Risks of Methamidophos Use to Federally Listed California Red Legged Frog

(*Rana aurora draytonii*)

Pesticide Effects Determination

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1. Executive Summary

Background

Methamidophos is an organophosphate insecticide currently registered for use on four agricultural crops in California: tomato, potato, cotton and alfalfa grown for seed. It is applied by aircraft, groundspray, or irrigation at a rate of up to 1 lb a.i./acre up to four times per year. Methamidophos is very soluble and very mobile and may move through the environment and be transported away from the site of application by run-off or spray drift. Methamidophos is not persistent in terrestrial or aerobic aquatic environments but may be more persistent in anaerobic aquatic environments where it will be associated with the aqueous phase. Studies show that bioaccumulation of methamidophos in fish is insignificant.

Methamidophos exhibits a range of toxicity from practically non-toxic to plants, to very highly toxic to avian species. Methamidophos is considered:

- very highly to highly toxic to avian species on an acute oral basis (LC_{50}= 42 ppm)
- slightly toxic to very highly toxic to avian species on a subacute dietary basis
- highly toxic to mammals on an acute oral basis (LD_{50}=7.9 mg/kg-bw)
- highly toxic to bees on an acute contact basis (LD_{50} =1.37 µg/bee)
- slightly toxic to freshwater fish on an acute basis (LC_{50} =25,000 µg/L)
- very highly toxic to aquatic invertebrates on an acute basis (EC_{50}= 26 µg/L)
- practically non-toxic to plants (EC_{50}>50 ppm)

The California red-legged frog inhabits a mosaic of aquatic and upland habitat that it requires to complete its life history. This assessment considers direct and indirect effects on the frog and its critical habitat. To ensure clarity and ease of understanding this assessment, the lifecycle of the frog was separated into an aquatic phase and a terrestrial phase, as the exposure and effects modeling for these two ecosystems are different. The aquatic phase includes eggs, larvae, tadpoles, juveniles, and adults. Although juveniles and adults spend a significant amount of time in terrestrial habitats, they also use the aquatic portion of their habitat, especially during breeding. The terrestrial phase evaluation includes juveniles and adults. Components of the ecosystem addressed in the assessment include aquatic plants, aquatic invertebrates, fish, terrestrial plants, terrestrial invertebrates, and terrestrial vertebrates (e.g. small mammals,) in addition to the various life stages of the frog itself.

Aquatic Phase

Direct, acute effects to the aquatic phase CRLF are not expected as there are no 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 methamidophos use is not likely to adversely affect the aquatic phase CRLF, or its critical habitat.

Terrestrial Phase

Methamidophos 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, small amphibians and fish 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.

Methamidophos Effects Determination Summary

Assessment Endpoint	Effects determination	Basis for Determination
Aquatic Phase (Eggs, larvae, tadpoles, juveniles, and adults)		
Direct 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 and Critical Habitat 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

Assessment Endpoint	Effects determination	Basis for Determination
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 (23-26 counties). Use is documented in all months except November, December, January. 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 for mammals, insects, birds.
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.

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. This assessment was completed in accordance with the August 5, 2004 Joint Counterpart Endangered Species Act (ESA) Section 7 Consultation Regulations specified in 50 CFR Part 402 (USFWS/NMFS 2004; FR 69 47732-47762). 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 procedures outlined in the *Overview of the Ecological Risk Assessment Process in the office of Pesticide Programs* (U.S. EPA 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 methamidophos on potatoes, tomatoes, and alfalfa for seed. 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. It is one in a series of endangered species effects determinations for pesticide active ingredients involved in this litigation.

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 methamidophos 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 methamidophos 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 methamidophos 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 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 methamidophos 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 methamidophos.

If a determination is made that use of methamidophos 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 overlay of CRLF habitat with methamidophos use) and further evaluation of the potential impact of methamidophos 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 methamidophos is expected to directly impact living organisms within the action area (defined in Section 2.7), critical habitat analysis for methamidophos 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 jeopardize the continued existence of the species. Evaluation of actions related to use of methamidophos 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 and jeopardize the continued existence of the species have been identified by the Services and are discussed further in Section 2.6.

2.2 Scope

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 methamidophos in accordance with the approved product labels for California is "the action" being assessed.

Methamidophos was first registered in the United States in 1972 under the trade name Monitor. It was used principally on potatoes, cotton, and cole crops to control a broad spectrum of insects by inhibiting cholinesterase through contact. A Registration Standard, which describes the terms and conditions for the continued registration of methamidophos, was issued for methamidophos in 1982. In 1997, the technical registrant, Bayer Corporation, voluntarily cancelled all uses of methamidophos except for use on cotton, potatoes, and tomatoes (in California, a special local need 24(c) label only). In 1998, a special local need registration was issued for use on alfalfa grown for seed in California. By December 1999, the registrant had also voluntarily phased-in closed mixing and loading systems for all remaining uses to address potential worker exposures. Use of methamidophos on cotton was canceled based on the Interim Reregistration Eligibility Decision (IRED) published in 2002 (April 7), scheduled to be phased out within 5 years (67 FR 63423-4, Oct. 11, 2002).

> "Therefore, EPA expects that registrant will implement the risk mitigation measures as soon as possible. The IRED document describes, in detail, what is necessary measures, such as submission of label amendments for end-use products and submission of any required data. Mitigation measures for methamidophos include a phase out of methamidophos use on cotton by 2007. Should a registrant fail to implement any of the risk mitigation identified in the IRED document, the Agency may take regulatory action to address risk concerns from the use of methamidophos."

However, because the labels have not yet been amended to reflect the mitigation measures outlined in the IRED, cotton use will again be considered in this assessment as the published label is considered the most current description of legal, registered uses.

This ecological risk assessment is for currently registered uses of methamidophos 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 identified major degradates of methamidophos are S-methyl phosphoramidothioate (CAS Reg. No. 17808-29-6), O,S-dimethyl phosphorothioate (DMPT, CAS Reg. No. 42576-53-4), methyl mercaptan, dimethyl disulfide, and methyl disulfide. These degradates are not considered in this assessment due to (1) their rapid dissipation in the environment as shown in laboratory studies (hours to days), (2) the tendency of methyl mercaptan, dimethyl disulfide and methyl sulfide to partition to air, and (3) lack of toxicity data on S-methyl phosphoramidothioate and DMPT on which to base an assessment. It is anticipated that LOCs will be exceeded based on parent methamidophos alone.

2.2.2 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).

Methamidophos does not have any registered products that contain multiple active ingredients.

2.3 Previous Assessments

2.3.1 Methamidophos Assessments

Assessments of potential ecological risks were conducted to support the Re-registration Eligibility Decision (RED) and IRED for methamidophos in 1998[1] and 2002[2], respectively. Acute and chronic risks to birds and mammals, bees and other non-target beneficial insects, and some risk to freshwater and estuarine invertebrates were identified. To mitigate ecological risks to terrestrial birds and mammals, and to freshwater and estuarine invertebrates, EPA took the following actions in 2002:

- Phased out and canceled the cotton use over 5 years.
- For cotton during the phase-out period, reduced the maximum number of applications to 2 per season.
- For tomatoes, reduced the maximum number of applications to 4 per season.

[1] http://www.epa.gov/pesticides/op/methamidophos/efed1abc.pdf
[2] http://www.epa.gov/oppsrrd1/op/methamidophos.htm

The RED and IRED summarized methamidophos hazard to organisms as:

- very highly to highly toxic to avian species on an acute oral basis
- slightly toxic to very highly toxic to avian species on a subacute dietary basis
- highly toxic to mammals on an acute oral basis
- highly toxic to bees on an acute contact basis
- slightly toxic to freshwater fish on an acute basis
- very highly toxic to aquatic invertebrates on an acute basis

On March 31, 2004 EPA released an assessment of the potential effects of methamidophos to 26 listed Environmentally Significant Units (ESUs) of Pacific salmon and steelhead. That assessment concluded that methamidophos would have no effect on the species under consideration. While methamidophos 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. Methamidophos is among the first group of ten chemicals to be completed. For information regarding the other chemicals in this group[3] please see the relevant document.

2.4 Stressor Source and Distribution

Methamidophos is a colorless crystal with a melting point of 44.9ºC. The technical product (40%) is a colorless to pale yellow liquid with a mercaptan-like odor. Methamidophos is miscible in water, and soluble in isopropanol (>200 g/L at 20ºC), dichloromethane (>200 g/L at 20ºC), hexane (0.1 – 1 g/L), and toluene (2-5 g/L).

Case number: 0043
CAS registry number: 10265-92-6
OPP chemical code: 101201
Empirical formula: $C_2H_8NO_2PS$
Molecular weight: 114.12 g/mol
Vapor Pressure: 3.5×10^{-5} mm Hg at 25 ^{o}C
Trade and other names: Monitor, Tamaron
Technical registrants: Bayer CropScience

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

2.4.1 Environmental Fate Assessment

Information from laboratory studies indicates that methamidophos is not persistent in aerobic environments but may be more persistent in anaerobic aquatic environments where it will be associated with the aqueous phase. Terrestrial field dissipation studies for methamidophos and acephate (methamidophos is the major degradate of acephate) indicated that methamidophos was not persistent.

Aerobic soil metabolism is the main degradation process for methamidophos. Methamidophos degraded with a calculated half-life of 14 hours in a sandy loam soil at greater than the currently registered application rate (nominal application rate of 6.5 ppm, compared to the expected 0.5 ppm from the maximum label rate of 1 lb ai/A), producing the intermediate degradate S-methyl phosphoramidothioate, which is itself rapidly metabolized by soil microorganisms to carbon dioxide and microbial biomass (half-life of < 5 days). Supplemental information also identifies DMPT as a major degradate which is also rapidly degraded in soil (half-life of < 4 days). Methamidophos photodegrades rapidly on soil irradiated with a mercury vapor lamp (dark control-corrected half-life 63 hours); however, in sterile aqueous solutions, methamidophos photodegrades slowly (dark control-corrected half-life > 200 days) and is stable against hydrolysis at acid pHs. Hydrolysis degradates at neutral and alkaline pHs include O-desmethyl, DMPT, and the volatile degradate dimethyldisulfide.

Supplemental information showed that methamidophos degraded in anaerobic sandy loam sediment: pond water systems in the laboratory with a DT_{50} (degradation time in which 50% degrades)of 41 days. Observed major degradates in the same study were DMPT and O-desmethyl methamidophos, but their persistence could not be determined due to incomplete material balances after 3 months of anaerobic incubation. Carbon 14 [^{14}C] labeled residues were distributed between the water and sediment fractions with the majority of residues observed in the water phase in a ratio of approximately 10 to 1. This study was repeated with a silty clay sediment with similar results (incomplete mass accounting due to loss of methane), DT_{50} 7-14 days, and DT_{90} 58-93 days; the calculated half-life was 19.4 days. There are no acceptable data for the aerobic aquatic metabolism of methamidophos.

Soil dissipation of methamidophos (O,S-dimethyl phosphoramidothioate) was conducted under U.S. field conditions in four replicate bare plots of loamy sand soil from Washington. Dissipation of methamidophos was rapid in this study. The average measured zero time concentration was 322 parts per billion (ppb). Under field conditions in the loamy sand soil, methamidophos had a log-linear half-life of 0.49 days in soil. The observed DT_{50} of methamidophos was 0.33-1 days. No major transformation products were identified. In the 0-15 centimeter (cm) soil layer, two minor transformation products were identified: S-methyl phosphoramidothioate (O-desmethyl methamidophos) was a maximum average of 27.1 ppb and O,S-dimethyl phosphorothioate was a maximum average of 14.3 ppb each at day zero. In the 0-15 cm soil layer, no transformation products were detected after 1 day. In the 15-30 cm soil layer, dimethyl

phosphorothiate was detected once at 3.7 ppb at 3 days (single replicate). No transformation products were detected in the 30-46 cm soil layer.

Laboratory studies showed that bioaccumulation of methamidophos in largemouth bass was insignificant; the maximum bioconcentration factor of 0.09 times the water concentration in whole fish occurred on day 28 and decreased to <0.014 ppm in the fish (quantification limit) after one day depuration.

2.4.2 Environmental Transport Assessment

Potential transport mechanisms 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). Methamidophos was not included in these monitoring studies. 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, and modeled estimated concentrations in water and air are considered in evaluating the potential for atmospheric transport of methamidophos 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 perimeter less than 1,000 feet (the range for AgDRIFT and AGDISP Tier 1 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 far-field 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.

Methamidophos is very soluble (>200 grams per liter (g/L); 2.0×10^5 parts per million (ppm)) and very mobile octanol water coefficient (K_{oc} = 1.5) in the laboratory. Only one K_{oc} value is available, because methamidophos was adsorbed in only one of the five soils (a clay loam) used in the batch equilibrium studies. The methamidophos degradate DMPT is also very mobile (K_{oc} = 1.6); no data are available for O-desmethyl

methamidophos, but it is expected to have similar mobility as its parent compound. Because methamidophos and its degradates are not persistent under aerobic conditions, little methamidophos residue could be expected to leach to groundwater. If any methamidophos residues did reach ground water, they might be expected to persist based on an observed anaerobic aquatic DT_{50} of 41 days for methamidophos and undetermined persistence for DMPT and O-desmethyl methamidophos. Volatilization from soil or water is not expected to be a major route of dissipation for methamidophos because of its rapid metabolism in soil and its calculated Henry's constant (1.6×10^{-11} atm-m^3/mole).

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 Methamidophos) 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

Methamidophos is a restricted-use insecticide, which means that it can be used only by or under the direct supervision of applicators who have been trained and certified by the state in which the pesticide is applied. Use sites are limited to four crops in California: cotton, potatoes, and under FIFRA section 24c, tomatoes and alfalfa grown for seed.

Labeled Uses

For the current labeled uses, methamidophos is applied as a post-emergence foliar application during the growing season. Table 2-1 lists the current labels that define the

Federal Action, the labeled uses and their maximum application rates, maximum number of applications per season, and methods of application.

Table 2-1. Labeled Methamidophos Uses (all application timing is foliar)

Uses	Label	% ai	Max Application Rate (lb ai/Acre)	Max # of Applications per season	Application Interval	Method of Application
Alfalfa	CA980013	40	0.985	1	up to 3 days prior to placing bees in or around the field	Aircraft; Ground
Potatoes	264-729	40	1	4	Apply in a 7- to 10-day preventative program or as necessary	Aircraft, ground, sprinkler irrigation (chemigation for potatoes only)
Tomatoes (fresh market)	CA780163	40	1	4	7 to 10 days	Aircraft- Low volume spray
Tomatoes	CA780163	40	1	4	7 to 10 days	High volume ground sprayer- High volume spray
Cotton	CA-790188	40	1	2	as needed," "do not apply after 65% of the bolls are open	Chemigation by overhead irrigation systems

2.4.4.2. Use and Usage in California

Analysis of labeled use information is the critical first step in evaluating the federal action. The current label for methamidophos 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.

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[4], Doane (www.doane.com); the full dataset is not provided due to its proprietary nature), and the California's Department of Pesticide Regulation

[4] 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.

