Potential Risks of Labeled S-Methoprene Uses to the Federally Listed California Red Legged Frog

(*Rana aurora draytonii*)

Pesticide Effects Determination

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1.0 Executive Summary

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 the insect growth regulator S-methoprene on some agricultural areas (i.e. mosquito control during flooding and fire ant bait around citrus) and certain non-agricultural sites in California. In addition, this assessment evaluates whether these actions can be expected to result in the destruction or adverse modification of the species' critical habitat. The structure of this risk assessment is based on guidance outlined in the U.S. EPA's Guidance for Ecological Risk assessment (U.S. EPA 1998), the Services' Endangered Species Consultation Handbook (USFWS/NMFS 1998) and is consistent with procedures and methodology outlined in the Overview Document (U.S. EPA 20040 and reviewed by the U.S. Fish and Wildlife Service and National Marine Fisheries Service (USFWS/NMFS 2004).

The CRLF was listed as a threatened species by the USFWS in 1996. The species 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). A total of about 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 in California (USFWS, 1996).

Technical S-methoprene, isopropyl (E, E) – 11- methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate, is a long chain hydrocarbon ester (Farm Chemical Handbook, 1997). S-methoprene is soluble in water at 1.4 mg/L (at 25^0 C) and also soluble in organic solvents. The specific gravity of technical S-methoprene is 0.9261 at 20^0 C. S-methoprene has a moderate vapor pressure ($2.3x10^{-5}$ mm Hg @ 25°C) and Henry's Law Constant ($6.9x10^{-6}$ atm m^3/mole). Consequently S-methoprene has the potential to volatilize from water or moist soil. However, volatilization is mitigated by the affinity of S-methoprene for soils and sediments (Toxnet). S-methoprene showed rapid degradation in both sterile and nonsterile pond water exposed to sunlight, with more than 80% of applied S-methoprene being degraded within 13 days (US EPA, 1982). S-methoprene has a low persistence in soil (rapidly biodegrades), with a soil half-life of 10-14 days and a half-life in water of <1 day in sunlight and >4 weeks in darkness. The major degradate is methoxycitronellic acid (7-methoxy-3, 7-dimethyloctanoic acid). The Koc of 2,800 suggests that S-methoprene is relatively lipophilic and upon application to water can be expected to adsorb to suspended solids and sediments. The Koc value also suggests that S-methoprene, if applied to soil, is slightly mobile on FAO scale and will tend to reside in the top few centimeters with potentially little leaching or ground water exposure (Hansch, *et al*., 1995). An estimated bioconcentration factor of 3,400 suggests that the potential for bioconcentration in aquatic organisms is very high (HSDB, 2002).

S-methoprene is used throughout the United States on indoor and commercial non-agricultural use sites, residential uses, agricultural areas, building perimeters, and wetlands. Although this compound is widely used, especially for public health pest control, the scope of this assessment is limited to California and the overall action areas that are important in the sustained protection of the CRLF. Table 1.0 shows registered uses of S-methoprene in California that may result in exposure scenarios that might impact non-target organisms and the CRLF. The S-methoprene formulations that are widely used include slow release forms such as briquets, sand mix, and granulars, as well as liquid formulations (EC and FLC). In order to insure efficacy, these formulations are usually applied directly to aquatic areas that can include stagnant slow moving shallow water bodies, lakes, freshwater wetlands and marshes, swamps, as well as, any place that contains freshwater and is suitable for mosquito development (old tires, man made depressions, fountains, etc).

Table 1.0 Registered S-Methoprene Uses That May Have Ecological Impact to Non-Target Fish and Wildlife in California

Food Crop	Rice, caneberries, date palms, citrus, small fruits (bogs, unspecified agricultural crops, unspecified orchards
Aquatic Non-Food Industrial	Drainage systems, sewage systems
Aquatic Non-Food Outdoor	Intermittently flooded areas/water, streams/rivers/channeled water, lakes, ponds, reservoirs, swamps/marshes/wetlands /stagnant water.
Aquatic Non-Food Residential	Ornamental ponds/aquaria, swimming pool water systems
Terrestrial Non-Food Crop	Wide area/general outdoor treatment (public health use), compost piles, ornamental herbaceous flowering/foliage/vine plants, rights-of-way, agricultural and nonagricultural uncultivated areas, ornamental woody, recreational areas
Forestry	Forest trees

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

Since, CRLFs exists within freshwater and terrestrial habitats, the potential for S-methoprene exposure to this frog, its food sources, and its habitat, is assessed by considering the aquatic and terrestrial life phases separately. The Agency has estimated high-end exposures in aquatic habitats by evaluating direct applications of S-methoprene to water (liquid or sustainable release formulations for mosquito control). In developing peak aquatic estimated environmental concentrations the Agency has relied on extrapolated levels (0.485 – 22.0 ug/L) from label information, as well as, adjusted maximum measured microcosm levels (0.35 – 4.21 ug/L) from the five S-methoprene formulations (briquete, XR briquete, granular/pellets, sand mix, and liquid). In order to evaluate an upper bound element of risk for terrestrial estimates, a 100% application of liquid and granular formulations to a terrestrial site are used with the T-REX model.

Risk quotients (RQs) are derived as quantitative estimates of potential high-end risk. Acute and chronic RQs are compared to the Agency's levels of concern (LOCs) to identify instances where S-methoprene use within the action area has the potential to adversely affect the CRLF and its designated critical habitat via direct toxicity or indirectly based on direct effects to its food supply (i.e., freshwater invertebrates, algae, fish, frogs, terrestrial invertebrates, and mammals) or habitat (i.e., aquatic plants and terrestrial upland and riparian vegetation). When RQs for a particular type of effect are below LOCs, the pesticide is determined to have "no effect" on the subject species. Where RQs exceed LOCs, a potential to cause adverse effects is identified, leading to a conclusion of "may affect." If a determination is made that use of S-methoprene use within the action area "may affect" the CRLF and its designated critical habitat, additional information is considered to refine the potential for exposure and effects, and the best available information is used to distinguish those actions that "may affect, but are not likely to adversely affect" (NLAA) from those actions that are "likely to adversely affect" (LAA) the CRLF and its critical habitat.

In addition to evaluating the parent compound and its exposure in the environment, the Agency considered degradate exposure. Degitz *et al*. (2003) did additional studies on the developmental toxicity of S-methoprene and its degradates (S-methoprene acid, S-methoprene epoxide, 7-methoxycitronellal, and 7-methoxycitronellic acid) to frog embryos (*Xenopus laevis*) and found that exposure to 0.5 mg/L of parent compound did not result in developmental effects. However, several degradates did produce developmental effects at 1.25 mg/L (S-methoprene acid), 2.5 mg/L (S-methoprene epoxide acid), 5 mg/L S-methoprene epoxide and 2.5 mg/L (7-methoxycitronellal). La Clair, (1998) noted that the lowest concentration of S-methoprene exposed to sunlight shown to cause malformations was 7.5 mg/L, which is 1,700 times greater than the level found under typical applications of S-methoprene. Degitz *et al*. 2003, noted that typical field application of sustained-release formulations of S-methoprene result in S-methoprene concentrations that do not exceed 0.01 mg/l, suggesting that S-methoprene-mediated developmental toxicity to amphibians may be overstated. According to Ankley (1998) it is unlikely that degradation products would accumulate to levels that would affect amphibian development.

Based on the best available information, the Agency has assessed the potential for direct and indirect risk to CRLF from S-methoprene exposure. The conclusion is that there is a "may affect", but "not likely to adversely affect" determination for the CRLF from the use of S-methoprene. The assessment endpoints (Table 1.1) where this determination is made include the following:

- 1) Survival, growth, and reproduction of CRLF individuals via effects to food supply (*i.e.* freshwater fish and invertebrates, non-vascular plants);

This assessment point reflects an LOC exceedance (0.07) for acute endangered species concerns (LOC = 0.05) calculated from one of the upper bound extrapolated sustainable release formulations (20% granular), although, the microcosm field values for the same formulation did not exceed this LOC concern. In order to evaluate this exceedance, the Agency also calculated the chance of individual exposure using the Individual Effects Chance Model (Version 1.1). These calculations suggest that the chance of individual effect from this granular extrapolated exposure is about 1in 988,000, which may be considered as a highly unlikely event. As an additional test of possible risk, the use of acute and chronic fish and invertebrate toxicity data produced RQs for the other formulations (using extrapolated and microcosm exposure values) that did not exceed LOCs for direct or indirect effects to the aquatic-phase CRLF. Therefore, the Agency concludes a "**may affect" but "not likely to adversely affect**" reading for this assessment point.

The Agency acknowledges that S-methoprene is highly efficacious to Dipteran insect larvae and that the use of this compound can result in a decline in emerging adult populations. However, according to information of the CRLFs diet in Section 2.5.3 these insects are not included in their diet. The Agency assumes that the CRLF is an opportunistic feeder and will supplement its diet with available invertebrates and small animals. Therefore, the Agency concluded **"no habitat modification"** from S-methoprene use. Although there is widespread overlap of potential S-methoprene with watersheds of the CRLF the Agency has also determined that there is no potential for modification of CRLF designated critical habitat (aquatic or terrestrial plants) from the use of S-methoprene because this compound does not have herbicidal qualities or mode of action. Further information on the results of the effects determination are included as part of the Risk Description in Section 5.2.

Table 1.1 Effects Determination Summary for Direct and Indirect Effects of S-methoprene on the California Red-legged Frog

Assessment Endpoint	Effects Determination	Basis
Aquatic-Phase (Eggs, Larvae, Tadpoles, Adults)		
1. Survival, growth, and reproduction of CRLF individuals via direct effects on aquatic phases	No Effect	Acute RQs do not exceed LOC for direct effects using acute and chronic fish data. There is widespread overlap of potential S-methoprene with watersheds of the CRLF.
2. Survival, growth, and reproduction of CRLF individuals via effects to food	May Affect , But not Likely to	LOC exceedance for granular formulation (0.07) to aquatic invertebrates. However,

Assessment Endpoint	Effects Determination	Basis
supply (*i.e.* freshwater fish and invertebrates, non-vascular plants)	Adversely Affect	exposure was an extrapolated value, microcosm value did not exceed LOC. There is widespread overlap of potential S-methoprene use with watersheds of the CRLF.
3. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat, cover, and/or primary productivity (*i.e.,* aquatic plant community)	No Effect	S-methoprene is a larvicide and does not kill plants. Although there is the potential for aquatic exposure, aquatic plants are not at risk.
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.	No Effect	S-methoprene is not toxic to plants and does not have herbicidal qualities.
Terrestrial Phase (Juveniles and adults)		
5. Survival, growth, and reproduction of CRLF individuals via direct effects on terrestrial phase adults and juveniles	Not Likely to Adversely Affect	S-methoprene does not exceed an equivalent LOC for acute or chronic toxicity LC50 values, based on available avian and mammal data. Most applications are granular formulations and the liquid applications are directed to water.
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)	Not likely to adversely affect	RQs for possible dietary items (small mammals, adult insects) are less than the LOCs. Based on the non-selective feeding behavior of adult CRLF and low magnitude of anticipated individual effects to potential prey items, S-methoprene is not expected to indirectly affect the terrestrial form of the CRLF. Although Dipterian populations may decline momentarily in the area where S-methoprene is used, these organisms are not expected to be a major component of the CRLFs diet.
7. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat (*i.e.,* riparian vegetation)	Not Likely to Adversely Affect	S-methoprene is not toxic to plants, plants are not at risk.

Table 1.2 Effects Determination Summary for the Critical Habitat Impact Analysis

Assessment Endpoint	Effects Determination	Basis
Aquatic Phase PCEs (Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)		

Assessment Endpoint	Effects Determination	Basis
Aquatic Phase PCEs ***(Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)***		
Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile and adult CRLFs.	No Habitat Modifications	Since S-methoprene does not control plants at the application sites, there is no potential for impacts to aquatic and terrestrial plants that comprise these habitats.
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[2].	No Habitat Modifications	Given that S-methoprene is not intended to control plants on the application sites, there is no potential for impacts to aquatic and terrestrial plants that comprise these habitats.
Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.	No Habitat Modifications	S-methoprene does not affect plant life and aquatic chemical (DO) components that are necessary for aquatic CRLF growth and development are not affected by S-methoprene exposure.
Reduction and/or modification of aquatic-based food sources for pre-metamorphoses (*e.g.*, algae)	No Habitat Modifications	Although S-methoprene is applied to water bodies, it does not have the potential for impacts to aquatic plants that comprise these habitats (non herbicidal properties).
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 drip line surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance	No Habitat Modifications	S-methoprene is not intended to control terrestrial plants at the application sites. This compound is not an herbicide and rapidly degrades in the environment through photolysis and biodegradation (7-10 days).
Elimination and/or disturbance of dispersal habitat: Upland or riparian dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal	No Habitat Modifications	Given that S-methoprene is not intended to control plants on the application sites, there is no potential for impacts to terrestrial plants that comprise these habitats.
Reduction and/or modification of food sources for terrestrial phase juveniles and adults	No Habitat Modifications	Although S-methoprene is toxic to Dipterian insects this does not pose acute risk to the CRLF. Frogs are opportunistic feeders and should supplement their diet with other terrestrial organisms. Dipterans

Assessment Endpoint	Effects Determination	Basis
Aquatic Phase PCEs (Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)		
		are not listed as a component of the CRLFs diet.
Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	No Habitat Modifications	Although S-methoprene is toxic to Dipterian insects this does not pose acute risk to the CRLF. Frogs are opportunistic feeders and should supplement their diet with other terrestrial organisms. Dipterans are not listed as a component of the CRLFs diet.

[1] Physico-chemical water quality parameters such as 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.

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.

2.0 Problem Formulation

Problem formulation provides a strategic framework for the risk assessment. By identifying the important components of the problem, it focuses the assessment on the most relevant life history stages, habitat components, chemical properties, exposure routes, and endpoints. 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 Document (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 the potential for S-methoprene exposure to amphibians from direct application to aquatic (i.e., marshes, ponds) and terrestrial areas in order to combat human pest (such as mosquitoes, fire ants, etc.). 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 registration of S-methoprene 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 S-methoprene 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 S-methoprene 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 S-methoprene 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 S-methoprene.

If a determination is made that use of S-methoprene 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 S-methoprene use) and further evaluation of the potential impact of S-methoprene 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 S-methoprene is expected to directly impact living organisms within the action area (defined in Section 2.7), critical habitat analysis for S-methoprene 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 S-methoprene 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 (*i.e.*, the FIFRA regulatory action) is an approved product label. The label is a legal document that stipulates how and where a given pesticide may be used. Product labels (also known as end-use labels) describe the formulation type (*e.g.*, liquid or granular), acceptable methods of application, approved use sites, and any restrictions on how applications may be conducted. Thus, the use or potential use of S-methoprene in accordance with the approved product labels for California is "the action" relevant to this ecological risk assessment.

Although it is recognized that S-methoprene is used throughout the United States, the scope of this assessment is limited to areas in California applicable to the protection of the CRLF and its designated critical habitat. Further discussion of the action area for the CRLF and its critical habitat is provided in Section 2.7. The current California uses of S-methoprene that will be assessed in this evaluation include direct applications to water bodies, as well as uses around certain agricultural areas such as rice and citrus. Although not used to combat agricultural pests, S-methoprene is used for mosquito control around flooded rice fields and berry bogs, and fire ant control around orchards like citrus. Sites of concern that receive S-methoprene include swamps, wetlands, turf, rights of ways, industrial parks, landscape maintance, lakes, and intermittently flooded areas. S-methoprene is formulated as flowable concentrates, soluble concentrates, and granular, pelleted/tabeted, and bait/solids (briquetes). Application methods include aircraft, high and low volume ground spray, and granular/dust application. Although this compound degrades in the environment via photolysis and biodegradation, concern has been raised over the expected environmental concentrations that can occur through the use of such formulations as the briquet and granular (sustainable release). The S-methoprene briquetes (submerged in water) have been reported as having relatively long half-lives, with a mean degradation of the briquettes at 19% by weight after 150 days and full degradation after 1.5 years (Boxmeyer *et al*., 1997).

S-methoprene undergoes environmental degradation after exposure to ultraviolet (UV) sunlight, as well as microbial breakdown. This is a concern to the Agency because of the similarities of these degradates and retinoic acid which is a significant component in vertebrate development, especially amphibians. The S-methoprene degradates are as follows: S-methoprene acid, S-methoprene epoxide, 7-methoxycitronellal, 7-methoxycitronellic acid and will be discussed in Section 4.0.

The Agency does not routinely include an evaluation of mixtures of active ingredients, either those mixtures of multiple active ingredients in product formulations or tank mixtures. In the case of the product formulations of different active ingredients, each active ingredient is subject to an individual risk assessment for regulatory decisions regarding the active ingredient on a particular use site. If effects data are available for a formulated product containing more that one active ingredient, they must 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).

S-methoprene has registered products that contain multiple active ingredients. Analysis of the available acute oral mammalian LD_{50} data for multiple active ingredient products relative to the single active ingredient is provided in Appendix A. The results of this analysis show that an assessment based on the toxicity of the single active ingredient of S-methoprene is appropriate.

2.3 Previous Assessments

S-methoprene was first registered by EPA as a conventional, chemical pesticide in 1975. The Agency issued a Registration Standard for S-methoprene in 1982, and subsequently reclassified S-methoprene as a biochemical pesticide. The Agency completed the Reregistration Eligibility Decision (RED) in 1991 (EPA, 1991) and reregistration of the active ingredient and all end-uses was completed in 1997. Tolerances (40CFR 180.359) and exemption from tolerances (40 CFR 180.1033 and 185.41500 have been established for S-methoprene in or on a number of food commodities. S-methoprene is also recognized by FDA as a feed additive for use in cattle feeds to control horn flies (40 CFR 186.4150).

