that are more conservative than the registrant-submitted data and that are found to be scientifically sound based on a review of the paper are used quantitatively. In addition, effects data for taxa that are directly relevant to the California Red-Legged Frog (i.e., aquatic-phase and terrestrial-phase amphibian data) were also considered over the use of surrogate taxa effects data, if available. The degree to which open literature data are used quantitatively or qualitatively is dependent on whether the information is scientifically sound and whether it is quantitatively linked to the assessment endpoints (e.g., maintenance of California Red-Legged Frog survival, reproduction, and growth) identified in Section 2.7 (Tables 2.7 and 2.8). For example, endpoints such as behavior modifications are likely to be qualitatively evaluated, because quantitative relationships between degree and type of behavior modifications and reduction in species survival, reproduction, and/or growth are usually not available.

Table 4.1. Acephate measurement endpoints and values selected for use in RQ calculations for the effects determination.

Assessment Endpoint [a]	Measures of Effect	Species	Toxicity Value	Study classification (Selection basis)	Reference
Survival and reproduction of individuals and communities of freshwater fish in close proximity to sites	Freshwater fish acute 96-hr LC_{50}	Rainbow trout	832 ppm ai	Supplemental (most sensitive)	MRID 40098001 (Mayer, 1986) as calculated using TOXANAL
	Freshwater fish early life-stage NOAEC	Rainbow trout	5.76 ppm ai	Extrapolated using most sensitive acute 96-h LC_{50} for Rainbow trout (832 ai) divided by 144 (highest rainbow trout ACR for organophosphates)	Section 4.1.1.2.
Survival and reproduction of individuals and communities of freshwater invertebrates in close proximity to sites	Freshwater invertebrate acute 96-h LC_{50} (for copepods 48-h LC_{50} or EC_{50} where the effect measured is surrogate)	*Daphnia magna*	acute 48-hr EC_{50} = 1.1 ppm ai	Supplemental (Most sensitive)	MRID 47116601 (McCann, 1978)
	Freshwater invertebrate reproductive NOAEC	*Daphnia magna*	0.15 ppm ai	Supplemental (Most sensitive)	MRID 44466601 (McCann, 1978)
Standing crop or biomass and growth of aquatic plants in close proximity to sites	Freshwater green algae, cyanobacteria or diatom 96-h IC_{50} for biomass.	Skeletonema costatum diatom	5-day EC_{50} >50 ppm ai NOEC = 5.0 ppm ai	Supplemental (Most sensitive)	MRID 40228401 (Mayer, 1986)
	Freshwater green algae, cyanobacteria or diatom 96-h NOAEC (or EC_{05}) for biomass				
Abundance (i.e., survival, reproduction, and growth) of individuals and populations of birds in close proximity to sites. (b)	Avian (single dose) acute oral LD_{50}	Dark eyed junco	106 mg ai/kg-bw	Supplemental (Most sensitive)	MRID 00093911 (Zinkl, 1981)
	Avian subacute 5-day dietary LC_{50}	Japanese quail	dietary sub-acute LC_{50} = 718 ppm ai	Supplemental (Most sensitive)	Smith, G.J., 1987.
	Avian reproduction NOAEL	Mallard duck	Reproductive study NOEL = 5 ppm ai [e]	Acceptable (Most sensitive)	MRID 00029691 (Beavers, 1979)

Assessment Endpoint [a]	Measures of Effect	Species	Toxicity Value	Study classification (Selection basis)	Reference
Abundance (i.e., survival, reproduction, and growth) of individuals and populations of mammals in close proximity to sites	Mammalian acute oral (single dose) LD_{50}	Meadow Vole	Acute oral weight adjusted LD_{50} = 180. 5 mg ai/kg bw	Acceptable (Most sensitive [c])	Rattner and Hoffman, 1984, see section 4.1.5.1 for details on tox value derivation
	Mammalian reproductive NOAEC or NOAEL	Rat	3- generation reproductive study NOAEL = 50 mg ai/kg bw-day diet [d]	Acceptable (Most sensitive)	MRID 40323401, 40605701
Survival of beneficial insect populations in close proximity to sites	Honey bee acute contact LD_{50}	Honey bee	acute contact LD_{50} = 1.20 ug ai/bee	Acceptable (Most sensitive)	MRID 00014714, 44038201 (Atkins et al, 1971)
Survival and growth of terrestrial plants in close proximity to sites	6a. Seedling emergence EC_{25}	Onion, ryegrass, corn, wheat, buckwheat, soybean, lettuce, flax, tomato, radish	>3.96 lb ai/A	Acceptable	Porch, J.R., *et al.*, 2003; MRID 46173203
	6b. Seedling emergence NOAEC		3.96 lb ai/A		
	6c. Vegetative vigor EC_{25}		>3.96 lb ai/A	Acceptable	Porch, J.R., *et al.*, 2003; MRID 46173204
	6d. Vegetative vigor NOAEC		3.96 lb ai/A		

[a] Assessment endpoints and measures of effect from Table 2.7 and 2.8.
[b] Note: Since, acute toxicity studies in birds demonstrated acute toxicity (LC_{50} or LD_{50}) values higher than the highest concentrations tested and resulted in no mortalities, acute risk quotients will not be derived for birds.
[c] As compared to other mammalian toxicity values when adjusted for body weight using allometric equations.
[d] Parental and pup weight, food consumption, litter size, mating performance and viability are all most sensitive measured effects.
[e] Based on reduced number viable embryos, live 3-week embryos.

Table 4.2. <u>Methamidophos</u> measurement endpoints and values selected for use in RQ calculations in this effects determination.

Assessment Endpoint [(a)]	Measures of Effect	Species	Toxicity Value	Study classification (Selection basis)	Reference
Survival and reproduction of individuals and communities of freshwater fish in close proximity to sites	Freshwater fish acute 96-hr LC_{50}	Rainbow trout	25 ppm ai	Supplemental (most sensitive)	MRID 00041312 (Nelson & Roney, 1979)
	Freshwater fish early life-stage NOAEC	Rainbow trout	0.1736 ppm ai	Extrapolated using most sensitive acute 96-h LC_{50} for Rainbow trout (25 ppm ai) divided by 144 (highest rainbow trout ACR for organophosphates)	Section 4.1.1.2.
Survival and reproduction of individuals and communities of freshwater invertebrates in close proximity to sites	Freshwater invertebrate acute 96-h LC_{50} (for copepods 48-h LC_{50} or EC_{50} where the effect measured is surrogate)	*Daphnia magna*	acute 48-hr EC_{50} = 0.026 ppm ai	Supplemental (Most sensitive)	MRID 00041311 (Nelson & Roney 1979)
	Freshwater invertebrate reproductive NOAEC	*Daphnia magna*	0.0045 ppm ai	Supplemental (Most sensitive)	MRID 46554501 (Kern et. al., 2005)
Standing crop or biomass and growth of aquatic plants in close proximity to sites	Freshwater green algae, cyanobacteria or diatom 96-h IC_{50} for biomass.	Skeletonema costatum diatom	5-day EC_{50} >50 ppm ai NOEC = 5.0 ppm ai	Supplemental (Most sensitive)	MRID 40228401 (Mayer, 1986)[1]
	Freshwater green algae, cyanobacteria or diatom 96-h NOAEC (or EC_{05}) for biomass				
Abundance (i.e., survival, reproduction, and growth) of individuals and populations of birds in close proximity to sites. (b)	Avian (single dose) acute oral LD_{50}	Common grackle	4.1 mg ai/kg-bw	Supplemental (Most sensitive)	MRID 00144428 (Lamb, 1972)
	Avian subacute 5-day dietary LC_{50}	Bobwhite quail	dietary sub-acute LC_{50} = 42 ppm ai	Supplemental (Most sensitive)	MRID 00093904 (Beavers & Fink,1979)
	Avian reproduction NOAEL	Mallard duck	Reproductive study NOEL = 3 ppm ai[3]	Acceptable (Most sensitive)	MRID 00014114 (Beavers & Fink, 1978)

Assessment Endpoint [(a)]	Measures of Effect	Species	Toxicity Value	Study classification (Selection basis)	Reference
Abundance (i.e., survival, reproduction, and growth) of individuals and populations of mammals in close proximity to sites	Mammalian acute oral (single dose) LD_{50}	mouse	Acute oral LD_{50} = 16.2 mg ai/kg bw	Acceptable (Most sensitive [(c)])	MRID 00014047 (1968)
	Mammalian reproductive NOAEC or NOAEL	Rat	3- generation reproductive study NOAEL = 0.5 mg/kg bw [5] (10 ppm)	Acceptable (Most sensitive)	MRID 00148455, 41234301 (1984)
Survival of beneficial insect populations in close proximity to sites	Honey bee acute contact LD_{50}	Honey bee	acute contact LD_{50} = 1.37 ug ai/bee	Acceptable (Most sensitive)	MRID 00036935 (Atkins et al, 1975)
Survival and growth of terrestrial plants in close proximity to sites	6a. Seedling emergence EC_{25}	Onion, ryegrass, corn, wheat, buckwheat, soybean, lettuce, flax, tomato, radish	>4.0 lb ai/A	Acceptable	MRID 46655802 Christ and Lam, 2005
	6b. Seedling emergence NOAEC		4.0 lb ai/A		
	6c. Vegetative vigor EC_{25}		>4.0 lb ai/A	Acceptable	MRID 46655802 Christ and Lam, 2005
	6d. Vegetative vigor NOAEC		4.0 lb ai/A		

[1] Most sensitive measure of effect in study that NOAEC is based on

[2] Most sensitive measure of effect in study that NOAEC is based on.

[3] Most sensitive measure of effect in study that NOAEC is based on.

[4] Since there are no aquatic plant studies for methamidophos, acephate RED was used to provide information on aquatic plant endpoint.

[5] Decrease in number of births, pup viability and body weight. There does not appear to be a palatability problem in the studies (personal communication Nancy McCarroll, HED, 2/10/98).

4.1.1 Toxicity to Freshwater Aquatic Animals

In the following sections relative acute toxicity of acephate and its major degradate, methamidophos, to aquatic animals is categorized using the scheme listed in Table 4.3.

Table 4.3. Categories of 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

4.1.1.1 Freshwater Fish: Acute Exposure (Mortality) Studies

Acute fish testing with acephate fulfilled data requirements (§72-1). There are no data from fish early life stage chronic testing (§72-4).

Parent acephate

Acephate technical grade active ingredient (TGAI) acute toxicity results exist for several cold water and warm water freshwater fish species, including Rainbow Trout, Bluegill Sunfish, Brook Trout, Atlantic Salmon, Cutthroat Trout, Yellow Perch, Channel Catfish, and Fathead Minnow. A complete list of all the acute freshwater fish toxicity data for acephate is provided in Appendix A. For twelve studies, the acute freshwater fish 96-h LC_{50} values for technical grade acephate range from >50 to >1,000 ppm ai (Appendix A) and of these twelve studies only one had definitive 96-h LC_{50} values (Rainbow trout values 96-h **LC_{50} = 832** ppm (Mayer, 1986)). Data from the Mayer study was reviewed and the LC50 was calculated. There is another more sensitive LC50 for trout from Mayer, but a definitive 96-h LC_{50} value above 100 ppm ai for a pesticide is not required unless exposure concentrations above 100 ppm are expected to occur under actual use conditions, which it does not for acephate labeled uses (Section 3.2.3). Based on this data, acephate is categorized at most as slightly toxic acutely to freshwater fish to practically non-toxic (Table 4.3). The most sensitive freshwater fish acute 96-h LC_{50} value of 832 ppm ai with rainbow trout (*Oncorhynchus mykiss*) (MRID 40098001, Mayer, 1986) was selected as the measurement endpoint for characterizing acute risks to freshwater vertebrate prey of the CRLF and for characterizing acute direct risks to the CRLF aquatic-phase (Table 4.1). No sublethal effects were reported as part of this study. This study is considered to be supplemental because of a lack of raw data to run a statistical analysis and only 5 fishes per concentration level was tested (no replicates).

Acephate formulation (75% wettable powder) acute toxicity test results were also available for several cold water and warm water freshwater species including Rainbow Trout, Bluegill Sunfish, Brook Trout, Largemouth Bass, Cutthroat Trout, Goldfish, Yellow Perch, Channel Catfish, Fathead Minnow, and Mosquito Fish (Appendix A). For these fourteen studies the 96-h LC_{50} values range from >100 to 6,000 ppm ai. Like the studies with acephate TGAI, most of these LC_{50} values were not definitive values. However, based on the limited data it does not appear that acephate as the 75% wettable powder formulation is more toxic than the TGAI.