Pesticide Use Reporting (CDPR PUR) database[5] . 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 methamidophos by county in this California-specific assessment were generated using CDPR PUR data. Usage data are averaged together over the years 2002 to 2005 to calculate average annual usage statistics by county and crop for methamidophos, including pounds of active ingredient applied and base acres treated.

Methamidophos use is not distributed evenly in the state of California. Table 2-2. displays the average amount of the active ingredient applied annually in each county, with reported methamidophos use between 2002 and 2005. Only 24 of the 58 counties in California reported use of methamidophos between 2002 and 2005.

Table 2-2. Average annual pounds of methamidophos applied, the total number of records from 2002-2005 and the average annual acres treated.

County	Sum of Average Annual Pounds Applied	Sum of Number Records Pounds	Sum of Average Annual Acres Treated	Sum of Number Records Area
Alameda	0.03	1	-	0
Colusa	82.04	3	83.0	3
Fresno	12,783.91	758	15,852.7	758
Imperial	1,095.66	106	1,781.8	106
Kern	1,874.91	131	1,404.5	105
Kings	3,644.61	101	4,587.3	101
Los Angeles	310.20	21	391.9	21
Madera	26.51	2	33.5	2
Merced	841.86	98	1,347.2	98
Modoc	2,593.75	202	3,007.4	202
Monterey	158.13	81	250.6	81
Orange	54.90	9	75.0	9
Riverside	149.22	6	166.9	6
Sacramento	250.65	40	258.3	40
San Diego	876.72	68	1,128.3	68
San Joaquin	1,073.43	144	1,294.8	144
San Luis Obispo	18.59	3	18.8	3
San Mateo	13.88	6	14.0	6
Santa Barbara	429.38	85	546.7	85
Santa Clara	0.12	2	-	0
Siskiyou	1,123.24	110	1,358.5	110
Solano	703.44	50	743.2	50
Stanislaus	201.37	12	243.8	12
Sutter	1,388.22	76	1,535.0	76
Ventura	103.87	30	130.1	30
Yolo	4,395.68	304	4,540.7	300

[5] 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.

County	Sum of Average Annual Pounds Applied	Sum of Number Records Pounds	Sum of Average Annual Acres Treated	Sum of Number Records Area
			54.25*	4
Grand Total	34,194.30	2449	40,793.5	2420

* square feet (not acres)

The average annual acreage treated with methamidophos is these counties is summarized by crop use and county in California in Table 2-3. The number of acres treated with methamidophos annually in California is approximately 40,277 acres; the highest acreage treated annually occurs in Fresno County (approximately 15,852.7 acres), followed by Kings County (approximately 4,587.3 acres), Yolo (approximately 4,527.3 acres) and Modoc County (approximately 3,007.4 acres). Of the 24 counties with reported use, the highest use occurred in Fresno County where it was applied on alfalfa for seed, cotton, and tomato, followed by Kings County, where the reported uses were cotton and alfalfa for seed. Except for Imperial, the primary use for the remaining counties was on potato and tomato crops.

Table 2-3 Average annual acres treated by county in California, 2002-2005 (Cotton excluded)

County	Alfalfa	Cotton	Potato	Tomatoes	Grand Total
Colusa				83.0	83.0
Fresno	6,624.2	8,347.0		881.5	15,852.7
Imperial	996.0	275.8	108.9	-	1,380.6
Kern		143.8	635.1	625.7	1,404.5
Kings	4,048.0	539.3		-	4,587.3
Los Angeles			391.9	-	391.9
Madera		33.5		-	33.5
Merced		7.5		1,339.7	1,347.2
Modoc	3.5		3,003.9	-	3,007.4
Monterey				161.0	161.0
Orange				75.0	75.0
Riverside		18.6	148.3	-	166.9
Sacramento				256.8	256.8
San Diego			132.5	995.8	1,128.3
San Joaquin			297.0	997.8	1,294.8
San Luis Obispo			18.8	-	18.8
Santa Barbara			546.7	-	546.7
Siskiyou			1,354.8	-	1,354.8
Solano				743.2	743.2
Stanislaus				243.8	243.8
Sutter				1,535.0	1,535.0
Ventura			6.0	76.5	82.5
Yolo	30.3			4,4970	4,527.3
				54.25*	54.3

County	Alfalfa	Cotton	Potato	Tomatoes	Grand Total
Grand Total	11,702.0	9,365.3	6,643.6	12,511.6	40,276.8

* square feet (not acres)

Source: CDPR PUR 2007

Alfalfa

On alfalfa, one pre-bloom application per crop season at a maximum single application rate of 0.985 lb a.i./acre is allowed by the label (Table 2-1). Methamidophos applications on alfalfa occur from April through November in California, but most applications occur June through September (Figure 2-2).

Annually in California from 2001-2005 on average 10,908 pounds of active ingredient were applied to approximately 11,702.0 acres of alfalfa (Table 2-3); pounds applied annually ranged from 6,631 to 18,570 (Figure 2-3). The average application rate was 0.77 lbs ai/acre (Table 2-3). From 2002 through 2005, methamidophos was reportedly applied to alfalfa grown for seed in the following counties: Fresno, Imperial, Kings, Modoc, Sutter, and Yolo (Table 2-3).

Potatoes

On potatoes, methamidophos may be applied at a maximum single application rate of 1 lb a.i./acre: at a maximum of four applications per year the seasonal maximum rate is 4 lbs a.i./acre (Table 2-1). Methamidophos applications on potatoes occur year round in California but most applications occur January through October (Figure 2-2).

On average in California from 2002-2005, 6,555 pounds of a.i. was applied annually to 6,643.6 acres of potatoes (Table 2-3); pounds applied annually ranged from approximately 3,270 to 7,100. The average single application rate was 0.79 lb a.i./acre (Table 2-3). From 2002 through 2005, methamidophos was reportedly applied to potatoes in the following counties: Imperial, Kern, Los Angeles, Modoc, Riverside, San Diego, San Joaquin, San Luis Obispo, Santa Barbara, Siskiyou, Tulare, and Ventura (Table 2-2).

Tomatoes

Considering tomatoes grown both for the fresh market and for processing, the average annual pounds of methamidophos applied in California from 2002-2005 was 11,600 lbs and ranged from approximately 6,740 to 15,830 pounds annually (Figure 2-3). Methamidophos applications on tomatoes occur in California from March through October with most applications occurring May through July, and October (Figure 2-2).

Tomatoes (fresh market)

On tomatoes grown for the fresh market, the maximum single application rate is 1 lb a.i./acre at a maximum of 4 applications per crop cycle (season) the maximum application is 4 lbs a.i./acre per season (Table 2-1). There is a seven to ten day spray interval between applications. For the years 2002-2005, annual average of 4,211 lbs a.i. were applied to 5,790 acres (Table 2-3). The average single application rate was 0.76 lb

a.i./acre (Table 2-3). From 2002 through 2005, methamidophos was reportedly applied to tomatoes in the following counties: Fresno, Imperial, Kern, Madera, Merced, Monterey, Orange, Sacramento, San Diego, San Joaquin, Stanislaus, Ventura, and Yolo

Tomatoes (processing)
On tomatoes grown for processing the application rate and interval is the same as those grown for the fresh market (Table 2-1). For the years 2002-2005, an annual average of 5,125 lb a.i. were applied to an average 6,046 (Table 2-3). The average application rate was 0.85 lb a.i./acre (Table 2-3). From 2002 through 2005, methamidophos was reportedly applied to tomatoes grown for processing in the following counties: Colusa, Fresno, Kern, Merced, Sacramento, San Joaquin, Solano, Stanislaus, Sutter, and Yolo. The value for methamidophos use on tomatoes for processing in Yolo county is not included in this assessment due to a data transcription error in the data set which results in an estimate of over 400 lbs a.i./acre as an application rate.

Cotton

In 2000, 17,646 lb a.i. was applied to 2.2% of California's cotton acreage (23,153 acres). In 1998, 114,377 lb a.i. were applied to 116,850 acres (11.35%), and in 1999, 17,900 lb a.i. were applied to 24,861 acres (2.5%). Thus, there was a decline in usage from 1998 to 2000.

Table 2-4 below presents the maximum application rate and the range of the 95th percentile of the application rate. Most applications were at or below the rates on the label.

Table 2-4. Methamidophos Typical Usage (lb. ai./A) in California between 2002-2005

Site Name	Maximum Application Rate	Minimum of 95 Percentile Application Rate	Maximum of 95 Percentile Application Rate
Alfalfa	11.87	0.77	0.80
Broccoli	0.50	0.50	0.50
Brussels Sprout	0.99	0.99	0.99
Cabbage	0.80	0.80	0.80
Cantaloupe	0.40	0.40	0.40
Cotton	1.03	0.59	0.99
Greenhouse Flower	0.51	0.51	0.51
Greenhouse Transplants	0.12	0.12	0.12
Potato	1.54	0.79	0.99
Research Commodity	1.06	0.99	1.06
Sugarbeet	0.77	0.76	0.76
Tomato	7.05	0.79	1.09
Tomato, Processing	10.41	0.79	1.10
Unknown	0.99	0.99	0.99

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.

It is important to consider the timing of pesticide application relative to the life-cycle of the CRLF. The figure below shows the average amount (pounds) of methamidophos applied to each registered use, by month from 2003 to 2005 as reported in the California PUR database.

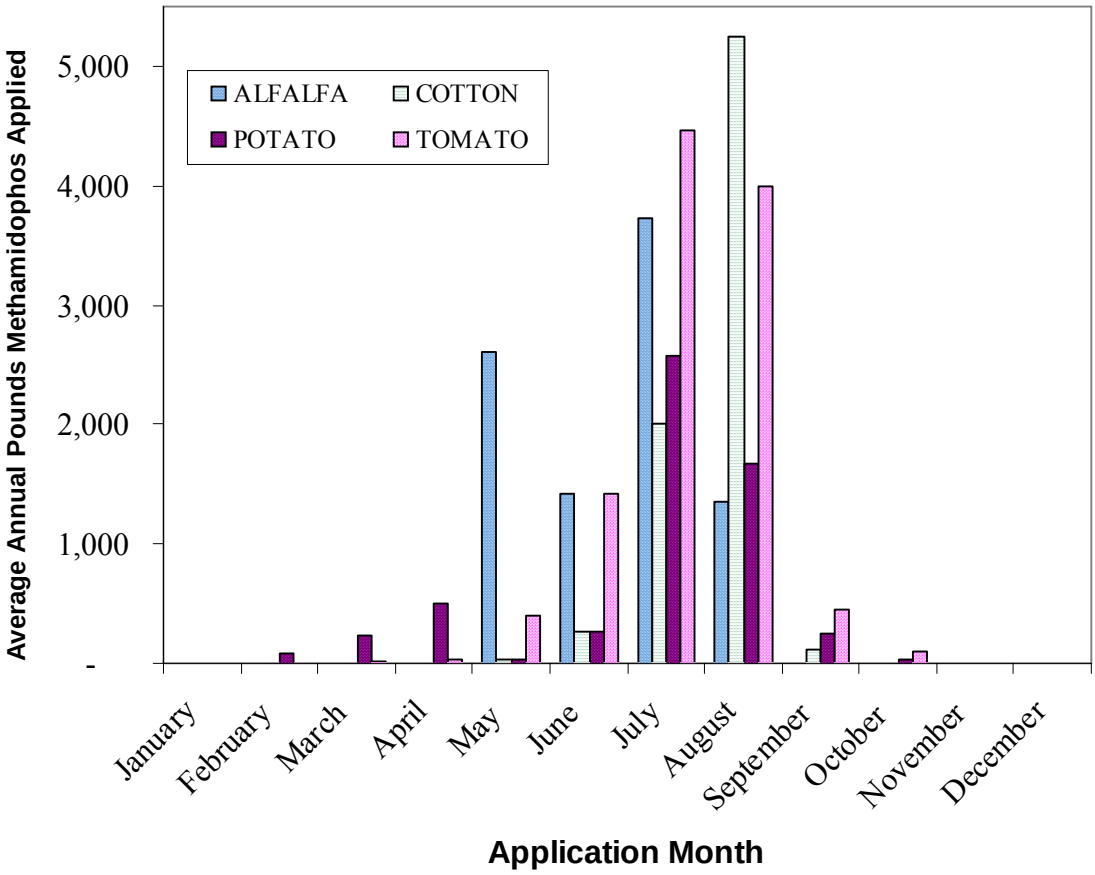


Figure 2A. Timing of Methamidophos Application: Average number of pounds of active ingredient applied in California for each registered crop, per month, between January 2003 through December 2005. Source: CDPR PUR 2007

In addition to considering the amount applied each month, the figure below show that the amount applied to each use varies annually and may not follow a predictable trend, although the total quantity applied each year has been decreasing over the last decade.

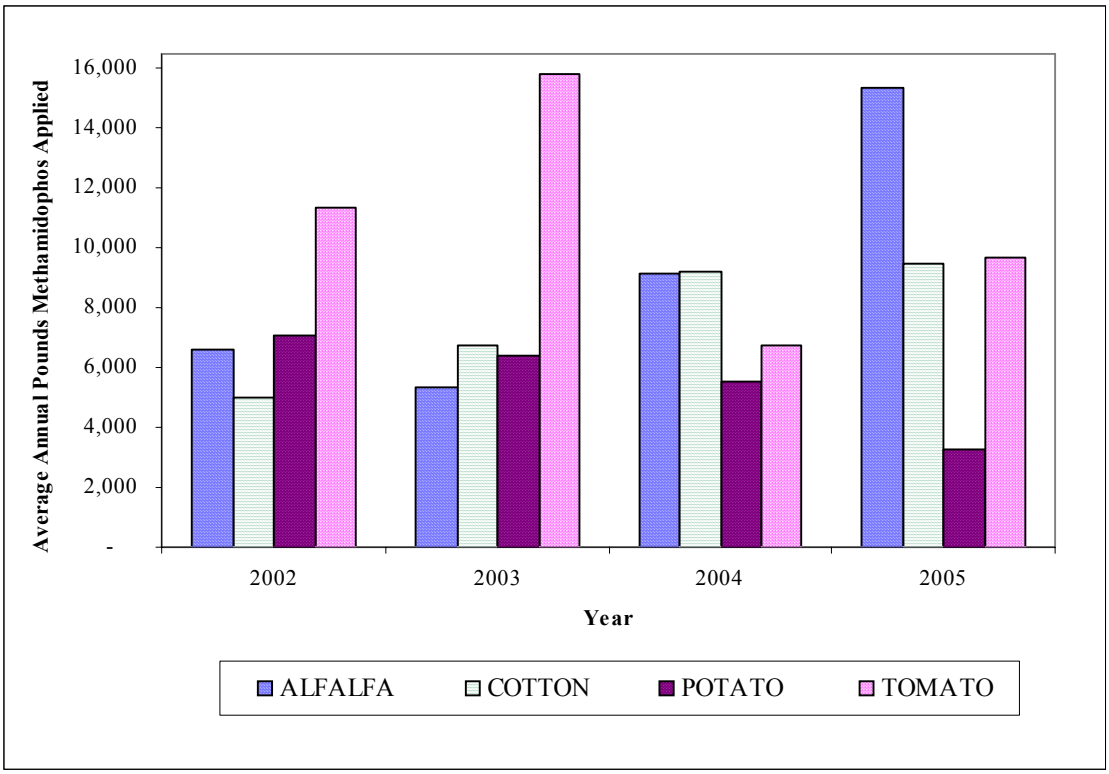


Figure 2B. Pounds of Methamidophos Applied Each Year by Crop .Source: CDPR PUR 2007

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.D)	**Core Areas [2,7] (Figure 2.D)**	**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)	**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	**Cottonwood Creek (8)**	--	✓	

Table 2.5. California Red-legged Frog Recovery Units with Overlapping Core Areas and Designated Critical Habitat				
Recovery Unit [1] (Figure 2.D)	**Core Areas [2,7] (Figure 2.D)**	**Critical Habitat Units [3]**	**Currently Occupied (post-1985) [4]**	**Historically Occupied [4]**
Foothills and Western Sacramento River Valley (2)	Putah Creek-Cache Creek (9)	--		✓
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	✓	
	Watsonville Slough- Elkhorn Slough (partial) (19)	SCZ-2 [5], MNT-1 [5]	✓	
	Carmel River-Santa Lucia (20)	MNT-2	✓	
	Estero Bay (22)	--	✓	
	Arroyo Grande Creek (23)	SLO-8	✓	
	Santa Maria River-Santa Ynez River (24)	--	✓	
Diablo Range and Salinas Valley (6)	**East San Francisco Bay (partial) (16)**	MER-1A-B	✓	
	--	SNB-1, SBB-2	✓[6]	
	Santa Clara Valley (17)	--	✓	
	Watsonville Slough- Elkhorn Slough (partial)(19)	--	✓	
	Carmel River-Santa Lucia (partial)(20)	--	✓	
	Gablan Range (21)	SNB-3	✓	
	Estrella River (28)	SLO-1	✓	
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]	

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

Recovery Unit [1] (Figure 2.D)	Core Areas [2,7] (Figure 2.D)	Critical Habitat Units [3]	Currently Occupied (post-1985) [4]	Historically Occupied [4]
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
[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.

