Risks of S-methoprene Use to Federally Listed California Tiger Salamander (CTS) (*Ambystoma californiense*)

Pesticide Effects Determinations
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μg/kg	Symbol for "micrograms per kilogram"
μg/L	Symbol for "micrograms per liter"
°C	Symbol for "degrees Celsius"
AAPCO	Association of American Pesticide Control Officials
a.i.	Active Ingredient
AIMS	Avian Monitoring Information System
Acc#	Accession Number
amu	Atomic Mass Unit
BCB	Bay Checkerspot Butterfly
BCF	Bioconcentration Factor
BEAD	Biological and Economic Analysis Division
bw	Body Weight
CAM	Chemical Application Method
CARB	California Air Resources Board
AW	Alameda Whipsnake
CBD	Center for Biological Diversity
CCR	California Clapper Rail
CDPR	California Department of Pesticide Regulation
CDPR-PUR	California Department of Pesticide Regulation Pesticide Use Reporting Database
CFWS	California Freshwater Shrimp
CI	Confidence Interval
CL	Confidence Limit
CTS	California Tiger Salamander
CTS-CC	California Tiger Salamander Central California Distinct Population Segment
CTS-SB	California Tiger Salamander Santa Barbara County Distinct Population Segment
CTS-SC	California Tiger Salamander Sonoma County Distinct Population Segment
DS	Delta Smelt
EC	Emulsifiable Concentrate
EC_{05}	5% Effect Concentration
EC_{25}	25% Effect Concentration
EC_{50}	50% (or Median) Effect Concentration

ECOTOX	EPA managed database of Ecotoxicology data
EEC	Estimated Environmental Concentration
EFED	Environmental Fate and Effects Division
e.g.	Latin *exempli gratia* ("for example")
EIM	Environmental Information Management System
EPI	Estimation Programs Interface
ESU	Evolutionarily significant unit
et al.	Latin *et alii* ("and others")
etc.	Latin *et cetera* ("and the rest" or "and so forth")
EXAMS	Exposure Analysis Modeling System
FIFRA	Federal Insecticide Fungicide and Rodenticide Act
FQPA	Food Quality Protection Act
ft	Feet
GENEEC	Generic Estimated Exposure Concentration model
HPLC	High Pressure Liquid Chromatography
IC_{05}	5% Inhibition Concentration
IC_{50}	50% (or median) Inhibition Concentration
i.e.	Latin for *id est* ("that is")
IECV1.1	Individual Effect Chance Model Version 1.1
KABAM	$\underline{K}_{OW}$ (based) <u>A</u>quatic <u>B</u>io<u>A</u>ccumulation <u>M</u>odel
kg	Kilogram(s)
kJ/mole	Kilojoules per mole
km	Kilometer(s)
K_{AW}	Air-water Partition Coefficient
Kd	Solid-water Distribution Coefficient
KF	Freundlich Solid-Water Distribution Coefficient
K_{OC}	Organic-carbon Partition Coefficient
K_{OW}	Octanol–water Partition Coefficient
LAA	Likely to Adversely Affect
lb a.i./A	Pound(s) of active ingredient per acre
LC_{50}	50% (or Median) Lethal Concentration
LD_{50}	50% (or Median) Lethal Dose
LOAEC	Lowest Observable Adverse Effect Concentration
LOAEL	Lowest Observable Adverse Effect Level
LOC	Level of Concern
LOD	Level of Detection