2.4 Stressor Source and Distribution

2.4.1 Environmental Chemistry and Fate Assessment

Technical S-methoprene is a long chain hydrocarbon ester, characterized as a pale yellow liquid with a faint fruity odor and has a boiling point of 100^0 C at 0.05 mm of Hg (Farm Chemical Handbook, 1997). Samples of S-methoprene that have been stored in glass for four years at 70^0 F did not show any appreciable chemical decomposition. Technical

S-methoprene is soluble in water at 1.4 mg/L (at 25^0 C) and also soluble in organic solvents. The specific gravity of technical S-methoprene is 0.9261 at 20^0 C. This compound is intended only for reformulation into and end-use pesticide and, therefore, is considered to be a “manufacturing-use “product (Toxnet).

S-methoprene has a moderate vapor pressure (2.3×10^{-5} mm Hg @ 25°C) and Henry’s Law Constant (6.9×10^{-6} atm m^3/mole). Consequently S-methoprene has the potential to volatilize from water or moist soil. However, volatilization is mitigated by the affinity of S-methoprene for soils and sediments (Toxnet). S-methoprene showed rapid degradation in both sterile and nonsterile pond water exposed to sunlight, with more than 80% of applied S-methoprene being degraded within 13 days (US EPA, 1982).

S-methoprene has a low persistence in soil (rapidly biodegrades), with a soil half-life of 10-14 days and a half-life in water of <1 day in sunlight and >4 weeks in darkness. S-methoprene degrades in both sterile and nonsterile pond water with exposure to sunlight (80% of applied S-methoprene is degraded after13 days). The major degradate is methoxycitronellic acid (7-methoxy-3, 7-dimethyloctanoic acid). S-methoprene rapidly degrades in plants, with a half-life of 1–2 days in alfalfa when applied at a rate of 1 pound per acre. In rice, the half-life is less than 1 day. In wheat, its half-life was estimated to be 3 to 7 weeks, depending on the level of moisture in the plant. The Koc of 2,300 suggests that S-methoprene is relatively lipophilic and upon application to water can be expected to adsorb to suspended solids and sediments. The high Koc value also suggests that S-methoprene, if applied to soil, is slightly mobile and will tend to reside in the top few centimeters with potentially little leaching or ground water exposure (Hansch, *et al*., 1995). An estimated bioconcentration factor of 3,400 suggests that the potential for bioconcentration in aquatic organisms is very high (HSDB, 2002). Uncharacterized S-methoprene residues accumulate in edible tissues of bluegill sunfish and crayfish at maximum bioconcentration factors of 457 and 75, respectively (rates of depuration are unknown).

Table 2.0 Summary of S-Methoprene Environmental Chemistry and Fate Properties

Study	**Value (units)**	**Major Degradates** *Minor Degradates*	**MRID #**	**Study Status**
Hydrolysis	Stable to hydrolysis at 20^0 C at pH 5-9, for 21-30 days	No degradates	00010439	Acceptable
Direct Aqueous Photolysis	The photolysis half-life of S-methoprene (0.5 and 0.01 ppm) was less than 1 day (Laboratory). After 7 days, 12% and 5% of applied compound remained in solution. S-methoprene is rapidly degraded in pond water exposed to natural sunlight; complete degradation occurs within 13 days post treatment.	The trans-2:cis-2 ratio changed as a result of photoisomerization from 97:3 to 46:54 . Fifty minor photolysis were found (all<10% of applied). These included methoxcitronellal (9%), methoxcitronellic acid (7%), 92E)-4,5-epoxy-11-methoxy-3,7,11-trimethyl-2-dodecenoate (4%), and 8-methoxy-4,8-	00010443, 00010441, 00010442, 05008622, 00010542, 05008610, 00010440	Acceptable

Study	Value (units)	Major Degradates *Minor Degradates*	MRID #	Study Status
		dimethyl-2-nonanone (4%).		
Soil Photolysis	Photodegradation was rapid (24% in 6 hours) on silica gel TLC plates.	Methoxcitronellal (14% of applied).	00010542, 05008610	Acceptable
Aerobic Soil Metabolism	S-methoprene applied at 1 lb/A is degraded rapidly in aerobic sandy loam and silt loam with a half-life of about 10 and 14 days, respectively. Half-life of S-methoprene in silt loam was about 14 days.	CO2 was major product (49% of applied)	00010420, 00010541, 00010874, 05008315	Acceptable
Anaerobic Aquatic Metabolism	No data available			
Aerobic Aquatic Metabolism	S-methoprene EC persists in FW and salt water for 132 days (dark condition) at 4.5^0 C (half life 28-35 days with 2-15% of S-methoprene remaining at 132 days).	Isomerization of trans-S-methoprene to cis-S-methoprene did not occur. Degradates included 7-Methoxycitronellic acid (29% of applied)	05009396, 00010974, 00010975, 05008622, 00010442	Acceptable
$K_{d\text{-}ads}$ / $K_{d\text{-}des}$ (mL/g) $K_{oc\text{-}ads}$ / $K_{oc\text{-}des}$ (mL/g)	Log Kow 5.50 2,300	Degrdates not measured	42290001, Hansch, *et al.*, 1995 EPI Suite	
Terrestrial Field Dissipation	No data available			
Aquatic Field Dissipation	Rice field: S-methoprene at 0.12, 0.31, and 0.81 ppm was present in water samples 1 hour after treatment with 0.5, 1.0, and 2.0 lbs ai/A, respectively. S-methoprene not detected in the water column after 72 hours	Degradates were not measured.	00010436, 00010437, 00010438, 00011484, 00010433, 00010417, 00011091, 00011092, 00012729, 05008625, 00011485, 00010434	Acceptable
Leaching	Not mobile when applied to sand, sandy loam, silt loam, and clay loam. All S-methoprene that was recovered was in the top 3 cm of soil column.	No degradates were measured.	00010444, 00010507	Acceptable
Accumulation	Fish: Day 21 the maximum ^{14}C levels in edible tissue was 2.78 ppm corresponding to a bioaccumulation factor of 457. ^{14}C remained at 1.67 ppm (bioaccumulation factor of 253) in the edible tissue at the end of the 42-day experiment. Crayfish:	Degradates not measured.	00012785	Acceptable

Study	Value (units)	Major Degradates *Minor Degradates*	MRID #	Study Status
	Accumulation in edible tissues of fish and crayfish at maximum bioaccumulation factors of 457 and 75, respectively. Elimination of 93-95% of methoprene residues within 14 days precludes bioaccumulation in fish			

2.4.2. Environmental Transport Assessment

This risk assessment is intended to be used to evaluate the potential for S-methoprene exposure to the aquatic and terrestrial-phase of the CRLF. S-methoprene is applied to bodies of shallow water as a liquid and as a sustainable release formulation for mosquito control. Label instructions for these formulations specify application to water in order to achieve maximum efficacy. Unlike adulticides that are used to combat adult mosquitoes (i.e. synthetic pyrethroids), S-methoprene is not applied as fine droplets in order to create a mist that is intended for drift over a target site. Instead, S-methoprene is applied directly to water in order to be efficacious to Dipterans larval forms. In addition to applications to aquatic areas, S-methoprene is also applied to land and around certain crops as a granular formulation. Although not used to combat agricultural pests, S-methoprene is applied in citrus orchards as bait for fire ant control. Therefore, in deciding on the environmental transport scenario for developing estimated environmental concentrations (aquatic and terrestrial), the Agency has relied on transport mechanisms that can be depicted as upper bound exposure to aquatic and terrestrial organisms. All aquatic EECs are estimates of direct application to water. This upper bound scenario precludes concern and modeling for runoff or drift that might occur for applications to terrestrial sites. T-REX is used to evaluate terrestrial application of liquid and granular formulation and possible exposure to avian species (surrogate for terrestrial-phase CRLF), as a upper bound maximum application.

The potential exposure to aquatic organisms from S-methoprene use from granular, briquets, pellets, and sand mix applications is approached by estimating expected release rates that has been calculated from label information and expected efficacy. In addition to these values, the Agency will also use adjusted field microcosm residue data that has been generated by the registrant. Both approaches will be used to generate the risk quotient (RQ) values for this CRLF assessment.

2.4.3 Mechanism of Action

S-methoprene is an analog of the insect juvenile hormone (JH) that is responsible for regulating larval growth. Since this regulatory mode of action does not result in direct toxicity to target organisms, the Agency considers S-methoprene to be a biological pesticide where control of target pests is through disruption of primary gene regulation at the onset of metamorphosis, thus preventing larvae from developing into adults (Hersher *et al.*, 1998, Degitz *et al*., 2003). The retention of juvenile characters in insect larval stages is controlled by JH which is present in larvae up to their transformation from pupae to adult stage where titer levels decline. S-methoprene mimics the JH by binding to corresponding receptors thus prolonging the larval stage and preventing these organisms from reaching adult stage and reproducing.

2.4.4 Use Characterization

Analysis of labeled use information is the critical first step in evaluating the federal action. The current label for S-methoprene represents the FIFRA regulatory action; therefore, labeled use and application rates specified on the label form the basis of this assessment. The assessment of use information is critical to the development of the action area and selection of appropriate modeling scenarios and inputs. Table 2.1 shows usage information that is disallowed, as well as, allowed in California. The table also shows the registered uses that the are not expected to have an ecological impact (i.e. indoor uses, pets, etc) and those uses that have the potential for environmental risk (i.e. rice, freshwater aquatic areas, marshes, etc.).

The Agency's Biological and Economic Analysis Division (BEAD) provides an analysis of both national- and county-level usage information (BEAD: CITATIONS HERE) using state-level usage data obtained from USDA-NASS[1], Doane (www.doane.com; the full dataset is not provided due to its proprietary nature), and the California's Department of Pesticide Regulation Pesticide Use Reporting (CDPR PUR) database[2] . CDPR PUR is considered a more comprehensive source of usage data than USDA-NASS or EPA proprietary databases, and thus the usage data reported for S-methoprene by county in this California-specific assessment were generated using CDPR PUR data. Usage data are averaged together over the years 2001 to 2005 to calculate average annual usage statistics by county and crop for S-methoprene, including pounds of active ingredient applied and base acres treated. California State law requires that every pesticide application be reported to the state and made available to the public.

S-methoprene is registered on a variety of sites including flooded fields, rice, caneberries, swamps, marshes, wetlands, the perimeter of buildings, livestock, indoor pet uses, commodity storage, waste treatment, culverts, drains and any thing that may contain water applicable for mosquito growth.

For the purpose of this assessment, EEC will be generated for uses that may have the potential for impact to fish and wildlife. These will include all formulations that are applied directly to water (i.e. granular, briquets, and liquid formulation) or broadcast over post or pre-flooded areas. Since, S-methoprene exposure from indoor applications are less likely to impact aquatic and terrestrial organisms, these uses will not be included in this assessment. The use of S-methoprene in a briquet and/or granular form was identified as a potential aquatic concern in earlier reviews. Since these formulations function as

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

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

slow release mechanisms for delivering S-methoprene at a relatively constant concentration over a period of time (i.e. 30 – 150 days), their appears to be the potential for impact to non-target aquatic organisms. The granule and pellet formulations are about 425 microns in size and are applied by ground or aerial equipment. The use of granules is advantageous in areas of dense plant cover because of more efficient foliage penetration. The briquets weigh about 5.4 – 36.2 g and are usually applied by hand to small areas (i.e. culverts, drains, etc.). Liquid S-methoprene formulations include emulsifiable concentrates (EC) and flowable concentrates (FLC) and are also applied directly to water. Tables 2.1 shows the average amount of S-methoprene used annually in California during 2002 – 2005. The four listed categories reflect the highest S-methoprene usage throughout the state with Public Health Pest Control accounting for about 97% of the the total. Table 2.2 shows the formulations that were used. The sustainable release forms (briquets, granular, pelleted/tableted, and impregnated material) accounted for about 69% of the total use.

Table 2.1 Summary of CDPR PUR Usage Data from 2002 - 2005 for S-Methoprene

Use	**Average Amount of S-Methoprene Used During this Time Period (lbs)**
Landscape Maintance	20.03
Public Health Pest Control	8466.70
Regulatory Pest Control	10.01
Structural Pest Control	206.77

Table 2.2 Summary of CDPR PUR Formulation Data from 2002- 2005 for S-Methoprene

Formulation	**Average Amount of S-Methoprene Used Relative to Formulation During this Time Period (lbs)**
Briquets	3609.16
Pelleted/Tableted	2307.96
Granular	82.85
Impregnated Material	0.015
Emulsifiable Concentrate	184.39
Pressurized Liquid	11.60
Soluble Concentrate	859.41
Flowable Concentrate	1631.81
Ready-to-Use Solution	1.12

The S-methoprene uses considered in this risk assessment represent currently registered products as noted from 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, misreported uses, or misuse. Historical uses, mis-reported uses, and misuse are not considered part of the federal action and, therefore, are not considered in this assessment. Appendix B, Table B.2 shows the major formulations of S-methoprene that were used in California (2001-2005) and the total amount applied. Table 2.3 shows maximum rates and formulations that were used in this risk assessment for the sustainable release formulations and the liquid that was applied to rice and caneberry bogs. The amount of S-methoprene that was expected to be released into the aquatic environment was extrapolated from label information and efficacy data. These rates reflect upper bound scenarios of the expected amount of S-methoprene (see calculations Appendix I).

Table 2.3 Maximum Rate of S-Methoprene Sustainable Release Formulations that Are Applied Directly to Water

Use	**Formulation**	**Max. % active ingredient**	**Max. Rate (lbs ai/A/day)**
Woodland pools, swamps, rice fields, storm drains, etc.	Briquet Briquet XR	2.1 8.62	0.0058 0.014
Woodland pools, swamps, berry bogs, rice fields, irrigated crop lands, etc.	Granular Sand mix	4.25 3.0	0.06 0.017
Use	**Formulation**	**Max. % active ingredient**	**Max Rate (lbs ai/A)**
Woodland pools, berry bogs, rice fields, irrigated crop lands, etc.	Liquid	20.0	0.013

The values noted in Table 2.4 reflect maximum application of the granular formulation to terrestrial areas where the CRLF may be found. Although frogs should not feed on granulars this scenario was included as an upper bound scenario.

Table 2.4 Maximum Rate of S-Methoprene Formulations that Are Applied to Terrestrial Site

Use	**Formulation**	**Max. % active ingredient**	**Max. Rate (lbs ai/A)**	**Max. Rate (lbs ai/A)**
Citrus (bait)	Granular	4.25	0.3	0.0075

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 Appendix C.

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

2.5.1 Distribution

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

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

The distribution of CRLFs within California is addressed in this assessment using four categories of location including recovery units, core areas, designated critical habitat, and known occurrences of the CRLF reported in the California Natural Diversity Database (CNDDB) that are not included within core areas and/or designated critical habitat (see Figure 3. 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

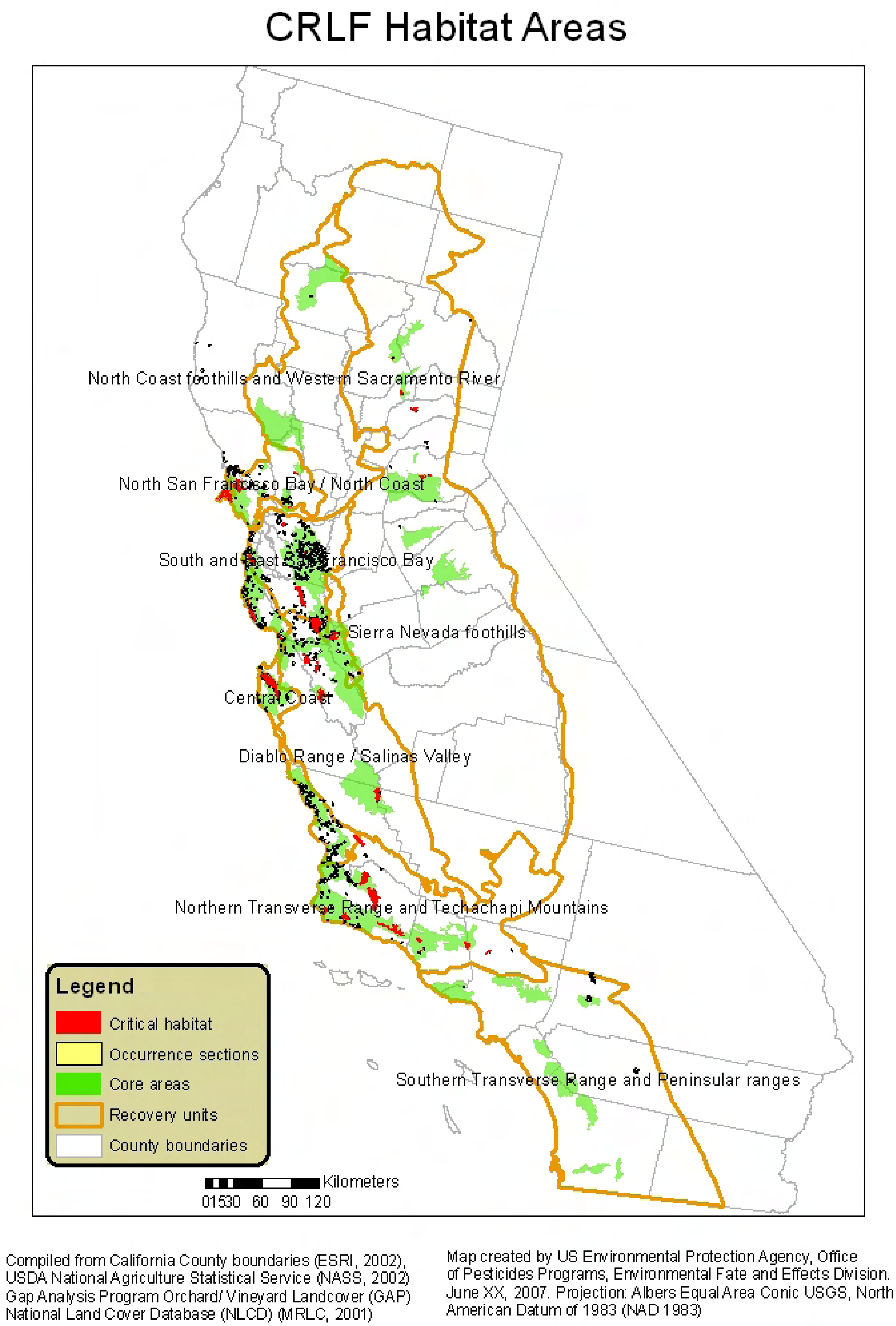


Figure 1. Distribution of the CRLF Range and Designated Critical Habitat.

current range and have been determined by USFWS to be important in the preservation of the species. Designated critical habitat is generally contained within the core areas, although a number of critical habitat units are outside the boundaries of core areas, but within the boundaries of the recovery units. Additional information on CRLF occurrences from the CNDDB is used to cover the current range of the species not included in core areas and/or designated critical habitat, but within the recovery units.