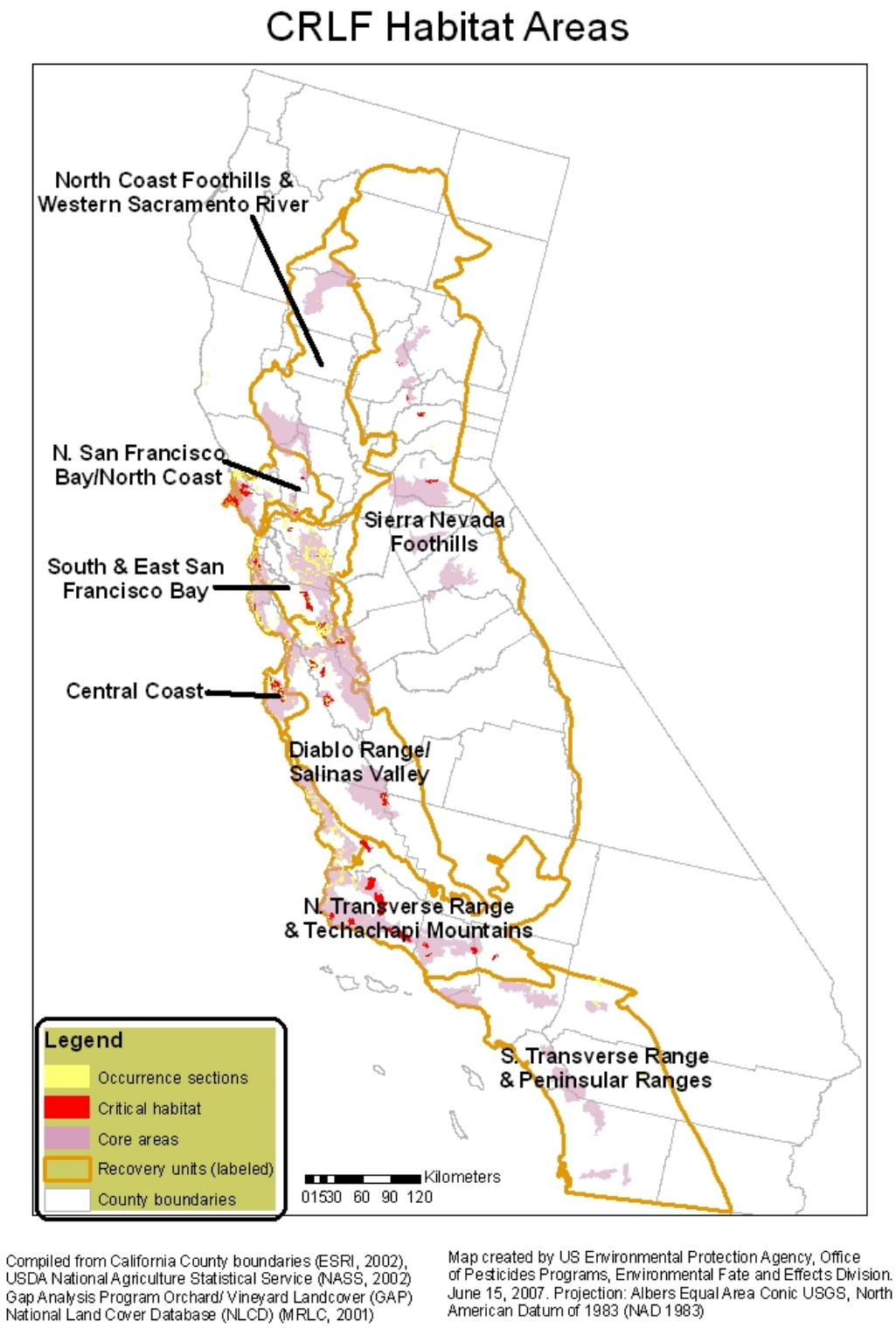


Figure 2C. CRLF Habitat areas

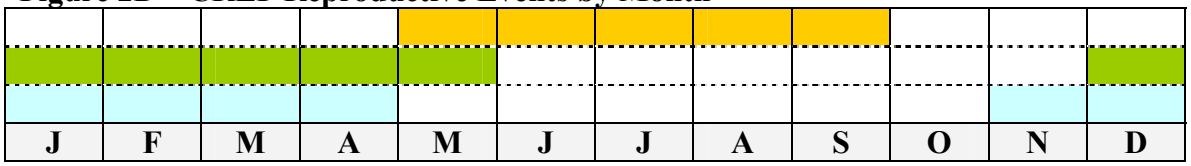
Other Known Occurrences from the CNDBB

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 2D depicts CRLF annual reproductive timing.

Figure 2D – 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 I.

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

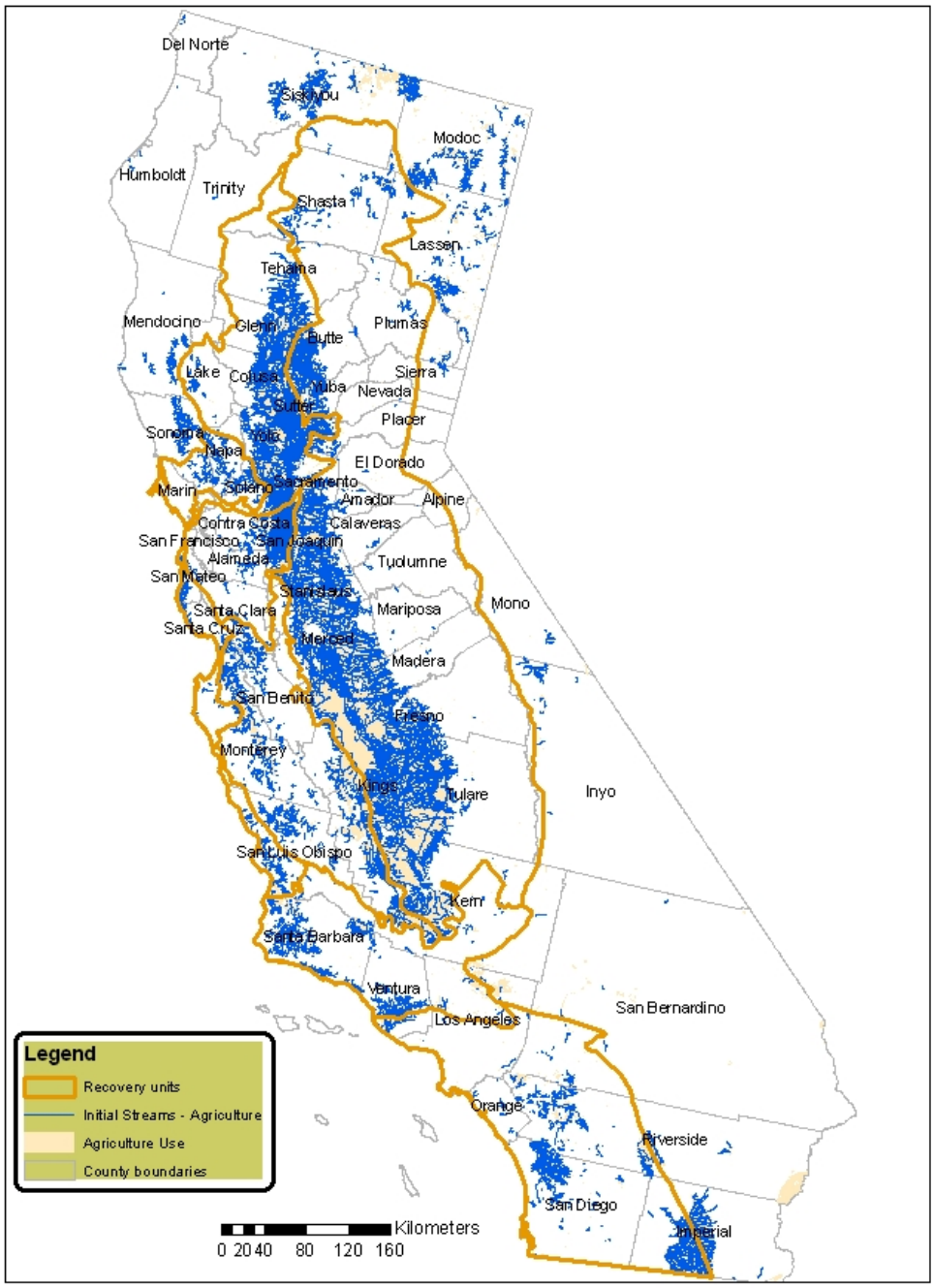
The definition of action area requires a stepwise approach that begins with an understanding of the federal action. The federal action is defined by the currently labeled uses for methamidophos. An analysis of labeled uses and review of available product labels was completed. This analysis indicates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

- Tomato
- Potato
- Alfalfa, for seed production
- Cotton

After 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 typically based on available land cover data. Local land cover data

available for the state of California were analyzed to refine the understanding of potential methamidophos use. The overall conclusion of this analysis is that there is an overlap between the use areas and known occurrences and critical habitat of the CRLF and therefore no areas are excluded from the final action area based on usage and land cover data. 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 2E.

Methamidophos Agriculture - Initial Area of Concern



Compiled from California County boundaries (ESRI, 2002), USDA National Agriculture Statistical Service (NASS, 2002) Gap Analysis Program Orchard/ Vineyard Landcover (GAP) National Land Cover Database (NLCD) (MRLC, 2001)

Map created by US Environmental Protection Agency, Office of Pesticides Programs, Environmental Fate and Effects Division. June XX, 2007. Projection: Albers Equal Area Conic USGS, North American Datum of 1983 (NAD 1983)

Figure 2E Initial Area of Concern for Methamidophos

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 methamidophos to determine which routes of transport are likely to have an impact on the CRLF.

The exceedances are then used to describe how far outside the initial area of concern effects may be seen. For example, AgDRIFT modeling can be used to define how far from the initial area of concern an effect to non-target terrestrial plants may be expected. Other processes considered in expanding the initial area of concern can include downstream distance where concentrations are expected to be above the LOC, long-range transport, and secondary exposure through biological vectors. The process of expanding the initial area of concern is repeated for all taxa where exceedances of the LOC occur, and the greatest expansion of the initial area of concern is considered the action area.

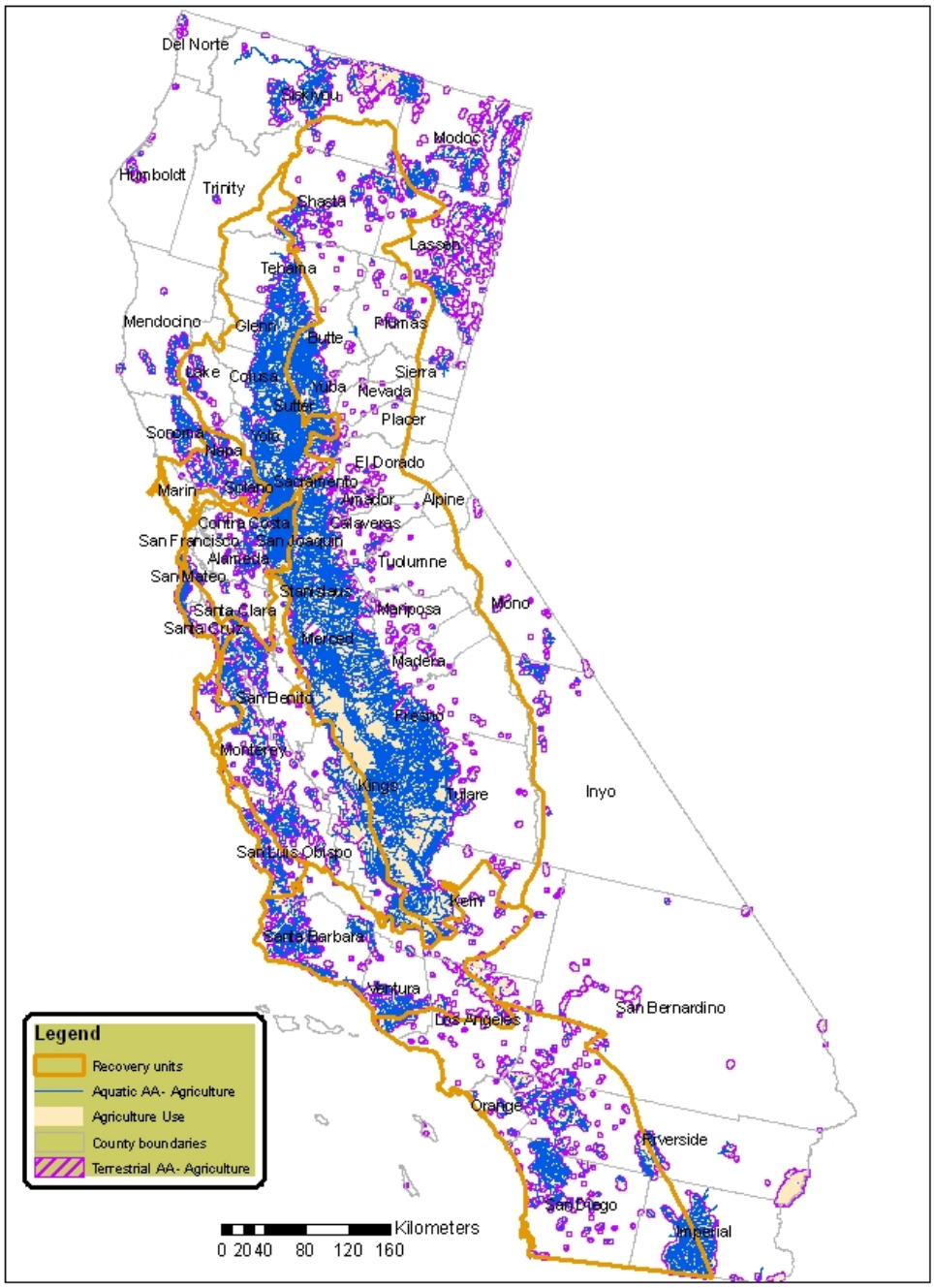
Review of the environmental fate data of as well as physico-chemical properties of methamidophos indicates that run-off and spray drift are likely to be the dominant mechanisms by which methamidophos is transported off-site. Methamidophos was detected in 10% of 168 samples taken in a 2002 air monitoring study in Fresno County, with a maximum value of 2.8 parts-per-trillion (ppt) by volume[6]. Methamidophos was not one of the pesticides included in eight long-range transport studies in the Sierra Nevada mountains. However, based on its low persistence, it is not anticipated that meaningful quantities of volatilized or resuspended methamidophos will be transported by the air route. Additionally, ground water transport is considered unlikely due to the non-persistence of methamidophos and its degradates, even when their mobility is considered.