LOEC	Lowest Observable Effect Concentration
LOQ	Level of Quantitation
m	Meter(s)
MA	May Affect
MATC	Maximum Acceptable Toxicant Concentration
m^2/day	Square Meters per Days
ME	Microencapsulated
mg	Milligram(s)
mg/kg	Milligrams per kilogram (equivalent to ppm)
mg/L	Milligrams per liter (equivalent to ppm)
mi	Mile(s)
mmHg	Millimeter of mercury
MRID	Master Record Identification Number
MW	Molecular Weight
n/a	Not applicable
NASS	National Agricultural Statistics Service
NAWQA	National Water Quality Assessment
NCOD	National Contaminant Occurrence Database
NE	No Effect
NLAA	Not Likely to Adversely Affect
NLCD	National Land Cover Dataset
NMFS	National Marine Fisheries Service
NOAA	National Oceanic and Atmospheric Administration
NOAEC	No Observable Adverse Effect Concentration
NOAEL	No Observable Adverse Effect Level
NOEC	No Observable Effect Concentration
NRCS	Natural Resources Conservation Service
OPP	Office of Pesticide Programs
OPPTS	Office of Prevention, Pesticides and Toxic Substances
ORD	Office of Research and Development
PCE	Primary Constituent Element
pH	Symbol for the negative logarithm of the hydrogen ion activity in an aqueous solution, dimensionless
pKa	Symbol for the negative logarithm of the acid dissociation constant, dimensionless
ppb	Parts per Billion (equivalent to µg/L or µg/kg)

ppm	Parts per Million (equivalent to mg/L or mg/kg)
PRD	Pesticide Re-Evaluation Division
PRZM	Pesticide Root Zone Model
ROW	Right of Way
RQ	Risk Quotient
SFGS	San Francisco Garter Snake
SJKF	San Joaquine Kit Fox
SLN	Special Local Need
SMHM	Salt Marsh Harvest Mouse
TG	Tidewater Goby
T-HERPS	Terrestrial Herpetofaunal Exposure Residue Program Simulation
T-REX	Terrestrial Residue Exposure Model
UCL	Upper Confidence Limit
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
USFWS	United States Fish and Wildlife Service
USGS	United States Geological Survey
VELB	Valley Elderberry Longhorn Beetle
WP	Wettable Powder
wt	Weight

1.0 Executive Summary

1.1 Purpose of Assessment

The purpose of this assessment is to evaluate potential direct and indirect effects on the California Tiger Salamander (*Ambystoma californiense*) (CTS) arising from FIFRA regulatory actions regarding use of S-methoprene, an insect juvenile hormone (JH) responsible for regulating larval growth, on agricultural (i.e. mosquito control during flooding and fire ant bait around citrus) and non-agricultural sites in California. In addition, this assessment evaluates whether these actions can be expected to result in modification of designated critical habitat for the CTS. This assessment was completed in accordance with the U.S. Fish and Wildlife Service (USFWS) and National Marine Fisheries Service (NMFS) *Endangered Species Consultation Handbook* (USFWS/NMFS, 1998) procedures outlined in the Agency's Overview Document (USEPA, 2004), and consistent with a suit in which S-methoprene was alleged to be of concern to the CTS (*Center for Biological Diversity (CBD) vs. EPA et al.* (Case No. 07-2794-JCS).

The CTS was listed as a threatened species by the USFWS in 1996. There are currently three CTS Distinct Population Segments (DPSs): the Sonoma County (SC) DPS, the Santa Barbara (SB) DPS, and the Central California (CC) DPS. Each DPS is considered separately in the risk assessment as they occupy different geographic areas. The main difference in the assessment will be in the spatial analysis. The CTS-SB and CTS-SC were down-listed from endangered to threatened in 2004 by the USFWS; however, the down-listing was vacated by the U.S. District Court. Therefore, the Sonoma and Santa Barbara DPSs are currently listed as endangered while the CTS-CC is listed as threatened. CTS utilize vernal pools, semi-permanent ponds, and permanent ponds, and the terrestrial environment in California. The aquatic environment is essential for breeding and reproduction and mammal burrows are also important habitat for aestivation.