Methamidophos, major degradate

There is only a single acute 96-h LC_{50} study with a freshwater fish and the major degradate, methamidophos TGAI, which was with a warm water carp (*Cyprinus carpio*) (Appendix A). The definitive 96-h LC_{50} value of 68 ppm methamidophos for the carp is more toxic than the definitive 96-h LC_{50} value observed for acephate freshwater fish (1,100 ppm ai). Acute test results of formulations with methamidophos are also more toxic than acephate TGAI or acephate formulations. Methamidophos 96-h LC_{50} values for formulations ranged from 12 to 38 ppm for two species, Rainbow Trout and Bluegill Sunfish, whereas for these same species the acephate TGAI results ranged from >50 to 1,100 ppm ai and for acephate formulations >150 to 2,050 ppm ai.

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

Parent Acephate

Similar to the acute data, chronic freshwater fish toxicity studies would be used to assess potential direct effects to the CRLF because direct chronic toxicity guideline data for frogs do not exist. Since there are no chronic data for freshwater fish, an acute to chronic ratio (ACR) was determined. Acephate is an organophosphate insecticide. The EFED toxicity database was accessed to derive an acute to chronic ratio of all organophosphate insecticides that have an acute LC50 and an early life stage fish study for rainbow trout. Rainbow trout is usually the most sensitive fish species among pesticides and is the most sensitive fish acute endpoint for acephate. Nineteen organophosphates were found that have both an acute and chronic study for rainbow trout. The ACR ranged from 5.4 for Terbufos to 144.0 for Dichlorvos. In order to provide the most conservative estimate for the chronic freshwater fish NOEC for acephate, the ACR of 144 will be used to estimate the NOEC for rainbow trout. The estimated chronic NOEC for rainbow trout as derived from and ACR of 144 and a LC50 of 832 ppm is **5.76 ppm or 5760 ppb.**

The following section presents the methodology used in deriving an avian ACR for organophosphates, the group to which acephate belongs, that was used to extrapolate a chronic fish NOAEC for acephate. The resulting early life stage for freshwater fish NOAEL was used as a surrogate for the aquatic-phase amphibian (U.S. EPA 2006). Of the organophosphates, 12 were evaluated for this extrapolation Table 4.4. The EFED toxicity database was accessed to derive an acute to chronic ratio of all organophosphate insecticides that have an acute LC50, an early life stage fish study for rainbow trout, and have been reviewed previously for scientific soundness. Rainbow trout is usually the most sensitive fish species among pesticides and is the most sensitive fish acute endpoint for acephate. A species and chemical specific ACR would ideally be determined which will then be used in the final organophosphate ACR derivation.

The estimated fish (aquatic phase amphibians) chronic NOAEC for acephate is derived as follows. The (acephate) rainbow trout LC_{50} used in this assessment is 832 ppm ai. The largest acute-to-chronic ratio from the organophosphates is 144 for Dichlorvos. This ratio is used to calculate the final NOEC for acephate.

750(acute)/5.2(chronic) = 144 = ACR ratio for Dichlorvos

$$\text{Estimated NOEC for acephate} = \frac{\text{LC50}}{\text{NOEC}} = \frac{\text{832 ppm}}{\text{est. NOEC}} = 144$$

Estimated Trout NOEC for acephate = 832/144 = **5.8 ppm ai**

The table below (4.4) shows the inputs for the organophosphates that were considered for the acephate ACR.

Methamidophos degradate

As with acephate, methamidophos does not have a chronic fish study. Therefore an ACR was also done to determine the estimated Trout NOEC for methamidophos.

$$\text{Estimated NOEC for methamidophos} = \frac{\text{LC50}}{\text{NOEC}} = \frac{\text{25 ppm}}{\text{est. NOEC}} = 144$$

Estimated Trout NOEC for methamidophos = 25/144 = **0.1736 ppm ai**

The table below shows the inputs for the organophosphates that were considered for the acephate ACR.

Acute to Chronic Table for Organophosphates

Table 4.4. Acephate Acute to Chronic Ratio for Rainbow Trout NOEC

Chemical	**96-hr LC_{50} (ppm ai)**	**MRIDs**	**NOAEC (ppm ai)**	**MRIDs**	**ACR**	**Acephate NOEC (PPM ai)**
Azinphos methyl	0.0088	03125193	0.00029	00145592	30.344	27.41
Coumaphos	0.890	40098001	0.0117	43066301	76.068	10.93
Dichlorvos	0.750	43284702	0.0052	43788001	144.23	5.76
Dimethoate	7.500	TN 1069*	0.430	43106303	17.441	47.70
Disulfoton	1.850	40098001	0.220	41935801	8.4090	98.94
Fenamiphos	0.068	40799701	0.0038	41064301	17.894	46.49
Fenitrothion	2.000	40098001	0.046	40891201	43.478	19.13
Fenthion	0.830	40214201	0.0075	40564102	110.66	7.518
Fonofos	0.050	00090820	0.0047	40375001	10.638	78.20
Isofenphos	1.800	00096659	0.153	00126777	11.764	70.72
Phosmet	0.105	40098001	0.0032	40938701	32.812	25.35
terbufos	0.0076	40098001	0.0014	41475801	5.4285	153.26

* TN 1069 is test number for EPA's Animal Biology Lab, McCann, 1977

4.1.1.3 Freshwater Fish: Sublethal Effects and Additional Open Literature Information

In addition to submitted studies, data were located in the open literature that report sublethal effect levels to freshwater fish that are less than the selected measures of effect summarized in Table 4.1.

Some sublethal effects to fish were found in open literature for acephate.

One study (Zinkl, 1987) found that the percentage of ChE inhibition that suggests poisoning by acephate or methamidophos is greater than 70% since brain ChE inhibition is at least this much in some trout that did not die. There is persistent ChE depression

(Brain ChE activity remains depressed 8 days after a 24-hour exposure to 25 mg/L of methamidophos and 15 days after exposure to 400 mg/L of acephate) which suggests sublethal effects such as inability to sustain physical activity in search of food, eluding predators, and maintaining position in flowing water would occur. However, additional studies are needed to conclude whether these sublethal effects do occur.

Several studies (Geen, 1981; Schoettger,1976, MRID 14861; Boscor, 1975, MRID 14637; Rabeni, 1979, MRID 14547) indicate no significant adverse effects on fish and benthic invertebrates from acephate applications.

The above field studies and laboratory data suggest that acephate and other organophosphate insecticides may not directly cause mortality to fish or benthic invertebrates. The amount of ChE inhibition that may cause mortality to fish (excess of 70% inhibition) suggests fish species may be somewhat resistant to adverse effects from acephate. Data are inconclusive as to whether there are behavior modifications to aquatic organisms from acephate or other organophosphate exposure.

4.1.2 Toxicity to Amphibians – Aquatic Phase

Amphibian toxicity data were used to assess potential direct and indirect effects to the CRLF. Direct effects to amphibians other than CRLF resulting from exposure to acephate may indirectly affect the CRLF via reduction in available food.

A summary of acute and chronic amphibian data, including published data in ECOTOX is provided below in Sections 4.1.3.1 through 4.1.3.3.

4.1.2.1 Amphibians: Acute Exposure Studies

The most sensitive study (MRIDs 00093943, 05019255, Lyons, 1976) found the Green Frog larvae/tadpole *(Rani clamitans)* 24 hr. **LC_{50} to be 6433 ppm (5857-6775).** This study is categorized as supplemental because of lack of raw data, dose-response data was not reported, ten tadpoles per treatment level was tested, with only one test vessel per treatment level (no replicates), and this study being a non-guideline study. Although the study was run for 96-hour period, only a 24-hour toxicity endpoint was derived because a linear dose-response pattern was not obtained. A behavior bioassay suggested that concentrations up to 500 ppm produced no observable differences between the treatment and control groups.

Another study of green frog larvae/tadpole was tested with acephate (MRID 4404290, Hall, 1980) up to 5 ppm for bio-concentration. Neither bio-accumulation nor toxicity was noted at 5 ppm concentration level.

A study (ECOTOX 11134, Geen, 1984) tested an amphibian, salamander, with acephate and found a 96-hour LC_{50} to be 8816 ppm. Exposure of egg masses to acephate concentrations of 798 ppm did not show any significant differences with the control to the time of hatching.

Acephate is classified as practically non-toxic to aquatic-phase amphibians on an acute basis.

Additional information can be found in Appendix A.

4.1.3 Toxicity to Freshwater Invertebrates

Freshwater aquatic invertebrate toxicity data were used to assess potential indirect effects of acephate to the CRLF. Direct effects to freshwater invertebrates resulting from exposure to acephate may indirectly affect the CRLF via reduction in available food. As discussed in Section A.5.1 of Attachment A, CRLF aquatic-phase is presumed to be algae grazers but there is some uncertainty in that assumption. Therefore, aquatic invertebrates are also assumed to be a food source for CRLF aquatic-phase.

A summary of acute and chronic freshwater invertebrate data, including published data in ECOTOX is provided below in Sections 4.1.3.1 through 4.1.3.3.

4.1.3.1 Freshwater Invertebrates: Acute Exposure Studies

The most sensitive acceptable study (MRID 47116601, McCann, 1978) found the *Daphnia magna* **LC_{50} to be 1.11 ppm ai (0.65-1.88).** The probit slope is 1.62. The range of LC_{50} toxicity for freshwater invertebrates is 1.11 to >1,000 ppm. One other *Daphnia magna* was tested and the LC_{50} is 71.8 ppm.

A complete list of all the acute freshwater invertebrate toxicity data for acephate is provided in Appendix A.

Acephate classification ranges from moderately toxic to practically non-toxic to freshwater invertebrates on an acute basis.

4.1.3.2. Freshwater Invertebrates: Chronic Exposure Studies

A submitted freshwater invertebrate life-cycle study (MRID 44466601, McCann, 1978) using *Daphnia magna* was reviewed. The control had 35% mortality of the adults and the treatments range from 10% to 35% mortality for adults with the highest concentration level having 10% mortality. Since this is a 21 day static test, it is assumed that the mortalities come from handling the organisms. There is a dose response trend of offspring per adult per day. With the dose response trend and because methamidophos daphnia life study has a more sensitive NOEC of 4.5 ppb, it was decided to make this study supplemental and not invalid since there is some useful information in this study.

The **NOEC is found to be 150 µg ai/L (0.150 ppm)** for 21-day test. The NOEC is based on reduction in numbers of young at 375 ppb and higher.

4.1.3.3. Freshwater Invertebrates: Open Literature Data

In addition to submitted studies, data were located in the open literature that report sublethal effect levels to freshwater invertebrates; however, they are less sensitive than the selected measures of effect summarized in Table 4.1 and 4.2.

4.1.4. Toxicity to Birds

As previously discussed, no guideline tests exist for frogs; therefore, birds are used as surrogate species for amphibians including frogs (U.S. EPA, 2004). The available open literature has no information on acephate toxicity to terrestrial-phase amphibians. Avian toxicity from open literature shows that acute and chronic ecotoxicity endpoints are generally less sensitive than the registrant submitted avian studies. A summary of acute and chronic avian data, including sublethal effects, is provided below.

4.1.4.1 Birds: Acute Exposure (Mortality) Studies

Avian acute toxicity studies were used to assess potential direct effects to the CRLF because direct acute toxicity guideline data on frogs are unavailable. Acephate toxicity has been evaluated in some avian species, including mallard duck, bobwhite quail, dark-eyed junco, common grackle, starling, redwing blackbird, and Japanese quail and the results of these studies demonstrate a moderate range of sensitivity. The range of acute oral LD_{50} values for acephate ranges from 106 mg a.i./kg-bw to 350 mg a.i./kg bw. The range of subacute dietary LC_{50} is from 718 ppm to >5000ppm; therefore, acephate is categorized as moderately toxic to avian species on an acute oral basis to birds and as practically non-toxic to moderately toxic to avian species on a subacute dietary basis.

<u>Acute Oral LD_{50} and Avian sub acute dietary endpoint analysis</u>

The most sensitive acute oral LD_{50} value is 106 mg/kg-bw for the dark eyed junco (MRID 00093911, Zinkl, 1981). However, there are uncertainties in using this value for risk assessment for the California Red-Legged Frog (CRLF). There were 5 dose groups with a geometric progression of 1.4X (EPA recommends 2X progression between doses). Only 4 birds were tested in each dose group (EPA recommends 10 birds per dose group). The 106 mg/kg-bw dose group had 2 birds out of 4 that died. No confidence interval and no probit slope were calculated. This study compared the LD_{50} value of birds fed larvae laced with acephate with birds that were given acephate by gavage. The birds initially refused to ingest larvae that contained 16 µg acephate/larvae; however, the birds were willing to consume larvae containing five µg acephate. The study found that acephate given by gavage without larvae produced more inhibition than the larvae-fed birds. The study also concludes that the higher the dose, the more ChE inhibition is found in the birds. Increased time of exposure may prolong the time for recovery from ChE inhibition. Feeding the birds larvae containing acephate may decrease the activity of the acephate when compared to the gavage. The birds fed for five days recovered in 12 to 22 days.