Recovery Units

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

Core Areas

USFWS has designated 35 core areas across the eight recovery units to focus their recovery efforts for the CRLF (see Figure 1). Appendix E 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 S-methoprene occur (or if labeled uses occur at predicted exposures less than the Agency's LOCs) within an entire recovery unit, that particular recovery unit would not be included in the action area and 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 Appendix C (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.

Other Known Occurrences from the CNDBB

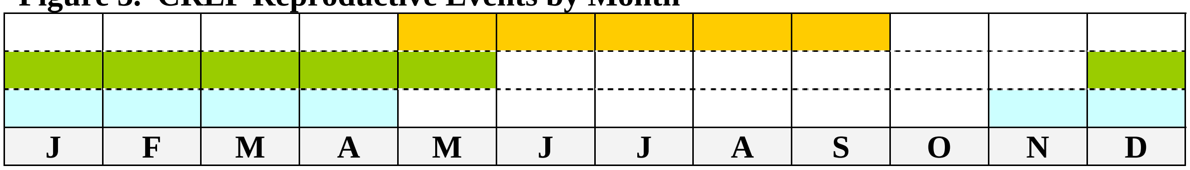
The CNDDB provides location and natural history information on species found in California. The CNDDB serves as a repository for historical and current species location sightings. Information regarding known occurrences of CRLFs outside of the currently occupied core areas and designated critical habitat 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.

Figure 2. Recovery Unit and Core Area Designations for CRLF

2.5.2 Reproduction

CRLFs breed primarily in ponds; however, they may also breed in quiescent streams, marshes, and lagoons (Fellers, 2005a). According to the Recovery Plan (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 3 depicts CRLF annual reproductive timing.

Figure 3. 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 CRLF utilizes habitat with perennial, or near perennial water (Jennings *et al.*, 1997). Dense vegetation close to water, shading, and water of moderate depth are habitat features that appear especially important to CRLF (Hayes ans Jennings, 1998). 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 also show that CRLFs do not frequently inhabit vernal pools, as conditions in these habitats generally are not suitable (Hayes and Jennings, 1998).

CRLFs also frequently breed in artificial impoundments such as stock ponds, although additional research is needed to identify habitat requirements within artificial ponds (USFWS, 2002). Adult CRLFs use dense, shrubby, or emergent vegetation closely associated with deep-water pools bordered with cattails and dense stands of overhanging vegetation (http://www.fws.gov/endangered/features/rl_frog/rlfrog.html#where).

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

During dry periods, the CRLF is rarely found far from water, although it will sometimes disperse from its breeding habitat to forage and seek other suitable habitat under downed trees or logs, industrial debris, and agricultural features (UWFWS, 2002). According to

Jennings and Hayes (1994), CRLFs also use small mammal burrows and moist leaf litter as habitat. In addition, CRLFs may also use large cracks in the bottom of dried ponds as refugia; these cracks may provide moisture for individuals avoiding predation and solar exposure (Alvarez, 2000).

2.6 Designated Critical Habitat

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

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 Appendix D for a full explanation on this special rule. One of the exemptions is the 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.

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 the use of S-methoprene 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 S-methoprene is expected to directly impact living organisms (Dipterians) within the action area, critical habitat analysis for S-methoprene is limited in a practical sense to those PCEs of critical habitat that are biological or that can be reasonably linked to biologically mediated processes.

2.7 Action Area

For listed species assessment purposes, the action area is considered to be the area affected directly or indirectly by the federal action and not merely the immediate area involved in the action (50 CFR 402.02). It is recognized that the overall action area for the national registration of S-methoprene is likely to encompass considerable portions of the United States based on the large array of non-agricultural uses, as appropriate. 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 S-methoprene may be expected to have on the environment, the exposure levels to S-methoprene that are associated with those effects, and the best available information concerning the use of S-methoprene 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 aquatic and terrestrial uses for S-methoprene. The Agency completed an analysis and review of labeled uses that showed that, for S-methoprene, the aquatic uses listed in Table 2.5 are considered as part of the federal action evaluated in this assessment.

Table 2.5 Summary of the Uses Considered as Part of the Federal Action Evaluated in this Assessment

Use Category	Uses
Agricultural	Rice, caneberries, date palms, citrus, small fruits (bogs, unspecified agricultural crops, unspecified orchards
Non-Agricultural	Drainage systems, sewage systems, Salt/brackish water sites, intermittently, Ornamental ponds/aquaria, swimming pool water systems flooded areas/water, streams/rivers/channeled water, lakes, ponds, reservoirs, forest trees, compost piles, swamps/marshes/wetlands/stagnant water, wide area/general outdoor treatment (public health use), ornamental herbaceous flowering/foliage/vine plants, rights-of-way, agricultural and nonagricultural uncultivated areas, ornamental woody, recreational areas

S-methoprene is also registered for use on pets (shampoos and collars), stored grains, hospital premises, cemeteries, industrial waste disposal systems, and other indoor uses. These uses are not evaluated for this risk assessment because the likelihood for environmental exposure is assumed to be negligible since these are indoor uses and warrant a determination of "no effect" (Table 2.6). Although, pet shampoo can be washed down a drain and eventually reach a treatment plant and an adjacent body of water, the risk to the CRLF from this application is assumed to result in "no effect". The Agency has arrived at this conclusion because the assessment strategy used for evaluating risk from aquatics uses represents a 100% direct application of S-methoprene to water scenario. The maximum percent active of S-methoprene in pet shampoo is relatively low at 0.50%. Any modeling of a Down-the-Drain scenario for this use would result in considerable dilution of the active ingredient. The issue of down-stream dilution was not evaluated in this assessment because the Agency has focused on a direct application to water as an upper bound scenario for developing this risk assessment.

Table 2.6 Summary of S-Methoprene Uses that are not Considered as part of the Federal Action Evaluated in the Assessment

Use Category	Uses
Non-AgriculturalFood	Compost, compost piles, ornamental herbaceous flowering/foliage/vine plants
	Food/feed storage area-full, cereals, mushroom houses/mushroom casing soil, eating establishments, commercial shipping containers-feed/food-empty, food processing plant premises/equipment, dairy cattle, beef/range/feeder cattle
Indoor Non-Food	Stored tobacco, commercial transportation facilities, tobacco processing plant premises/equipment, commercial/institutional/industrial premises/equipment (indoors), horses, ponies, farm premises (indoor)
Indoor Medical	Hospitals/medical institutions premises (human/veterinary)
Indoor Residential	Kennels and/or pet sleeping quarters, household/domestic dwellings indoor premises, cats, dogs, pet shampoos
Terrestrial Non-Food Areas	barns, barnyards, auction barns, cemeteries, zoos.
Terrestrial Food Crop	Stored commodities: Legume vegetables, Corn (field and pop), Sunflower, Cotton, Peanuts, Birdseed, Canola, Cereal grains, Oats, Rice, Sorghum, Wheat, Millet, Cocoa
Aquatic Non-Food Outdoor	Industrial waste disposal system, Sewage system

The current labels for S-methoprene represent the FIFRA regulatory action; therefore, labeled use and application rates specified on the label form the basis of this assessment. The assessment of use information is critical to the development of the action area and selection of appropriate modeling scenarios and inputs. In order to assess the potential risk to aquatic organisms and the CRLF from exposure to S-methoprene at these sites, the Agency has completed an assessment by using a upper bound scenarios of direct application to shallow water (1ft) at the maximum liquid application rate to an aquatic area (0.5853 lbs ai/A) and to rice (0.013 lbs ai/A). The maximum application of the different sustainable release formulations (briquete at 0.0058 lbs ai/A/day; briquete XR at 0.014 lbs ai/A/day; granular at 0.06 lbs ai/A/day) were extrapolated and also evaluated for this assessment. An evaluation of potential S-methoprene exposure to the terrestrial-phase CRLF was also completed by using the maximum application rate for a liquid formulation (0.5829 lbs ai/A) to foliage (ornamental woody plants) as well as the application of granular S-methoprene to dry areas around citrus (0.3 lbs ai/A). The T-REX model was used for this portion of the assessment which includes calculations of dietary exposure for multiple classes of birds and mammals.

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 S-methoprene use. The overall conclusion of this analysis is that S-methoprene use is widespread and not confined to particular regions. According to label instructions, S-methoprene can be used anywhere mosquitoes are considered to be a potential public health threat. The initial area of concern is defined as all land cover types that represent the labeled uses described above. Since S-methoprene is used throughout California and generally covers all areas where water may be present, a land cover map would show the entire state.

Once the concern is defined, the next step is to compare the extent of that concern 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 S-methoprene to determine which routes of transport are likely to have an impact on the CRLF. In the case of S-methoprene, the exposure routes that are most likely to affect non-target organisms are direct applications to water of liquid formulation and sustainable release formulations (granular/briquette/pellet) with constant release rates. Direct application of liquid formulations to land and granular application to land will also be considered in this assessment for possible impact to terrestrial-phase CRLF.

LOC exceedances are used to describe how far effects may be seen from the initial area of concern. Since the Agency is evaluating direct application to water as a upper bound scenario, factors such as spray drift, downstream run-off, atmospheric transport, etc. are not a part of this assessment since these exposure routes present a lower potential for exposure. The LOCs used in the analysis were the endangered species LOC for acute effects (0.05 for aquatic animals; 0.1 for terrestrial animals) and the chronic LOC (1 for aquatic and terrestrial animals).

2.8 Assessment Endpoints and Measures of Ecological Effect (Mortality, Growth, and Reproduction)

Assessment endpoints are defined as "explicit expressions of the actual environmental value that is to be protected."[3] Selection of the assessment endpoints is based on valued entities (e.g., CRLF, organisms important in the life cycle of the CRLF, and the PCEs of its designated critical habitat), the ecosystems potentially at risk (e.g. aquatic areas, riparian vegetation, and upland and dispersal habitats), and the routes by which ecological receptors are exposed to S-methoprene-related contamination (e.g., direct contact, etc).

As discussed in USEPA (2000) a quantitative component-based evaluation of mixture toxicity requires data of appropriate quality for each component of a mixture. In this mixture evaluation an LD_{50} with associated 95% confidence interval (CI) is needed for the formulated product. The same quality of data is also required for each component of the mixture. Given that the formulated products for methoprene do not have LD50 data available it is not possible to undertake a quantitative or qualitative analysis for potential interactive effects. However, because the active ingredients are not expected to have similar mechanisms of action, metabolites, or toxicokinetic behavior, it is reasonable to conclude that an assumption of dose-addition would be inappropriate. Consequently, an assessment based on the toxicity of methoprene is the only reasonable approach that employs the available data to address the potential acute risks of the formulated products.

Most S-methoprene formulations only contain a single active ingredient (e.g. S-methoprene). Available toxicity data for aquatic organisms did not show any significant differences between formulated product and the technical active ingredient. For aquatic species in which comparative data are available, the confidence intervals for technical and formulation overlap suggesting that the toxicity of technical S-methoprene and the formulations are similar. Toxicity data on avian species is only available for the technical active ingredient.

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

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

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 S-methoprene is provided in Table 2.7. 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.

Table 2.7 Summary of Assessment Endpoints and Measurements of Ecological Effects for Direct and Indirect Effects of S-Methoprene on the CRLF.

Assessment Endpoint	Measures of Ecological Effects[4]
Aquatic-Phase CRLF (Eggs, larvae, juveniles, and adults)[a]	
Direct Effects	
1. Survival, growth, and reproduction of CRLF	1a. Amphibian acute LC_{50} (ECOTOX) or most sensitive fish acute LC_{50} (guideline or ECOTOX) if no suitable amphibian data are available 1b. Amphibian chronic NOAEC (ECOTOX) or most sensitive fish chronic NOAEC (guideline or ECOTOX) 1c. Amphibian early-life stage data (ECOTOX) or most sensitive fish early-life stage NOAEC (guideline or ECOTOX)
Indirect Effects and Critical Habitat Effects	
2. Survival, growth, and reproduction of CRLF individuals via indirect effects on aquatic prey food supply (*i.e.,* fish, freshwater invertebrates, non-vascular plants)	2a. Most sensitive fish, aquatic invertebrate, and aquatic plant EC_{50} or LC_{50} (guideline or ECOTOX) 2b. Most sensitive aquatic invertebrate and fish chronic NOAEC (guideline or ECOTOX)
3. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat, cover, food supply, and/or primary productivity (*i.e.*, aquatic plant community)	3a. Vascular plant acute EC_{50} (duckweed guideline test or ECOTOX vascular plant) 3b. Non-vascular plant acute EC_{50} (freshwater algae or diatom, or ECOTOX non-vascular)
4. Survival, growth, and reproduction of CRLF individuals via effects to riparian vegetation	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)

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

Assessment Endpoint	Measures of Ecological Effects[4]
Terrestrial-Phase CRLF (Juveniles and adults)	
Direct Effects	
5. Survival, growth, and reproduction of CRLF individuals via direct effects on terrestrial phase adults and juveniles	5a. Most sensitive bird[b] or terrestrial-phase amphibian acute LC_{50} or LD_{50} (guideline or ECOTOX) 5b. Most sensitive bird[b] or terrestrial-phase amphibian chronic NOAEC (guideline or ECOTOX)
Indirect Effects and Critical Habitat Effects	
6. Survival, growth, and reproduction of CRLF individuals via effects on terrestrial prey (*i.e.,*terrestrial invertebrates, small mammals , and frogs)	6a. Most sensitive terrestrial invertebrate and vertebrate acute EC_{50} or LC_{50} (guideline or ECOTOX)[c] 6b. Most sensitive terrestrial invertebrate and vertebrate chronic NOAEC (guideline or ECOTOX)
7. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat (*i.e.*, riparian and upland vegetation)	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)

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 S-methoprene 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. 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 S-methoprene effects data are available.

Assessment endpoints and measures of ecological effect selected to characterize potential modification to designated critical habitat associated with exposure to S-methoprene are provided in Table 2.8. 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 S-methoprene on critical habitat of the CRLF are described in Table 2.7. Some components of these PCEs are associated with physical abiotic features (*e.g.*, presence and/or depth of a water body, or distance between two sites), which are not expected to be measurably altered by use of pesticides. Assessment endpoints used for the analysis of designated critical habitat are based on the adverse modification standard established by USFWS (2006).

Table 2.8 Summary of Assessment Endpoints and Measures of Ecological Effects for Primary Constituent Elements of Designated Critical Habitat

Assessment Endpoint	Measures of Ecological Effect
Aquatic-Phase CRLF PCEs (Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)	
Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile and adult CRLFs.	a. 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.	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 CRLF PCEs (Upland Habitat and Dispersal Habitat)	
Elimination and/or disturbance of upland habitat; ability of habitat to support food source of CRLFs: Upland areas within 200 ft of the edge of the riparian vegetation or dripline surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance	a. 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.	

[a] Physico-chemical water quality parameters such as salinity, pH, and hardness are not evaluated because these processes are not biologically mediated and, therefore, are not relevant to the endpoints included in this assessment.

2.9 Conceptual Model

2.9.1 Risk Hypotheses

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

The labeled use of S-methoprene within the action area may:

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

2.9.2 Diagram

The conceptual model is a graphic representation of the structure of the risk assessment. It specifies the stressor (S-methoprene), 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 4 and 6, and the conceptual models for the aquatic and terrestrial PCE components of critical habitat are shown in Figures 5 and 7. 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.