1.2 Scope of Assessment

1.2.1 Uses Assessed

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 area that is important in the sustained protection of the CTS. Table 1.0 shows registered uses of S-methoprene in California to which the CTS and other non-target organisms upon which it depends (i.e., for dietary and habitat requirements) may be exposed. The S-methoprene formulations that are widely used in California include slow release forms such as briquets, sand mix, and granulars, as well as liquid formulations (EC and FLC). In order to ensure 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, manmade depressions, fountains, *et*).

Table 1.0 Registered S-methoprene Uses 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

1.2.2 Environmental Fate Properties of S-methoprene

Technical methoprene, isopropyl (E, E) – 11- methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate, is a long chain hydrocarbon ester (Farm Chemical Handbook, 1997) and is soluble in water at 1.4 mg/L (at 25^0 C) and also in organic solvents. The specific gravity of technical methoprene is 0.9261 at 20^0 C. Methoprene has a moderate vapor pressure ($2.3x10^{-5}$ mm Hg @ 25°C) and Henry's Law Constant of $6.9x10^{-6}$ atm m^3/mole. Consequently methoprene has the potential to volatilize from water or moist soil; however, volatilization is mitigated by the affinity of methoprene for soils and sediments (Toxnet, 2003). Vapor phase methoprene may be degraded by reaction with photochemically produced hydroxyl radicals and ozone in the atmosphere. The half-lives for these reactions are calculated to be 1.5 hours (hydroxyl radicals) and 48 minutes (ozone) (Toxnet, 2003). The photodegradation of methoprene is expected to be very rapid due to its absorbance in the environmental spectrum (λ >290 nm) (Toxnet, 2003).

Methoprene showed rapid degradation in both sterile and nonsterile pond water exposed to sunlight, with more than 80% of applied methoprene being degraded within 13 days (US EPA, 1982). Schooley *et al.* (1975a) studied the dissipation of methoprene in pond water and sewage at dose rates of 0.001 and 0.01 mg/L, respectively and found that methoprene showed a half-life of approximately 30 hours at 0.001mg/L and 40 hours at 0.01 mg/L in pond water, and a 60-70 hour half-life in sewage. The breakdown of methoprene from the aquatic environment is rapid with >90% of the initial methoprene parent compound, applied as emulsifiable concentrates (ECs), flowable concentrates, or briquets at 0.05-2.0 lbs ai/A, dissipating from a variety of aquatic sites within 3 days after application (Schaefer and Dupras, 1973).

Methoprene is relatively immobile when applied to soil and is unlikely to leach (Koc = 2,800) (Hansch, *et al.*, 1995). Biodegradation of methoprene has been reported to be relatively fast in a variety of soils and environmental conditions (Toxnet, 2003). 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 Food and Agricultural Organization (FAO, 2005) 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).

Figure 1-0. Structure of S-methoprene

1.2.3 Evaluation of S-methoprene Degradates

Methoprene undergoes ester hydrolysis O-demethylation and oxidative cleavage of the C-4 double bond resulting in the major degradate methoxycitronellic acid (7-methoxy-3, 7-dimethyloctanoic acid) and S-methoprene acid, S-methoprene epoxide, 7-methoxycitronellal, and 7-methoxycitronellic acid. Each degradate accounts for < 10% of the total. Degitz *et al.*, 2003 studied the developmental toxicity of S-methoprene and the resulting degradates (S-methoprene acid, S-methoprene epoxide, 7-methoxycitronellal, and 7-methoxycitronellic acid) to frog embryos (*Xenopus laevis*) and found that S-methoprene concentrations > 2 mg/L (slightly higher than solubility) did not cause developmental effects. Various degradates, however, did produce developmental effects at very high concentrations as follows: > 1.25 mg/L S-methoprene acid, and ≥ 2.5 mg/L S-methoprene epoxide and 7-methoxycitronellal. No developmental effects were noted at > 30 mg/L for 7-methoxycitronellic acid. It was also concluded that field application of S-methoprene sustained – release formulations do not typically exceed 0.01 mg/l suggesting that concerns for S-methoprene mediated developmental toxicity to amphibians may be unwarranted (Degitz *et al.*, 2003). According to Ankley (1998) it is unlikely that degradation products would accumulate to levels that would affect amphibian development and that the parent compound and degradates are not potential developmental toxicants to *X. laevis*. Toxicity studies on degradates are limited but a 96 hour rainbow trout study on methoprene acid produced an LC_{50} = 36.9 mg/L suggesting slight toxicity (MRID 203343). Since degradates are not found in significant amounts in the environment (< 10%) and do not appear toxic at environmental expected levels, this assessment is therefore focused on the parent compound, S-methoprene.