The next lowest LD_{50} value is 109 mg/kg-bw (86-139 mg ai/kg-bw)for bobwhite quail (MRID 43939301, Campbell, 1992). This study was conducted with a granular formulation (15% ai). The probit slope is 5.4. The formulation LD_{50} = 734 mg/kg (86-139 mg/kg formulation). This study followed EPA guidelines and is an acceptable study for the formulation. The LD_{50} value (109 mg/kg-bw) from this bobwhite study will be used in the T-REX model for assessing effects to CRLF (terrestrial-phase).

There are a total of six acute oral studies with acephate using four different species, with the range of LD_{50} being from 106 mg/kg-bw to 350 mg/kg-bw. Additional information may be found in Appendix A.

4.1.4.2. Birds: Chronic Exposure (Reproduction) Studies

Avian reproduction studies indicate that when parents are fed between 5 and 20 ppm technical grade acephate, the survival of embryos and chicks are adversely affected.

Effects seen in a study on northern bobwhite quail at 80 ppm include reduced body weight, number of eggs laid, eggs set, viable embryos, number of embryos alive at 3 weeks, number of normal hatchlings, and 14-day old survivors. The NOEL is 20 ppm for the bobwhite quail (MRID 00029692, Beavers, 1979).

Effects seen in a study on mallard ducks at 20 ppm include a reduced number of viable embryos and live 3 week embryos. The NOEL for the mallard is 5 ppm (MRID 00029691, Beavers, 1979).

4.1.4.3. Avians: Sublethal Effects and Additional Open Literature Information

Vyas (ECOTOX 40313) reported that acephate (representing all organophosphates) affected adult migratory white-throated sparrows (*Zonotrichia albicollis*). Adult birds exposed to 256 ppm acephate a.i. were not able to establish a preferred migratory orientation and exhibited random activity. All juvenile treatment groups displayed a seasonally correct southward migratory orientation. The author hypothesized that acephate may have produced aberrant migratory behavior by affecting the memory of the adult's migratory route and wintering ground. The "experiment reveals that an environmentally relevant concentration" (similar to 0.5 lb ai/A application) of an OP such as acephate "can alter migratory orientation, but its effect is markedly different between adult and juvenile sparrows. Results suggest that the survival of free-flying adult passerine migrants may be compromised following organophosphorus pesticide exposure." Although birds are surrogate species for frogs, it is uncertain as to whether this aberrant behavior in birds can translate over to another aberrant behavior with frogs in the absence of additional data.

ECOTOX 40343, Vyas, 1996. The effects of a 14-day dietary exposure of acephate on cholinesterase activity in three regions; basal ganglia, hippocampus, and hypothamulus were examined in the brain of the white-throated sparrow, *Zonotrichia albicollis*. All three regions experienced depressed cholinesterase activity between 0.5-2 ppm ai acephate. The regions exhibited cholinesterase recovery at 2-16 ppm ai acephate; however, cholinerase activity dropped and showed no recovery at higher dietary levels (> 16 ppm acephate) which suggests that each region maintains its own ChE activity level integrity until the brain is saturated so that the differences of the regions is nil. Each region of the brain is responsible for different survival areas such as a foraging and escaping predators, memory and spatial orientation, food and water intake, reproduction and several others. Evidence indicated that the recovery is initiated by the magnitude of depression, not the duration. In general, as acephate concentration increased, depression in ChE activity among brain regions increased and differences of ChE activity among the three brain regions decreased. The pattern of ChE depression in different regions of the brain following low level exposure may prove to be a critical factor in the survival of the bird. The authors hypothesized that adverse effects to birds in the field may occur at pesticide exposure levels customarily considered negligible.

Zinkl, 1978. Several large acreages of forest were sprayed with acephate at 0.5, 1.0 or 2.0 lb. ai/A application rates. There was no brain ChE inhibition on day zero after application. Birds collected from the 2 lb ai/A plots from day one thru six post spray showed ChE inhibition. Brain ChE inhibition was shown in birds 33 days after treatment but not 89 days after treatment. Birds seemed to have more inhibition of ChE in summer application when compared to the fall application in the 1 lb. ai/A plots (30-50% and 25-40% depression, respectively). The greatest ChE inhibition occurred in dark-eyed juncos (65%) collected 15 days after treatment. In the 2 lb. ai/A plots, dark-eyed juncos and golden-crowned kinglets had 54% ChE inhibition. Of the 14 species collected, only pine siskins *(Siinus pinus)* did not show any ChE inhibition. Symptoms of organophosate poisoning were observed such as a warbling vireo salivating profusely, an American robin having difficulty maintaining a perching position, and a mountain chickadee having visible tremors. All of these observations were made in the 1 lb. ai/A plots. The authors concluded that since marked ChE inhibition did not occur on day zero, but was evident up to 33 days after application, there was either an accumulative effect that was detected later or acephate was converted to a more potent ChE inhibitor such as methamidophos. Spraying the forest with 0.5, 1.0 or 2.0 lb. ai/A caused marked and widespread, and prolonged ChE depression in passerine birds.

ECOTOX 39518, MRID 40329701. Zinkl, 1980, Zinkl, 1979. Acephate was sprayed in a forest at 0.5 lb ai/A. Eleven species of birds had ChE inhibition that ranged on average from 20 to 40%. The maximum depression of ChE found in chipping sparrows was 57% at day six. Western tanager species was found to have significant inhibition up to 26 days after application. Brain residue analysis of a western tanager collected on day three contained 0.318 ppm of acephate and 0.055 ppm of methamidophos.

MRID 05014922 and 00163173. (Bart, 1979). Acephate was applied in this study on June 13 at 0.55 kg/ha (0.5 lb ai/A) on two 200 hectare plots. Authors measured the presence of the red-eyed vireos by the number of their particular songs. Significant ($P<0.05$) decline in number of red-eyed vireos was observed. The decline was concentrated in the interior of the treated plots rather than spread throughout. This would conclude that acephate affected the decline of the number of red-eyed vireos. No birds were tested and it is not known whether the declines in vireos were due to direct effects or indirect effects such as killing off the food items.

MRID 00141694. (Rudolph, 1984) Kestrels were dosed with 50 mg/kg of 75% acephate formulation. Serum ChE was 37% inhibited and returned to predosed levels eight days later. Then the birds were dosed again and the serum ChE activity was inhibited at 42%; brain ChE was at 26% inhibition. The kestrel prey-catching activity was not altered from the acephate at 50 mg/kg-bw dose level.

MRID 40644802. (Richmond, 1979) Site: Wallowa-Whitman National Forest. Applications of 1.12 (1.0 lb ai/A) and 2.24 (2.0 lb ai/A) kg/ha were made on forest plots in Oregon. Extensive inhibition of brain ChE activity (commonly at 30-50%) for up to 33 days for 11 of the 12 species of birds that were collected was observed. The highest frequency of ChE inhibition was observed on day two post spray. Two species of birds had observable population decreases. Some birds on the plots treated with 1.12 kg/ha had 65% ChE inhibition which is thought to be fatal. At both plots, birds were found with coordination problems, salivating profusely, and inability to fly. These behaviors were observed up to 20 days after application in the 2.24 kg/ha plot. It was also observed that breeding pairs for the warbling vireo and yellow-rumpled warbler were decreased. The authors hypothesized that application of acephate at rates of 1.12 and 2.24 kg/ha can cause sickness and death to forest birds.

MRID 00093909. (McEwen, 1981) Site:WY, UT and AZ rangeland. In 1979 and 1980, the birds and small mammals collected up to 24 days after application had reduced ChE activity. Reduction of 20% or more is indicative of exposure to brain ChE inhibitor. Of the birds collected in AZ, 24.5% had reduced ChE activity >20%. The birds with the most ChE inhibition were the last ones collected (21-24 days post treatment). In 1981, horned larks and lark buntings were collected in WY on a 12,000 acre plot that was treated with acephate at the rate of 0.105 kg/ha. More than 20% ChE inhibition was found in 19% of the horned larks and 25% of the lark buntings. Deer mice were also collected in WY. They were found to have ChE inhibition that ranged from 12.7% to 14.6%.

4.1.5. Toxicity to Mammals

Rat or mouse toxicity values are obtained from the Agency's Health Effects Division (HED) as substitute for wild mammal testing. Toxicity data on small mammals are used in this assessment to assess the effect of acephate exposure on their availability as food items for the CRLF. While the relative percent composition of mammal in the frog’s diet is uncertain, gut content studies have found the CRLF gut to contain 50% mammal content (USFWS, 2002). It is necessary to consider the affect of acephate on this potentially significant food source, as population level effects to mammals may result in indirect effects to the CRLF. Additional information can be found in Appendix A.

4.1.5.1. Mammals: Acute Exposure (Mortality) Studies

Mammalian toxicity studies reviewed by the Agency indicate that acephate is characterized as moderately toxic to small mammals on an acute oral basis (LD_{50}=866 mg/kg bw, rat, MRID 00014675; LD_{50}=321 mg/kg bw, meadow vole, Rattner and Hoffman, 1984). However, for the degradate, toxicity studies indicate that methamidophos is highly toxic to small mammals on an acute oral and dermal basis (MRID 00014044, 00014047, 00014048).

The meadow vole appears (by numbers alone) to be more sensitive than the rat. In order to provide the vole toxicity input into the T-REX exposure model, the LD_{50} value needs to be adjusted. Below are the steps to compute the adjusted value.

In order for the terrestrial exposure and the LD_{50} toxicity value for mammals to be imputed into the T-REX model for determination of RQ to mammals, the LD_{50} value of the meadow vole must be converted to an adjusted LD_{50}. The dose-based LD_{50} (mg/kg-bw) or NOAEL (mg/kg-bw) values from acceptable or supplemental toxicity studies are adjusted for the size of the animal tested compared with the size of the animal being assessed (e.g., 350-gram rat) are relative to the animal's body weight (mg residue/kg bw) because consumption of the same mass of pesticide residue results in a higher body burden in smaller animals compared with larger animals. Adjusted mammalian LD_{50}s (mg/kg-bw) are used to calculate dose-based acute risk quotients for 15-, 35-, and 1000-gram mammals. The following equations are used for the adjustment (U.S. EPA 1993):

Adjusted mammalian LD_{50} where:

$$Adj.\,NOAEL\ or\ LD_{50} = NOAEL\ or\ LD_{50}\left(\frac{TW}{AW}\right)^{(0.25)}$$

Adj. LD_{50} = adjusted NOAEL or LD_{50} (mg/kg-bw)
LD_{50} = endpoint reported from mammal study (mg/kg-bw)
TW = body weight of tested animal (35g vole)
AW = body weight of assessed animal (350 g rat)

Thus, the meadow vole LD_{50} of 321 mg/kg-bw x $(35g/350)^{0.25}$ = **180.5 mg/kg-bw** = adjusted LD_{50} for meadow vole input for T-Rex exposure model.

The meadow vole adjusted LD_{50} is more sensitive than the rat LD_{50} (866 mg/kg-bw). Therefore the meadow vole LD_{50} will be the value use in the T-REX terrestrial exposure model.

4.1.5.2. Mammals: Chronic Exposure (Reproduction) Studies

Laboratory data indicate that acephate and its degradate, methamidophos, may pose chronic risk to mammals by affecting the reproductive capacity of mammals. Acephate fed to female rats at 500 ppm were found to have significant adverse effects when compared to controls of parental and pup body weight, food consumption, litter size, and mating performance and viability. The NOEL for the rat reproductive study is 50 ppm (MRID 40323401, MRID 40605701).

4.1.5.3 Mammals: Sublethal Effects and Additional Open Literature Information

ECOTOX 39518, MRID 40329701. (Zinkl, 1980,). There is a marked inhibition of brain ChE activity in squirrels after aerial treatment of forests at rates of 0.57 kg/ha (0.51 lb/A) of Orthene but no mortality noted.

ECOTOX 35459, Stehn, 1976. Increased ingestion of arthropods by insectivorous mammals has been reported following acephate application. This signifies a direct pathway for substantial exposure to acephate due to consumption of dead and dying insects.

4.1.6 Toxicity to Insects

Toxicity data on insects are used in this assessment to assess the effect of acephate exposure on their availability as food items for the CRLF. Insect toxicity from open literature shows that acute ecotoxicity endpoints are generally less sensitive than the registrant submitted bee studies. Additional information can be found in Appendix A.