These data suggest that the Action Area will be defined by spray drift perimeters from the aquatic and terrestrial exposure analysis, and by downstream dilution analysis of the ecological pond concentrations.

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 (Figure 2F).

[6] http://www.cdpr.ca.gov/docs/empm/pubs/tac/tacstdys.htm

Methamidophos Agriculture - Action Area (AA)



Compiled from California County boundaries (ESRI, 2002), USDA National Agriculture Statistical Service (NASS, 2002) Gap Analysis Program Orchard/ Vineyard Landcover (GAP) National Land Cover Database (NLCD) (MRLC, 2001)

Map created by US Environmental Protection Agency, Office of Pesticides Programs, Environmental Fate and Effects Division. June XX, 2007. Projection: Albers Equal Area Conic USGS, North American Datum of 1983 (NAD 1983)

Figure 2F Action Area for Methamidophos

Action Area Calculation

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 methamidophos, 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 5-4.

Minimum exposure = (RQ/Listed species LOC)*(1 lb/acre).

In the case of methamidophos, the target exposure is 0.00066 lb/acre, due to acute effects on avian species (including reptiles and terrestrial amphibians, acute RQ = 493).

The distance from the use site (sprayed field) needed to achieve the target exposure of 0.00066 lb/acre was calculated with the Gaussian Far-Field extension of the AgDISP model. The input parameters for AgDISP are given below; all other parameters were the default values.

Table 2-6. 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	0.083
Active fraction	0.033
Surface Canopy	None
Specific Gravity, Carrier	1.19
Deposition type	Terrestrial point
Initial Average Deposition	0.00066 lb/acre

The result of this analysis is that a perimeter of 7,241 feet from the edge of the sprayed field is needed to bring the acute mammal RQ to below the LOC of 0.1. Thus, the Action Area extends to a distance of 7,241 feet from the edge of fields sprayed with methamidophos.

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."[7] Selection of the assessment endpoints is based on valued entities (e.g., CRLF, organisms important in the life cycle of the CRLF, and the PCEs of its designated critical habitat), the ecosystems potentially at risk (e.g,. water bodies, riparian vegetation, and upland and dispersal habitats), the migration pathways of methamidophos (e.g., runoff, spray drift, etc.), and the routes by which ecological receptors are exposed to methamidophos-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 methamidophos is provided in Table 2.6.

Table 2.6 Summary of Assessment Endpoints and Measures of Ecological Effects for Direct and Indirect Effects of methamidophos on the California Red-legged Frog

Assessment Endpoint	Measures of Ecological Effects[8]	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} 1b. none available 1c. Rainbow trout (Acute-Chronic-Ratio)

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

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} 2a2. -*Daphnia magna* acute 48-hr 2a3. Skeletonema costatum algae (5-day) 2b1. *Daphnia magna* NOAEC 2b2. none available – use rainbow trout (Acute-Chronic-Ratio)
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
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)[9]	4a and b. Tier I seedling emergence and vegetative vigor
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 junco acute oral LD_{50} 5b. Mallard duck Reproductive study NOEL
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} 6a2. Rat Acute oral LD_{50} 6b1. None available 6b2. Rat 3- generation reproductive study NOAEL
7. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat (*i.e.,* riparian vegetation)	7a. Distribution of EC_{25} for monocots (seedling emergence, vegetative vigor, or ECOTOX 7b. Distribution of EC_{25} for dicots (seedling emergence, vegetative vigor, or ECOTOX)[5]	7a. and b. Tier I seedling emergence and vegetative vigor

[a] Adult frogs are no longer in the "aquatic phase" of the amphibian life cycle; however, submerged adult frogs are considered "aquatic" for the purposes of this assessment because exposure pathways in the water are considerably different that exposure pathways on land.
[b] Birds are used as surrogates for terrestrial phase amphibians.
[c] Although the most sensitive toxicity value is initially used to evaluate potential indirect effects, sensitivity

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

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 methamidophos 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 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 methamidophos effects data are available.

Assessment endpoints and measures of ecological effect selected to characterize potential modification to designated critical habitat associated with exposure to methamidophos are provided in Table 2.e. 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 methamidophos on critical habitat of the CRLF are described in Table 2.7. Some components of these PCEs are associated with physical abiotic features (e.g., presence and/or depth of a water body, or distance between two sites), which are not expected to be measurably altered by use of pesticides. Assessment endpoints used for the analysis of designated critical habitat are based on the adverse modification standard established by USFWS (2006).

Table 2.7. Summary of Assessment Endpoints and Measures of Ecological Effect for Primary Constituent Elements of Designated Critical Habitat	
Assessment Endpoint	**Measures of Ecological Effect[10]**
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)
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.[11]	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)
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)
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)
Terrestrial Phase PCEs (Upland Habitat and Dispersal Habitat)	
Elimination and/or disturbance of upland habitat; ability of habitat to support food source of CRLFs: Upland areas within 200 ft of the edge of the riparian vegetation or dripline surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance	a. Distribution of EC_{25} values for monocots (seedling emergence, vegetative vigor, or ECOTOX) 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.
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.	

[10] All toxicity data reviewed for this assessment are included in Appendix A and G.
[11] Physico-chemical water quality parameters such as salinity, pH, and hardness are not evaluated because these processes are not biologically mediated and, therefore, are not relevant to the endpoints included in this assessment.

2.9 Conceptual Model

2.9.1 Risk Hypotheses

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

- Labeled uses of methamidophos within the action area may directly affect the CRLF by causing mortality or by adversely affecting growth or fecundity;
- Labeled uses of methamidophos within the action area may indirectly affect the CRLF by reducing or changing the composition of food supply;
- Labeled uses of methamidophos 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 methamidophos 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 methamidophos, therefore methamidophos will have NO EFFECT on the CRLF based on these endpoints. For more information on plant toxicity studies, see Appendix A.

- Labeled uses of methamidophos 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 methamidophos 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 methamidophos 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 methamidophos 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 methamidophos 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 (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.F and 2.G, and the conceptual models for the aquatic and terrestrial PCE components of critical habitat are shown in Figures 2.H and 2.I. *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-persistent nature of methamidophos. Likewise, groundwater transport is considered unlikely due to the non-persistence of methamidophos, even when its mobility 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.

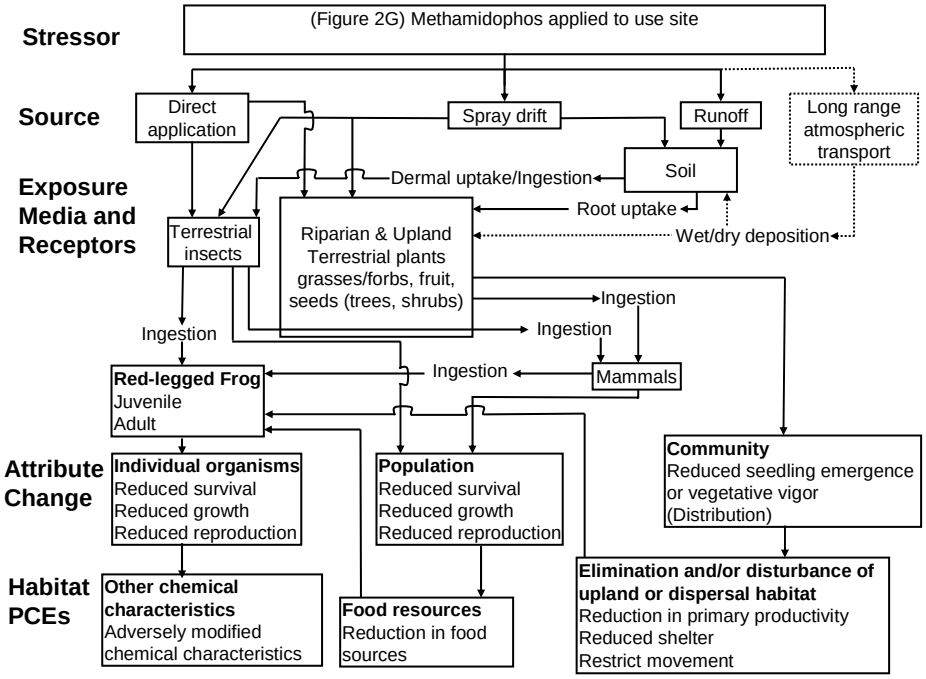


Figure 2G . Conceptual Diagram for Terrestrial Phase Effects on CRLF

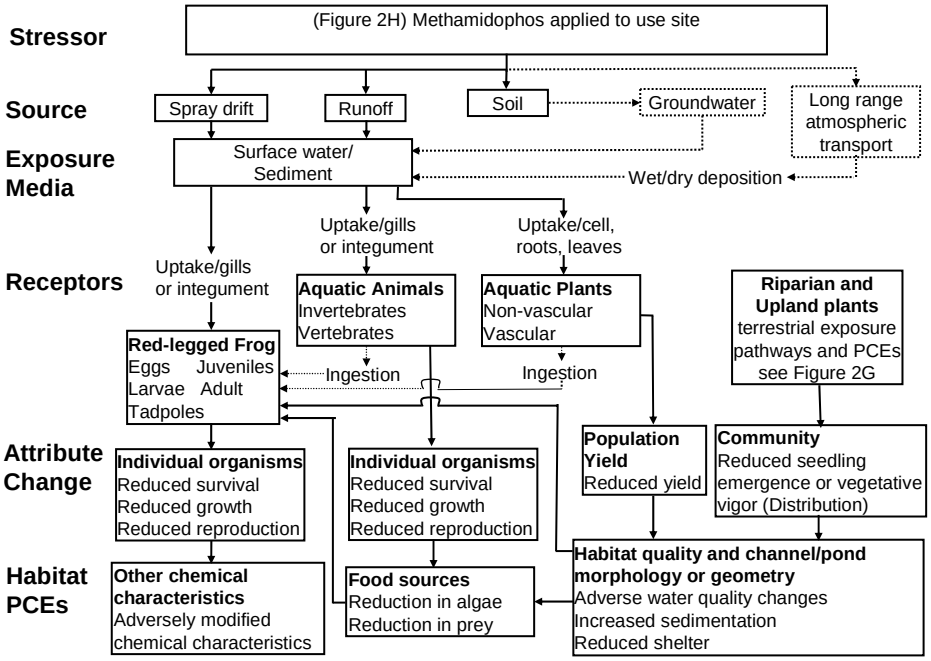


Figure 2H . Conceptual Diagram for Effects on Aquatic Phase CRLF

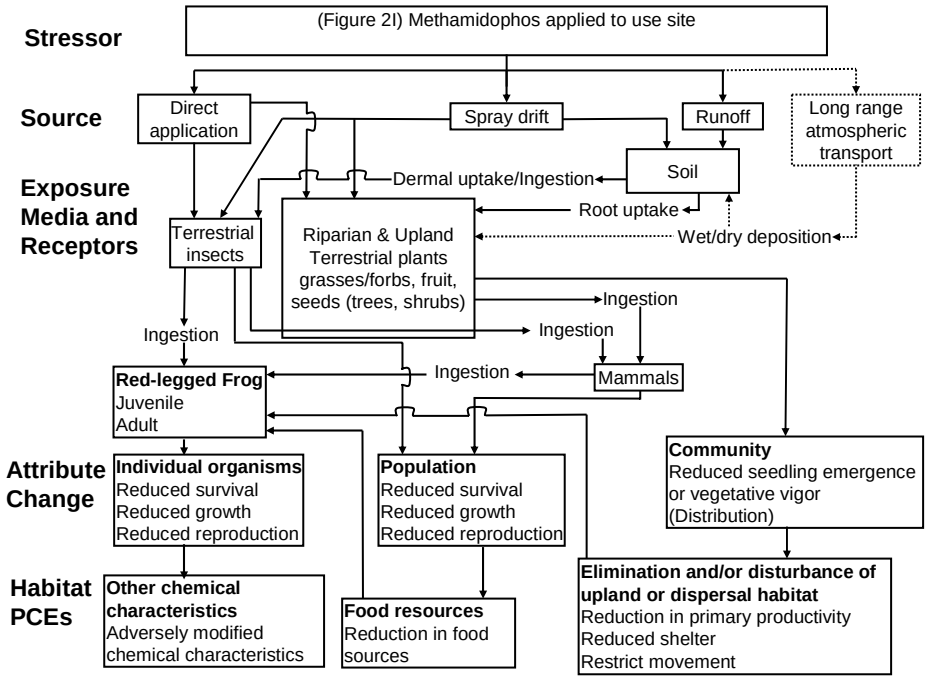


Figure 2 I . Conceptual Diagram for Effects Terrestrial Critical Habitat

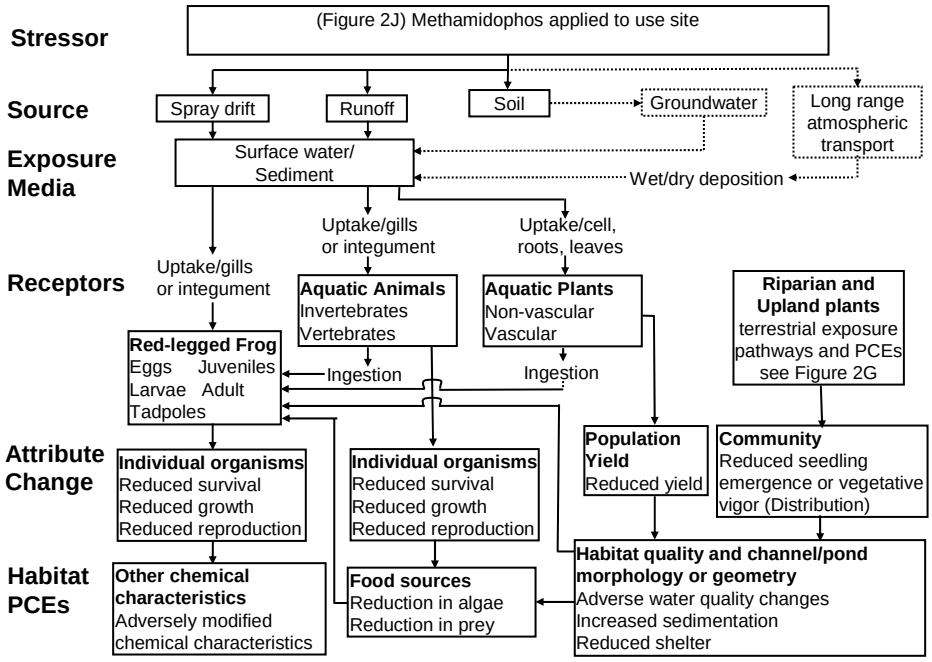


Figure 2 J . Conceptual Diagram for Effects on Aquatic Critical Habitat

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 assessment (USEPA 1998).