1.3 Assessment Procedures

A description of routine procedures for evaluating risk to the San Francisco Bay Species is provided in Attachment I.

1.3.1 Exposure Assessment

1.3.1. a Aquatic Exposures

S-methoprene is most efficacious when applied directly to water. The formulations that are evaluated in this assessment include granular, briquette, pelleted/tableted, emulsifiable concentrate, and flowable concentrate. Aquatic exposure and corresponding Estimated Environmental Concentrations (EECs) were derived based on label information, and a modified Pesticide Root Zone Model coupled with the Exposure Analysis Model System (PRZM/EXAMS) for granular and multiple Liquid applications to water. Since the objective in applying S-methoprene to the aquatic environment is to avoid drift by using a course spray (large droplets), the AgDRIFT and AGDISP models were not used in this assessment. Based on the PRZM/EXAMS modeling for S-methoprene use patterns, peak EECs range from 1.61 to 9.46μg/L. Extrapolated EECs for the sustainable release Briquete were 2.1 – 6.2 ug/L. These estimates are compared with analysis of available microcosm residue studies that range from <0.20 to 4.24 ug/L. No S-methoprene monitoring data were reported by NAWQA for California surface waters with agricultural watersheds.

1.3.1. b Terrestrial Exposures

To estimate S-methoprene exposures to terrestrial species resulting from uses involving S-methoprene applications, the T-REX model is used for foliar and granular uses (Appendix H). KABAM ($\underline{K}_{OW}$ (based) Aquatic BioAccumulation Model) v.1.0 is used to estimate potential bioaccumulation of S-methoprene residues in an aquatic food web and subsequent risks these residues pose to organisms consuming aquatic species (Appendix F).

1.3.2 Toxicity Assessment

The assessment endpoints include direct toxic effects on survival, reproduction, and growth of individuals, as well as indirect effects, such as reduction of the food source and/or modification of habitat. Primary constituent elements (PCEs) were used to evaluate whether S-methoprene has the potential to modify designated critical habitat. The Agency evaluated registrant-submitted studies and data from the open literature to characterize S-methoprene toxicity. The most sensitive toxicity value available from acceptable or supplemental studies for each taxon relevant for estimating potential risks to the assessed species and/or their designated critical habitat was used.

The Agency has summarized available acute freshwater fish studies and concluded that S-methoprene is highly to moderately toxic (0.37-1.52 mg/L) to warm water species and highly to practically non-toxic (0.76-106 mg/L) to coldwater fish. 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. S-methoprene is non-toxic to plants and is practically non toxic to avian and mammalian species on an acute basis (refer to Section 4.0).

1.3.3 Measures of Risk

Acute and chronic risk quotients (RQs) are compared to the Agency's Levels of Concern (LOCs) to identify instances where S-methoprene use has the potential to adversely affect the assessed species or adversely modify their designated critical habitat. When RQs for a particular type of effect are below LOCs, the pesticide is considered to have "no effect" on the species and its designated critical habitat. Where RQs exceed LOCs, a potential to cause adverse effects or habitat modification is identified, leading to a conclusion of "may affect". If S-methoprene use is concluded to be a "may affect" the assessed species, and/or may cause effects to designated critical habitat, the best available additional information is considered to refine the potential for exposure and effects, and distinguish actions that are Not Likely to Adversely Affect (NLAA) from those that are Likely to Adversely Affect (LAA).