4.1.6.1. Acute toxicity to bees

Analysis of the results of honey bee acute contact studies indicate that acephate is highly toxic to bees and beneficial insects on an acute contact basis (MRID 00014714, MRID 44038201)**.** The study indicated an **LD_{50} of 1.2 ug/honey bee**. Further studies indicated that acephate is highly toxic to bees from two hours to 96 hours after foliar application at rates of 1 lb/A and from 2 hours to 24 hours at 0.5 lb ai/A rate (Appendix A)**.**

EPA also reviewed a study (MRID 05004012) that tried to determine a toxicity ratio of acephate. By comparing the sensitivity of beneficial predator insects to that of the pest tobacco budworm, one would be able to determine the selectivity of toxicity to the beneficial or pest insect. The ratio is calculated using the LC_{50} values for the pest divided by the LC_{50} values for the beneficial insect; a ratio greater than 1 represents that acephate is more toxic to the predator than to the pest. Green lacewing had a calculated ratio of 6.4 and the ratio for the parasitic wasp was 10.0. Acephate is more toxic to these two beneficial predators than it is to the pest.

An acute contact toxicity study for methamidophos, a degradate of acephate, on bees indicates that methamidophos is highly toxic to bees on an acute contact basis (MRID 00036935). The LD_{50} was 1.37 ug/bee.

4.1.6.2 Insects: Sublethal Effects and Additional Open Literature Information

ECOTOX 35475. Stoner,1985. All bee colonies that were fed 10 ppm acephate lost queens early in the study and the affected colonies were unable to rear new queens. Acephate appears to be systemic in nurse bees, causing glandular secretions fed to queens to be toxic. The study implied infrequent encounters by honey bee foragers with acephate on crops at levels of 1 ppm (1 ppm is NOAEC level) or less should be harmless. However, foragers may be expected to encounter levels greater than 1 ppm in the field because of 6-9 day residue persistence and residual systemic activity of acephate in plants for up to 15 days. Acephate fed to worker bees via sugar syrup showed up in the royal jelly for the queen, indicating that acephate is systemic to bees. Although these concentrations of 1 ppm or less were harmless to the worker bees, levels at 0.1 ppm showed significant reduction of the surviving brood. Consequently, the study concluded that acephate is a hazard to honey bees because of its high contact toxicity, and because of its systemic nature.

MRID 00099762. (Johansen, 1977). Orthene was found to be more detrimental to honey bee populations than carbaryl. Brood cycles of some colonies were found to be permanently broken, and all of the bees were dead within 45-48 days after exposure. Depression in the numbers of wild foraging bees at all treated plots was apparent. Measured seed and fruit production of northern bluebells (*Mertensia paniculata*) were significantly reduced from lack of pollination due to acephate when compared to control.

MRID 00099763. (Johansen, 1977). Severe impacts on yellow jacket wasps and ants at rates of application of 1 and 2 lb ai/A sprayed on forest. Temperature seems to affect the exposure of wasps in that cooler temperature (39^{o}F) causes wasps not to forage out of nests and therefore not be exposed as much, whereas warmer temperatures (59^{o}F) increases the activity of wasps and the exposure to acephate.

4.1.7. Summary of Effects Assessment

Of the numerous studies evaluated, the lowest, and therefore most sensitive, toxicity endpoint was chosen to assess the possibility of direct or indirect effect to the frog, as determined by calculation of the RQ.

4.1.7.1 Direct Effects

The acute mortality endpoint for the rainbow trout, with a 96-hr LC_{50} = 110ppm ai, is the most sensitive value of the available data, and will be used as a surrogate (measurement endpoint) for calculating direct effects (risk) to the aquatic-phase frog. Because there were no data available for chronic toxicity associated with long term (relative to life-cycle) exposure, an acute to chronic ratio was calculated using the relationship between the acute and chronic toxicity of other organophosphate pesticides, as described above. The chronic exposure endpoint for the aquatic phase of the frog life-cycle is estimated to be 0.150 ppm for the rainbow trout.

There are three avian toxicity tests used to calculate the risk to the terrestrial phase of the CRLF. For acute exposure, the mortality endpoint for dark eyed junco, LD_{50}=106 mg/kg-bw was used. Dietary effects were calculated based on data from tests with the Japanese quail, LC_{50}=718 ppm. Finally, risks due to chronic exposure were calculated using the exposure level where no effects to embryo mortality were observed for chronic exposure of the mallard duck to acephate, NOAEL= 5 ppm.

4.1.7.2. Indirect Effects

Based on the life history of the frog, impacts to CRLF prey or habitat could indirectly affect the CRLF. To assess the risk of indirect effects from prey, the toxicity endpoints for small mammals, birds (other frogs as food item), terrestrial arthropods, and aquatic invertebrates were used.

The most sensitive acute mammal endpoint is mortality of the rat upon acute exposure to acephate, LD_{50}=866 mg/kg-bw. The most sensitive mammalian reproduction endpoint is adverse affects to parental and pup weight, food consumption, litter size, mating performance and viability upon chronic exposure of a rat to acephate, NOAEL=50 mg/kg-bw.

The most sensitive acute endpoint is mortality of the dark eyed junco, LD_{50}=106 mg/kg-bw was used. Dietary effects were calculated based on data from tests with the Japanese quail, LC_{50}=718 ppm. Finally, risks due to chronic exposure were calculated using the exposure level where no effects to embryo mortality were observed for chronic exposure of the mallard duck to acephate, NOAEL= 5 ppm.

The most sensitive acute aquatic invertebrate endpoint is the daphnid 48-hr EC_{50}=1.1 ppm a.i. and the chronic endpoint used to estimate risk to aquatic prey items is the daphnid NOAEC= 0.015 ppm.

Acephate is highly toxic to bees up to 96 hours after foliar application. Comparative toxicity between beneficial and non-beneficial insects shows that Acephate may be more harmful to beneficial insects than insect pests.

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

The Agency uses the probit dose response relationship as a tool for providing additional information on the potential for acute direct effects to individual listed species and aquatic animals that may indirectly affect the listed species of concern (U.S. EPA, 2004). As part of the risk characterization, an interpretation of acute RQ for listed species is discussed. This interpretation is presented in terms of the chance of an individual event (i.e., mortality or immobilization) should exposure at the EEC actually occur for a species with sensitivity to acephate 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 (i.e., freshwater fish used as a surrogate for aquatic-phase amphibians and freshwater invertebrates). 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. The upper and lower bounds of the effects probability are based on available information on the 95% confidence interval of the slope. A statement regarding the confidence in the estimated event probabilities is also included. Studies with good probit fit characteristics (i.e., statistically appropriate for the data set) are associated with a high degree of confidence. Conversely, a low degree of confidence is associated with data from studies that do not statistically support a probit dose response relationship. In addition, confidence in the data set may be reduced by high variance in the slope (i.e., large 95% confidence intervals), despite good probit fit characteristics.

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

4.1.7.4. Review of Ecological Incident Information System (EIIS)

In general, although there are many reported incidents of toxic effects to non-target plants and animals from acephate, the majority of these reports are not clearly documented or else acephate was applied in combination with other pesticides and it is not possible to determine which pesticide primarily caused the undesirable effect. A more detailed account of these reports can be found in Appendix C. The majority of acephate specific incidents reported were associated with bee kills. Some reports were also associated with bird and fish kills, and damage to plants, but the exact causes of the reported incidents are uncertain.

The EIIS database show the following reported incidents that are associated with acephate use. More details of the incidents can be found in Appendix C. The two avian incidents have a probable certainty incident. The plant incidents are localized residential uses with a mixture of other active ingredients and in an aerosol container. Fish incidents are not reported below but can be found in Appendix C. In each of the incidents, the fish kills resulted from a mixture of other active ingredients that are known to be more toxic to fish than acephate.

Avians –

1998 in SC – 24 dead boat-tailed grackles collected and methamidophos residues found within them attributed to acephate use on fire ants

2005 in GA - 50 boat-tailed grackles were found dead. Acephate residues found within some of them. Acephate was used to control fire ants.

Plant Injury –

There are no data or information available to ascertain the extent of the damage or the type of damage for the plants. Thus it is not known whether acephate caused the damage. There are some alleged mortalities for plants from the use of a rose and spray mixture. It is not certain as to whether the mixture caused the mortalities since the data reported to EPA is very sparse.

1994 in PA - Orthenex Rose and Flower Spray (aerosol) is alleged to have cause damage to ornamentals and/or flowers.

1998 in FL - There was an allegation of plant damage from the use of Ortho Systemic Rose and Floral Spray on ornamentals.

1998 in PA - There was an allegation of plant damage from the use of Ortho Orthenex™ Insect and Disease Control Formula III on ornamentals.

1999 in DC - There was an allegation of plant damage from the use of Isotox Insect Killer Formula IV. Product was sprayed on a dwarf Alberta pine with the results that the pine is dying.

1999 in IN - There was an allegation of plant damage from the use of Ortho Orthenex™ Insect and Disease Control Formula III on ornamentals. The report indicated that the flowering almond and hibiscus were dying.

1999 in TX - There was an allegation of plant damage from the use of Ortho Orthenex™ Insect and Disease Control Formula III on ornamentals. There was an allegation of plant damage from the use of Ortho Orthenex™ Insect and Disease Control Formula III on ornamentals. The report indicated that the homeowner applied this product on 40 – 50 bushes used as hedge per recommendation of county extension agent. About 95% of the bushes died.

1999 in GA - There was an allegation of plant damage from the use of Ant-Stop Orthene™ Fire Ant Kill. Product was applied on spots of the lawn resulting in “burnt spots”.

Bee Kills

Washington State reported 7 incidents of bee kills from 1992 to 2002. Most of the incidents indicate that 40 to 60 colonies were killed off per incident.

5. Risk Characterization

Risk characterization is the integration of the exposure and effects characterizations to determine the potential ecological risk from varying acephate use scenarios within the action area and likelihood of direct and indirect effects on the California Red Legged frog. The risk characterization provides estimation and description of the likelihood of adverse effects; articulates risk assessment assumptions, limitations, and uncertainties; and synthesizes an overall conclusion regarding the effects determination (i.e., “no effect,” “likely to adversely affect,” or “may affect, but not likely to adversely affect”) for the California Red Legged frog.

5.1 Risk Estimation

Risk was estimated by calculating the ratio of estimated environmental concentrations (EECs; see Tables 3.3 through 3.8) and the appropriate toxicity endpoint (see Tables 2.7, 4.1, 4.2). 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 (Table 5.1.). Appendix E describes the categories of toxicity.

Table 5.1. Levels of Concern for Terrestrial and Aquatic Organisms

Taxa	Acute LOC	Chronic LOC
Avian[1] (terrestrial phase amphibians)	0.1	1
Mammalian[2]	0.1	1
Terrestrial plants[3]	1	
Aquatic Animals[4] (aquatic phase amphibians)	0.05	1
Insects [5]	0.05	1

Used in RQ calculations:
[1] LD_{50} and estimated NOEL
[2] LD_{50} and NOEC
[3] EC25
[4] LC/EC_{50} and estimated and reproductive NOEC
[5] LD_{50} per EFED’s CRLF Steering Committee

Aquatic screening level RQs are based on the most sensitive endpoints and modeled surface water concentrations from the following scenarios of acephate: citrus, cotton, lettuce, row crops (beans, celery, peppers), turf (landscape maintenance), almond (surrogate for pistachio), fruit trees, and cole crops (broccoli, cauliflower, Brussels sprouts).

5.1.1 Aquatic Direct and Indirect Effects

5.1.1.2 Direct Effects

There is no direct acute risk to CRLF aquatic-phase from the use of acephate. Tier 1 EECs (Table 3.3) were below the LOC. The very low RQs (Tables 5.2, 5.3) for direct effects to fish (surrogate for CRLF) indicate that direct effects are not expected even for applications much higher than the maximum labeled rate. Therefore, a Tier 2 analysis was not conducted for fish.

5.1.1.3 Indirect Effects

Indirect risk to aquatic phase CRLF is driven by acephate use on cotton and almond with an acute RQ of 0.13 for prey items (aquatic invertebrates, Table 5.5).

The only acute RQs above the LOC in the aquatic phase were for invertebrates exposed to methamidophos as a degradate of acephate (estimated as 18% of the modeled acephate EEC). This suggests that the CRLF may be at indirect risk in the aquatic phase due to reduction in prey base (invertebrates). Aquatic plant RQ were also below LOC, therefore no indirect effects mediated via aquatic plants are expected, either as they affect food supply or habitat.

Chronic effects to all aquatic taxa were below LOC for both parent acephate and its degradate methamidophos.