2.10.1 Exposure Analysis

Exposure in Aquatic Phase

Risks (direct effects) to the aquatic phase CRLF will be assessed by comparing modeled surface water exposure concentrations of methamidophos to acute and chronic (early life stage hatching success and growth) effect concentrations for aquatic phase amphibians (or surrogate freshwater fish) from laboratory studies (Tables 2.6 and 2.7). Risks to aquatic dietary food resources (aquatic invertebrates, algae) of the aquatic phase CRLF or risks to aquatic habitat that support the CRLF will also be assessed by comparing modeled surface water exposure concentrations of methamidophos to laboratory established effect levels appropriate for the taxa (Tables 2.6 and 2.7).

Surface water methamidophos concentrations will be quantified using a model, PRZM-EXAMS., For the screening assessment, the standard EXAMS water body of 2 meters maximum depth, and 20,000 cubic meters volume, will be used. Loading of

methamidophos into the surface water via run-off and spray drift is considered. Agricultural scenarios appropriate for labeled methamidophos uses (California potatoes, cotton, tomatoes, and alfalfa) will be used to account for local soils, weather and growing practices which impact the magnitude and frequency of methamidophos 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.

Measurement endpoint values which will be used to evaluate risks (direct effects) to the CRLF and to its aquatic prey and habitat (i.e., aquatic animals and plants) (Table 2.6 and Table 2.7) will be derived from registrant submitted laboratory toxicity studies, and studies from the scientific literature (ECOTOX database). If there are data gaps (e.g., no fish early life stage NOAEC), the best available method for extrapolating a value for the missing data will be used. Such extrapolation methods range from development of simple empirical models like acute-to-chronic ratios using methamidophos data for other taxa, or for the same taxa but based on other organophosphates to more complex empirical models such as ACE (acute effects) and ICE (chronic survival), or quatitative structure activity models (QSARs). The need to use such models (i.e., identification of data gaps), and which model to use will be determined as part of the Effects Analysis.

Surface water exposure concentrations and measurement endpoints will be compared quantitatively for RQ values. The RQs will be interpreted according to established Agency guidance (Levels of Concern).

Exposure in Terrestrial Phase

Risks to the terrestrial phase CRLF will be assessed by comparing modeled exposure to effect concentrations from laboratory studies. Risks to other Listed and non-Listed species will be assessed in the same way.

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 (ref). 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 6.5 days, the maximum found in Willis and McDowell (1987) will be substituted for the default 35-day value.

Exposure of terrestrial plants will be quantified using the TerrPlant model, which automates exposure comprising run-off and spray drift.

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 its habitat. Direct effects to the red-legged frog are based on toxicity information for freshwater fish and birds, which are generally used as a surrogate for aquatic and terrestrial phase amphibians, respectively. Effects on the CRLF and other potentially affected animals and plants will be assessed using toxicity endpoints derived from laboratory toxicity studies, and from the scientific literature (ECOTOX database). Assessment endpoints to be considered are mortality, and adverse effects on growth and reproduction. Sub-lethal effects will be considered if any are described in the laboratory studies or literature; effects that are not related to mortality, growth or reproduction may be considered only qualitatively.

Methamidophos' 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 ACRs (acute to chronic ratio) for methamidophos.

Indirect effects to the red legged frog are assessed by looking at available toxicity information of the frog's prey items and habitat requirements (freshwater invertebrates, freshwater vertebrates, aquatic plants, terrestrial invertebrates, terrestrial vertebrates, and terrestrial plants).

Exposure concentrations and effects thresholds will be compared quantitatively, and Risk Quotients (RQ) calculated if quantitative endpoints have been established. The RQs will be interpreted according to established Agency guidance (Levels of Concern).

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 methamidophos. To define the extent of the Action Area, the following exposure assessment tools will be used: PRZM-EXAMS, TREX, AgDrift, AgDISP (with far-field Gaussian extension), and ArcView, 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, methamidophos 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 methamidophos 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 methamidophos 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 for the land uses identified for methamidophos 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 methamidophos in California include cotton, tomatoes, potatoes, and alfalfa grown for seed. The relevant labels are EPA Reg. No. 264-729 (Monitor 4 Liquid Insecticide) for the use on potatoes, 24(c) label CA-790188 for cotton, 24(c) label CA-780163 for tomatoes, and 24(c) label CA-980013 for alfalfa grown for seed. The application rates, intervals, and frequency are summarized in Table 3-1.

Table 3-1. Label Use rates for Methamidophos in California

Use	Label	Application Rate, lb/acre	Number of applications allowed	Application Interval	Application Type
Potatoes	264-729	0.75 to 1.0 (1.5 to 2 pints product)	4 at maximum rate (implied by maximum seasonal rate of 8 pints)	"Apply in a 7- to 10-day preventative program or as necessary"	Aerial, Ground, Chemigation
Tomatoes	CA-780163	0.75 – 1.0	4	7 to 10 days	Aerial, Ground
Alfalfa for seed	CA-980013	1.0	"Do no make more than one pre-bloom application per crop season"	"up to 3 days prior to placing bees in or around the field"	Aerial, Ground
Cotton	CA-790188	1.0	2 per season	"as needed," "do not apply after 65% of the bolls are open"	Chemigation

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 and critical habitat in Figure 2.C.

3.2.1. Conceptual Model of Exposure

Aquatic exposure of the CRLF within the action area is estimated with the PRZM-EXAMS model consistent with the Overview Document (EPA, 2004). Estimated Environmental Concentrations (EECs) are produced using the standard farm pond of 20,000 cubic meters volume. Watersheds where methamidophos 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 diluting the pond concentration with flow from streams outside the use area.

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 LOC, a spray drift perimeter is calculated (using AgDrift model) that will reduce the pond concentration to below the LOC.

3.2.2 Existing Monitoring Data

There is very little useful water monitoring data for methamidophos, due to its non-persistent nature. The California Surface Water database and NAWQA have no data on methamidophos. The assessment will be based on modeled concentrations as described in section 3.2.1.

3.2.3 Modeling Approach

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. Where LOCs for direct/indirect effects and/or 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 (26) California-specific PRZM scenarios are available for this 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 PRZM scenarios were chosen for this assessment for each crop (potato, tomato, alfalfa, cotton) that represent agricultural areas in California. All scenarios are non-irrigated meaning that only natural precipitation drives the potential for run-off to the farm pond. The potato scenario was developed specifically for the CRLF assessments, and so may not be conservative for a national assessment, however it is representative of Kern County. Finally, the alfalfa scenario was developed for the organophosphate cumulative assessment, and so may not be conservative for a national assessment, however it is representative of the Central Valley. 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

The estimated water concentrations from surface water sources were calculated using Tier II 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 for all uses was set at March 1, to coincide with the frog's reproductive season, and a period of higher rainfall, so that exposure due to run-off was not underestimated.

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.

Table 3-2 Summary of PRZM/EZAMS Environmental Fate Data Used for Aquatic Exposure Inputs for Methamidophos CRLF Assessment		
Fate Property	**Value**	**MRID (or source)**
Molecular Weight	141.2	Calculated from structure
Henry's constant	1.62 x10 E-11 atm-m3/mole	MRID
Vapor Pressure	1.73 x 10 E-5 torr	MRID
Solubility in Water	200,000 mg/l	MRID 43661003
Photolysis in Water	200 days	MRID 00150610
Aerobic Soil Metabolism Half-lives	1.75 days	MRID 41372201
Hydrolysis	27 days	MRID 00150609
Aerobic Aquatic Metabolism (water column)	3.5 days	Per Input Parameter Guidance, 2x soil input value
Anaerobic Aquatic Metabolism (benthic)	19.4 days	MRID 46934002
Koc	0.88 ml/g	MRID 40504811
Application Efficiency	95 % for aerial 99 % for ground	Default value[c]
Spray Drift Fraction[b]	5 % for aerial 1 % for ground	Default value
Application method (CAM)	2	Foliar spray
Incorporation depth	0 cm	Foliar spray

Master Record Identification (MRID) is record tracking system used within OPP to manage data submissions to the Agency. Each data submission if given a unique MRID number for tracking purposes.
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 table below (3.3) presents the results of the PRZM-EXAMS modeling.

Table 3-3. Modeled Aquatic Exposures for Methamidophos with No Irrigation and March 1 as First Application Date

Crop	Aerial/Ground Application	Drift, %	Peak, ppb	21-day avg, ppb	60-day avg, ppb
Tomato 4 apps @ 7 days	A	5	11.6	5.8	2.7
		0	8.8	3.1	1.2
	G	1	9.7	3.8	1.6
		0	9.1	3.3	1.3
Potato 4 apps @ 7 days	A	5	5.2	3.5	1.9
		0	1.7	0.60	0.22
	G	1	2.5	1.1	0.55
		0	1.8	0.63	0.23
Seed Alfalfa 1 application	A	5	6.4	2.5	0.99
		0	4.2	1.5	0.61
	G	1	4.8	1.8	0.71
		0	4.3	1.6	0.63
Cotton 2 apps at 7 days	A	5	5.6	2.9	1.3
		0	3.0	1.1	0.44
	G	1	3.6	1.5	0.62
		0	3.1	1.2	0.46

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 and critical habitat in Figure 2.C.

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. Off-field exposure of animals is estimated with the AgDrift and AgDISP model.

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, 4 applications spaced at 7 days (or 1 application for alfalfa). The decay rate used on foliage and other food items was 6.5 days (Willis & McDowell, 1987, p. 45). Direct risk to the CRLF was bounded using 20-gram and 100-gram avian weight classes, since the weight of the frog falls in between these weights

(Fellers & Guscio, 2004). 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.

Indirect risk to the CRLF through effects on its prey base was estimated in two ways.

First, indirect effects via larger prey (small amphibian and mammal) were estimated conservatively using the 20-gram weight class for the amphibian and the 15-gram weight class for the mammal. The short-grass food category was used since it provides the highest dose. The dose (in lb/acre) needed to bring all RQs below their respective LOC (0.1 for acute, 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 perimeter 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.

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). The LOC for terrestrial invertebrates (insects) is 0.05.

3.3.3. Model Inputs

TREX model inputs included application rate (1 lb/acre) number of applications (1 to 4), application interval (7 days), and foliar decay rate (6.5 days).

3.3.4 Results

See Appendix C for T-REX details of EEC calculations. Summaries are given here

Direct Effects

Table 3-4 and 3-5 presents the results of the TREX analysis for direct effects.

Table 3-4. Potato and tomato EEC (ppm) at 1 lb ai/A appied 4 times with 7 day interval (maximum exposure)

Food items	20 gram bird	100 gram bird
Broadleaf plants/sm Insects	277.56	158.28
Fruits/pods/seeds/lg insects	30.84	17.59

Table 3-5. Alfalfa for Seed EEC (ppm) at 1 lb ai/A appied once (minimum exposure)

Food items	20 gram bird	100 gram bird
Broadleaf plants/sm Insects	153.75	87.68
Fruits/pods/seeds/lg insects	17.08	9.74

Indirect Effects

Table 3-6 and 3-7 presents the results of the TREX analysis for indirect effects.

Table 3-6. Potato and tomato EEC (ppm) at 1 lb ai/A appied 4 times with 7 day interval (maximum exposure)

Food Items	20 gram bird	15 gram mammal
Short Grass	493.45	157.94
Tall Grass	226.16	72.39
Broadleaf plants/sm Insects	277.56	88.84
Fruits/pods/seeds/lg insects	30.84	9.87

Table 3-7. Alfalfa for Seed EEC (ppm) at 1 lb ai/A appied once (minimum exposure)

Food Items	20 gram bird	15 gram mammal
Short Grass	273.34	228.82
Tall Grass	125.28	104.88
Broadleaf plants/sm Insects	153.75	128.71
Fruits/pods/seeds/lg insects	17.08	14.30

4. Effects Assessment

This assessment evaluates the potential for methamidophos to adversely affect the California Red-Legged Frog (CRLF). As described in Agency's Overview Document (U.S. USEPA, 2004) and evaluation by the U.S. Fish and Wildlife Service (USFWS/NMFS, 2004), the most sensitive endpoint for each taxa is evaluated. As previously discussed in Section 2.7, assessment endpoints for the CRLF include 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.

According to the Recovery Plan for the California Red-Legged Frog, CRLF are sensitive to salinity. When the eggs are exposed to salinity levels greater than 4.5 parts per thousand, there is 100% mortality. Therefore, this assessment will not evaluate estuarine species.

For this assessment, evaluated taxa include freshwater fish (surrogate for aquatic phase of CRLF), freshwater aquatic invertebrates, birds (surrogates for terrestrial phase of CRLF), small mammals, terrestrial invertebrates, algae, and terrestrial plants. Given that the frog's prey items and habitat requirements are dependent on the availability of small mammals and frogs, insects, algae, aquatic invertebrates; toxicity information for aquatic and terrestrial plants (habitat) and food items are also discussed. Acute (short-term) and chronic (long-term) toxicity information is characterized based on registrant-submitted studies and a comprehensive review of the open literature on methamidophos. In addition to registrant-submitted and open literature toxicity information, indirect effects to CRLF, via impacts to aquatic terrestrial plant community structure and function are also evaluated based on community-level threshold concentrations. Other sources of information, including use of the acute probit dose response relationship to establish the probability of an individual effect and reviews of the Ecological Incident Information System (EIIS), are conducted to further refine the characterization of potential ecological effects associated with exposure to methamidophos. Currently, no guideline tests exist for frogs, and no frog data were available for methamidophos; thus, surrogate species, freshwater fish and birds, are used as described in the Overview Document (U.S. EPA, 2004). In addition, section 4.3 discusses available frog toxicity data for other organophosphates. A summary of the available ecotoxicity information, the community-level endpoints, use of the probit dose response relationship, and the incident information for methamidophos are provided in Sections 4.1 through 4.4, respectively.