1.4 Summary of Conclusions

Based on the best available information, the Agency makes a May Effect/ But Not Likely to Adversely Affect determination for CTS from the use of S-methoprene. Additionally, the Agency has determined that there is not the potential for modification of designated critical habitat from the use of the chemical. 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 EECs, the Agency has relied on extrapolated levels from label information, and model generated values from the five S-methoprene formulations (briquete, XR briquete, granular/pellets, sand mix, and liquid). All calculated aquatic RQ values are below the Agency's listed species LOC indicating no direct risk to aquatic-phase CTS from S-methoprene aquatic residues. The assessment further shows that exposure of S-methoprene terrestrial uses will not directly or indirectly result in adverse effects to terrestrial-phase CTS. A summary of the risk conclusions and effects determinations for the aquatic- and terrestrial-phase CTS and its designated critical habitatis are presented in Table 1-1. Use-specific determinations are provided in Tables 1-2 and 1-3. Further information on the results of the effects determination is included as part of the Risk Description in Section 5.3.

Table 1-1 Effects Determination Summary for Effects of S-methoprene on the CTS

Species	Effects Determination	Basis for Determination
California Tiger Salamander Salamander (*Ambystoma californiense*)		**Potential for Direct Effects**
	No Effect	This assessment shows that the use of S-methoprene will not result in direct effects to the CTS from acute or chronic toxicity. Lines of evidence include: • Acute RQs do not exceed LOC for direct effects using acute and chronic fish data. • The likelihood of an individual effect (mortality) is approximately 1 in 418×10^6 based on aquatic exposure.
		Potential for Indirect Effects
	May Effect but Not Likely to Adversely Effect	***Food sources*** Risk assessment shows that terrestrial invertebrates and vertebrate prey populations will not be reduced. Lines of evidence include: • Acute RQs do not exceed LOC for direct effects using acute avian and mammalian data (T-REX). • The likelihood of an individual effect is about 1 in 294,000 (avian). Risk assessment shows that aquatic invertebrate prey propulations will not be reduced. Lines of evidence include: • Acute RQs do not exceed LOC for direct effects using acute freshwater aquatic invertebrate data. • One RQ from granular formulation exposure exceeded acute LOC (extrapolated value) • The likelihood of an individual effect is about 1 in 988,000.

		Habitat Modifications Risk assessment shows that S-methoprene will not modify the habitat of this species by reducing available burrows. This conclusion is based on acute and chronic RQs values for mammals that do not exceed the LOC.

Table 1-2 Use Specific Summary of the Potential for Adverse Effects to Aquatic Taxa

Uses	Potential for Effects to Identified Taxa Found in the Aquatic Environment:							
	CTS-CC, SC, and SB and Freshwater Vertebrates		Freshwater Invertebrates		Vascular Plants		Non-vascular Plants	
	Acute	Chronic	Acute	Chronic	Acute	Chronic		
Woodland pools, swamps, irrigated crop lands, etc.	No	No	No	No	No	No	No	No
Rice	No	No	No	No	No	No	No	No

Table 1-3 Use Specific Summary of the Potential for Adverse Effects to Terrestrial Taxa

Uses	Small Mammals		Small Birds		CTS-CC, CTS-SC, CTS-SB and Amphibians		Invertebrates (Acute)	Dicots	Monocots
	Acute	Chronic	Acute	Chronic	Acute	Chronic			
Orchards, pastures, terrestrial food, rangeland.	No	No	No	No	No	No	No	No	No

Based on the conclusions of this assessment, and the NLAA findings, a formal consultation with the U.S. Fish and Wildlife under Section 7 of the Endangered Species Act should be initiated.