Table 5.2. Tier 1 Acute Risk Quotients for Acephate

Use	EEC	**RQs for Direct and Indirect Effects,** *Rainbow trout, LC_{50} = 832,000 ppb*	**RQs Indirect Effects,** *Prey Item: Daphnia magna, EC_{50} = 1,110 ppb*	**RQs Direct Effects**, *Green frog LD_{50} = 6,433,000 ppb*
Cotton, Aerial, 6 applications of 1 lb/acre spaced at 3 days	Peak, 85.4 ppb	< 0.05	**0.08 ***	< 0.05

* Exceeds LOC of 0.05

Table 5.3. Tier 1 Chronic Risk Quotients for Acephate

Use	Chronic RQ		
Cotton, Aerial, 6 applications of 1 lb/acre spaced at 3 days	EEC	**RQs for Direct and Indirect Effects,** *Rainbow trout, NOAEC = 5,800 ppb*	**RQs Indirect Effects,** *Prey Item: Daphnia magna, NOAEC = 150 ppb*
	21-day, 43.6 ppb 60-day, 18.4 ppb	< 0.05	**2.9 ***

* Exceeds LOC of 1.0

Table 5.4. Risk Quotients: Acute Aquatic Acephate Exposure (Tier 2)

Crop/Use	**Application Method (**Air/ Ground**)**	**Acephate Peak EEC (ppb)**	**RQs for Direct and Indirect Effects,** *Rainbow trout, LC_{50} = 832,000 ppb*	**RQs Indirect Effects,** *Prey Item: Daphnia magna, EC_{50} = 1,110 ppb*	**RQs Direct Effects**, *Green frog LD_{50} = 6,433,000 ppb*
Citrus	G	0.55	< 0.05	< 0.05	< 0.05
Cotton	A	19.2			
	G	13.4			
Lettuce	A	16.7			
	G	15.0			
Row Crop (beans, celery, peppers)	A	9.7			
	G	5.9			
Turf (Bermudagrass for seed)	A	4.5			
	G	2.5			
Almond (pistachio)	A	18.1			
	G	12.9			
Fruit trees	A	14.0			
	G	7.3			
Cole Crops	A	13.4			
	G	11.3			

Table 5.5. Risk Quotients: Acute Aquatic Methamidophos (Acephate degradate) Exposure (Tier 2)

Crop/Use	**Application Method (**Air/ Ground**)**	**Acephate Peak EEC (ppb)**	**Methamidophas Adjusted Peak EEC (ppb)**	**RQ for Indirect Effects,** *Prey Item: Daphnia magna(EC_{50} = 26 ppb)*	**RQ for Direct and Indirect Effects**, *Aquatic Life Phase: Rainbow trout (LC_{50} = 25,000 ppb)*
Citrus	G	0.55	0.099	< 0.05	< 0.05
Cotton	A	19.2	3.46	**0.13**	
	G	13.4	2.41	**0.09**	
Lettuce	A	16.7	3.00	**0.12**	
	G	15.0	2.70	**0.10**	
Row Crop (beans, celery, peppers)	A	9.7	1.75	**0.067**	
	G	5.9	1.06	< 0.05	
Turf (Bermudagrass for seed)	A	4.5	0.81	< 0.05	
	G	2.5	0.45	< 0.05	
Almond (pistachio)	A	18.1	3.26	**0.13**	
	G	12.9	2.32	**0.089**	
Fruit trees	A	14.0	2.52	**0.097**	
	G	7.3	1.31	**0.0504**	
Cole crops	A	13.4	2.41	**0.093**	
	G	11.3	2.03	**0.078**	

EEC=Expected Environmental Concentration; ppb=Parts per billion; Risk Quotients (**RQ**) in bold type exceed LOC

Table 5.6. Risk Quotients: Chronic Aquatic Exposure to Acephate and Methamidophos (Tier 2)

Crop/Use	**Application Method (**Air/ Ground**)**	**Acephate EEC (ppb)**		**Methamidophos EEC (ppb)**		**RQs for Direct Effects** Rainbow Trout		**RQs for Indirect Effects** Daphnia	
		21-day	**60-day**	**21-day**	**60-day**	**Acephate** *NOAEC =5,800 (ppb)*	**Meth.** *NOAEC =0.1736 (ppb)*	**Acephate** *NOAEC =150 (ppb)*	**Meth.** *NOAEC =4.5 (ppb)*
Citrus	G	0.33	0.20	0.06	0.04	< 1	< 1	< 1	< 1
Cotton	A	12.8	6.7	2.30	1.21				
	G	7.5	3.5	1.35	0.63				
Lettuce	A	10.7	5.0	1.93	0.9				
	G	9.0	4.1	1.62	0.74				
Row Crop	A	5.3	2.4	0.95	0.43				
	G	3.3	1.5	0.59	0.27				
Turf	A	2.7	1.3	0.49	0.23				
	G	1.6	0.76	0.29	0.13				
Almond	A	13.6	7.5	2.45	1.35				
	G	8.1	4.0	1.46	0.72				
Fruit Trees	A	9.7	5.0	1.75	0.9				
	G	4.6	2.1	0.83	0.38				
Cole Crops	A	9.0	4.4	1.62	0.79				
	G	7.1	3.4	1.28	0.61				

5.1.2 Terrestrial Phase Direct Effects

Maximum usage of acephate (cotton)

Direct acute risk to terrestrial-phase of CRLF is driven by acephate use on cotton with an acute RQ of 0.34 to 6.82 (dose-based) and 0.07 to 0.66 (dietary based, Table 5.7). Direct chronic risk to CRLF terrestrial-phase is driven by acephate use on cotton with RQs ranging from 10 to 94.

Exposures and RQ values for terrestrial organisms are given in the T-REX output below. The CRLF is represented by a bird ("avian") of 20 or 100 grams body weight, which consumes small insects or large insects. Acute, dose-based RQs are reported by the size of the animal and their diet. The acute RQs ranges from 0.34 for a 100-gram frog consuming large insects to 6.82 for a 20-gram frog consuming small insects, respectively. These RQs are above the Listed Species LOC, 0.1. Dietary-based acute RQs are above the LOC for small insects only (RQ = 0.66).

Chronic risk to the CRLF is also represented by the avian taxa. Chronic RQ for birds eating small insects and large insects range from 10 to 94, well above the LOC (1).

Minimum usage of acephate (sewage disposal)

For the minimum exposure of CRLF, (sewage disposal areas, Table 5.8), dose-based acute RQs are above LOC (0.1) for consumption of small insects. The chronic dietary RQ is also above LOC for the CRLF.

Tables 5.7 and 5.8 provide the RQs for direct and indirect effects to the CRLF.

Table 5.7. T-REX Inputs for Analysis of Direct and Indirect Effects to CRLF (Maximum Use, Cotton)

Chemical Name:	Acephate
Use	Cotton
Formulation	Orthene 75
Application Rate	1 lbs a.i./acre
Half-life	8.2 days
Application Interval	3 days
Maximum # Apps./Year	6
Length of Simulation	1 year

Table 5.7a Dietary-based EECs (ppm)	**Kenaga Values**
Short Grass	837.5
Tall Grass	383.9
Broadleaf plants/sm Insects	471.1
Fruits/pods/seeds/lg insects	52.3

Table 5.7b
RQs associated with Direct Effect as Represented by Avian species, as surrogate for the CRLF

	20 g Acute Dose-based	**100 g Acute Dose-based**	**Acute Dietary**	**Chronic Dietary**
Broadleaf plants/sm insects	**6.8**	**3.1**	**0.66**	**94.2**
Fruits/pods/seeds/lg insects	**0.76**	**0.34**	0.07	**10.5**

Table 5.7c Indirect Effects as Represented by Prey Item - *RQs*

	15 g Mammal Dose-based Acute	**15 g Mammal Dose-based Chronic**	**Dietary-based Mammal Chronic RQ**	**20 g Avian Acute Dose-based**	**Avian Acute Dietary**	**Avian Chronic Dietary**
Short Grass	**2.01**	**145.3**	**16.8**	**12.15**	**1.17**	**167.5**
Tall Grass	**0.92**	**66.6**	**7.7**	**5.57**	**0.53**	**76.77**
Broadleaf plants/sm insects	**1.13**	**81.7**	**9.4**	**6.83**	**0.66**	**94.22**
Fruits/pods/lg insects	**0.13**	**9.08**	**1.1**	**0.76**	0.07	**10.47**

Table 5.8.
T-REX Inputs for Analysis of Direct and Indirect Effects to CRLF (Minimum Use, Sewage Disposal)

Chemical Name:	Acephate
Use	Sewage disposal
Formulation	Orthene 75
Application Rate	0.13 lbs a.i./acre
Half-life	8.2 days
Application Interval	na
Maximum # Apps./Year	1
Length of Simulation	1 year

Table 5.8a Dietary-based EECs (ppm)	**Kenaga Values**
Short Grass	31.2
Tall Grass	14.3
Broadleaf plants/sm Insects	17.55
Fruits/pods/seeds/lg insects	1.95

Table 5.8b
***RQs* associated with Direct Effect as Represented by Avian species, as surrogate for the CRLF**

	20 g Acute Dose-based	**100 g Acute Dose-based**	**Acute Dietary**	**Chronic Dietary**
Broadleaf plants/sm insects	**0.25**	**0.11**	0.02	**3.5**
Fruits/pods/seeds/lg insects	0.03	0.01	<0.01	0.39

Table 5.8c Indirect Effects as Represented by Prey Item - *RQs*

	15 g Mammal Dose-based Acute	**15 g Mammal Dose-based Chronic**	**Dietary-based Mammal Chronic RQ**	**20 g Avian Acute Dose-based**	**Avian Acute Dietary**	**Avian Chronic Dietary**
Short Grass	0.07	**5.41**	0.62	**0.45**	0.04	**6.24**
Tall Grass	0.03	**2.48**	0.29	**0.21**	0.02	**2.86**
Broadleaf plants/sm insects	0.04	**3.05**	0.35	**0.25**	0.02	**3.51**
Fruits/pods/lg insects	<0.01	0.34	0.04	0.03	<0.01	0.39

5.1.3. Individual Effects Calculation for Direct Acute Effect on CRLF

The risk of mortality to the CRLF is based on the avian (bird) taxon in T-REX, where the CRLF is represented by a 20-gram or 100-gram bird that consumes small insects or large insects. The individual chance of effects is calculated from the probit-slope response relationship, using an Excel spreadsheet (IEC v1.1, June 22, 2004).

The acute toxicity data used is for the bobwhite quail. The bobwhite LD50 is 109 mg/kg-body weight. Individual effect probabilities were calculated for the cotton scenario (six applications of 1 lb a.i./acre spaced at 3 day intervals). A lower application rate, as is applied to sewage disposal areas, was modeled at a rate of 0.13 lb/A. The results are shown in Table 5.9.

Table 5.9a. Individual Effects Chance for Mortality of CRLF

Exposure scenario	TREX risk quotient	Chance of effect (1-in-…)
Listed Species Threshold	(LOC, 0.1)	9.38 E+11
Cotton, 20-g bird, small insects (max exposure)	6.8	Approaching 1
Cotton, 100-g bird, small insects (max exposure)	3.1	Approaching 1
Cotton, 20-g bird, large insects (max exposure)	0.76	3.38
Cotton, 100-g bird, large insects (max exposure)	0.34	57
0.13 lb/A, 20-g bird, small insects (min. exposure)	0.25	297
0.13 lb/A, 100-g bird, large insects	0.03	2.76E+11
0.13 lb/A, 20-g bird, small insects	0.11	1.25E+5
0.13 lb/A, 100-g bird, large insects	0.01	8.86E+18

Table 5.9b. Individual Effects Probability Calculation for Prey Items

Organism	slope	Threshold (LOC or RQ)	Chance of Effect, 1 in …
Daphnia magna at LOC	4.5 (default)	0.05 (LOC)	4.18E+8
Daphnia magna at maximum RQ		0.13 (RQ)	2.99E+4
Honey bee at LOC		0.05 (LOC)	4.18E+8
Honey bee, Large insect RQ (cotton)		5.5 (RQ)	1
Honey bee, Small insect RQ (cotton)		50 (RQ)	1
Honey bee, Large insect RQ (sewage)		0.2	1,210
Honey bee, Small insect RQ (sewage)		1.86	1.13
15 gm mammal at LOC		0.1 (LOC)	2.94E+5
15 gm mammal at RQ		1.13 (RQ)	1.68

These results show that the risk to an individual CRLF is high (approaching 100% mortality) if exposed to the maximum cotton exposure scenario, and consuming a diet of small insects. Risks are also large for a diet of large insects (1-in-3.38, or 30% for the 20-g bird, and 1-in-57, or 1.75% for the 100-g bird).

For the low exposure scenario (0.13 lb/acre, applied once) the risk is appreciable for a 20-g bird consuming small insects (1-in-297, or 0.34%).

The lowest RQ that gives an individual chance of effect approaching 100% is 3.1. The RQ for 20-g birds consuming small insects, if exposure is 2 applications of 1 lb a.i./acre, spaced at 3 day intervals, is 3.42.

These results indicate that the chance of individual mortality for a CRLF, as represented by 20-g and 100-g birds, is considerable even for low application rates (0.34%) and approaches 100% at exposures well below the maximum allowed on the label.

5.1.4. Indirect Effects, Terrestrial Phase

Indirect effects on the CRLF in the terrestrial phase of its life cycle might be due to loss of prey (insects, small mammals, small frogs, small birds) or effects on plants that provide habitat. Because no adverse effects on terrestrial plants are expected, no indirect effects on the CRLF mediated via plants are expected.