4.1 Evaluation of Ecotoxicity Studies: Aquatic and Terrestrial

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

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

Data that pass the ECOTOX screen are evaluated along with the registrant-submitted data, and may be incorporated qualitatively or quantitatively into this endangered species assessment. In general, effects data in the open literature that are more conservative than the registrant-submitted data are considered. In addition, data for taxa that are directly relevant to the California Red-Legged Frog (i.e., aquatic-phase and terrestrial-phase amphibians) were also considered. The degree to which open literature data are quantitatively or qualitatively characterized is dependent on whether the information is relevant to the assessment endpoints (i.e., maintenance of California Red-Legged Frog survival, reproduction, and growth) identified in Section 2.7. For example, endpoints such as behavior modifications are likely to be qualitatively evaluated unless quantitative relationships between modifications and reduction in species survival, reproduction, and/or growth are available.

Table 4.1 summarizes the most sensitive ecological toxicity endpoints for the CRLF, based on an evaluation of both the submitted studies and the open literature, as previously discussed. A brief summary of submitted and open literature data considered relevant to this ecological risk assessment for the CRLF is presented below. Additional information is provided in Appendix A, A1 and G.

Table 4.1 Methamidophos 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	25,000 µg/L ai	Supplemental (most sensitive)	MRID 00041312 (Nelson & Roney, 1979)
	Freshwater fish early life-stage NOAEC	Rainbow trout	173.6 µg/L 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} = 26 µg/L ai	Supplemental (Most sensitive)	MRID 00041311 (Nelson & Roney 1979)
	Freshwater invertebrate reproductive NOAEC	*Daphnia magna*	4.5 µg/L 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} >50,000 µg/L ai NOEC = 50,000 µg/L 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} = 7.92 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).

Table 4.2 Levels of Concern for Terrestrial and Aquatic Organisms

Taxa	Listed Species 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

Used in RQ calculations:
[1] LD_{50} and estimated NOAEL
[2] LD_{50} and NOAECL
[3] NOAEC
[4] LC/EC_{50} and estimated and reproductive NOAEC

4.2. Evaluation of Aquatic Effects

No guideline tests exist for frogs. The available open literature has no information on methamidophos toxicity to aquatic-phase amphibians. Fish toxicity from open literature shows that acute and chronic ecotoxicity endpoints are generally less sensitive than the

registrant submitted fish studies. A summary of acute and chronic freshwater fish data, including sublethal effects, is provided below.

4.2.1 Toxicity to Freshwater Fish

4.2.1.1. Freshwater Fish: Acute Exposure (Mortality) Studies

Freshwater fish acute toxicity studies were used to assess potential direct effects to the CRLF. Methamidophos toxicity has been evaluated in some freshwater fish species, including rainbow trout, bluegill sunfish, and carp, and the results of these studies demonstrate a narrow range of sensitivity. The range of acute freshwater fish LC_{50} values for methamidophos is from 25,000 to 68,000 µg/L; therefore, methamidophos is categorized as slightly (>10,000 to 100,000 µg/L) toxic to freshwater fish on an acute basis. The freshwater fish acute **LC_{50}** value of **25,000 µg/L** is based on a static 96-hour toxicity test using rainbow trout (*Oncorhynchus mykiss*) (MRID 00041312, Nelson, 1979). No sublethal effects were reported as part of this study. A complete list of all the acute freshwater fish toxicity data for methamidophos is provided in Appendix A.

4.2.1.2. Freshwater Fish: Chronic Exposure (Growth/Reproduction) Studies

Since there are no chronic data for freshwater fish, an acute to chronic ratio (ACR) was determined. Methamidophos is an organophosphate insecticide. The EFED 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 was chosen since the most sensitive fish acute endpoint for methamidophos is rainbow trout. Nineteen organophosphates were found that have both an acute and chronic study for rainbow trout. The ACR ranged from 0.28 for oxydemeton-methyl to 511.0 for sulprofos. In order to provide the most conservative estimate for the chronic freshwater fish NOEC for methamidophos, the ACR of 511 will be used to estimate the NOEC for rainbow trout. The estimated chronic NOEC for rainbow trout as derived from and ACR of 511 and a LC50 of 25 is **0.0489 ppm or 48.9 µg/L.**

The following section presents the methodology used in deriving an avian ACR for organophosphates, the group to which methamidophos belongs, that was used to extrapolate a chronic fish NOAEC for methamidophos. 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 methamidophos. 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 methamidophos is derived as follows. The (methamidophos) rainbow trout LC_{50} used in this assessment is 25 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 methamidophos.

Estimated Trout NOEC for methamidophos = 25,000/144 = **1.736 µg ai/L**

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

Acute to Chronic Table for Organophosphates

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

Chemical	**96-hr LC_{50} (µg ai/L)**	**MRIDs**	**NOAEC (µg ai/L)**	**MRIDs**	**ACR**
Azinphos methyl	8.8	03125193	0.29	00145592	30.344
Coumaphos	890	40098001	11.7	43066301	76.068
Dichlorvos	750	43284702	5.2	43788001	144.23
Dimethoate	7,000	TN 1069*	430	43106303	17.441
Disulfoton	1,850	40098001	220	41935801	8.4090
Fenamiphos	68	40799701	3.8	41064301	17.894
Fenitrothion	2,000	40098001	46	40891201	43.478
Fenthion	830	40214201	7.5	40564102	110.66
Fonofos	50	00090820	4.7	40375001	10.638
Isofenphos	1,800	00096659	153	00126777	11.764
Phosmet	105	40098001	3.2	40938701	32.812
terbufos	7.6	40098001	1.4	41475801	5.4285

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

4.2.1.3. Freshwater Fish: Sublethal Effects and Additional Open Literature Information

The open literature ECOTOX did not identify any data that report sublethal effect levels to freshwater fish that are less sensitive than the selected measures of effect summarized in Table 4.1 for methamidophos. Appendix G provides the reasons for the rejection of studies identified using the ECOTOX database.

4.2.2. Toxicity to Freshwater Invertebrates

Freshwater aquatic invertebrate toxicity data were used to assess potential indirect effects of methamidophos to the CRLF. Adverse effects to freshwater invertebrates resulting from exposure to methamidophos may indirectly affect the CRLF via reduction in available food. As discussed in the CRLF Life History, Attachment 1, the CRLF aquatic-

phase larvae (tadpoles) is presumed to be an algae grazer consuming diatoms, algae, and detritus. 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. Most frequently encountered were: carabid (11) and tenebrionid (9) beetles; water striders (9); lycosid spiders (7); larval neuropterans (*e.g.,* alderflies) (7). 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 ECTOX is provided below in Sections 4..2..2.1 through 4.2.2.3.

4.2.2.1 Freshwater Invertebrates: Acute Exposure Studies

The most sensitive acceptable study (MRID 00041311, Nelson, 1979) found the *Daphnia magna* **LC_{50} to be 26 µg ai/L (200–34).** Two other *Daphnia magna* were tested with the LC_{50} found to be 27 to 50 µg ai/L (MRID 00041311, 00014110) which is similar to the most sensitive endpoint. Appendix A provides a summary and description of other freshwater invertebrate studies not used in the RQ calculations. Methamidophos is classified as very highly toxic to freshwater invertebrates on an acute basis.

4.2.2.2. Freshwater Invertebrates: Chronic Exposure Studies

A submitted freshwater invertebrate life-cycle study (MRID 46554501, Kern, 2005) using *Daphnia magna* was reviewed. Despite there being some questions regarding the concentration levels of study, the reviewer believes that the results are acceptable enough to use for risk assessment.

The **NOEC is found to be 4.49 µg ai/L (0.0045 ppm)** for 21-day dry weight, 21-day immobility, and 21-day reproduction endpoint. The LOEC is 53 µg ai/L (0.053 ppm) for all of the above endpoints.

4.2.2.3. Freshwater Invertebrates: Open Literature Data

In addition to submitted studies, data were located in the open literature[12] that report effect levels to freshwater invertebrates that are less than the selected measures of effect summarized in Table 4.1. This sensitive endpoint was not used since the mortality in the controls ranged from 60% to 80% which indicate the study to be not very sound. No sublethal effects to freshwater aquatic invertebrates were found in open literature for methamidophos.

[12] Juarez, L.M., J. Sanchez, 1989. Toxicity of the Organophosphorous Insecticide Methamidophos (O,S-Dimethyl Phosphoramidothioate) to Larvae of the Freshwater Prawn, *Macrobachium rosenbergii* (DeMan) and the Blue Shrimp, *Penaeus stylirostris* Stimpson. Bull. Environ. Contam. Toxicol. (1989) 43:302-309.

4.3. Toxicity to Birds

There are no registrant submitted nor open literature data on methamidophos 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.

4.3.1. Birds: Acute Exposure (Mortality) Studies

Avian acute toxicity studies were used to assess potential direct effects to the CRLF. Methamidophos 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 narrow range of sensitivity. The range of acute oral LD_{50} values for methamidophos is from 1.78 mg/kg-bw to 29.5 mg/kg-bw. The range of subacute dietary LC_{50} is from 42 ppm to 1650 ppm; therefore, methamidophos is categorized as very highly to highly toxic to avian species on an acute oral basis (<10 mg/kg-bw to 10-50 mg/kg-bw) to birds and as slightly toxic to very highly toxic to avian species on a subacute dietary basis.

4.3.2. Acute Oral LD_{50}

Based on professional judgment, the lower 95% confidence limit on the **acute oral LD_{50} of 4.1 mg/kg-bw** (MRID 00144428) for the common grackle was selected to evaluate acute oral risks to birds and terrestrial-phase amphibians. The common grackle study was selected because it had the most scientifically sound lowest acute oral value. Though classified as supplemental, the study covered a larger portion of the dose-response curve (i.e., 6 doses) and control results indicated handling and environmental conditions were sound. To address concerns that the results were potentially not as precise as a guideline study because fewer birds were tested the 95% lower confidence limit on the LD_{50} (4.1 mg/kg-bw) was selected for use rather than the mean LD_{50} study result (6.7 mg/kg-bw). (note: however, it is unknown if fewer common grackles would need to be tested to achieve the same precision as with mallards and bobwhite quail in guideline studies). For a more detailed discussion of studies considered but not selected for use in RQ calculations, see Appendix A1.

4.3.3. Avian sub acute dietary endpoint analysis

The most sensitive avian LC_{50} is an acceptable bobwhite quail study (MRID 00093904, Beavers, 1979) with an **LC_{50} of 42 ppm** (34 – 52). The study shows a dose response slope of 3.4. Noted in the study, was the observation that the birds were too sick to eat when exposed to methamidophos. Another bobwhite study (MRID 00014064) reported that repellency was observed at 826 ppm. Other bobwhite studies show LC_{50} values of 57.9 and 59 ppm which is near the most sensitive LC_{50} value, thus supporting the selection of the chosen LC_{50} used in RQ calculations. The mallards tend to be less sensitive with LC_{50} values ranging from 848 to 1650 ppm. The Japanese quail LC_{50} has a

LC_{50} value of 92 ppm which is comparable in magnitude to the bobwhite studies. Methamidophos is considered to be very highly toxic to quail and slightly toxic to mallard ducks on a sub acute dietary basis.

A complete list of all the acute bird toxicity data for methamidophos is provided in Appendix A.

4.3.4 Birds: Chronic Exposure (Reproduction) Studies

Similar to the acute data, chronic avian toxicity studies would be used to assess potential direct effects to the CRLF because direct chronic toxicity guideline data for frogs do not exist. The most sensitive avian reproductive study is a bobwhite quail (MRID 00014114, Beavers, 1978) with a **NOEL of 3 ppm** and a LOEL of 5 ppm. The NOEL was based on eggshell thickness, embryo viability, embryo development, hatchability, and survivability of hatchlings. There does not appear to be a palatability problem in this study (personal communication Nancy McCarroll, HED, 2/10/98).

4.3.5 Birds: Sublethal Effects and Additional Open Literature Information

In addition to submitted studies, data on sublethal effects data were located in the open literature on birds but effects are observed at similar exposure rates or less sensitive than those selected as measures of effect summarized in Table 4.1. Stromborg (ECOTOX ref. 40022) shows northern bobwhites to have eggs laid affected by methamidophos at 7.8 ppm and NOEL of 5 ppm. This would confirm reproductive endpoint of 3 ppm selected.

4.4 Toxicity to Mammals

Toxicity data on small mammals is used in this assessment to assess their availability as a food items for the CRLF.

4.4.1. Mammals: Acute Exposure (Mortality) Studies

The mouse studies (MRID 00014047, 1968; MRID00014048, 1968) have similar LD_{50} values as the most sensitive rat studies (00014044, 1968) with LD_{50} of 16.2 mg/kg-bw and 18 mg/kg-bw for the mouse and 15.6 mg/kg-bw (male) and 13.0 mg/kg-bw (female), respectively. Since the CRLF diet includes small mammals like a small mouse and the adjusted LD_{50} (7.92 mg/kg-bw) value is more sensitive than the rat LD_{50}(13.0 mg/kg-bw) the LD_{50} value chosen is from the mouse study (MRID 00014048, 1968) with **LD_{50} of 16.2 mg/kg-bw**.

4.4.2. Mammals: Chronic Exposure (Reproduction) Studies

A two-generation rat reproductive study (MRID 00148455, 41234301; 1984) found the **NOAEL to be 0.5 mg/kg/day (10 ppm)** and the LOAEL to be 1.65 mg/kg/day (33 ppm). The NOEL was based on decrease in number of births, pup viability and pup body weight.

4.4.3. Mammals: Sublethal Effects and Additional Open Literature Information

In addition to submitted studies, no data more sensitive than the selected measures of effect summarized in Table 4.1 were located in the open literature.

4.5 Toxicity to Insects

Toxicity data on insects is used in this assessment to assess their availability as a food items for the CRLF.

A honey bee acute contact study (MRID 00036935, Atkins, 1975) found an **LD_{50} of 1.37 µg/bee**. The dose response slope is 10.32. Methamidophos is categorized as highly toxic to bees on an acute contact basis.

4.6 Toxicity to Plants

4.6.1 Toxicity to Aquatic Plants

Aquatic plant toxicity studies were used as one of the measures of effect to evaluate whether methamidophos may affect primary production. Primary productivity is essential for indirectly supporting the growth and abundance of the CRLF aquatic phase. In addition to providing cover, other aquatic plants harbor a variety of aquatic invertebrates that CRLF may eat.

<u>Aquatic Plants: Laboratory Data</u>

There are no aquatic plant studies submitted for methamidophos. There are no aquatic plant studies found in ECOTOX literature database. Acephate, another organophosphate and of which methamidophos is the primary degradate, was found to have an aquatic plant study, *Skeletonema costatum*, which is a marine diatom. **The EC_{50} is greater than 50 ppm** (Mayer, 1986; MRID 40228401). This 96-hr static study was found to have an EC_{50} value greater than 50,000 ppb; it appears that methamidophos is practically nontoxic to aquatic plants. This study is considered to be supplemental due to lack of available raw data.