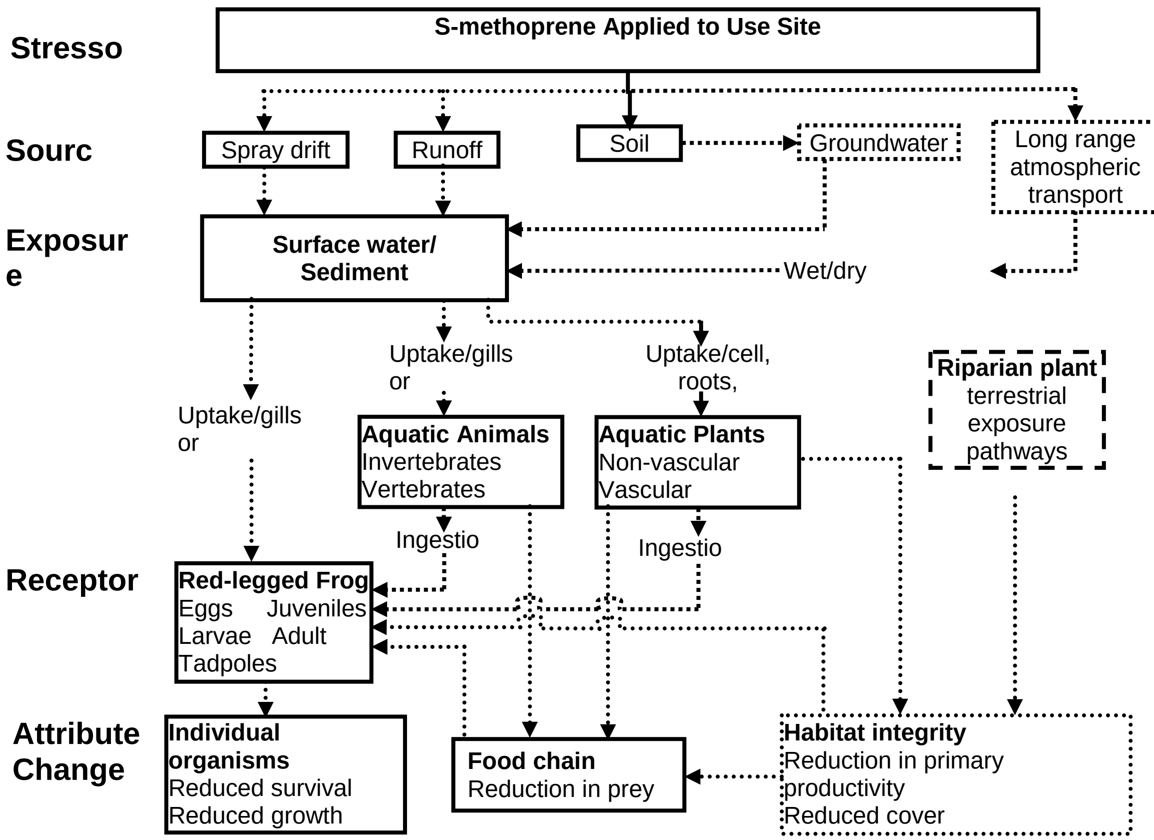

Figure 4.0 Conceptual Model for S-Methoprene Effects on Aquatic Phase of the Red-Legged Frog

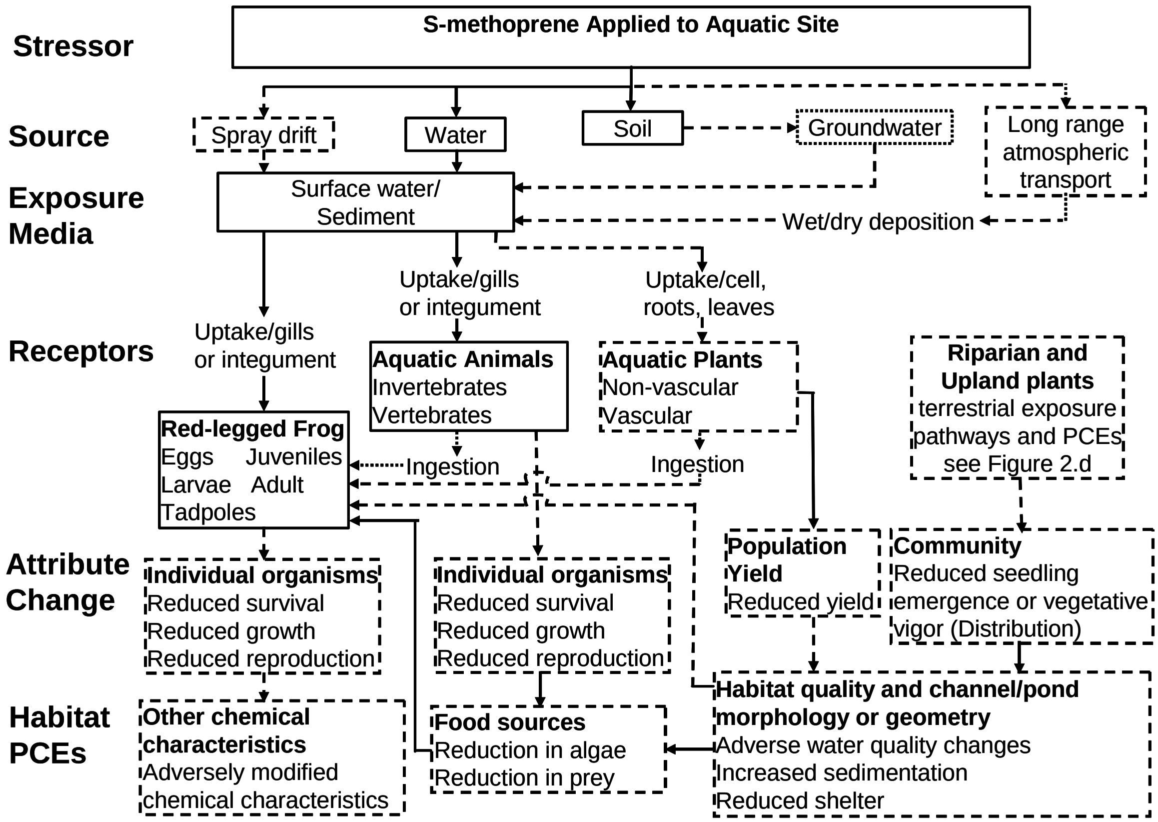

Figure 5.0 Conceptual Model for S-Methoprene Effects on Aquatic Component of the Red-Legged Frog Critical Habitat

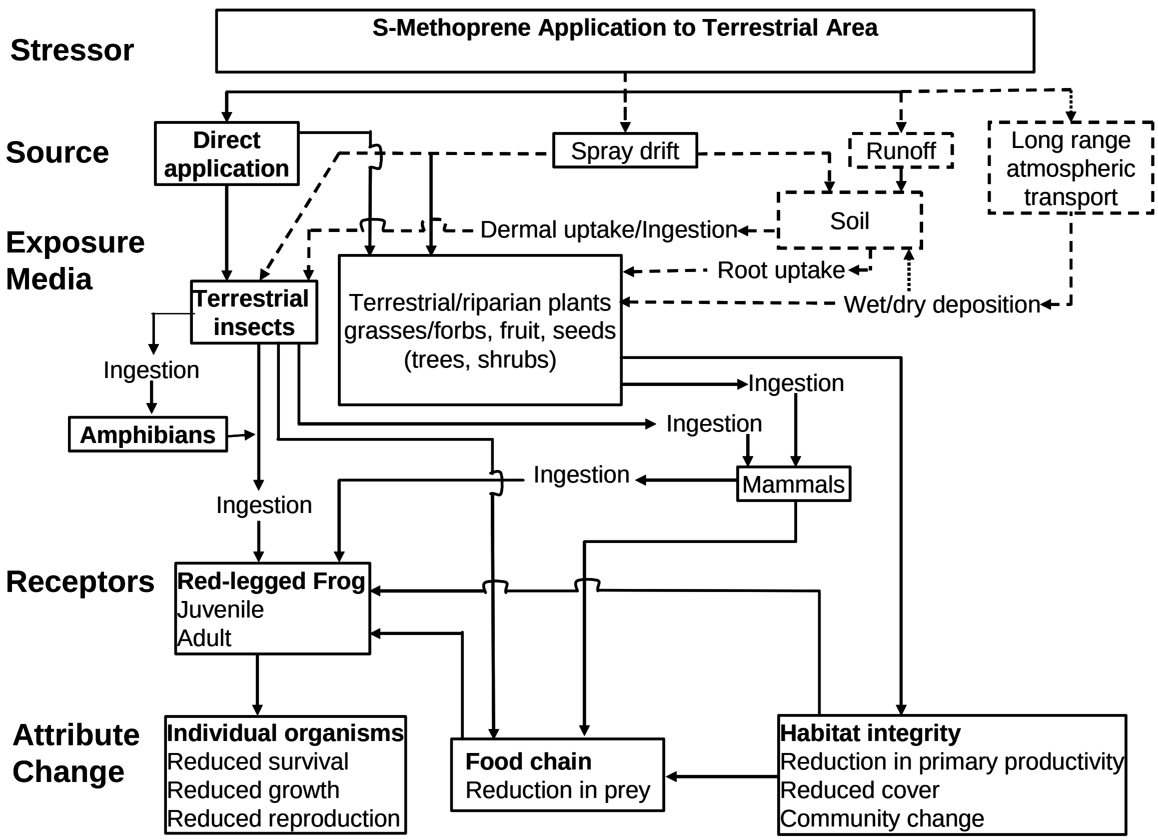

Figure 6.0 Conceptual Model for S-Methoprene Effects on Terrestrial Phase of the Red-Legged Frog

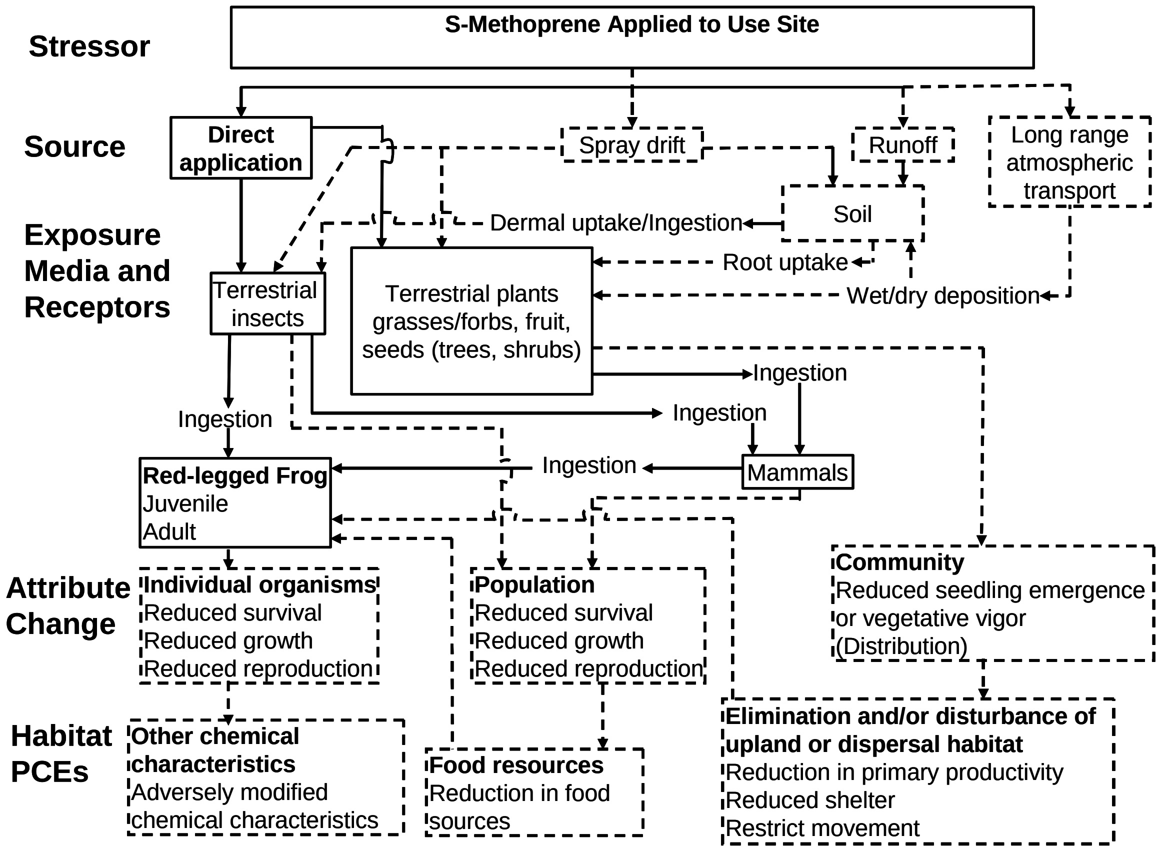

Figure 7.0 Conceptual Model for S-Methoprene Effects on Terrestrial Component of the Red-Legged Frog Critical Habitat

2.10 Analysis Plan

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

The maximum label application rates for use of S-methoprene on aquatic areas use sites in California were selected for modeling environmental concentrations for the screening-level deterministic (risk-quotient based) portion of this assessment. The most sensitive toxicity endpoints from surrogate test species are used to estimate treatment-related effects on growth, and survival and reproduction. Estimated environmental concentrations (EECs) used for this assessment are based solely on S-methoprene parent compound.

The following sections characterize the use, environmental fate, and ecological effects of S-methoprene and, using a risk quotient (ratio of exposure concentration to effects concentration) approach, estimate the potential for adverse effects on non-target terrestrial and aquatic animals. The assessment is then refined by exploring the potential for direct and/or indirect effects to the CRLF and/or the modification of its designated critical habitat from S-methoprene use in California to make our effects determinations.

2.10.1 Measures to Evaluate Risk Hypotheses and Conceptual Model

2.10.1.1 Measures of Exposure

The environmental fate properties and use pattern for S-methoprene suggest that runoff and spray drift are not the principal potential transport mechanisms of S-methoprene to the aquatic and terrestrial habitats of the CRLF. The relevant exposure pathway is direct application of S-methoprene to water and/or to land as a liquid formulation or as a sustainable release rate formulation.

Measures of exposure are based on aquatic and terrestrial models that predict estimated environmental concentrations (EECs) of S-methoprene using maximum labeled application rates and methods of application. The scenario used in this risk assessment to predict aquatic EECs is direct application of S-methoprene formulations to 1ft of shallow water. The sustainable release forms of S-methoprene were approached through calculations and extrapolations of expected environmental release rate from label information and efficacy studies. In addition the Agency also used adjusted field microcosm concentrations that were submitted by the registrant for these formulations. Exposure estimates for the terrestrial-phase CRLF and terrestrial invertebrates and mammals (serving as potential prey) assumed to be in the target area or in an area exposed to S-methoprene use are derived using the T-REX model (version 1.3.1, 12/07/2006). The T-REX model incorporates the Kenega nomograph, as modified by Fletcher *et al.* (1994), which is based on a large set of actual field residue data. The upper limit values from the nomograph represented the 95th percentile of residue values from actual field measurements (Hoerger and Kenega, 1972). For modeling purposes, direct exposures of the CRLF to S-methoprene through contaminated food are estimated using the EECs for the small bird (20 g) which consumes small insects. Dietary-based and dose-based exposures of potential prey (small mammals) are assessed using the small mammal (15 g) which consumes short grass. The small bird (20g) consuming small insects and the small mammal (15g) consuming short grass are used because these categories represent the largest RQs of the size and dietary categories in T-REX that are appropriate surrogates for the CRLF and one of its prey items. Estimated exposures of terrestrial insects to S-methoprene are bound by using the dietary based EECs for small insects and large insects. These approaches are parameterized using relevant reviewed registrant-submitted environmental fate data.

2.10.1.2 Measures of Effect

Measures of effect are obtained from a suite of registrant-submitted guideline studies conducted with a limited number of surrogate species and/or from acceptable open literature studies (EPA 2004, USFWS/NMFS 2004). The acute measures of effect routinely used for listed and non-listed animals in screening level assessments are the LD_{50}, LC_{50} or EC_{50}, depending on taxa (see Table 2.8). LD stands for "Lethal Dose", and LD_{50} is the amount of a material, given all at once, that is estimated to cause the death of 50% of a group of test organisms. LC stands for “Lethal Concentration” and LC_{50} is the concentration of a chemical that is estimated to kill 50% of a sample population. EC stands for “Effective Concentration” and the EC_{50} is the concentration of a chemical that is estimated to produce some measured effect in 50% of the test population. Endpoints for chronic measures of exposure for listed and non-listed animals are the NOAEL or NOAEC. NOAEL stands for “No Observed-Adverse-Effect-Level” and refers to the highest tested dose of a substance that has been reported to have no harmful (adverse) effects on a test population. The NOAEC (*i.e.*, “No-Observed-Adverse-Effect-Concentration”) is the highest test concentration at which none of the observed results were statistically different from the control.

Table 2.8. Acute and Chronic Measures of Effect.

TAXA	ASSESSMENT	MEASURE OF EFFECT
Aquatic Animals (*Freshwater fish and inverts.*	Acute	Lowest tested EC_{50} or LC_{50} (acute toxicity tests)
	Chronic	Lowest NOAEC (early life-stage or full life-cycle tests)

TAXA	ASSESSMENT	MEASURE OF EFFECT
Terrestrial Animals *Birds*	Acute/Subacute	Lowest LD_{50} (single oral dose) and LC_{50} (subacute dietary)
	Chronic	Lowest NOAEC (21-week reproduction test)
Terrestrial Animals *Mammals*	Acute	Lowest LD_{50} (single oral dose test)
	Chronic	Lowest NOAEC (two-generation reproduction test)

3.0 Exposure Assessment

3.1 Measure of Aquatic Exposure

Although, this compound has several indoor uses, this risk assessment will be focused on those outdoor uses that have the potential for S-methoprene exposure to fish and wildlife and the CRLF. Since S-methoprene is efficacious to Dipteran larvae, the effective mode of application to potential breeding areas is a direct application to water for liquid formulations, as well as, sustainable release rate formulations (briquets, granular). Target sites are any area or site of standing water. Non agricultural and agricultural land that is flooded is also registered for S-methoprene use. Since the compound can be applied to rice and caneberry flooded fields, the Agency derived EEC values by using the Tier I rice model (Appendix G). In order to evaluate exposure from direct application to water from application of sustainable release formulations, the Agency has used extrapolated values from label information that reflect direct application to 1 ft of water (Appendix I). Granular formulations were assessed by extrapolating S-methoprene release rate relative to the size of the granular and its expected length of efficacy in the environment. The aquatic EECs represent upper bound water column values, calculated without any consideration for S-methoprene degradation (photolysis or biodegradation) or adsorption to particulate/ sediment.