Small mammals and frogs that might be eaten by the CRLF are represented in the TREX analysis as the 15-gram mammal and the 20-g bird, respectively. Both prey items are assumed to eat short-grass food items, as this provides the highest dose, and therefore the most protective assessment.

5.1.4.1 Acute Effects

The small mammal RQ is 0.49 (Table 5.7), and the small bird RQ is 12.1 (Table 5.7), both above the LOC of 0.1 for Listed species. The dietary-based RQ (Table 5.7) is 1.2 for birds (above LOC), and was 16.7 for mammals (Table 5.9).

Acute effects on insects are calculated (Appendix L) using the acute contact LD50 for the honey bee (1.2 micrograms per bee) divided by 0.128 grams body weight, to obtain an LD50 in ppm. Exposures are the small insect and large insect from the TREX dietary analysis. The LD50 is then (1.2 micrograms)/(0.128 grams) = 9.4 ppm. The dietary-based EECs are 471 ppm (small insect) and 52 ppm (large insect). The resulting RQ values are 50 and 5.5, respectively, and are well above the LOC for terrestrial invertebrates (0.05).

5.1.4.1. Chronic Effects

The chronic avian RQ is 167 (Table 5.7) , and the chronic mammal RQ is 145 (dose-based, Table 5.9) or 17 (dietary-based, Table 5.9). All of the RQs are above the LOC (1).

Based on these RQ values, it is presumed that the CRLF will be indirectly affected by adverse effects (both acute and chronic) on animals in its prey base.

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 California Red Legged frog.

If the RQs presented in the Risk Estimation (Section 5.1.2) show no indirect effects, and LOCs for the CRLF are not exceeded for direct effects (Section 5.1.1), a "no effect" determination is made based on acephate's use within the action area. If, however, indirect effects are anticipated and/or exposure exceeds the LOCs for direct effects, the Agency concludes a preliminary "may affect" determination for the CRLF. 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 and potential community-level effects to aquatic plants and terrestrial plants growing in semi-aquatic areas. 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.

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

- Significance of Effect: Insignificant effects are those that cannot be meaningfully measured, detected, or evaluated in the context of a level of effect where "take" occurs for even a single individual. "Take" in this context means to harass or harm, defined as the following:

    - Harm includes significant habitat modification or degradation that results in death or injury to listed species by significantly impairing behavioral patterns such as breeding, feeding, or sheltering.

    - Harass is defined as actions that create the likelihood of injury to listed species to such an extent as to significantly disrupt normal behavior patterns which include, but are not limited to, breeding, feeding, or sheltering.

- Likelihood of the Effect Occurring: Discountable effects are those that are extremely unlikely to occur. For example, use of dose-response information to estimate the likelihood of effects can inform the evaluation of some discountable effects.

- Adverse Nature of Effect: Effects that are wholly beneficial without any adverse effects are not considered adverse.

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

5.2.1 Direct Effects to the California Red Legged Frog

The federal action is all labeled uses. In order to compare the location of the labeled uses with the areas important to the frog, the potential use areas in California were over laid with the core areas, critical habitat and known occurrence areas of the CRLF. The result of this layering is the ability to discern areas of overlap between potential use and the CRLF life-cycle.

5.2.1.1. Aquatic Phase

Risk Quotients for freshwater fish (surrogates for the CRLF) are below LOC for both acute and chronic effects (Tables 5.2 thru 5.6).

5.2.1.2. Terrestrial Phase (Direct Effects)

Risk Quotients for terrestrial-phase CRLF, as represented by 20-gram and 100-gram birds, exceed LOC for both acute and chronic (reproductive) effects (Table 5.7 and 5.8). Acute RQs range from 0.34 to 6.8 for CRLF for maximum exposure from cotton (1 lb ai/A applied 6 times with 3 day interval) and from 0.11 to 0.25 (small insect food source) for a minimum exposure of 0.13 lb ai/A applied once onto sewage disposal areas. RQs for large insect food source are below LOC (0.1). Chronic RQs range from 10 to 94 for CRLF for maximum exposure from cotton (1 lb ai/A applied 6 times with 3 day interval) and from 0.39 to 3.5 for a minimum exposure of 0.13 lb ai/A applied once onto sewage disposal areas. Both mortality and adverse reproductive effects to the CRLF (from a small insect diet) are anticipated based on labeled uses of acephate and risk quotients. RQs for CRLF from large insect diet are below acute and chronic LOC.

Refinement of RQ for CRLF terrestrial phase

Birds are currently used as surrogates for reptiles and terrestrial-phase amphibians. However, reptiles and amphibians are poikilotherms (body temperature varies with environmental temperature) while birds are homeotherms (temperature is regulated, constant, and largely independent of environmental temperatures). Therefore, reptiles and amphibians (collectively referred to as herptiles in this guidance) 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 or reptiles on a daily dietary intake basis, assuming similar caloric content of the food items. This can be seen when comparing the estimated caloric requirements for free living iguanid lizards (Iguanidae) (**EQ 1**) to passerines (song birds) (**EQ 2**) (U.S. EPA, 1993):

$$\textbf{iguanid FMR (kcal/day)= 0.0535 *(bw in g)}^{0.799} \qquad \textbf{(EQ 1)}$$

$$\textbf{passerine FMR (kcal/day) = 2.123 *(bw in g)}^{0.749} \qquad \textbf{(EQ 2)}$$

With relatively comparable exponents (slopes) to the allometric functions, one can see that, given a comparable body weight, the free living metabolic rate of birds can be 40 times higher than reptiles, though the requirement differences narrow with high body weights. Consequently, use of avian food intake allometric equation as a surrogate to herptiles is likely to result in an over-estimation of exposure for reptiles and terrestrial-phase amphibians.

There is a current need to evaluate dietary exposure to terrestrial-phase amphibian species (e.g., California Red-Legged Frog, CRLF) and an anticipated need to evaluate dietary exposure for amphibians and reptiles in the future for the purpose of conducting endangered species effects determinations. Therefore, T-REX (version 1.3.1.) has been altered to allow for an estimation of food intake for herptiles (T-HERPS) using the same basic procedure that T-REX uses to estimate avian food intake.

A comparison is made between the T-REX model which uses the bird as a surrogate for the CRLF and the T-HERPS model which calculates the allometric functions for amphibians.

Cotton (maximum exposure) Discussion

T-REX model shows that the ranges of direct affects to birds as surrogate for CRLF is from 0.34 to 6.8 for dose-based acute, from 0.07 to 0.66 (LOC for listed terrestrial animals) for dietary acute, and from 10 to 94 for chronic dietary.

T-HERPS model show the ranges of RQ for amphibians that was corrected for body weight, metabolic rates and caloric intake requirements from avian data. The ranges of RQ for T-HERPS is from <0.01 to 4.8 (LOC for listed terrestrial animals) for the dose-based acute, 0.02 to 1.17 for dietary acute, and from 8.3 to 168 for chronic dietary.

The refinement of cotton models show a slight decrease in RQs in T-HERPS. All the chronic LOCs for CRLF is exceeded and many of the acute LOCs are exceeded (see Tables 5-10 and 5-11).

Sewage Disposal Areas (minimum exposure) Discussion

T-REX model shows that the ranges of direct affects to birds as surrogate for CRLF is from 0.11 to 0.25 (small insect diet) for dose-based acute, <0.05 (LOC for listed terrestrial animals) for dietary acute, and from 0.39 to 3.5 for chronic dietary.

T-HERPS model show the ranges of RQ for amphibians that was corrected for body weight, metabolic rates and caloric intake requirements from avian data. The only the small herbivore mammal diet RQ for T-HERPS exceeds the LOC with an RQ of 0.18, from <0.01 to 4.8 (LOC for listed terrestrial animals) for the dose-based acute, all of dietary acute RQs is below LOC, and from 4 of the 7 food items are above LOC for chronic dietary.

The refinement of sewage disposal areas models show a slight decrease in RQs in T-HERPS. For four out of seven food items, the chronic LOCs for CRLF is exceeded and only the small herbivore mammal diet RQ exceeds the acute LOC (see Tables 5-10 and 5-11).

Results of the T-HERPS model are below:

Table 5-10 Summary of T-HERPS Risk Quotient Calculations Based on Upper Bound Kenaga EECs for cotton (Maximum Exposure)

Acute and Chronic RQs are based on the Upper Bound Kenaga Residues.

The maximum single day residue estimation is used for both the acute and reproduction RQs.

RQs reported as "0.00" in the RQ tables below should be noted a <0.01 in your assessment. This is due to rounding and significa figure issues in Excel.

Upper Bound Kenaga Residues For RQ Calculation

Chemical Name:	Acephate
Use	Cotton
Formulation	Orthene
Application Rate	1 lbs a.i./acre
Half-life	8.2 days
Application Interval	3 days
Maximum # Apps./Year	6
Length of Simulation	1 year

Endpoints

Avian	Bobwhite quail	LD50 (mg/kg-bw)	109.00
	Japanese Quail	LC50 (mg/kg-diet)	718.00
	Bobwhite quail	NOAEL(mg/kg-bw)	0.00
	Mallard duck	NOAEC (mg/kg-diet)	5.00

Dietary-based EECs (ppm)	Kenaga Values
Short Grass	837.49
Tall Grass	383.85
Broadleaf plants/sm Insects	471.09
Fruits/pods/seeds/lg insects	52.34
Small herbivore mammals	551.86
Small insectivore mammals	34.49
Small terrestrial phase amphibians	16.35

Terrestrial Herpetofauna Results

Weight Class	Body Weight (g)	Ingestion (Fdry) (g bw/day)	Ingestion (Fwet) (g/day)	% body wgt consumed	FI (kg-diet/day)
Small	1.4	0.017	0.1	3.9	5.44E-05
Mid	37	0.212	1.4	3.8	1.41E-03
Large	238	0.893	6.0	2.5	5.96E-03

Body Weight (g)	Adjusted LD50 (mg/kg-bw)
1.4	109.00
37	109.00
238	109.00

Dose-based EECs (mg/kg-bw)	Herpetofaunal Size Classes and Body Weights		
	small (g)	mid (g)	large (g)
	1.4	37	238
Short Grass	32.54	31.98	20.96
Tall Grass	14.91	14.66	9.61
Broadleaf plants/sm Insects	18.30	17.99	11.79
Fruits/pods/seeds/lg insects	2.03	2.00	1.31
Small herbivore mammals	N/A	522.03	81.16
Small insectivore mammals	N/A	32.63	5.07
Small terrestrial phase amphibian	N/A	0.62	0.41

Dose-based RQs (Dose-based EEC/adjusted LD50)	Amphibian/Reptile Acute RQs for Small, Medium, and Large Species (grams)		
	1.4	37	238
Short Grass	0.30	0.29	0.19
Tall Grass	0.14	0.13	0.09
Broadleaf plants/sm insects	0.17	0.17	0.11
Fruits/pods/seeds/lg insects	0.02	0.02	0.01
Small herbivore mammals	N/A	4.79	0.74
Small insectivore mammals	N/A	0.30	0.05
Small terrestrial phase amphibian	N/A	0.01	0.00

Dietary-based RQs (Dietary-based EEC/LC50 or	RQs	
	Acute	Chronic
Short Grass	1.17	167.50
Tall Grass	0.53	76.77
Broadleaf plants/sm Insects	0.66	94.22
Fruits/pods/seeds/lg insects	0.07	10.47
Small herbivore mammals	0.77	110.37
Small insectivore mammals	0.05	6.90
Small terrestrial phase amphibian	0.02	3.27

Note: To provide risk management with the maximum possible information, it is recommended that both the dose-based and concentration-based RQs be calculated when data are available

Table 5-11 Summary of T-HERPS Risk Quotient Calculations Based on Upper Bound Kenaga EECs for sewage disposal areas (Minimum Exposure)

Acute and Chronic RQs are based on the Upper Bound Kenaga Residues.

The maximum single day residue estimation is used for both the acute and reproduction RQs.

RQs reported as "0.00" in the RQ tables below should be noted as <0.01 in your assessment. This is due to rounding and significant figure issues in Excel.