4.6.2. Terrestrial Plants

Phytotoxicity tests of methamidophos exposure to numerous plant species (seedling emergence and vegetative vigor) 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 methamidophos is 1.0 lb/A and it is relatively short-lived in the environment. Based on the results of the submitted terrestrial plant toxicity tests, it appears that seedlings and emerged plants are not sensitive to methamidophos and effects to both aquatic and

terrestrial plants will not be considered in this assessment. For more information on plant toxicity studies, see Appendix A and A1.

4.7 Aquatic and Terrestrial Field Studies

4.7.1. Terrestrial Field Studies

Perritt (MRID 41548803) considered the aerial application of Monitor 4 on cotton at 1 lb ai/A with 8 day intervals applied 7 times in Alabama. Thirty percent of the placed carcasses were found. EFED concluded that thirty-four casualties were found during the study at eight test fields. Ten of the casualties were found during pre-application periods, and six were found post application under circumstances that did not indicate that exposure to Monitor 4 Spray was a potential cause of mortality. Only one casualty was found under circumstances suggesting that it was likely treatment related. Cause of death could not be determined for another seventeen casualties, but exposure to Monitor 4 Spray could not be precluded as a potential cause of mortality and therefore the study is classified as supplemental.

Die-offs of sage grouse (*Centrocercus urophasiannus*) were noted in 1981 near potato fields sprayed with methamidophos (Blus et al, 1989). Five intoxicated sage grouse were collected and inhibition of brain ChE activity ranged from normal to 61%. Although methamidophos half-life is <4 days, low levels of methamidophos may persist for several weeks in plants. Thus, intoxicated grouse may be exposed to additional residues when ChE reversal is initiated and the grouse resumes feeding on the contaminated foliage. According to the authors, these findings suggest that OP insecticides may adversely affect sage grouse populations whose summer range include cropland. The authors also noted that this study may provide some evidence for the claim that pesticides are partly responsible for the declining populations of upland game birds in the U.S. and Europe.

Adult radio-equipped hens were released near potato fields and compared with radio-equipped hens in Tule Lake National wildlife Refuge during the summers of 1990 – 1992 (Grove et al, 1998). Hens were monitored after methamidophos application to potato fields and later captured. Measurements of Brain AChE were taken. Direct toxicity of the radio- equipped adult hens did not occur. Two juveniles (not radio-equipped) were found dead as a result of methamidophos exposure. Brain AChE activity inhibition in the captured hens ranged from 19% to 62%. Six of the pheasants had inhibition of brain AChE that is greater than 55%. Twenty-five of the 41 adult pheasants captured within 20 days of spray application had detectable methamidophos residues on food items taken from their upper GI tract. Seven of the adults had food items that ranged from 0.18 to 2.10 ppm (wet basis). Hens captured near potato fields that were sprayed appear to have lost weight when compared to controls. It appears that the application of methamidophos have impacted the availability of food items for the birds and juveniles. None of the radio-equipped hens died as a direct result of methamidophos exposure or predation. In addition, authors concluded that most of the nesting failures of radio-equipped hens occurred prior to insecticide applications.

In a study comparing methamidophos and permethrin, Temple and Palmer (1995) conclude that methamidophos applications (1 lb ai/A) have equal or less adverse impact on avian reproduction than the permethrin insecticide (which is practically not toxic to vertebrates) which was used as the control. This study was limited to the European Starling reproduction and did not address the other species in the area. This study also is designed not to look at acute toxicity but focused on reproductive endpoints. There was some avian mortalities in the study but it is not apparent if these mortalities are chemical related. Fourteen percent of the post application blood samples $\geq$ 50% ChE inhibition. These findings suggest that animals that have greater exposure to contaminated food, or are more sensitive to OP pesticides than are starlings, could die from ChE inhibition.

4.7.2 Aquatic Field Studies

In a field study evaluating the effects of acephate and methamidophos, (Hussain, et al. 1985), backswimmer (aquatic insect) and rainbow trout displayed ChE inhibition for 4 hours before recovery began. This suggests that aquatic insects and fish that are exposed to acephate/methamidophos may not recover by spontaneous reactivation of AchE. Therefore aquatic insects or fish may be stressed for some time because of physiological effects caused by inhibition of AchE.

4.8 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 methamidophos 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.

4.9 Incident Database Review

A number of incidents have been reported in which methamidophos has been associated with some type of environmental effect. Incidents are maintained and catalogued by EFED in the Ecological Incident Information System (EIIS). As of the writing of this assessment, 17 incidents are in EIIS for methamidophos spanning the years 1985 to 2000. Most (11/17, 65%) of the incidents involved bee kills. Of the remaining 6 incidents, 4 involved bird mortalities and 2 involved plants. One plant incident involved another herbicide that may have caused the plant damage and another incident involved having methamidophos residues on a crop that does not have any established tolerances for methamidophos. These incidents are summarized in Appendix E.

5. Risk Characterization

Risk characterization is the integration of the exposure and effects characterizations to determine the potential ecological risk from varying methamidophos 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 is estimated by calculating the ratio of the expected environmental concentration and the appropriate toxicity endpoint. This value is the risk quotient (RQ), which is then compared to pre-established levels of concern (LOC) for each category evaluated. The RQ methodology, LOCs , and specific details of the calculations are contained in Appendix F. The highest EECs and most sensitive endpoints are used to determine the screening level RQ. Using these two values theoretically results in a conservative estimate of risk. Risk quotients are presented in 5.1.1. (direct effect) and in 5.1.2. (indirect effect).

Table 5.1. Levels of Concern for Terrestrial and Aquatic Organisms

Taxa	Listed species 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	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

5.1.1 Direct Effects

5.1.1.1 Aquatic Phase.

Direct effects to the CRLF in the aquatic phase were estimated using exposure estimates from PRZM-EXAMS and surrogate fish toxicity. For acute effects, the fish LC50 endpoint was used. For chronic effects, there were no data (fish early life stage study) for methamidophos. Therefore a chronic endpoint (NOAEC) for the fish was estimated from the Acute-to-Chronic ratios for other organophosphate insecticides. See

section 4.1 for details. There are no LOC exceedences for direct acute or chronic risk to CRLF aquatic-phase from the use of methamidophos, based on RQ's calculated using freshwater fish (LC_{50} = 25,000 ppb) as a surrogate for the aquatic phase of the frog. Tables 5-2 and 5-3 below give the exposures, endpoints and risk quotients for acute and chronic effects, respectively.

Table 5-2. Acute Risk Quotients for Fish in Freshwater Environments

Crop	Application	Peak EEC, ppb	Risk Quotient
Potato	Aerial	5.2	0.00021
	Ground	2.5	0.0001
Tomato	Aerial	11.6	0.0046
	Ground	9.7	0.00039
Alfalfa	Aerial	6.4	0.00026
	Ground	4.8	0.00019
Cotton	Aerial	5.6	0.00022
	Ground	3.6	0.00014

Table 5-3. Chronic Risk Quotients for Fish (NOEC = 48.9 ppb) in Freshwater Environments

Crop	Application	21-day average EEC, ppb	Risk Quotient
Potato	Aerial	3.5	0.07
	Ground	1.1	0.02
Tomato	Aerial	5.8	0.12
	Ground	3.8	0.08
Alfalfa	Aerial	2.5	0.05
	Ground	1.8	0.04
Cotton	Aerial	2.9	0.06
	Ground	1.5	0.03

Notes: (a) Estimated from acute-to-chronic ratio for other OP insecticides.
(b) Exceeds Chronic LOC (1)

5.1.1.2 Terrestrial Phase.

Direct effects to the terrestrial phase CRLF were estimated using TREX, assuming that the frog was represented by a bird weighing 20 or 100 grams, and consumed a diet of small insects. Invertebrates make up the bulk of the CRLF diet. Indirect risk to the CRLF via effects on prey items, such as the tree frog and mouse are considered below (section 5.1.2).

Tables 5-4 and 5-5 below summarizes the direct risks to the CRLF. The TREX results are given in Appendix C.

Table 5-4. Summary of Direct, Acute and Chronic Risks to Terrestrial Phase CRLF, as represented by effects to avian species. Potato and tomato EEC (ppm) at 1 lb ai/A applied 4 times with 7 day interval (maximum exposure)

Effect	Endpoint	Size Class (grams)	Food Item	EEC (ppm)	RQ (a)
Acute	LD50 (3.67) (mg/kg-bw)	20	Small insect	277	**75.7**
			Large insect	31	**8.4**
	LD50 (4.67) (mg/kg-bw)	100	Small insect	158	**33.9**
			Large insect	18	**3.8**
Subacute Dietary	LC50 (42) (ppm)	--	Small insect	244	**5.8**
			Large insect	27	**0.64**
Reproductive	NOAEC (3) (ppm)	--	Small insect	244	**81.2**
			Large insect	27	**9.0**

Notes: (a) Bold RQ values exceed LOC for listed species.
(b) LOC for acute is 0.1 and for chronic is 1.0

Table 5-5. Summary of Direct, Acute and Chronic Risks to Terrestrial Phase CRLF, as represented by effects to avian species. Alfalfa EEC (ppm) at 1 lb ai/A appied once (minmum exposure)

Effect	Endpoint	Size Class (grams)	Food Item	EEC (ppm)	RQ (a)
Acute	LD50 (3.67) (mg/kg-bw)	20	Small insect	153.7	**41.9**
			Large insect	17.1	**4.7**
	LD50 (4.67) (mg/kg-bw)	100	Small insect	87.7	**18.8**
			Large insect	9.7	**2.1**
Subacute Dietary	LC50 (42) (ppm)	--	Small insect	135	**3.2**
			Large insect	15	**0.36**
Reproductive	NOAEC (3) (ppm)	--	Small insect	135	**45.0**
			Large insect	15	**5.0**

Notes: (a) Bold RQ values exceed LOC for listed species.
(b) LOC for acute is 0.1 and for chronic is 1.0

The RQ's in the table above are based on protective assumptions, modeled with highest and lowest labeled uses and consumption of the most contaminated part of the frog's diet (i.e. small insect and large insect). Even with a minimal number of applications (1), the calculated RQ's still exceed the LOC for Listed species.

Both acute and chronic direct effects are expected from terrestrial phase exposure.

5.1.2 Indirect Effects.

5.1.2.1. Aquatic Phase

In the aquatic phase, CRLF larvae are thought to be algal grazers, like other amphibians (Recovery Plan, p. 16). Aquatic plant data indicate no significant difference from

controls. Therefore no RQs are calculated. Because there are no adverse effects expected on aquatic plants, there is No Effect on the CRLF based on these endpoints.

Sub-adult and adult CRLF consume invertebrates. Since acute RQs for freshwater invertebrates range up to 0.45 (Table 5-6), 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 below), indirect risk to the CRLF via effects on aquatic invertebrates is considered "NLAA."

Adverse or toxic effects to other aquatic animals were estimated using acute and chronic endpoints for the appropriate test species. Tables 5-6 and 5-7 below give the exposures, endpoints and risk quotients for acute and chronic effects, respectively. The chronic RQ for invertebrtates exceeds the LOC for the tomato use with aerial application, only. Thus, adverse reproductive effects are expected for this use.

Table 5-6. Acute Risk Quotients for Invertebrates (LC_{50} = 26 ppb)in Freshwater Environments

Crop	Application	Peak EEC, ppb	RQ
Potato	Aerial	5.2	**0.20**
	Ground	2.5	**0.10**
Tomato	Aerial	11.6	**0.45**
	Ground	9.7	**0.37**
Alfalfa	Aerial	6.4	**0.25**
	Ground	4.8	**0.18**
Cotton	Aerial	5.6	**0.22**
	Ground	3.6	**0.14**

Notes: (a) bold RQs exceed Listed Species LOC (0.05)
(b) Maximum RQ/LOC ratio for Action Area Downstream Dilution Analysis is 0.45/0.05 = 8.9.

Table 5-7. Chronic Risk Quotients for Invertebrates (NOEC = 4.5 ppb) in Freshwater Environments

Crop	Application	21-day average EEC, ppb	RQ
Potato	Aerial	3.5	0.78
	Ground	1.1	0.24
Tomato	Aerial	5.8	**1.29 (a)**
	Ground	3.8	0.84
Alfalfa	Aerial	2.5	0.40
	Ground	1.8	0.40
Cotton	Aerial	2.9	0.64
	Ground	1.5	0.33

Notes: (a) Exceeds Chronic LOC (1)

5.1.2.2. Terrestrial Phase

As described in the Exposure Assessment, indirect effects to CRLF through its diet are assessed via adverse toxic effects on its prey items, namely small birds, mammals, amphibians (represented by the bird) , and terrestrial invertebrates. The frog and mouse are represented in TREX by a 20-gram bird and 15-gram mammal, respectively. The short grass food item category was chosen because it gives the highest, and therefore most conservative exposure. Table 5-8 below summarizes the acute and chronic risks to the CRLF via effects on these prey items. The complete TREX output is given in Appendix C.

Table 5-8. Summary of Acute and Chronic Risks to Terrestrial Phase Prey Animals that Consume Short Grass Food Category (Maximum Use, Tomato and Potato)

Prey	Risk Category	EEC (ppm)	RQ (a)	Listed Species LOC	LOC/RQ for Action Area
20-g bird or frog	Acute, Dose-based	493	**134.5**	0.1	0.0007 (b)
	Chronic, Dietary	433	**144.4**	1	0.007
15-g mammal	Acute, dose-based	413	**23.8**	0.1	0.004
	Chronic, Dietary	433	**43.3**	1	0.02
	Chronic, dose-based	413	**375.9**	1	0.003

Notes: (a) Bold RQs indicate values above Listed species LOC.
(b) Lowest LOC/RQ ratio will be used to calculate terrestrial Action Area.

Indirect effects to the CRLF through the invertebrate portion of its diet may be estimated by comparing the contact LD50 for the honey bee (1.37 micrograms/bee) to the EEC calculated for large insects by TREX (27 micrograms/gram)*(0.128 grams body weight for the bee) = 3.46 micrograms. The RQ is then EEC/LD50 = 3.46/1.37 = **2.52**. The small insect EEC (244 ppm) is used to bound this estimate, giving an LD50 of (244)*(0.128) = 31.2 micrograms. **The RQ is then 31.2/1.37 = 22.8**. Both of these RQ values are well above the LOC (0.05), so toxic effects on terrestrial invertebrates are presumed. The label for methamidophos (EPA Reg. No. 264-729) does indicate that the product is "highly toxic to bees," and that it should not be applied if bees are visiting an adjacent field. Indirect effects to the CRLF via adverse effects on its terrestrial prey base are expected as multiple components of the CRLF diet, including invertebrates, mammals, and birds, may be affected..

5.1.2.3 Effects on Critical Habitat

Effects on Critical Habitat that will be considered are limited to those that are biologically mediated. PCE #2 (alteration of chemical quality) is affected by contamination with methamidophos. PCE #5 (alteration of upland habitat) may be affected by loss of prey items, and by loss of mammals burrows for shelter due to adverse effects on small mammals. PCE #7 (Alteration or elimination of the CRLF's food sources or prey base) is affected in the terrestrial environment via effects on prey animals and insects.