Table 3.0 Maximum Rate of S-Methoprene Formulations that are Applied Directly to Water (Extrapolated Values from Label Information)

Use	Formulation	App Intervals (days)	Max. Rate (lbs ai/A/day)	Peak EEC (ppb)	21-day EEC (ppb)	60-day EEC (ppb)
Woodland pools, swamps, rice fields, storm drains, etc.	Briquet Briquet XR	150 30	0.0058 0.014	2.0 5.04	2.0 5.04	2.0 5.04
Woodland pools, swamps, berry bogs, rice fields, irrigated crop lands, etc.	Granular Sand mix	7 7	0.06 0.017	0.06 0.017	.06 0.017	0.06 0.017
Woodland						

Use	Formulation	App Intervals (days)	Max. Rate (lbs ai/A/day)	Peak EEC (ppb)	21-day EEC (ppb)	60-day EEC (ppb)
pools, berry bogs, rice fields, irrigated crop lands, etc.	Liquid	7	0.013	4.81	-	-

In addition to extrapolated values, the Agency has also used microcosm generated field data submitted by the registrant for various slow release formulations (Appendix I). In order to be comparative to the formulations used in the extrapolated exercise, the microcosm values have been adjusted. This approach provides a range of EEC values that can be used in the development of aquatic risk quotients (RQ).

Table 3.1 Adjusted Environmental Concentrations of S-Methoprene Found in Freshwater Microcosm

Use	Formulation	Peak EEC (ppb)	21-day EEC (ppb)	60-day EEC (ppb)[1]
Woodland pools, swamps, rice fields, storm drains, etc.	Briquet Briquet XR	4.24 3.37	0.13 0.96	0.13 0.96
Woodland pools, swamps, berry bogs, rice fields, irrigated crop lands, etc.	Granular Sand mix	0.06 0.017	0.06 0.017	0.06 0.017
Woodland pools, berry bogs, rice fields, irrigated crop lands, etc.	Liquid	2.21	0.255	0.203

[1] Study was not conducted beyond 35 days. Therefore values for the 60-day EEC will be the values recorded at 35 days.

3.1.1 Monitoring Data

S-methoprene has a limited set of surface water monitoring data relevant to the CRLF assessment. No surface water monitoring studies which specifically targeted S-methoprene use (application period and/or sites) were available for analysis as part of this assessment. Generally, targeted monitoring data are collected with a sampling program designed to capture, both spatially and temporally, the maximum use of a particular pesticide. Because none of the available regional monitoring studies were designed specifically for S-methoprene, they are considered ‘non-targeted’. Typically, sampling frequencies employed in monitoring studies are insufficient to document peak exposure values. This coupled with the fact that these data are not temporally or spatially correlated with pesticide application times and/or areas limit the utility of these data in estimating exposure concentrations for risk assessment. Monitoring data can be used to set lower bounds on the occurrence in the environment, since concentrations were at least as high as those found in the monitoring studies. For these reasons, baseline risk assessments rely on model-generated values for estimating acute and chronic exposure values, and the non-targeted monitoring data are typically used for qualitative characterizations.

3.2. Measure of Terrestrial Exposure

3.2.1 Terrestrial Exposure Modeling

The EEC values used for terrestrial animal exposure are derived from the Kenaga nomograph, as modified by Fletcher *et al.* (1994), based on a large set of actual field residue data. The upper limit values from the nomograph represent the 95th percentile of residue values from actual field measurements (Hoerger and Kenaga, 1972). The Fletcher *et al.* (1994) modifications to the Kenaga nomograph are based on measured field residues from 249 published research papers, including information on 118 species of plants, 121 pesticides, and 17 chemical classes. These modifications represent the 95th percentile of the expanded data set. Risk quotients are based on the most sensitive LC_{50} and NOAEC for birds (Bobwhite quail and mallard duck) and LD_{50} for mammals (based on lab rat studies).

S-methoprene label instructions show that the terrestrial uses for the liquid formulations (EC and FLC) are chemigation to ornamental woody plants, lawns, low pressure spray around building premises, and application to fire ant mounds. Since, the maximum application to a terrestrial site (ornamental woody plants) is 0.5829 lbs ai/A, the Agency derived EECs (Table 3.2) and assumed this to be a upper bound scenario. Using the T-REX model (version 1.3.1, December 22, 2006) with maximum input values (4 applications at 0.5829 lb a.i./A with a 7-day application interval) the Agency was able to estimate terrestrial exposure for avian and mammalian species.

Table 3.2 Estimated Environmental Concentrations (in mg/kg; parts per million (ppm) on Potential Food Items Following Label-Specified Applications (4 Applications at 0.5829 lb a.i./Acre, a 7-Day Application Interval) of S-Methoprene Using T-REX

DIETARY-BASED EECs	Estimated Environmental Concentrations (ppm)	
	Upper Bound	Mean
Short Grass	262	93
Tall Grass	120	39
Broadleaf Plants/Small Insects	147	49
Fruits/Pods/Seeds/Large Insects	16	8

Table 3.3 characterizes S-methoprene granular LD_{50}/square foot using the T-REX model. In order to evaluate risk from exposure to granular formulations, the Agency has modeled a scenario with maximum application to the area around citrus orchards. The label

information indicates that prior to flooding or in anticipation of flooding, granular S-methoprene can be broadcast to a terrestrial area like citrus (0.3 lbs ai/A). Although frogs do not feed on granular particles this scenario was included as a upper bound scenario. The values noted in Table 3.3 reflect maximum application of the granular formulation to terrestrial areas where the CRLF may be found. A complete description of the input parameters and output is contained in **Appendix H**

Table 3.3 Characterization of S-Methoprene Granular LD50/Square Foot Using T-REX for a 20 g bird (Granular Weight = 0.43 mg)

Estimation of the number of granules needed to achieve toxicity thresholds	
No. of granules needed to achieve adjusted LD50	114984.17
No. of granules needed to achieve Acute LOC exceedance (1/2 adjusted LD50)	57492.09
No. of granules needed to achieve Endangered Species LOC exceedance (1/10 adjusted LD50)	11498.42
Minimum Foraging Area Needed to Allow for Ingestion of Sufficient Mass of a.i. to Achieve LOC Exceedance	
Foraging area (square feet) needed to achieve LOC exceedance assuming 100% feeding efficiency	0.66
Foraging area (square feet) needed to achieve LOC exceedance assuming 50% feeding efficiency	1.33
Foraging area (square feet) needed to achieve LOC exceedance assuming 10% feeding efficiency	6.65

Table 3.4 shows acute and chronic RQ values for food items (e.g. small and large insects) that terrestrial-phase CRLF may utilize. The values are based on upper bound Kenaga values for T-REX and show no acute risk to avian species or chronic risk to mammalians. Avian reproductive study did not show a LOEC but the NOAEC was found to be 32 ppb.

Table 3.4 Acute and Chronic RQs for Terrestrial-Phase CRLF (Based on Upper Bound Kenaga Values from T-REX).

DIETARY CATEGORY	Acute Avian RQ: Dose-Based		Acute Avian RQ: Dietary-Based	Mammalian Chronic RQ: Dietary-Based
	20 g	100 g		
Broadleaf plants/small insects	0.09	0.07	0.01	0.0
Fruits/pods/seeds/large insects	0.02	0.01	0.00	0.2

Bolded RQs exceed the Agency's endangered species LOC

3.2.2 Terrestrial Plant Exposure

S-methoprene is non-toxic to plants. Residues studies on wheat have shown that this compound does not translocate in plants and is not picked-up from soil.

4. 0 Effects Assessment

Based on the available data, S-methoprene is characterized as acutely very high to moderately toxic to freshwater fish. S-methoprene is highly toxic to freshwater invertebrates on an acute basis and chronically toxic to the developing juveniles (growth effects). Aquatic predatory insects appear to show moderate acute toxicity after S-methoprene exposure. Terrestrial organisms appear to be less sensitive to S-methoprene exposure. Avian and mammal species show practically no acute toxic effects after exposure to S-methoprene. Toxicity data for terrestrial invertebrates that are chronically exposed to S-methoprene are not currently available. See Table 4.0 for the assessment endpoints used in this assessment (*i.e.*, the most sensitive acute and chronic endpoints for each taxon assessed here).

Table 4.0 Summary of Specific Assessment Endpoints Considered in This Assessment.

TAXA	MEASURE OF EFFECT		
Survival, growth and/ or reproduction of:	**Species**	**Toxicity**	**Endpoint**
Freshwater Fish	***Acute***		
	Rainbow trout (*Oncorhynchus mykiss*)	96 hr LC_{50} = 0.76 mg/L	Mortality
	Chronic		
	Fathead minnow (*Pimephales promelas)*	NOAEC = 0.048 mg/L	Growth affected
Freshwater Invertebrates	***Acute***		
	Daphnia magna	EC_{50} = 0.33 mg/L	Mortality
	Chronic		
	Daphnia magna	NOAEC = 0.051 mg/L	Growth Effects
Birds	***Acute***		
	Mallard duck (*Anas platyrhynchos*) Mallard duck (*Anas platyrhynchos*)	LC_{50}>10,000 ppm LD_{50}>2,000 mg ai/kg	Mortality Mortality
	Chronic		
	Mallard duck (*Anas platyrhynchos*)	NOAEC at 3 and 30 ppm.	No reproductive effects
Mammals	***Acute***		
	Rat (Rattus norvegicus)	LD_{50} >5,000 mg/kg	Mortality
	Chronic		
	Rat (*Rattus norvegicus*)	NOEL = 2,500 ppm	No adverse effects
Aquatic Insect	***Acute***		
	Water boatman *(Corisella decolor sp.*)	24 hr LC50 = 1.20 mg/L	Mortality

4.1 Evaluation of Terrestrial Ecotoxicity Studies

4.1.1 Terrestrial Plant Exposure

S-methoprene is non-toxic to plants. Residues studies on wheat have shown that this compound does not translocate in plants and is not picked-up from soil.

4.1.2 Bird and Mammal Hazard Assessment

4.1.2.1 Avian Toxicity Studies

An acute oral toxicity study using the technical grade of the active ingredient (TGAI) is required to establish the toxicity of S-methoprene to birds. The preferred test species is either mallard duck (*Anas platyrhynchos*; a waterfowl) or Bobwhite quail (*Colinus virginianus*; an upland game bird). Results of these studies are summarized below in Table 4.0. S-methoprene has been shown to be practically non-toxic to avian species as noted in the mallard duck and Bobwhite quail acute studies where toxicity ranges from LD_{50} >2,000 mg/kg to LC_{50} = 10,000 ppm. No reproductive effects at 3 and 30 ppm for Bobwhite quail (Table 4.1).

Table 4.1 Summary of Avian Toxicity for S-Methoprene

Test Species	% ai	Endpoint	Toxicity Category	MRID No. Author/Year	Study Classification1
Acute Toxicity					
Mallard duck (*Anas platyrhynchos*)	Tech.	LD_{50}>2,000 mg ai/kg	Practically non-toxic	003202508 Fink/1972	Acceptable
Bobwhite quail *(Colinus virginianus)*	Tech	LC_{50} > 10,000 ppm	Practically non-toxic	003202509 Fink/1972	Acceptable
Mallard duck (*Anas platyrhynchos*)	Tech.	LC_{50}>10,000 ppm	Practically non-toxic	003202509 Fink/1972	Acceptable
Chronic Toxicity					
Bobwhite quail *(Colinus virginianus)*	Tech.	No reproductive effects at 3 and 30 ppm.		003202511 Fink&Reno/ 1973	Acceptable

4.1.2.2 Mammal Studies

Summaries of the most sensitive toxicity values (acute and chronic) for mammals are shown in Table 4.2. The acute oral LD_{50} for the racemic and S-methoprene in rats is >10,000 (Hallesy *et al.*, 1972) and 5,000 mg/kg (Shindeler and Brown, 1984), respectively. The acute dermal LD_{50} for both the racemic and the S-methoprene in rabbits is >2,000 mg/kg (Hamiliton, 1972; Brown 1984, respectively). A 2 year chronic feeding test showed that rats expose to S-methoprene at 0, 250, 1000, or 5,000 ppm in the daily diet did not exhibit any adverse health effects when compared to controls (Wazeter & Goldenthal, 1975). The data show that methoprene (racemic or S-methoprene) has an extremely low potential for acute toxicity to mammals via oral, dermal, or chronic routes of exposure. Data are available for evaluating reproductive effects of S-methoprene to mammals. The three-generation rat reproduction study showes a NOEL = 2,500 ppm and no reproductive effects. These results suggest that chronic exposure of mammals to S-methoprene is not expected to cause developmental toxicity.

Table 4.2 Summary of Mammalian Toxicity Studies for S-Methoprene and RS-Methoprene

Test Species	% ai	Test Duration	Endpoint	MRID No. Author/year	Study Classification
Rat *(Rattus norvegicus*)	RS-methoprene tech	14-day	LD_{50} >10,000 mg/kg	00024607 Hallesy *et al*./1972	Acceptable
Rat *(Rattus norvegicus*)	S-methoprene tech	14-day	LD_{50} >5,000 mg/kg	00150132 Shindler & Brown/ 1984	Acceptable
Rabbit	RS-methoprene tech	Acute dermal	LC_{50}>2,000 mg/kg	00024617 Hamiliton/ 1972	Acceptable
Rabbit	S-methoprene	Acute dermal	LC_{50}>2,000 mg/kg	00150133 Brown/1984	Acceptable
Rat *(Rattus norvegicus*)	RS-methoprene tech	90-days	NOEL = 500 ppm	00024612 Jorgenson & Sasmore/1972	Acceptable
Rat *(Rattus norvegicus*)	RS-methoprene tech	2-year	NOEL = 5,000 ppm	00010739 Wazeter & Goldenthal/1972	Acceptable
Rat *(Rattus norvegicus*)	RS-methoprene tech	Three generation reproduction	NOEL = 2,500 ppm	00010741 Kileen & Rapp/1974	Acceptable

4.1.1.3 Toxicity of S-Methoprene to Insects

Although S-methoprene is most toxic to Dipterans, it is also toxic to non-target species that include Hemiptera, Lepidoptera, and Coleopteran. Mosquitoes are very sensitive to methoprene exposure at about 0.001 mg/L (Lawler, 2000). The available acute toxicity tests that were conducted on non-target insects are presented in Table 4.3.

Table 4.3 Toxicity of RS-Methoprene to Insects

Species	% ai RS-methoprene	Results	Reference
Water boatman (*Corisella decolor sp*.)	10	96 hr LC_{50} = 1.65 mg/L	Miura, 1973
Backswimmers (*N. unifasciata*)	10	24 hr LC_{50} = 1.20 mg/L	Miura, 1973
Honey bee (Apis mellifera L.)	10	31 day feeding. No effect at 1000 ug/L methoprene	Barker and Waller, 1978
Diving Beetles (*Laccophilus sp.)*	10	72 hr LC_{50} = 2.0 mg/L	Miura, 1973
Mosquitoes larvae (Ochlerotatus nigromaculis)	10	Toxic effect at 1.0 ug/L	Miura, 1973

4.2 Evaluation of Aquatic Ecotoxicity Studies

4.2.2 Aquatic Hazard Assessment

4.2.1.1 Fish Toxicity Studies

The Agency has summarized available acute fish studies and concluded that S-methoprene is highly to moderately toxic (0.37-1.52 mg/L to warm water freshwater fish and highly to practically non-toxic (0.76-106 mg/L) to coldwater, freshwater fish. Acute toxicity on the metabolite ZR-1946 shows an LC_{50} = 36.9 mg/L suggesting slight toxicity Table 4.4).

Table 4.4 Summary of Fish Toxicity Studies for S-methoprene (Parent Compound, Metabolites, Formulation)

Species	(% ai)	Results	MRID	Classification
Freshwater Species				
Bluegill sunfish (*Lepomis macrochirus*)	Tech.	96hr LC_{50} =1.52 mg/L	00010388	Acceptable
	Tech	96hr LC_{50} > 0.37 mg/L	43351902	Supplemental
Rainbow trout (*Oncorhynchus mykiss*)	Tech.	96hr LC_{50}=1.6 mg/L	2033423	Supplemental
	Tech	96hr LC_{50} = 4.39 mg/L	2033423	Supplemental
	Tech	96hr LC_{50} > 50 mg/L	00010643	Acceptable
	Tech.	96hr LC_{50} =106 mg/L	2033423	Supplemental
	Tech	96 hr LC_{50} = 0.76 mg/L (0.24 – 1.2 mg/L)	43351901	Supplemental
	10	96hr LC_{50} = 28.4 mg/L	2033423	Supplemental
	4E	96hr LC_{50} = 6.0 mg/L	2033423	Supplemental
	Metabolite ZR-1946	96hr LC_{50} = 36.9 mg/L	2033423	Supplemental

Species	(% ai)	Results	MRID	Classification
	(methop rene acid)			
	10	96hr LC_{50} =52.7 mg/L	2033423	Supplemental
	10	96hr LC_{50}=1.55 mg/L; (1.10-2.41 mg/L)		Supplemental
	68.9	24hr LC_{50}>10.0 mg/L		Supplemental
	68.9	24hr LC_{50}=80.0 mg/L (80 -100 mg/L)		Supplemental

A chronic toxicity study on the fathead minnow produced a NOAEC value of 48 ug/L and a LOAEC of 84 ug/L. The effects that were noted included decreased weight and length of juveniles. No other effects were noted (Table 4.5).