Upper Bound Kenaga Residues For RQ Calculation

Chemical Name:	Acephate
Use	seagwe diposal
Formulation	Orthene
Application Rate	0.13 lbs a.i./acre
Half-life	8.2 days
Application Interval	0 days
Maximum # Apps./Year	1
Length of Simulation	1 year

Endpoints

Avian	Bobwhite quail	LD50 (mg/kg-bw)	109.00
	Japanese Quail	LC50 (mg/kg-diet)	718.00
	Bobwhite quail	NOAEL(mg/kg-bw)	0.00
	Mallard duck	NOAEC (mg/kg-diet)	5.00

Dietary-based EECs (ppm)	Kenaga Values
Short Grass	31.20
Tall Grass	14.30
Broadleaf plants/sm Insects	17.55
Fruits/pods/seeds/lg insects	1.95
Small herbivore mammals	20.56
Small insectivore mammals	1.28
Small terrestrial phase amphibians	0.61

Terrestrial Herpetofauna Results

Weight Class	Body Weight (g)	Ingestion (Fdry) (g bw/day)	Ingestion (Fwet) (g/day)	% body wgt consumed	FI (kg-diet/day)
Small	1.4	0.017	0.1	3.9	5.44E-05
Mid	37	0.212	1.4	3.8	1.41E-03
Large	238	0.893	6.0	2.5	5.96E-03

Body Weight (g)	Adjusted LD50 (mg/kg-bw)
1.4	109.00
37	109.00
238	109.00

Dose-based EECs (mg/kg-bw)	Herpetofaunal Size Classes and Body Weights		
	small (g)	mid (g)	large (g)
	1.4	37	238
Short Grass	1.21	1.19	0.78
Tall Grass	0.56	0.55	0.36
Broadleaf plants/sm Insects	0.68	0.67	0.44
Fruits/pods/seeds/lg insects	0.08	0.07	0.05
Small herbivore mammals	N/A	19.45	3.02
Small insectivore mammals	N/A	1.22	0.19
Small terrestrial phase amphibian	N/A	0.02	0.02

Dose-based RQs (Dose-based EEC/adjusted LD50)	Amphibian/Reptile Acute RQs for Small, Medium, and Large Species (grams)		
	1.4	37	238
Short Grass	0.01	0.01	0.01
Tall Grass	0.01	0.01	0.00
Broadleaf plants/sm insects	0.01	0.01	0.00
Fruits/pods/seeds/lg insects	0.00	0.00	0.00
Small herbivore mammals	N/A	0.18	0.03
Small insectivore mammals	N/A	0.01	0.00
Small terrestrial phase amphibian	N/A	0.00	0.00

Dietary-based RQs (Dietary-based EEC/LC50 or	RQs	
	Acute	Chronic
Short Grass	0.04	6.24
Tall Grass	0.02	2.86
Broadleaf plants/sm Insects	0.02	3.51
Fruits/pods/seeds/lg insects	0.00	0.39
Small herbivore mammals	0.03	4.11
Small insectivore mammals	0.00	0.26
Small terrestrial phase amphibian	0.00	0.12

Note: To provide risk management with the maximum possible information, it is recommended that both the dose-based and concentration-based RQs be calculated when data are available

5.2.2. Indirect Effects to the CRLF

5.2.2.1. Aquatic Phase

Sub-adult and adult CRLF consume invertebrates. Since acute RQs for freshwater invertebrates range up to 0.13 (Table 5.5), there is a "May Affect" finding. However, since the RQ is below the Acute Risk LOC (0.5), other factors must be considered in determining if this constitutes a "Likely to Adversely Affect" or "Not Likely to Adversely Affect" finding, as explained below in section 5.4.2. Based on the likelihood of individual effects on aquatic invertebrates (Table 5.9b above), indirect risk to the CRLF via effects on aquatic invertebrates is considered "NLAA."

5.2.2.2. Terrestrial Phase

Cotton (maximum exposure) Discussion

Risk quotients for two common prey animals (frog and small mammal and bird) greatly exceed both acute and chronic LOC (Table 5-8). These prey animals are anticipated to suffer adverse effects (mortality and reproductive effects) from labeled acephate uses. The acute RQ for a terrestrial invertebrate (honey bee), representing the bulk of the terrestrial phase CRLF diet, ranges from 5.5 to 50. Thus, adverse indirect effects to the CRLF, mediated via reduction in prey base, are anticipated.

The terrestrial-phase CRLF uses small mammal burrows for shelter. If populations of small mammals are reduced, as is anticipated from the acute RQs for individual effects on them, then there may be fewer burrows for the CRLF to exploit. In addition, there are chronic effects that may reduce the population of mammalian food item for CRLF. Thus, there may be an indirect effect on the CRLF through adverse effects to terrestrial phase habitat.

Sewage Disposal Areas (minimum exposure) Discussion

Risk quotients for common prey animals (frog and small mammal and bird) do exceed some of dosed-based acute but are discountable due to the low likelihood of effects to individual prey animals. Chronic RQs for birds and mammals are above LOC for all food items except for large insects (Table 5.8). These prey animals are anticipated to suffer adverse reproductive effects from labeled acephate uses. The acute RQ for a terrestrial invertebrate (honey bee), representing the bulk of the terrestrial phase CRLF diet, ranges from 0.20 to 1.86. Thus, adverse indirect acute effects "May Affect" the CRLF, mediated via reduction in prey base. Chronic effects (reproductive effects) "May Affect" CRLF and are not discountable.

5.3 Action Area

The Action Area for endangered species from the labeled use of a pesticide is defined by exceedence of the Level of Concern for any Listed species. Risk Quotients from the screening risk assessment are compared to the Listed Species LOCs for all taxa to determine the geographic extent of the Action Area.

If necessary, standard modeling assumptions are changed to determine the limits of LOC exceedence. For example, the spray drift assumption for aerial application can be lowered from the standard 5% until LOC is no longer exceeded, and that spray drift amount entered into AgDrift or AgDISP to determine the distance from the sprayed field to the standard pond that will lower RQ to below LOC. That distance around the sprayed field then determines the Action Area (assuming no secondary poisoning effects from movement of contaminated animals).

5.3.1. Aquatic Phase

The Action Area for effects on aquatic species consists of two parts. One is a spray drift perimeter around the use site, and the other is a downstream dilution factor. Both parts are intended to find the geographic extent of Listed species LOC exceedance.

5.3.1.1 Spray Perimeter.

The Action Area for effects on aquatic species was based on acute effects to Listed aquatic invertebrates, since these were the only LOCs exceeded (Tables 5-6 and 5-7). To be below the LOC for Listed aquatic invertebrates (0.05), the peak concentration in the EXAMS pond would need to (0.05)*(26 ppb) = 1.3 ppb, where 26 ppb is the EC50 for *Daphnia magna* for methamidophos, a degradate of acephate.

Tier 1 and Tier 2 exposure analyses showed that no LOCs are exceeded from parent acephate for invertebrates. Tier 2 exposure analysis for the degradate methamidophos showed RQs as high as 0.13 (Table 5.5). The likelihood of an individual effect on Daphnia at this RQ is very low (Table 5.9b), thus this effect is discountable, and no spray perimeter for indirect effects is needed.

5.3.1.2 Downstream Dilution

The downstream dilution analysis calculates how far downstream the EEC remains above the Listed species LOC, given flow contributions from both contaminated and uncontaminated streams in the watersheds of potential acephate use. The initial area of concern was defined by Figure 2.E., which shows all agricultural land in all counties in California where acephate is used. Flow contributions from streams in the corresponding watersheds are included in a GIS (Geographic Information System) analysis, until the pesticide concentrations (initially the EXAMS pond peak EEC) from contaminated and uncontaminated streams, weighted for flow, fall below the Listed species LOC.

The downstream dilution factor that must be achieved is defined by the maximum ratio between an RQ and its corresponding LOC. In the case of methamidophos as a degradate of acephate, this is the acute RQ for aquatic invertebrates from aerial application to cotton and almond (0.13), divided by into LOC (0.05) for a factor of 0.38. See Table 5.6.

5.3.2. Terrestrial Phase

The Action Area due to effects on Listed species is also defined by the geographic extent of LOC exceedence. Quantitative estimates of exposure of avian (including reptiles and terrestrial amphibians) and mammal species is done with the TREX model, which automates exposure analysis according to the Hoerger-Kenaga nomogram, as modified by Fletcher (1994).

For acephate, the Action Area was calculated on the basis of the smallest avian (20-gram body weight) or mammal (15-gram), consuming the most highly contaminated food category (short grass). This results in the highest RQs, and thus the most conservative estimate of the Action Area.

The lowest ratio between the LOC for Listed terrestrial avian and mammalian species (0.1 for acute effects and 1.0 for chronic effects) and the RQ, times the maximum single application rate, is used to determine the exposure (in lb/acre) that is below LOC, as shown in Table 5-8.

Exposure below LOC = (LOC/RQ)*(1 lb/acre).

In the case of methamidophos, the target exposure is 0.006 lb/acre, due to chronic effects on avian species (chronic RQ = 167).

The distance from the use site (sprayed field) needed to achieve the target exposure of 0.006 lb/acre was calculated with the Gaussian Far-Field extension of the AgDISP model. The input parameters for AgDISP are given below (Table 5-12); all other parameters were the default values.

Table 5-12. Input Parameters for AgDISP Gaussian Far-Field Extension Analysis

Input Parameter	Value
Release Height	15 feet
Wind Speed	15 mph
Spray Quality	ASAE very fine to fine
Non-Volatile fraction	0.032 (1.33 lb product in 5 gal = 42 lb water)
Active fraction	0.024 (nonvol frac x % a.i. = 75%)
Surface Canopy	None
Specific Gravity, Carrier	1
Deposition type	Terrestrial point
Initial Average Deposition	0.006 lb/acre

The result of this analysis is that a perimeter of 2,913 (0.55 miles) feet from the edge of the sprayed field is needed to bring the chronic avian RQ to below the LOC of 1. Thus, the Action Area extends to a distance of 2,913 feet from the edge of fields sprayed with acephate.

Figure 5A shows the full extent of the Action Area, based on the terrestrial effects distance of 2,913 feet and the downstream dilution factor of 0.38.

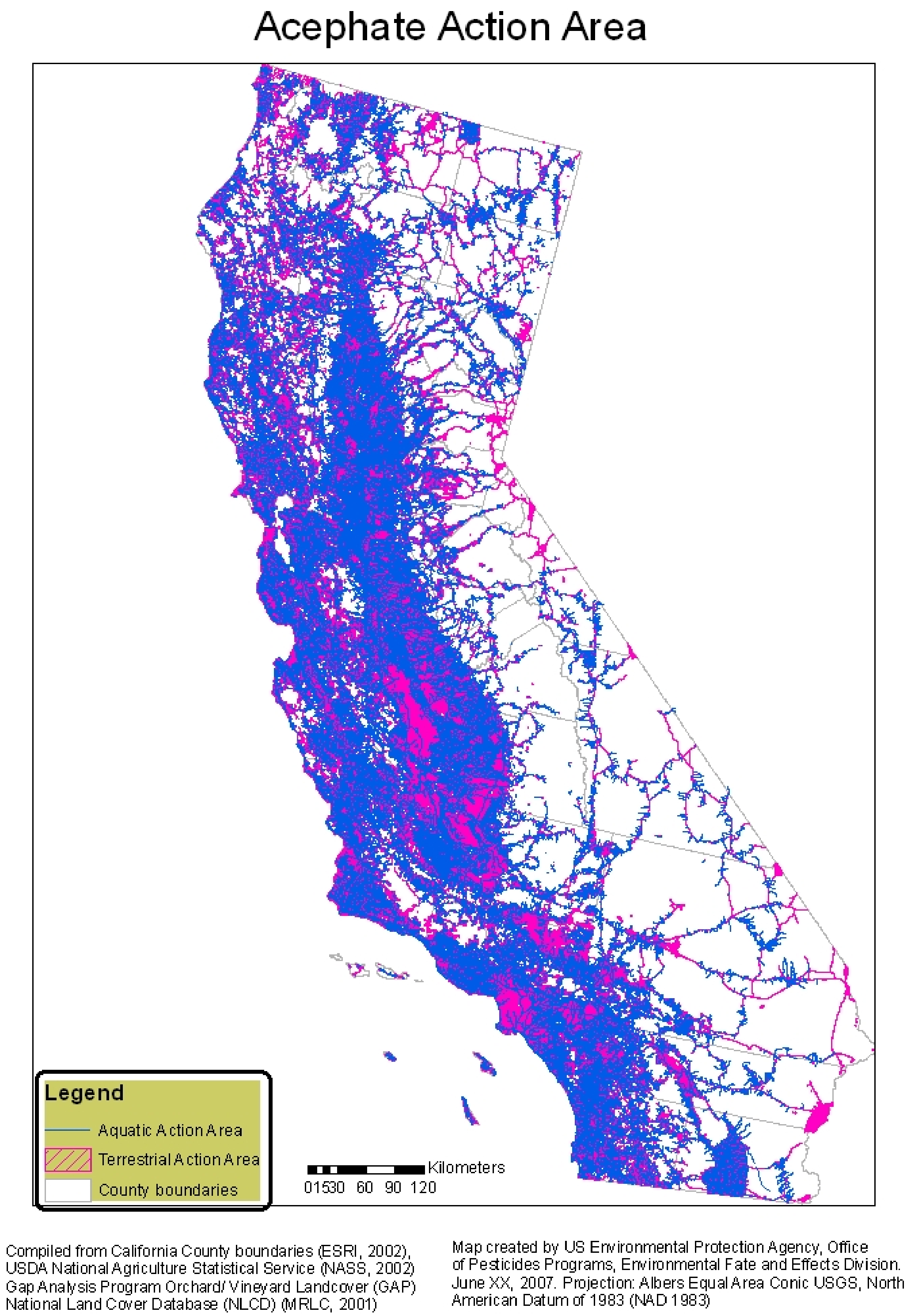


Figure 5A Action Area for Acephate

5.4 Listed Species Effect Determination for the California Red-Legged Frog

5.4.1. "May Affect" Determination

When the action area overlaps (spatially) the designated Core Areas and Critical Habitats a "may affect" determination is made. If there is no overlap, and thus no expected exposure, a "no effect" determination is made. Upon a "may affect" determination the probability of effect is considered and a "Likely to Adversely Affect" or "Not Likely to Adversely Affect" determination is made.