5.1.3 Individual Effect Chance Calculation

The chance of an individual mortality for a CRLF, based on the T-REX surrogate of a 20-gram or 100-gram bird, was calculated using the IECv1.1 Excel spreadsheet. Diet consisted of small or large insects. The slopes used were 7.4 for the acute toxicity data, based on a bobwhite quail study, and 3.4 for the subacute dietary study, based on Japanese quail. The results are given below in tables 5-9a and 5-9b. At the calculated risk quotients, the chance of individual mortality approaches 100%.

Table 5-9a Individual Effect Probability Calculation for CRLF

Surrogate Organism	Small Insect RQ	Chance of Effect, 1-in…	Large Insect RQ	Chance of Effect, 1-in…
20-gram bird	75.7	1	9.49	1
100-gram bird	33.9	1	4.25	1
Sub acute dietary	5.8	1	0.64	1
Level of Concern (slope 7.4)	0.1	1.47E+13	0.1	1.47E+13
Level of Concern (slope 3.4)	0.1	2970	0.1	2970

Table 5-9b Individual Effect 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, tomato aerial RQ	4.5 (default)	0.45 (RQ)	16.9
Daphnia magna, tomato ground RQ	4.5 (default)	0.37 (RQ)	38.5
Honey bee at LOC	10.32	0.05 (LOC)	4.74E+40
Honey bee, Large insect EEC	10.32	2.52 (RQ)	1
Honey bee, Small insect RQ	10.32	22.8 (RQ)	1
15-gram mammal	4.5 (default)	0.1 (LOC)	2.94E+5
15-gram mammal	4.5 (default)	23.8 (RQ)	1

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 methamidophos'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 CRLF

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 5-1 and 5-2).

5.2.1.2. Terrestrial Phase

Risk Quotients for terrestrial-phase CRLF, as represented by 20-gram and 100-gram birds, greatly exceed LOC for both acute and chronic (reproductive) effects (Table 5-4 and 5-5). Acute RQs range from 0.6 to 75.7 for CRLF for maximum exposure from tomato and potato (1 lb ai/A applied 4 times with 7 day interval) and from 0.36 to 41.9 for a minimum exposure of 1 lb ai/A applied once onto alfalfa fields. Chronic RQs range from 9.0 to 81 for CRLF for maximum exposure from tomato and potato (1 lb ai/A applied 4 times with 7 day interval) and from 5 to 45 for a minimum exposure of 1 lb ai/A applied once onto alfalfa fields. Both mortality and adverse reproductive effects to the CRLF are anticipated based on labeled uses of methamidophos and risk quotients.

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):

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

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

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.

T-REX model shows that the ranges of direct affects to birds as surrogate for CRLF is from 3.6 to 72.4 for dose-based acute, from 0.6 to 5.6(LOC for listed terrestrial animals) for dietary acute, and from 8.6 to 77.7 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 0.05 to 79.72 (LOC for listed terrestrial animals) for the dose-based acute, 0.19 to 6.5 for dietary acute, and from 2.7 to 91.0 for chronic dietary.

The refinement of models show a slight decrease in RQs in T-HERPS but the LOC for CRLF is still exceeded (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 Potato and Tomato (Maximum Exposure)

Table 5-10a. Upper Bound Kenaga, Acute Terrestrial Herpetofauna Dose-Based Risk Quotients

Size Class (grams)	Adjusted LD50	EECs and RQs									
		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects		Small Herbivore Mammals		Small Insectivore Mammal		Small Amphibians	
		EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
1.4	1.98	9.47	**4.78**	1.05	**0.53**	N/A	N/A	N/A	N/A	N/A	N/A
37	3.24	9.31	**2.87**	1.03	**0.32**	270.07	**83.37**	16.88	**5.21**	0.32	**0.10**
238	4.28	6.10	**1.42**	0.68	**0.16**	41.98	**9.80**	2.62	**0.61**	0.21	**0.05**

Table 5-10b. Upper Bound Kenaga, Subacute Terrestrial Herpetofauna Dietary Based Risk Quotients

LC50 (ppm)	EECs and RQs				
	Broadleaf Plants/ Small Insects	Fruits/Pods/ Seeds/ Large Insects	Small Herbivore Mammals	Small Insectivore Mammals	Small Amphibians

	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
42	243.71	**5.80**	27.08	**0.64**	285.50	**6.80**	17.84	**0.42**	8.46	**0.20**

Size class not used for dietary risk quotients

Table 5-10c. Upper Bound Kenaga, Chronic Terrestrial Herpetofauna Dietary Based Risk Quotients

NOAEC (ppm)	EECs and RQs									
	Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects		Small Herbivore Mammals		Small Insectivore Mammals		Small Amphibians	
	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
3	243.71	**81.24**	27.08	**9.03**	285.50	**95.17**	17.84	**5.95**	8.46	**2.82**

Size class not used for dietary risk quotients

Bold RQs exceed Listed species LOC

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

Table 5-11a. Upper Bound Kenaga, Acute Terrestrial Herpetofauna Dose-Based Risk Quotients

Size Class (grams)	Adjusted LD50	EECs and RQs									
		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects		Small Herbivore Mammals		Small Insectivore Mammal		Small Amphibians	
		EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
1.4	1.98	5.24	**2.65**	0.58	**0.29**	N/A	N/A	N/A	N/A	N/A	N/A
37	3.24	5.15	**1.59**	0.57	**0.18**	149.60	**46.18**	9.35	**2.89**	0.18	**0.06**
238	4.28	3.38	**0.79**	0.38	**0.09**	23.26	**5.43**	1.45	**0.34**	0.12	0.03

Table 5-11b. Upper Bound Kenaga, Subacute Terrestrial Herpetofauna Dietary Based Risk Quotients

LC50 (ppm)	EECs and RQs									
	Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects		Small Herbivore Mammals		Small Insectivore Mammals		Small Amphibians	
	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
42	135.00	**3.21**	15.00	**0.36**	158.15	**3.77**	9.88	**0.24**	4.69	**0.11**

Size class not used for dietary risk quotients

Table 5-11c. Upper Bound Kenaga, Chronic Terrestrial Herpetofauna Dietary Based Risk Quotients

NOAEC (ppm)	EECs and RQs									
	Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects		Small Herbivore Mammals		Small Insectivore Mammals		Small Amphibians	
	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ

3	135.00	**45.00**	15.00	**5.00**	158.15	**52.72**	9.88	**3.29**	4.69	**1.56**

Size class not used for dietary risk quotients

Bold RQs exceed Listed species LOC

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 0.45 (Table 5-6), 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 below), indirect risk to the CRLF via effects on aquatic invertebrates is considered "NLAA."

5.2.2.2. Terrestrial Phase

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 methamidophos uses . The acute RQ for a terrestrial invertebrate (honey bee), representing the bulk of the terrestrial phase CRLF diet, ranges from 2.5 to 22.8. 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 RQs for individual effects on them, then there may be fewer burrows for the CRLF to exploit. Thus, there may be an indirect effect on the CRLF through loss of terrestrial phase habitat.

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 exceedence.

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*.

Spray drift assumptions for aerial application were varied from the standard 5%, to determine if spray drift perimeters could delimit the Action Area. Table 3-3 shows the results of PRZM-EXAMS modeling runs with assumptions of 5%, 1% (default assumptions for aerial and ground application, respectively), or 0% drift.

In all cases, the LOC for acute effects on invertebrates is exceeded, both under default spray drift assumptions (1% or 5%), and when spray drift is set to 0%. Thus, no spray drift buffer can be set that will reduce EECs, and therefore RQs, to below LOC. A spray drift buffer to set the Action Area for aquatic effects therefore cannot be established.

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 methamidophos use. The initial area of concern was defined by Figure 2.E., which shows all agricultural land in all counties in California where tomatoes, potatoes, cotton, or alfalfa for seed are grown. 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, this is the acute RQ for aquatic invertebrates from aerial application to potatoes (0.45), divided by the LOC (0.05) for a factor of 8.9. 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 methamidophos, 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 0.0007 lb/acre, due to acute effects on avian species (acute RQ = 134.5).

The distance from the use site (sprayed field) needed to achieve the target exposure of 0.0007 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	0.083
Active fraction	0.033
Surface Canopy	None
Specific Gravity, Carrier	1.19
Deposition type	Terrestrial point
Initial Average Deposition	0.0007 lb/acre

The result of this analysis is that a perimeter of 7,241 feet from the edge of the sprayed field is needed to bring the acute avian RQ to below the LOC of 0.1. Thus, the Action Area extends to a distance of 7,241 feet from the edge of fields sprayed with methamidophos.

Figure 5A shows the full extent of the Action Area, based on the terrestrial effects distance of 7,241 feet and the downstream dilution factor of 8.9.

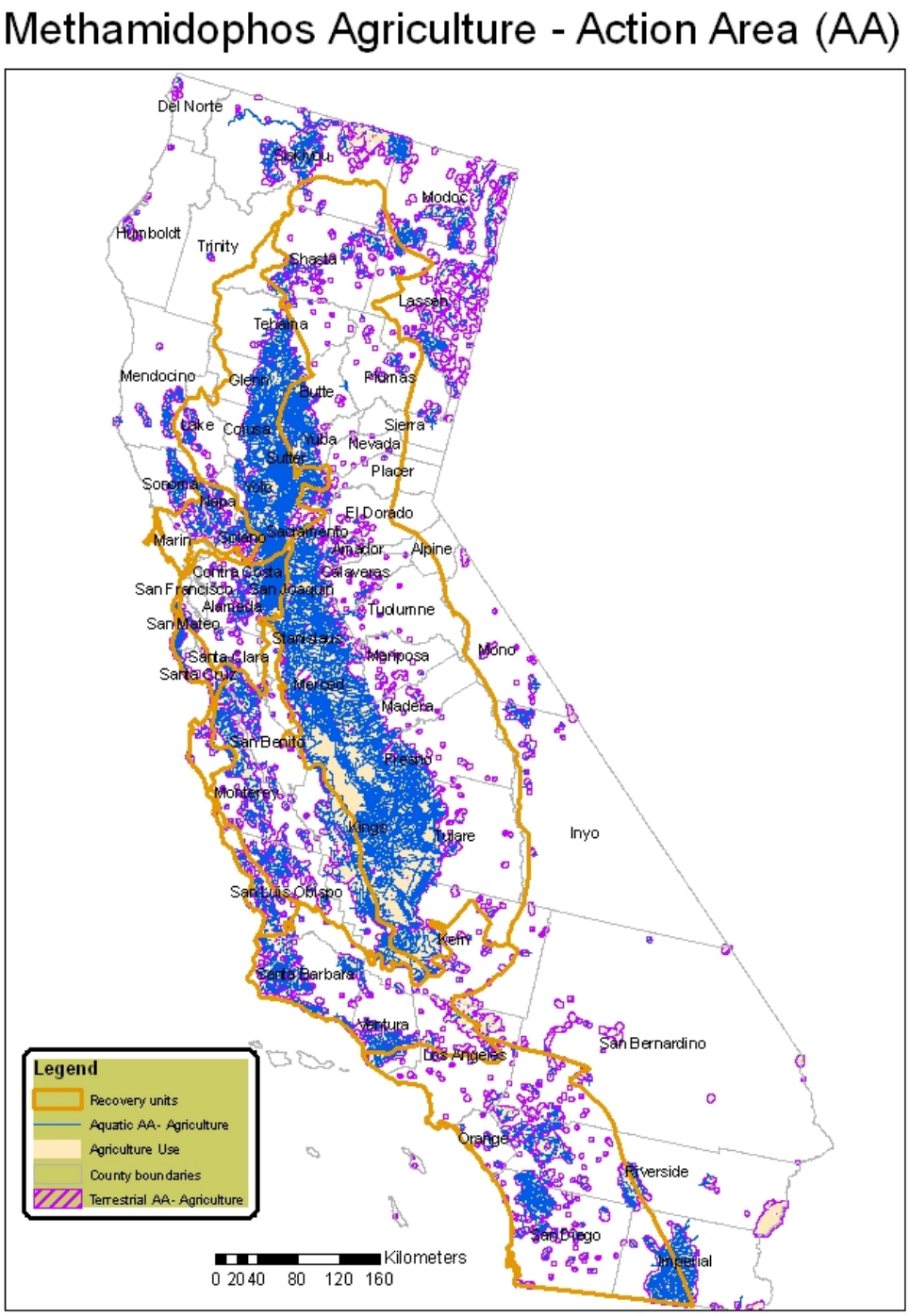


Figure 5A Action Area for Methamidophos

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 methamidophos use in California, the use of methamidophos "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 Methamidophos agriculture uses with a 7241 ft buffer.

Measure	RU1	RU2	RU3	RU4	RU5	RU6	RU7	RU8	Total
Initial Area of Concern (no buffer)									66,524 sq km
Action Area – Initial area of concern + buffer									105,492 sq km
Established species range area (sq km)	3654	2742	1323	3279	3650	5306	4917	3326	28,197
Overlapping area (sq km)	560	344	219	1175	1734	1647	2104	773	8,556
Percent area affected	*15%*	*13%*	*17%*	*36%*	*48%*	*31%*	*43%*	*23%*	*30%*
# Occurrence Sections	3	2	21	171	228	75	76	25	601

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. Risk quotients for animals that may serve as prey for the CRLF are also well above LOCs (Table 5-8). The risk quotient for a terrestrial invertebrate (honey bee), representing the bulk of the CRLF diet, is 2.9, well above the LOC of 0.05. **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 and estuarine 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. Methamidophos 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 methamidophos 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 Methamidophos Effects Determination Summary

Assessment Endpoint	Effects determination	Basis for Determination
Aquatic Phase (Eggs, larvae, tadpoles, juveniles, and adults)		
Direct 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
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 (23-26 counties). Use is documented in all months except November, December, January. 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 for mammals, insects, birds.
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 Revsisted

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 methamidophos 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 methamidophos 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 methamidophos 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 methamidophos 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 methamidophos within the action area may adversely modify the designated critical habitat of the CRLF by reducing or changing breeding and non-	Rejected. "No Effect" for aquatic plants and "NLAA" for indirect effects via invertebrates.

breeding aquatic habitat (via modification of water quality parameters, habitat morphology, and/or sedimentation)	
Labeled uses of methamidophos 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 methamidophos 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 methamidophos 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 methamidophos 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 methamidophos in terrestrial habitat is believed to have direct and indirect effects on CRLF.

6. Uncertainties

6.1. Exposure Assessment Uncertainties

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 = 1.75 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 (Koc) used was the highest and only quantified value obtained (0.88). 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 methamidophos on foliage and other food items for the TREX analysis was set at 6.5 days, rather than the default value of 35 days. This value was obtained from Willis & McDowell (1987) from a field experiment on citrus in Florida; this is the same reference used to obtain the default value of 35 days. The value of 6.5 days was the highest of the half-lives for methamidophos, so it is the most protective of the measured values.

6.2 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, averages 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).

6.3 Effects Assessment Uncertainties

6.3.1 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 methamidophos, 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.

6.3.2 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 methamidophos. 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 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.

6.5 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 methamidophos. 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 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.

6.7 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.

6.8 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.

6.9 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.

6.10 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.

6.11 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.

6.12 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.

6.13 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.

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