Table 4.5 Freshwater Fish: Chronic Exposure (Growth/Reproduction) Studies

Species	% ai	Effect	MRID	Classification
Fathead minnow *(Pimephales promelas)*	Tech	NOAEC = 48 ug/L LOAEC = 84 ug/L Growth effects	42811201	Supplemental

4.2.1.3 Toxicity to Aquatic Freshwater Invertebrates

Acute toxicity studies on *Daphnia magna* showed 48 hr EC_{50} = 0.33 – 0.36 mg/L suggesting that S-methoprene is highly toxic to freshwater invertebrates on an acute basis. Chronic toxicity to daphnid early life stages showed effects on growth at 0.051 mg/L (Table 4.6).

Table 4.6 Freshwater Invertebrates Acute Toxicity Studies with S-Methoprene

Species	% ai	Effect	MRID	Classification
Acute Toxicity				
Daphnia magna	Tech.	48 hr. EC50 = 0.36 mg/L (0.21-0.55 mg/L)	43163301	Supplemental
Daphnia magna	Tech.	48hr. EC50 = 0.33 mg/L (0.11 – 0.52 mg/L)	003203609	Acceptable
Chronic Toxicity				
Daphnia magna	Tech.	LOAEC = 0.051 mg/L	2033145	Supplemental

4.2.1.4 Field Studies: Non-target organisms

A comprehensive study on the effects of S-methoprene to nontarget aquatic organisms was conducted in Minnesota by the Metropolitan Mosquito Control District (Hershey *et* *al*.,1998). Wetlands in Wright County were sampled for three years (1988-1990) in order to evaluate natural variability in 179 genera of insect. After this baseline sampling, eight of the wetlands were treated six times during the spring and the summer at 3-week intervals (1991-1993) with S-methoprene at 0.05-0.058 kg a.i./ha (0.275 – 0.32 lbs ai/A) based on a 4% a.i. formulation as a 20-d release granule). Nine other sites were treated with *Bacillus thuringiensis israelensis* (Bti) and nine were left untreated. During the first year of treatment, S-methoprene exposure had minimal effects on nontarget insect groups. However, in the second and third years researchers noted a significant reduction in taxa richness of Tipulidae, Ceratopogonidae, and Stratiomyidae. Insect density was reduced by 57 – 83% and biomass reduction amounted to 50-83% during this test period (Niemi *et al.*, 1999). Examination of the reproductive success of red-winged blackbirds (*Agelaius phoeniceus*) did not indicate that S-methoprene exposure had an adverse impact. Ali (1991) evaluating S-methoprene (Altosid Liquid Larvicide 5%) efficacy against midges (Chironomidae) in experimental ponds found that at 0.28 kg a.i./ha (1.5 lbs ai/A) was effective against *tanytarsini* and *chironomini*. He also noted that this formulation had very little effect on chironomids when it was applied at 0.015 kg a.i./ha (0.075 lbs ai/A). Pinkney *et al*., (2000) investigated the non-target effects of S-methoprene (Altosid Liquid Larvicide 5%) in experimental ponds at the Patuxent Wildlife Research Center, Maryland. Researchers sprayed (0.011 kg a.i. kg/ha, 0.06 lbs ai/A) the ponds three times at 3-week intervals and insect-emergence was evaluated before and after spraying. Relative to controls, the emergence data showed only isolated cases of significant non-target insect reductions in the sprayed ponds, and the Hester-Dendy data showed no significant difference between the S-methoprene and control ponds. Norland and Mulla (1975) using experimental ponds, exposed caged mayfly nymphs (*Callibaetis pacificus*) to an emulsified concentration of S-methoprene (1.56 lbs a.i./A; 0.30 kg a.i./ha). Emergence was tracked at 4 hours and again at 4 days after treatment. The results show substantial decrease in the percentage emerging from exposure groups relative to controls.

Table 4.7 Overview of S-methoprene Field Studies and Effects to Non-Target Insects

Researcher	Amount of S-methoprene used	Results relative to non-target insects
Pinkeny et al., (2000)	0.011 kg a.i./ha (0.06 lbs ai/A); EC	Little to no effect to non-target insects.
Ali (1999)	0.28 kg a.i./ha (1.5 lbs ai/A); EC	*Tanytarsini* and *chironomini* populations effected.
	0.015 kg a.i./ha; EC	No effects
Hershey *et al*., 1998	1.1 – 13.20.058 kg a.i./ha (0.27 -0.32 lbs ai/A); 20-day slow release granule.	1st year there were no significant effects. 2nd and 3rd years population effects were noted for Tipulidae, Ceratopogonidae, and Stratiomyidae.
Norland and Mulla (1975)	0.3 kg a.i./ha (1.56 lbs	Caged mayflies. Substantial

	ai/A); EC	decrease in emergence.

5.0 Risk Characterization

5.1 Risk Estimation - Integration of Exposure and Effects Data

Risk characterization is the integration of exposure and ecological effects characterization to determine the potential ecological risk from registered uses of S-methoprene, and the likelihood of direct and indirect effects to CRLF in aquatic and terrestrial habitats. For the screening-level portion of this assessment, the deterministic risk quotient method is used to provide a metric of potential risks. The RQ is a comparison of exposure estimates to toxicity endpoints; estimated exposure concentrations are divided by acute and chronic toxicity values. The resulting unit less RQs are compared to the Agency's levels of concern (LOCs) (see Table 5.0). LOCs are used to indicate when the use of a pesticide, as directed on the label, has the potential to cause adverse effects on non-target organisms.

Table 5.0 Agency Levels of Concern (LOC).

Risk	Description	RQ	Taxa
Acute	Potential for acute risk to non-target organisms which may warrant regulatory action in addition to restricted use classification	acute RQ > 0.5	aquatic animals, mammals, birds
Acute Restricted Use	Potential for acute risk to non-target organisms, but may be mitigated through restricted use classification	acute RQ > 0.1	aquatic animals
		acute RQ > 0.2	mammals and birds
Acute Listed Species	Listed species may be potentially affected by use	acute RQ > 0.05	aquatic animals and terrestrial invertebrates
		acute RQ > 0.1	mammals and birds
Chronic	Potential for chronic risk may warrant regulatory action, listed species may potentially be affected through chronic exposure	chronic RQ > 1	all animals
Non-Listed and Listed Plant	Potential for effects in non-listed and listed plants	RQ > 1	all plants

5.2 Potential for Direct Effects

5.2.1 Aquatic-Phase of CRLF

Based on surrogate freshwater toxicity data and extrapolated and actual field EECs, the Agency has calculated RQ values to reflect a wide range of uncertainty. The extrapolated values are theoretical expected environmental water concentration and were calculated from label information regarding weight of briquete or granular, the amount of active ingredient present, and the expected efficacy of the formulation in the field. This approach reflects upper-bound values and assumes a steady state release rate, no degradation, and no adsorption to particulate. The aquatic areas that this approach is expected to simulate are stagnant water bodies of about 1 foot in depth. These areas are the most prolific breeding grounds for mosquitoes. Although S-methoprene is not used on agricultural crops, flooded agricultural lands are registered for S-methoprene applications (i.e. rice, caneberries). In addition to this extrapolated approach, the Agency is also using field concentrations (Table 5.1) that were generated in a controlled freshwater microcosm study (Judy and Howell, 1992).

Table 5.1 Acute and Chronic RQs for Aquatic Organisms Based on EECs from Extrapolated S-Methoprene Release Rates for Granular, Briquets, Sand Mix, and Liquid Formulations Applied to a Shallow (1 ft) 1 Acre Body of Water

Formulation	Peak EEC (ug/L)	Acute RQ	35 day EEC (ug/L)	Chronic RQ
Freshwater Fish		**LC_{50} = 760 ug/L**		**(NOAEC = 48 ug/L)**
Briquets	2.0	0.00	2.0	0.04
Briquet XR	5.04	0.01	5.04	0.10
Granular	22.0	0.03	22.0	0.46
Sand Mix	6.2	0.01	6.2	0.13
Liquid	0.5853	0.00	0.5853	0.01
Freshwater Invertebrates		**EC_{50} = 330 ug/L**		**NOAEC = 51 ug/L**
Briquets	2.0	0.01	2.0	0.04
Briquet XR	5.04	0.01	5.04	0.10
Granular	22.0	**0.07**	22.0	0.43
Sand Mix	6.2	0.02	6.2	0.12
Liquid	0.5853	0.00	0.5853	0.01

The extrapolated EEC values in Table 5.0 produced low RQs generated from the five S-methoprene formulations that are registered for use on a wide range of aquatic areas that may support mosquito populations (marshes, swamps, culverts, wetlands, flooded orchards, flooded agricultural fields, old tires, etc). These formulations are slow release in order to be efficacious over a period of time (7 – 150 days) when applied directly to water. Using the acute and chronic fish data as a surrogate for the aquatic-phase of the CRLF, the RQ values (acute RQs = 0.0 – 0.03; chronic RQs = 0.1 – 0.13) prove to be less than the acute endangered (acute LOC >0.05; chronic LOC>1.0), suggesting a “no effect” to these organisms. The Agency has also evaluated the EECs that were generated in the microcosm study where actual S-methoprene levels were measured over a period of time (35 days). The environmental concentrations that were generated in the microcosm study were adjusted to reflect the current maximum rates and percent active of the formulations in Table 5.2. These measured concentrations were generally lower than the extrapolated values and reflected some initial fluctuation in S-methoprene levels (an initial high release rate before a steady state was achieved). However, the RQs generated from the upper bound values from this field data also suggest no direct acute or chronic risk from S-methoprene exposure to the aquatic-phase of the CRLF.

Table 5.2 Acute and Chronic RQs for Aquatic Organisms Based on Maximum Adjusted EECs from Microcosm Treated With S-Methoprene Granular,Briquets, Sand Mix, and Liquid Formulations

Formulation	Peak EEC (ug/L)	Acute RQs	35 day EEC (ug/L)	Chronic RQs
Freshwater Fish		**LC_{50} = 760 ug/L**		**(NOAEC = 48 ug/L)**
Briquets	4.24	0.00	0.14	0.00
Briquet XR	3.37	0.00	0.96	0.02
Granular	2.10	0.00	0.21	0.00
Sand Mix	0.35	0.00	0.20	0.00
Liquid	2.21	0.00	0.20	0.00
Freshwater Invertebrates		**EC_{50} = 330 ug/L**		**NOAEC = 51 ug/L**
Briquets	4.24	0.01	0.14	0.00
Briquet XR	3.37	0.01	0.96	0.02
Granular	2.10	0.01	0.21	0.00
Sand Mix	0.35	0.00	0.20	0.00
Liquid	2.21	0.01	0.20	0.00

The probit dose-response slope can be used to calculate the chance of an individual event corresponding to the listed species acute LOCs and/or RQs. The analysis uses the EFED spreadsheet IEC (version 1.1.xls). It is important to note that the IEC model output can go as low as 1 x 10^{-16} in estimating the event probability for animals. This cut-off is a limit in the Excel spreadsheet environment and is not to be interpreted as an agreed upon lower bound threshold for concern for individual effects in any given listed species. If information is unavailable to estimate a slope from a study, a default slope assumption of 4.5 is used as per original Agency assumptions of typical acute toxicity dose-response slope cited in Urban and Cook (1986).

The slope for the LC_{50} of the most sensitive acute freshwater fish (rainbow trout; LC_{50} = 760 µg a.i./L) was not available. Therefore, the default slope of 4.5 was used in determining the chance of an individual effect. Using the acute endangered species LOC of 0.05, the chance of an individual mortality for aquatic-phase CRLF is ~ 1 in 418,000,000 suggesting "no effect" of S-methoprene direct exposure to the aquatic-phase CRLF (Table 5.3).

Table 5.3 Chance of Individual Acute Effects to Aquatic-Phase CRLF Using Surrogate Freshwater Fish Toxicity Data and the Probit Slope Response Relationship.

LOC OR USE SITE SCENARIO (RQ)	LOC OR RQ	PROBIT SLOPE		CHANCE OF AN INDIVIDUAL EFFECT
Acute Endangered Species LOC	0.05	Slope	4.5	~ 1 in 418,000,000
		Upper Bound	2.32	~ 1 in 418,000,000
		Lower Bound	6.15	~ 1 in 418,000,000

5.2.2 Terrestrial-Phase of CRLF

Based on surrogate avian toxicity data, the maximum allowable application rate (4 applications, 0.013 lbs a.i./acre/application, 7-day application interval), the body-weight scaling factor for S-methoprene from Mineau *et al.* (1996) of 1.0778, and upper bound Kenaga values from T-REX, there is no potential for direct adverse effects on terrestrial-phase CRLF individuals from S-methoprene use in California (see Appendix H). Using calculations based on bird ingestion rates and dietary and weight categories for the CRLF, Table 3.4 shows RQ values for acute avian dose-base risk at 0.07 – 0.09 for 100 – 20g birds that consume small insects and RQs = 0.01 – 0.02 for the same size group consuming large insects. The acute avian dietary-based RQs ranged from 0.0 – 0.1, while the mammalian chronic dietary-based RQ ranged from 0.0 – 0.2. These RQ values do not exceed the Agency's LOC for avian and mammalian endangered species concerns (acute LOC>0.1). Additionally, the granular LD_{50}/square foot results that were generated in T-REX also suggest that the broadcast application of S-methoprene granulars should not create an acute toxicity concern for the terrestrial-phase of the CRLF or to birds and mammals in general. The number of granules needed to achieve the adjusted LD_{50} are about 114,984 suggesting an unlikely event (Appendix H, Table H.2).

5.3 Potential for Indirect Effects (Decreased Availability of Food Items)

5.3.1 Aquatic-Phase of CRLF

Aquatic-phase CRLF are known to eat diatoms, algae, and detritus (larvae CRLF) and aquatic and terrestrial invertebrates (juvenile CRLF). Since S-methoprene is not toxic to plants, only the invertebrate food sources will be assessed for potential indirect effects to aquatic-phase CRLF. The one aquatic invertebrate LOC exceedance (0.07) calculated by the Agency was for the 20% granular formulation (Table 5.1). However, this exposure value was an extrapolated upper-bound and as a comparison, the corresponding adjusted microcosm field value did not exceed the LOC (Table 5.2). In order to evaluate this exceedance, the Agency also calculated the chance of individual aquatic invertebrate exposure and risk by using the Individual Effects Chance Model (Version 1.1). These calculations suggest that the chance of effects to an invertebrate food source from this granular extrapolated exposure is about 1in 988,000, which may be considered as a highly unlikely event. As an additional indicator of possible risk, the acute and chronic invertebrate RQs for the other formulations (using extrapolated and microcosm exposure values) did not exceed LOCs for direct or indirect effects to the aquatic-phase CRLF. Therefore, the Agency concludes a "may affect" but "not likely to adversely affect" for the assessment point regarding the survival, growth, and reproduction of CRLF individuals via effects to aquatic food supply.

5.3.2 Terrestrial-Phase of CRLF

Adult and juvenile CRLFs forage in aquatic and terrestrial habitats. The main food sources for juvenile terrestrial-phase CRLFs are thought to be aquatic and terrestrial invertebrates. In addition to aquatic and terrestrial invertebrates, adults also feed on fish, frogs, and small mammals. S-methoprene is classified as practically non-toxic to mammals and avian species, as well as, predatory insects. Since the acute avian dose-based RQs range from 0.01 - 0.09, the avian dietary-based RQs = 0.0 – 0.01, and the chronic mammalian dietary-based RQs = 0.0 – 0.2 there are no LOC exceedances for the terrestrial-phase of the CRLF. Therefore, there should not be an indirect affect to the CRLF from S-methoprene exposure to potential terrestrial food items. Although, S-methoprene is efficacious to Dipterian larvae, these organisms are not major components of the CRLFs diet. Since, these frogs appear to be opportunistic feeders, a decline in adult mosquitoes and black flies should not influence terrestrial feeding habits.

There were no endangered species exceedances for maximum application of liquid or granular formulations. This suggests that there is very low potential for direct adverse effects to terrestrial-phase CRLF from S-methoprene use in California. The slope for the LC_{50} of the most sensitive avian species (mallard duck LD50 >2,000 ppm) was not available. Therefore, the default slope of 4.5 was used in determining the chance of an individual effect. Using the acute endangered species LOC of 0.05, the chance of an individual mortality for terrestrial-phase CRLF is ~ 1 in 294,000 (Table 5.4).

Table 5.4 Chance of Individual Acute Effects to Terrestrial-Phase CRLF Using Surrogate Mallard Duck Toxicity Data and the Probit Slope Response Relationship.