Based on the action area for acephate use in California, the use of acephate "May Effect" the CRLF. Table 5.13 displays the proportion of the core area within each recovery unit that overlaps with the potential use areas.

Table 5.13 Terrestrial spatial summary results for acephate uses with 2,913-ft buffer.

Measure	**RU1**	**RU2**	**RU3**	**RU4**	**RU5**	**RU6**	**RU7**	**RU8**	**Total**
Initial Area of Concern (no buffer)									67,491 sq km
Established species range area (sq km)	3654	2742	1323	3279	3650	5306	4917	3326	28,197
Overlapping area (sq km)	1615	1855	983	2323	2918	4243	3576	2662	20,175
Percent area affected	*44*	*68*	*74*	*71*	*80*	*80*	*73*	*80*	*72*
# Occurrence Sections	9	3	64	270	272	108	89	24	865[1]

[1]*26 occurrence sections occur outside of Recovery Units.*

5.4.2 "Adverse Effect" Determination

Risk Quotients for direct, acute and chronic effects to the terrestrial-phase CRLF (Tables 5.4, 5.5, 5.10 and 5.11) are well above their respective LOCs for the high exposure (cotton).

At low exposure (sewage disposal areas) the direct, acute and chronic LOC is exceeded for small insect food items. Chronic Risk quotients for animals (mammals, birds and amphibians) that may serve as prey for the CRLF are also well above LOCs for three of four food categories. The risk quotient for a terrestrial invertebrate (honey bee), representing the bulk of the CRLF diet, is 5.5 to 50 for high exposure (cotton), well above the LOC of 0.05. For the low exposure (sewage disposal areas), the RQs are 0.2 and 1.86 which also provide an individual chance of effect of one in 1,210 and 1.13, respectively. **Thus, both direct and indirect adverse effects to the terrestrial-phase CRLF and its critical habitat are anticipated**.

Risk quotients for direct, acute and chronic effects to the aquatic-phase CRLF, as represented by freshwater fish, are below the LOC (Tables 5.2 and 5.3). Acute and chronic effects on the aquatic phase CRLF and its critical habitat are not anticipated.

Aquatic invertebrate acute RQs (tables 5.6 and 5.7) are below the acute LOC (0.5) for all uses, and the likelihood of individual effects is low (Table 5.9b). Thus adverse indirect effects on the CRLF due to loss of prey items are discountable, and therefore NLAA. Acephate is not toxic to aquatic plants, so no indirect effects to the CRLF via reduction in primary production as a food source are anticipated.

Based on this analysis, it is concluded that the labeled uses of acephate in California "may affect, and are likely to adversely effect" the California Red-Legged Frog, where the Action area overlaps its habitat, due to terrestrial effects.

Table 5.12 Acephate Effects Determination Summary

Assessment Endpoint	Effects determination	Basis for Determination
Aquatic Phase (Eggs, larvae, tadpoles, juveniles, and adults)		
Direct Effects and Critical Habitat Effects		
1. Survival, growth, and reproduction of CRLF	No Effect	All Acute and Chronic RQ are below the listed LOC for surrogate species (rainbow trout)
Indirect Effects		
2. Reduction or modification of aquatic prey base	May Affect, Not Likely to Adversely Affect	Acute LOC is exceeded for aquatic invertebrates, however effect is considered discountable based on low likelihood of individual effect.
3. Reduction or modification of aquatic plant community	No Effect	No LOC Exceedences for any plant species
4. Degradation of riparian vegetation	No Effect	No LOC Exceedences for any plant species. No adverse aquatic critical habitat modification is expected.
Terrestrial Phase (Juveniles and Adults)		
Direct Effects		
5. Survival, growth, and reproduction of CRLF	May Affect, Likely to Adversely Affect	Acute and Chronic LOC exceedences for birds, the surrogate species for direct effects to frogs. Initial Area of Concern overlaps habitat. Use is widespread (nearly all counties). Use is documented in all months. Probability of effect approaches 100% at calculated RQs.

Assessment Endpoint	Effects determination	Basis for Determination
Indirect Effects and Critical Habitat Effects		
6. Reduction or modification of terrestrial prey base	May Affect, Likely to Adversely Affect	Acute and Chronic LOC exceedences for multiple components of CRLF prey base (mammals, birds, and terrestrial invertebrates). LAA to terrestrial phase CRLF and its critical habitat based on acute RQs exceeding 0.5 and chronic RQs over LOC for mammals, insects, birds. Adverse terrestrial critical habitat modification is expected.
7. Degradation of riparian vegetation	No Effect	No plant LOC exceedences.

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 adverse modification to critical habitat.

5.5 Risk Hypotheses Revisited

Table 5.13 below revisits the risk hypotheses presented in section 2.9.1. The risk hypotheses were accepted or rejected in accordance with the "No Effect," "May Affect," and "Likely to Adversely Affect," or "Not Likely to Adversely Affect" findings in this assessment.

Table 5.13 Risk Hypotheses Revisited

Risk Hypothesis	Conclusions
Labeled uses of acephate within the action area may directly affect the CRLF by causing mortality or by adversely affecting growth or fecundity	Rejected for Aquatic exposure. "No Effect" finding. Accepted for Terrestrial exposure. "LAA" finding.
Labeled uses of acephate within the action area may indirectly affect the CRLF by reducing or changing the composition of food supply	Accepted for Terrestrial exposure. "LAA" finding. Rejected for Aquatic exposure. "NLAA" finding.
Labeled uses of acephate within the action area may indirectly affect the CRLF and/or adversely 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	Rejected. "No Effect" finding for aquatic plants.
Labeled uses of acephate within the action area may indirectly affect the CRLF and/or adversely 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	Rejected. "No Effect" finding for terrestrial plants.
Labeled uses of acephate within the action	Rejected. "No Effect" for aquatic plants

area may adversely 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)	and "NLAA" for indirect effects via invertebrates.
Labeled uses of acephate within the action area may adversely modify the designated critical habitat of the CRLF by reducing the food supply required for normal growth and viability of juvenile and adult CRLFs	Accepted for Terrestrial exposure. "LAA" finding via effects on vertebrate and invertebrate food items. Rejected for Aquatic exposure. "NLAA" finding for aquatic invertebrates, "No Effect" for aquatic plants.
Labeled uses of acephate within the action area may adversely 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	Accepted. Effects on small mammals may reduce number of burrows used for shelter.
Labeled uses of acephate within the action area may adversely 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	Accepted. Effects on small mammals may reduce number of burrows used for shelter.
Labeled uses of acephate within the action area may adversely modify the designated critical habitat of the CRLF by altering chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs	Accepted. Presence of acephate in terrestrial habitat is believed to have direct and indirect effects on CRLF.

6. 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 insecticide resistance, timing of applications, cultural practices, and market forces.

6.1.2 Usage Uncertainties

County-level usage data were obtained from California's Department of Pesticide Regulation Pesticide Use Reporting (CDPR PUR) database. Four years of data (2002 – 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. No methodology for removing outliers was provided by CDPR for 2001 and earlier pesticide data; therefore, this information was not included in the analysis because it may misrepresent actual usage patterns. CDPR PUR documentation indicates that errors in the data may include the following: a misplaced decimal; incorrect measures, area treated, or units; and reports of diluted pesticide concentrations. In addition, it is possible that the data may contain reports for pesticide uses that have been cancelled. The CPDR PUR data does not include home owner applied pesticides; therefore, residential uses are not likely to be reported. As with all pesticide use 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.

6.2. Exposure Assessment Uncertainties

Due to lack of appropriate PRZM scenarios for California, not all labeled uses were modeled for aquatic exposure. It is likely that the cotton use, at 6 lb a.i. per acre, provides the highest aquatic exposure estimate, including those not modeled, most of which have maximum rates of 1 to 2 lb a.i. per acre.

Landscape maintenance is known to be a major use of acephate. This exposure is described for the aquatic environment using the PRZM turf scenario. However, there are a number of application techniques, such as those described as gallons per pot or teaspoons per mound, for which it is difficult to compare exposure potential to agricultural uses that are given in pounds per acre. It is assumed that the greatest aquatic and terrestrial exposure potential is for agricultural uses that allow aerial application.

All exposure estimates were done with maximum application rates, minimum intervals, and maximum number of applications, to define the Action Area for the Federal action. Actual exposures will depend on actual use rates, which may be lower.

Aquatic exposure modeling inputs were based on the available guideline data. Some inputs (e.g., soil metabolism half-life = 2.3 days) were based on a single value, which by EFED policy is multiplied by 3 to account for uncertainty. The aquatic metabolism rates (both aerobic and anaerobic) were set by policy at 2 times the soil input value. The partition coefficient (Kd) used was the highest and only quantified value obtained (0.09). The use of values for the other soils (essentially, Kd = 0) would have resulted in somewhat higher exposure estimates.

Spray drift estimates were set at 1% for ground application and 5% for aerial application, per EFED policy. Actual spray drift from aerial application may be higher.

The decay half-life of acephate on foliage and other food items for the TREX analysis was set at 8.2 days, rather than the default value of 35 days. This value was obtained from Willis & McDowell (1987) from a field experiment on lemons in California; this is the same reference used to obtain the default value of 35 days. The value of 8.2 days was the highest of the half-lives for acephate, so it is the most protective of the measured values.

Methamidophos exposures in the aquatic environment were estimated as 18% of the modeled acephate concentrations. This is based on a maximum 23% formation of methamidophos in a Fresno loam soil, and a molecular weight conversion factor of 0.77. This approach is considered reasonable, as the fate and transport properties of acephate and methamidophos are very similar (very mobile and non-persistent). The use of more sophisticated techniques (e.g., parent-daughter kinetics modeling ans use of methamidophos-specific fate inputs) is unlikely to provide more defensible exposure estimates.

6.2.1 PRZM Modeling Inputs and Predicted Aquatic Concentrations

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 m3) 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. As previously discussed in Section 2.X and Attachment 1, 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).

6.2.2 Aquatic Exposure Estimates

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 a farmer's 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.

6.3.3 Residue Levels Selection

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.

6.3.4 Dietary Intake

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. Environmental Protection Agency, 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.

6.4 Effects Assessment Uncertainties

6.4.1 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, such as acephate, 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 California Red Legged Frog.

6.4.2 Extrapolation of Long-term Environmental Effects from Short-Term Laboratory Tests

The influence of length of exposure and concurrent environmental stressors to the California Red Legged Frog (i.e., urban expansion, habitat modification, decreased quantity and quality of water in CRLF habitat, predators, etc.) will likely affect the species' response to acephate. Additional environmental stressors may decrease the CRLF's sensitivity to the insecticide, although there is the possibility of additive/synergistic reactions. Timing, peak concentration, and duration of exposure are critical in terms of evaluating effects, and these factors will vary both temporally and spatially within the action area. Overall, the effect of this variability may result in either an overestimation or underestimation of risk. However, as previously discussed, the Agency's LOCs are intentionally set very low, and conservative estimates are made in the screening level risk assessment to account for these uncertainties.

6.4.3 Sublethal Effects

For an acute risk assessment, 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 assessment is exercised on a case-by-case basis and only after careful consideration of the nature of the sublethal effect measured and the extent and quality of available data to support establishing a plausible relationship between the measure of effect (sublethal endpoint) and the assessment endpoints.

6.4.4 Location of Wildlife Species

For this baseline terrestrial 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.

6.5. Use of avian data as surrogate for amphibian data.

Toxicity data for terrestrial phase amphibians was not available for use in this assessment. Therefore, avian toxicity data were used as a surrogate for risk estimation. There is uncertainty regarding the relative sensitivity of herptiles and birds to acephate. If birds are substantially more or less sensitive than the California red legged frog, then risk would be over or under estimated, respectively.

6.6. Assumptions Associated with the Acute LOCs

The risk characterization section of this endangered species assessment includes an evaluation of the potential for individual effects. 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 for the effects study corresponding to the taxonomic group for which the LOCs are exceeded.

6.8. Action Area

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.

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