LOC OR USE SITE SCENARIO (RQ)	LOC	PROBIT SLOPE		CHANCE OF AN INDIVIDUAL EFFECT
Acute Endangered Species LOC	0.05	Slope	4.5	~ 1 in 294,000

5.4 Potential for Adverse Effects on Designated Critical Habitat PCEs

For S-methoprene use, the assessment endpoints for designated critical habitat PCEs involve a reduction and/or modification of food sources necessary for normal growth and viability of aquatic-phase CRLFs, and/or a reduction and/or modification of food sources for terrestrial-phase juveniles and adults. Since these endpoints are also being assessed relative to the potential for indirect effects to aquatic- and terrestrial-phase CRLF, the effects determinations for indirect effects from the potential loss of food items will be the same as the effects determinations regarding the potential for adverse effects on designated critical habitat PCEs.

Based on the best available information, the Agency has assessed the potential for direct and indirect risk to CRLF from S-methoprene exposure. The conclusion is that there is a **“may affect”, but “not likely to adversely affect”** determination for the CRLF from the use of S-methoprene. The assessment endpoints (Table 1.1) where this determination is made include the following:

- 1) Survival, growth, and reproduction of CRLF individuals via effects to food supply (*i.e.* freshwater fish and invertebrates, non-vascular plants);

This assessment point reflects an LOC exceedance (0.07) for acute endangered species concerns (LOC = 0.05) calculated from one of the upper bound extrapolated sustainable release formulations (20% granular), although, the microcosm field values for the same formulation did not exceed this LOC concern. In order to evaluate this exceedance, the Agency also calculated the chance of individual exposure using the Individual Effects Chance Model (Version 1.1). These calculations suggest that the chance of individual effect from this granular extrapolated exposure is about 1in 988,000, which may be considered as a highly unlikely event. As an additional test of possible risk, the use of acute and chronic fish and invertebrate toxicity data produced RQs for the other formulations (using extrapolated and microcosm exposure values) that did not exceed LOCs for direct or indirect effects to the aquatic-phase CRLF. Therefore, the Agency concludes a **"may affect" but "not likely to adversely affect"** reading for this assessment point.

The Agency acknowledges that S-methoprene is highly efficacious to Dipteran insect larvae and that the use of this compound can result in a decline in emerging adult populations. However, according to information of the CRLFs diet in Section 2.5.3 these insects are not included in their diet. The Agency assumes that the CRLF is an opportunistic feeder and will supplement its diet with available invertebrates and small animals. Therefore, the Agency concluded "no habitat modification" from S-methoprene use. Although there is widespread overlap of potential S-methoprene with watersheds of the CRLF the Agency has also determined that there is no potential for modification of CRLF designated critical habitat (aquatic or terrestrial plants) from the use of S-methoprene because this compound does not have herbicidal qualities or mode of action. Further information on the results of the effects determination are included as part of the Risk Description in Section 5.2.

6.0 Assumptions, Limitations and Uncertainties

6.1 Direct and Indirect Effects

6.1.1 Aquatic-Phase

Overall, the uncertainties inherent in the exposure assessment tend to result in both an over-estimation and under-estimation of exposures. Among the most significant overestimation of the total mass of S-methoprene to a single aquatic area is the extrapolations of release rates for the sustainable release formulations (granulars, briquets, sand mix). The values were calculated from label information and the expected efficacy in the field. These were treated as upper-bound estimates because the Agency did not take into consideration such mitigating factors as degradation and adsorption to particulate and sediments. In addition the extrapolated values reflect a constant release rate which may not occur in the environment. However, the Agency did temper these uncertainties with field data from a microcosm study on similar formulations. After adjusting these values to reflect the current maximum rates, these EECs were also used in formulating a risk assessment.

Additional factors that may account for under-estimation of exposure in this modeling relative to the most vulnerable watersheds may include differences between pond volume, field size, and flow dynamics relative to habitat characteristics of the CRLF.

6.1.2 Modeling Assumptions and Uncertainties

Overall, the uncertainties addressed in this assessment cannot be quantitatively characterized. However, given the available data and the tendency to rely on conservative modeling assumptions, it is expected that the modeling results in high-end exposure estimates, particularly at the screening level.

In general, the simplifying assumptions used in this assessment appear from the characterization in Section 3.2.6 to be reasonable. There are also a number of assumptions that tend to result in over-estimation of exposure. Although these assumptions cannot be quantified, they are qualitatively described. For instance, modeling in this assessment for each S-methoprene use assumes that all applications have occurred concurrently on the same day. This is unlikely to occur in reality, but is a reasonable conservative assumption in lieu of actual data.

6.2. Uncertainties Related to Terrestrial Exposures

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. The field measurement efforts used to develop the Fletcher estimates of exposure involve highly varied sampling techniques. It is possible that much of these data reflect residues averaged over entire above ground plants in the case of grass and forage sampling.

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

Differences in assimilative efficiency between laboratory and wild diets suggest that current screening assessment methods do not account for a potentially important aspect of food requirements. Depending upon species and dietary matrix, bird assimilation of wild diet energy ranges from 23 – 80%, and mammal's assimilation ranges from 41 – 85% (U.S. 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.

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 the modeled treatment area.

6.3 Effects Assessment Uncertainties

6.3.1. Use of Surrogate Species to Represent Sensitivity to S-methoprene

Toxicity data for aquatic- or terrestrial-phase amphibians are not available for use in this assessment. Therefore, fish and avian toxicity data, respectively, are used as a surrogate for aquatic- and terrestrial-phase CRLFs. If the surrogate species are substantially more or less sensitive than the CRLF, then risk would be over- or under- estimated, respectively.

6.3.2. Age Class and Sensitivity of Effects Thresholds

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

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

6.3.3 Sublethal Effects

For the acute risk assessment, the screening risk assessment relies on the acute mortality endpoint. 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 is used to assess chronic risk. 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.

Some sublethal effects have been reported in toxicity studies. However, these effects typically occurred at levels above the lowest NOAEC in fish that was used to derive risk quotients. Also, no data are available to link the sublethal measurement endpoints to direct mortality or diminished reproduction, growth or survival that are used by OPP as assessment endpoints.

6.3.4 Impact of Multiple Stressors on the Effects Determination

The influence of length of exposure and concurrent environmental stressors to the CRLF (i.e., construction of dams and locks, fragmentation of habitat, change in flow regimes, increased sedimentation, degradation of quantity and quality of water in the watersheds of the action area, predators, etc.) will likely affect the species response to S-methoprene. Additional environmental stressors may affect sensitivity to this compound, 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 low, and conservative estimates are made in the screening level risk assessment to account for these uncertainties.

6.3.5 Potential Exposure to Pesticide Mixtures

As discussed in Section 2.2, this assessment evaluates potential effects resulting from exposure to S-methoprene and its degradates. In the environment, multiple chemical stressors may co-occur. Quantifying the uncertainty of the presence of multiple stressors is beyond the scope of this assessment; however, some studies have evaluated potential interactive effects of several limited pesticide mixtures.

As discussed further in **Appendix I,** acute oral toxicity data (i.e., LD_{50} values) from mammalian studies for formulated products that contain S-methoprene and one or more additional active ingredients were also evaluated for potential interactive effects. The LD_{50} values are potentially useful only to the extent that a wild mammal would consume plants or animals immediately after these dietary items were directly sprayed by the product. Given uncertainties associated with the differential rates of degradation, transport, etc. for the active ingredients in the formulation with increasing time post application, a qualitative discussion of potential acute mammalian risk of the multiple-ai product relative to the single S-methoprene active ingredient is completed (USEPA 2004). While a quantitative evaluation of the data is not possible with currently accepted scientific methods, as a screening tool, a qualitative analysis can be used to indicate if formulated products exhibit interactive effects (e.g., synergism or antagonism).

6.4. Use Data

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.

6.5 General Uncertainties

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.

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

- Enhanced information on the density and distribution of CRLF life stages within specific recovery units and/or designated critical habitat within the action area. This information would allow for quantitative extrapolation of the present risk assessment's predictions of individual effects to the proportion of the population extant within geographical areas where those effects are predicted. Furthermore, such population information would allow for a more comprehensive evaluation of the significance of potential resource impairment to individuals of the species.

- Quantitative information on prey base requirements for individual aquatic- and terrestrial-phase frogs. While existing information provides a preliminary picture of the types of food sources utilized by the frog, it does not establish minimal requirements to sustain healthy individuals at varying life stages. Such information could be used to establish biologically relevant thresholds of effects on the prey base, and ultimately establish geographical limits to those effects. This information could be used together with the density data discussed above to characterize the likelihood of adverse effects to individuals.

- Information on population responses of prey base organisms to the pesticide. Currently, methodologies are limited to predicting exposures and likely levels of direct mortality, growth or reproductive impairment immediately following exposure to the pesticide. The degree to which repeated exposure events and the inherent demographic characteristics of the prey population play into the extent to which prey resources may recover is not predictable. An enhanced understanding of long-term prey responses to pesticide exposure would allow for a more refined determination of the magnitude and duration of resource impairment, and together with the information described above, a more complete prediction of effects to individual frogs and potential modification to critical habitat.

6.6 Uncertainty Regarding Incidents that have Suggested S-Methoprene Affects

Insect Juvenile Hormone

Insect juvenile hormone (JH) is secreted by a pair of endocrine glands behind the brain called the corpora allata. This compound is a regulator of insect development and modifies the response to the molting hormone, 20-hydroxyecdysone. Most insect species contain only juvenile hormone JHIII. To date JH0, JHI, and JHII have been identified only in the Lepidoptera (butterflies and moths). The form JHB3 (JHIII bisepoxide) appears to be the most important JH in the mosquitoes or flies. In larval insects, the JH-JH receptor interaction insures that the outcome of a molt is to another larval stage, while the absence of the JH-JH receptor binding results in a pupae or adult molt (Riddiford, 1996). Therefore, JH maintains larval and nymph characteristics in preadult insect stages and suppresses metamorphosis from final larval to adult stage (Riddiford, 1996). Female insect sexual maturity is also regulated by JH. Produced at high levels during larval stages, JH is reduced to negligible amounts at the onset of the pupae stage. After or during the transformation to mature adult the JH level increases again and influences egg production. The development of immature insects to adult forms depends on a delicate endocrine balance and can be affected by externally-introduced JH. Responses of insects to this exogenous hormone may be expressed through a change in the rate of emergence, the cessation of ecdysis, and the development of abnormal morphological features in immature stages (Staal, 1975). S-methoprene is a pesticide that acts as an insect juvenile hormone mimic disrupting the development of insects and preventing the larvae from emerging as adults. Used primarily in mosquito management, S-methoprene also has the potential to provide control against midge (Ali, 1981; Lothrop and Mulla, 1998).

Toxicity to Crustaceans

Although S-methoprene is used in mosquito management, and was developed as an analogue to the insect juvenile hormone (JH) in order to disrupt larval development, there have been concerns over possible impact to aquatic crustaceans. In evaluating the scope of possible impact to crustaceans from S-methoprene exposure we must take into consideration the phylogenic close relationship between crustaceans and insects. This information has been reinforced through research on the Hox gene, which shows where the divergence in this gene can be traced in insects and crustaceans (Boore *et al*., 1998). Both insects and crustaceans have similarities in their early developmental stages and both have certain analogues compounds of similar function, insect JH and methyl farnesoate (MF), the unepoxidated form of the insect juvenile hormone, in crustaceans (Laufer and Biggers, 2001). Like JH in insects, the MF appears to be stimulatory to early postembryonic larval stages and inhibitory in the larval-juvenile transitions. Because of the conserved similarity of these two endocrine compounds in insects and crustaceans, there is also the potential for toxic concern from S-methoprene exposure to crustaceans.

Toxicity to Amphibians

Reports of declining amphibian populations, as well as incidents of malformations in frog (anuran) species across the United States have raised concern with various causal explanations. Numerous reports have described occurrences of frog deformities with links to UV radiation (Ankley *et a*l., 1998), trematode parasites (Johnson *et al*., 1999), and possible exposure to pesticides like S-methoprene. Since S-methoprene is used to combat mosquitoes in urban and suburban wetlands, application of this compound can coincide with anuran reproduction and subsequent early stages of tadpole development.

S-methoprene is an insect juvenile hormone (JH) analogue that can be converted to a retinoid analogue after exposure to bacterial action and/or ultraviolet (UV) sunlight (Harmon *et al*., 1995). This process occurs rapidly in an aquatic environment under normal sunlight and temperature with a half-life of 30 hr at 1.0 ppb and 40 hr at 10 ppb (Harmon *et al*., 1995). Since retinoids act as ligands during vertebrate development, several researchers have expressed concern that S-methoprene exposure may have the potential to cause developmental effects to amphibians, especially during metamorphosis. Although JH is not present in vertebrate species, there is some evidence that S-methoprene and its derivative, S-methoprene acid, are capable of binding to the retinoid X receptor (RXR) and therefore may be able to effect vertebrate gene transcription (Harmon *et al.*,1995). Using cell cultures CV-1 Scoff *et al.*, (2004) found that retinoids can affect two classes of receptors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs). The corresponding ligands that are formed were found to function as transcription factors for regulating gene activity that is important in embryonic development of body axes, brain and limbs. Schoff *et al*. (2004) noted that a degradate of S-methoprene, methoxy-S-methoprene acid, acted as a ligand for RXRs and was capable of activating transcription through RAR/RXR response elements. Working with frog embryos, Minucci *et al*., (1996) found that during early development in *Xenopus*, synthetic retinoids selective for RXR and RAR receptors caused striking malformations along the anterior-posterior axis. In evaluating this potential problem, Degitz *et al*. (2003) did additional studies on the developmental toxicity of S-methoprene and it's degradates (S-methoprene acid, S-methoprene epoxide, 7-methoxycitronellal, and 7-methoxycitronellic acid) to frog embryos (*Xenopus laevis*) and found that exposure to 0.5 mg/L of parent compound did not result in developmental effects. However, several degradates did produce developmental effects at 1.25 mg/L (S-methoprene acid), 2.5 mg/L (S-methoprene epoxide acid), 5 mg/L S-methoprene epoxide and 2.5 mg/L (7-methoxycitronellal). No developmental or teratogenic effects were noted at > 30 mg/L for 7-methoxycitronellic acid. La Clair, (1998) noted that the lowest concentration of S-methoprene exposed to sunlight shown to cause malformations was 7.5 mg/L, which is 1,700 times the level found under typical applications of S-methoprene. Degitz *et al*. 2003, suggested that typical field application of sustained-release formulations of S-methoprene result in S-methoprene concentrations that do not exceed 0.01 mg/l, suggesting that S-methoprene-mediated developmental toxicity to amphibians may be overstated. It is unlikely that S-methoprene degradation products would accumulate in the environment to concentrations that could affect amphibian development (Ankley, 1998).

Whether S-methoprene has played a role in the loss of the CRLF is unclear but appears to be unlikely. The concentrations that have been reported in the environment are usually below the toxicity threshold that has been established from laboratory studies or field studies as noted in this assessment. There is still uncertainty in our understanding of the long-term exposures and the additive role of predators, parasites, UV light, other pesticides and other stressors to the wellbeing of the CRLF.

7.0 Addressing the Risk Hypotheses

In order to conclude this risk assessment, it is necessary to address the risk hypotheses defined in Problem Formulation (Section 2.9). Based on the results of this assessment, several hypotheses can be rejected, meaning that they are not of concern for the CRLF. However, several of the original hypotheses cannot be rejected, meaning that the statements represent concerns in terms of effects of S-methoprene on the CRLF.

Based on the results of this assessment, the following hypotheses **<u>can</u>** be rejected:
The labeled use of S-methoprene within the action area may:

- directly affect the CRLF by causing mortality or by adversely affecting growth or fecundity;
- indirectly affect the CRLF by reducing or changing the composition of food supply;
- indirectly affect the CRLF or modify designated critical habitat by reducing or changing the composition of the aquatic plant community in the ponds and streams comprising the species' current range and designated critical habitat, thus affecting primary productivity and/or cover;
- indirectly affect the CRLF or modify designated critical habitat by reducing or changing the composition of the terrestrial plant community (i.e., riparian habitat) required to maintain acceptable water quality and habitat in the ponds and streams comprising the species' current range and designated critical habitat;
- modify the designated critical habitat of the CRLF by reducing or changing breeding and non-breeding aquatic habitat (via modification of water quality parameters, habitat morphology, and/or sedimentation);

- modify the designated critical habitat of the CRLF by reducing the food supply required for normal growth and viability of juvenile and adult CRLFs;
- modify the designated critical habitat of the CRLF by reducing or changing upland habitat within 200 ft of the edge of the riparian vegetation necessary for shelter, foraging, and predator avoidance.
- modify the designated critical habitat of the CRLF by reducing or changing dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal.
- modify the designated critical habitat of the CRLF by altering chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs.

8.0 Summary of Direct and Indirect Effects to the California Red Legged Frog and Modification to Designated Critical Habitat for the California Red Legged Frog

In fulfilling its obligations under Section 7(a)(2) of the Endangered Species Act, the information presented in this endangered species risk assessment represents the best data currently available to assess the potential risks of S-methoprene to the CRLF and its designated critical habitat. A summary of the risk conclusions and effects determination for the CRLF and its designated critical habitat, given the uncertainties discussed in Section 6. Based on the conclusions of this assessment, a formal consultation with the U. S. Fish and Wildlife Service under Section 7 of the Endangered Species Act **should not be initiated.**

Based on the best available information, the Agency has assessed the potential for direct and indirect risk to CRLF from S-methoprene exposure. The conclusion is that there is a "may affect", but "not likely to adversely affect" determination for the CRLF from the use of S-methoprene. The assessment endpoints (Table 8.0) where this determination is made include the following:

- 1) Survival, growth, and reproduction of CRLF individuals via effects to food supply (*i.e.* freshwater fish and invertebrates, non-vascular plants);

This assessment point reflects an LOC exceedance (0.07) for acute endangered species concerns (LOC = 0.05) calculated from one of the upper bound extrapolated sustainable release formulations (20% granular), although, the microcosm field values for the same formulation did not exceed this LOC concern. In order to evaluate this exceedance, the Agency also calculated the chance of individual exposure using the Individual Effects Chance Model (Version 1.1). These calculations suggest that the chance of individual effect from this granular extrapolated exposure is about 1in 988,000, which may be considered as a highly unlikely event. As an additional test of possible risk, the use of acute and chronic fish and invertebrate toxicity data produced RQs for the other formulations (using extrapolated and microcosm exposure values) that did not exceed LOCs for direct or indirect effects to the aquatic-phase CRLF. Therefore, the Agency concludes a "**may affect" but "not likely to adversely affect**" reading for this assessment point.

The Agency acknowledges that S-methoprene is highly efficacious to Dipteran insect larvae and that the use of this compound can result in a decline in emerging adult populations. However, according to information of the CRLFs diet in Section 2.5.3 these insects are not included in their diet. The Agency assumes that the CRLF is an opportunistic feeder and will supplement its diet with available invertebrates and small animals. Therefore, the Agency concluded **"no habitat modification"** from S-methoprene use. Although there is widespread overlap of potential S-methoprene with watersheds of the CRLF the Agency has also determined that there is no potential for modification of CRLF designated critical habitat (aquatic or terrestrial plants) from the use of S-methoprene because this compound does not have herbicidal qualities or mode of action. Further information on the results of the effects determination are included as part of the Risk Description in Section 5.2.

Table 8.0 Effects Determination Summary for Direct and Indirect Effects of S-methoprene on the California Red-legged Frog

Assessment Endpoint	**Effects Determination**	**Basis**
Aquatic-Phase (Eggs, Larvae, Tadpoles, Adults)		
1. Survival, growth, and reproduction of CRLF individuals via direct effects on aquatic phases	No Effect	Acute RQs do not exceed LOC for direct effects using acute and chronic fish data. There is widespread overlap of potential S-methoprene with watersheds of the CRLF.
2. Survival, growth, and reproduction of CRLF individuals via effects to food supply (*i.e.* freshwater fish and invertebrates, non-vascular plants)	May Affect , But not Likely to Adversely Affect	LOC exceedance for granular formulation (0.07) to aquatic invertebrates. However, exposure was an extrapolated value, microcosm value did not exceed LOC. There is widespread overlap of potential S-methoprene use with watersheds of the CRLF.
3. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat, cover, and/or primary productivity (*i.e.*, aquatic plant community)	No Effect	S-methoprene is a larvicide and does not kill plants. Although there is the potential for aquatic exposure, aquatic plants are not at risk.
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.	No Effect	S-methoprene is not toxic to plants and does not have herbicidal qualities.
Terrestrial Phase (Juveniles and adults)		
5. Survival, growth, and reproduction of CRLF individuals via direct effects on terrestrial phase adults and juveniles	Not Likely to Adversely Affect	S-methoprene does not exceed an equivalent LOC for acute or chronic toxicity LC50 values, based on available avian and mammal data. Most applications are granular formulations and the liquid applications are

Assessment Endpoint	Effects Determination	Basis
		directed to water.
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)	Not likely to adversely affect	RQs for possible dietary items (small mammals, adult insects) are less than the LOCs. Based on the non-selective feeding behavior of adult CRLF and low magnitude of anticipated individual effects to potential prey items, S-methoprene is not expected to indirectly affect the terrestrial form of the CRLF. Although Dipterian populations may decline momentarily in the area where S-methoprene is used, these organisms are not expected to be a major component of the CRLFs diet.
7. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat (*i.e.*, riparian vegetation)	Not Likely to Adversely Affect	S-methoprene is not toxic to plants, plants are not at risk.

Table 8.1 Effects Determination Summary for S-Methoprene Exposure to the California Red-Legged Frogs Critical Habitat

Assessment Endpoint	Effects Determination	Basis
Aquatic Phase PCEs (Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)		
Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile and adult CRLFs.	No Habitat Modifications	Since S-methoprene does not control plants at the application sites, there is no potential for impacts to aquatic and terrestrial plants that comprise these habitats.
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[2].	No Habitat Modifications	Given that S-methoprene is not intended to control plants on the application sites, there is no potential for impacts to aquatic and terrestrial plants that comprise these habitats.
Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.	No Habitat Modifications	S-methoprene does not affect plant life and aquatic chemical (DO) components that are necessary for aquatic CRLF growth and development are not affected by S-methoprene exposure.
Reduction and/or modification of aquatic-based food sources for pre-metamorphoses (*e.g.*, algae)	No Habitat Modifications	Although S-methoprene is applied to water bodies, it does not have the potential for impacts to aquatic plants that comprise these habitats (non herbicidal properties).

Assessment Endpoint	Effects Determination	Basis
Aquatic Phase PCEs ***(Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)***		
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 drip line surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance	No Habitat Modifications	S-methoprene is not intended to control terrestrial plants at the application sites. This compound is not an herbicide and rapidly degrades in the environment through photolysis and biodegradation (7-10 days).
Elimination and/or disturbance of dispersal habitat: Upland or riparian dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal	No Habitat Modifications	Given that S-methoprene is not intended to control plants on the application sites, there is no potential for impacts to terrestrial plants that comprise these habitats.
Reduction and/or modification of food sources for terrestrial phase juveniles and adults	No Habitat Modifications	Although S-methoprene is toxic to Dipterian insects this does not pose acute risk to the CRLF. Frogs are opportunistic feeders and should supplement their diet with other terrestrial organisms. Dipterans are not listed as a component of the CRLFs diet.
Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	No Habitat Modifications	Although S-methoprene is toxic to Dipterian insects this does not pose acute risk to the CRLF. Frogs are opportunistic feeders and should supplement their diet with other terrestrial organisms. Dipterans are not listed as a component of the CRLFs diet.

[1] Physico-chemical water quality parameters such as 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.

References

Altig, R. and R.W. McDiarmid. 1999. Body Plan: Development and Morphology. In R.W. McDiarmid and R. Altig (Eds.), Tadpoles: The Biology of Anuran Larvae.University of Chicago Press, Chicago. pp. 24-51.

Alvarez, J. 2000. Letter to the U.S. Fish and Wildlife Service providing comments on the Draft California Red-legged Frog Recovery Plan

Ali, A. 1991. Activity of a new formulations of S-methoprene against midges (Diptera: Chironomidae) in experimental ponds. *J Am Mosq Control Assoc* **7:616-620**.

Borst, D., Tsukimura, B., Laufer, H., Couch, E., 1994. Regional differences in methyl farnesoate production in the lobster mandibular organ. *Biol. Bull*. **186:9-16**.

Boxmeyer, C.E., Leach, S., Palchick, S.M. 1997. Degradation of Altosid XR briquettes under field conditions in Minnesota*. J Amer. Mosq. Control Ass*. **13: 275-277.**

Crawshaw, G.J. 2000. Diseases and Pathology of Amphibians and Reptiles *in:* Ecotoxicology of Amphibians and Reptiles; ed: Sparling, D.W., G. Linder, and C.A. Bishop. SETAC Publication Series, Columbia, MO.

Degitz, SJ, Durhan, EJ, Tietge, JE, Kosian, PA, Holcombe, GH, Ankley, GT. 2003. Developmental toxicity of S-methoprene and several degradation products in *Xenopus laevis. Aquatic Toxicology,* **64(1): 97-105.**

Ding, Q., Tobe, S. 1991. Production of farnesoic acid and methyl farnesoate by mandibular organs of the cray fish, *Procambarius clarkia*. *Insect Biochem***. 21:285-291.**

EPA. 1991. S-methoprene Reregistration Eligibility Document Facts.Washington, DC: US Environmental Protection Agency.

Fellers, G. M., et al. 2001. Overwintering tadpoles in the California red-legged frog (*Rana aurora draytonii*). Herpetological Review, 32(3): 156-157.

Fellers, G.M, L.L. McConnell, D. Pratt, S. Datta. 2004. Pesticides in Mountain Yellow-Legged Frogs (Rana Mucosa) from the Sierra Nevada Mountains of California, USA. Environmental Toxicology & Chemistry 23 (9):2170-2177.

Fellers, Gary M. 2005a. *Rana draytonii* Baird and Girard 1852. California Red-legged Frog. Pages 552-554. *In*: M. Lannoo (ed.) Amphibian Declines: The Conservation Status of United States Species, Vol. 2: Species Accounts. University of California Press, Berkeley, California. xxi+1094 pp. (http://www.werc.usgs.gov/pt-reyes/pdfs/Rana%20draytonii.PDF)

Fellers, Gary M. 2005b. California red-legged frog, *Rana draytonii* Baird and Girard. Pages 198-201. *In*: L.L.C. Jones, et al (eds.) Amphibians of the Pacific Northwest. xxi+227.

Hayes, M.P. and M.M. Miyamoto. 1984. Biochemical, behavioral and body size differences between *Rana aurora aurora* and *R. a. draytonii*. Copeia 1984(4): 1018-22.
Hayes and Tennant. 1985. Diet and feeding behavior of the California red-legged frog. The Southwestern Naturalist 30(4): 601-605.

Harmon, M.A., Boehn, M.F., Mangelsdorf, D.J. 1995. Activation of mammalian retinoid X receptors by the insect growth regulator S-methoprene. *Proc. Natl. Acad. Sci.* **92:6157-6160.**

Hershey, A.E., Shannon, L.J., Axler, R.P., Ernst, C., Mickelson, P. (1995) Effects of S-methoprene and Bti (*Bacillus thuringiensis var. israelensis*) on non-target insects *Hydrobiologia.* **308:219-227**.

HSDB. 2002. Hazardous Substance Data Bank: S-methoprene. National Library of Medicine, National Toxicology Program. Available at http://www.toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB Accessed December 17, 2002.

Jennings, M.R. and M.P. Hayes. 1985. Pre-1900 overharvest of California red-legged frogs (*Rana aurora draytonii*): The inducement for bullfrog (*Rana catesbeiana*) introduction. Herpetological Review 31(1): 94-103.

Jennings, Mark R. 1988. Natural history and decline of native ranids in California. Pp. 61-72. *In* Proceedings of the conference on California herpetology. H.F. DeLisle, P.R. Brown, B. Kaufman, and H.M. McGurty (eds). Southwestern Herpetologists Society Special Publication (4): 1-143.

Jennings, M.R. and M.P. Hayes. 1994. Amphibian and reptile species of special concern in California. Report prepared for the California Department of Fish and Game, Inland Fisheries Division, Rancho Cordova, California. 255 pp.

Jennings, M.R., S. Townsend, and R.R. Duke. 1997. Santa Clara Valley Water District California red-legged frog distribution and status – 1997. Final Report prepared by H.T. Harvey & Associates, Alviso, California. 22 pp.

Judy, D and Howell, B. 1992. Concentrations of methoprene found in freshwater microcosms treated with sustained release Altosid formulations. ABC Laboratories Columbia, Missouri. Study conducted for Zoecon corp. Dallas, Texas. **MRID#428112-02**

Johnson, P.T.J., Lunde, K.B., Ritchie, E.G., Launer, L.H. 1999. The effect of trematode infection an amphibian limb development and survivorship. *Science*. **284:803-804**.

Kupferberg, S. 1997. Facilitation of periphyton production by tadpole grazing: Functional differences between species. Freshwater Biology 37:427-439.

Kupferberg, S.J., J.C. Marks and M.E. Power. 1994. Effects of variation in natural algal and detrital diets on larval anuran (*Hyla regilla*) life-history traits. Copeia 1994:446-457.

Laufer, H., Bigger, W.J. 2001. Unifying concepts learned from methyl farnesoate for invertebrate reproduction and post-embryonic development. *Amer. Zool*. **41:442-457**.

LeNoir, J.S., L.L. McConnell, G.M. Fellers, T.M. Cahill, J.N. Seiber. 1999. Summertime Transport of Current-use pesticides from California's Central Valley to the Sierra Nevada Mountain Range,USA. Environmental Toxicology & Chemistry 18(12): 2715-2722.

McConnell, L.L., J.S. LeNoir, S. Datta, J.N. Seiber. 1998. Wet deposition of current-use pesticides in the Sierra Nevada mountain range, California, USA. Environmental Toxicology & Chemistry 17(10):1908-1916.

Metropolitian Mosquito Control District. 1996. An assessment of non-target effects of the mosquito larvicides, Bti and S-methoprene, in metropolitan area wetlands. St. Paul, MN, USA.

Minucci, S., Saint-Jeannet, J-P, Toyama, R., Scita, G., Deluca, L.M., Tiara, M., Levin, A.A., Ozato, K., Dawid, I.B. 1996. Retinoid X receptor-celective ligands produce malformations in *Xenopus* embryos. *Proc. Natl. Acad. Sci.* **93:1803-1807**.

Niemi, G.J., Axler, R.P., Hanowski, J.M., Hershey, A.E., Lima, A.R., Regal, R.R., Shannon, L, Whiteside, M (1990) Evaluation of the effects of S-methoprene and Bti (*Bacillus thurgiensis israelensis*) on non-target organisms: a summary of before-and-after sites in western Wright County **NRRI/TR-90/17.**

Norland, R.L., Mulla, M.S. 1975. Impact of Altosid on selected members of an aquatic ecosystem. *Environ Entomol* **4:145-152.**

Olmestead, A.W., LeBlanc, G.A. 2001. Temporal and quantitative changes in sexual reproductive cycle of the cladoceran Daphnia magna by a juvenile hormone analog. *J Exp Zool.* **290: 148-155.**

Pinkney, A.E., McGowan, P.C., Murphy, D.R., Lowe, T.P., Sparling, D.W., Ferrington, L.C. 2000. Effects of the mosquito larvicides temephos and S-methoprene on insect populations in experimental ponds. *Environ Toxicol Chem* **19:678-684.**

Quistad, G.B., Staiger, L.E., Schooley, D.A. 1975. Environmental degradation of the insect growth regulator S-methoprene (isopropyl 2E, 4E)-11-Methoxy-3,7,11-trimethyl-2,4-dodecadienoate), III. Photodecomposition. *J. Agr. Food Chem*. **23:299-303**
Riddiford, L.M. 1996. Juvenile hormone: the status of its "status quo" action. *Arch. Insect Biochem. Physiol*. **32:271-286**.

Rathburn, G.B. 1998. *Rana aurora draytonii* egg predation. Herpetological Review, 29(3): 165.

Reis, D.K. 1999. Habitat characteristics of California red-legged frogs (*Rana aurora draytonii*):Ecological differences between eggs, tadpoles, and adults in a coastal brackish and freshwater system. M.S. Thesis. San Jose State University. 58 pp.

Scoff, P.K. and Ankley, G.T. 2004. Effects of S-methoprene, its metabolites, and breakdown products on retinoid-activated pathways in transfected cell lines. *Environ Toxicol Chem* **23:1305-1310.**

Seale, D.B. and N. Beckvar. 1980. The comparative ability of anuran larvae (genera: *Hyla*, *Bufo* and *Rana*) to ingest suspended blue-green algae. Copeia 1980:495-503.

D.W. Sparling, G.M. Fellers, L.L. McConnell. 2001. Pesticides and amphibian population declines in California, USA. Environmental Toxicology & Chemistry 20(7): 1591-1595.

Staal, G.B. 1975. Insect growth regulators with juvenile hormone activity. *Ann. Rev. Entomol.* **20:417-460.**

Toxnet 2003. National Library of Medicine. National Institute of Health. S-methoprene. Available: http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~QhG32K:1:enex. (Accessed: September 2007).

U.S. Environmental Protection Agency (U.S. EPA). 1998. Guidance for Ecological Risk Assessment. Risk Assessment Forum. EPA/630/R-95/002F, April 1998.

U.S. EPA. 2004. Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs. Office of Prevention, Pesticides, and Toxic Substances. Office of Pesticide Programs. Washington, D.C. January 23, 2004.

U.S. Fish and Wildlife Service (USFWS). 1996. Endangered and threatened wildlife and plants: determination of threatened status for the California red-legged frog. Federal Register 61(101):25813-25833.

USFWS. 2002. Recovery Plan for the California Red-legged Frog (*Rana aurora draytonii*). Region 1, USFWS, Portland, Oregon. (http://ecos.fws.gov/doc/recovery_plans/2002/020528.pdf)

USFWS. 2006. Endangered and threatened wildlife and plants: determination of critical habitat for the California red-legged frog. 71 FR 19244-19346.

USFWS. Website accessed: 30 December 2006. http://www.fws.gov/endangered/features/rl_frog/rlfrog.html#where

U.S. Fish and Wildlife Service (USFWS) and National Marine Fisheries Service (NMFS). 1998. Endangered Species Consultation Handbook: Procedures for Conducting Consultation and Conference Activities Under Section 7 of the Endangered Species Act. Final Draft. March 1998.

USFWS/NMFS. 2004. 50 CFR Part 402. Joint Counterpart Endangered Species Act Section 7 Consultation Regulations; Final Rule. FR 47732-47762.

Wan, M. T. K. and Wilson, D. M. (1975). Impacts of "Altosid" Juvenile Hormone on Non-target Organisms in an Aquatic Environment of Interior British Columbia. *Rep.No.EPS 5-PR-75-9, Pollution Abatement Branch, Environ.Prot.Serv., Vancouver, B.C.* 46 p.

Wassersug, R. 1984. Why tadpoles love fast food. Natural History 4/84.