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 listed species and its resources (*i.e.*, food and habitat) are not expected to be uniform across the action area. In fact, given the 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 CTS life stages within the action area and/or applicable designated critical habitat. 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 assessed species.
- Quantitative information on prey base requirements for the assessed species. While existing information provides a preliminary picture of the types of food sources utilized by the assessed species, 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 species and potential 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. The structure of this risk assessment is based on guidance contained in U.S. EPA's *Guidance for Ecological Risk Assessment* (USEPA, 1998), the Services' *Endangered Species Consultation Handbook* (USFWS/NMFS, 1998) and is consistent with procedures and methodology outlined in the Overview Document (USEPA, 2004) and reviewed by the U.S. Fish and Wildlife Service and National Marine Fisheries Service (USFWS/NMFS/NOAA, 2004).

2.1 Purpose

The purpose of this endangered species assessment is to evaluate potential direct and indirect effects on individuals of the California Tiger Salamander (CTS) arising from FIFRA regulatory actions regarding use of S-methoprene for mosquito control (inhibitor of larval development) with direct application to shallow water bodies, as well as use around some agricultural areas such as citrus (fire ant control) and rice (mosquito control). This ecological risk assessment has been prepared consistent with a stipulated injunction in the case *Center for Biological Diversity (CBD) vs. EPA et al.* (Case No. 07-2794-JCS) entered in Federal District Court for the Northern District of California on May 17, 2010. In this assessment, direct and indirect effects to the CTS and potential modification to designated critical habitat for this species are evaluated in accordance with the methods described in the Agency's Overview Document (USEPA, 2004).

There are currently three CTS Distinct Population Segments (DPSs): the Sonoma County (SC) DPS, the Santa Barbara (SB) DPS, and the Central California (CC) DPS. Each DPS is considered separately in the risk assessment as they occupy different geographic areas. The main difference in the assessment will be in the spatial analysis. The CTS-SB and CTS-SC were downlisted from endangered to threatened in 2004 by the USFWS, however, the downlisting was vacated by the U.S. District Court. Therefore, the Sonoma and Santa Barbara DPSs are currently listed as endangered while the CTS-CC is listed as threatened. CTS utilize vernal pools, semi-permanent ponds, and permanent ponds, and the terrestrial environment in California. The aquatic environment is essential for breeding and reproduction and mammal burrows are also important habitat for aestivation.

In accordance with the Overview Document, provisions of the ESA, and the Services' *Endangered Species Consultation Handbook,* the assessment of effects associated with registrations of S-methoprene is based on an action area. The action area is the area directly or indirectly affected by the federal action, as indicated by the exceedance of the Agency's Levels of Concern (LOCs). It is acknowledged that the action area for a national-level FIFRA regulatory decision associated with a use of 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 CTS and their designated critical habitat within the state of California. As part of the "effects determination," one of the following three conclusions will be reached separately for each of the assessed species in the lawsuits regarding the potential use of S-methoprene in accordance with current labels:

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

Additionally, for habitat and PCEs, a "No Effect" or a "Habitat Modification" determination is made._A description of routine procedures for evaluating risk to the San Francisco Bay Species is provided in Attachment I.

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 CTS and its designated critical habitat. Further discussion of the action area for the CTS 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 cranberry 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 (briquettes). 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 briquette and granular (sustainable release). The S-methoprene briquettes (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 (< 10 hrs) after exposure to ultraviolet (UV) sunlight, as well as microbial breakdown. 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.

2.2.1 Evaluation of Degradates

Methoprene undergoes ester hydrolysis O-demethylation and oxidative cleavage of the C-4 double bond resulting in the major degradate methoxycitronellic acid (7-methoxy-3, 7-dimethyloctanoic acid) and S-methoprene acid, S-methoprene epoxide, 7-methoxycitronellal, and 7-methoxycitronellic acid. Each degradates accounts for < 10% of the total. Degitz *et al*., 2003 studied 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 S-methoprene concentrations > 2 mg/L (slightly higher than solubility) did not cause developmental effects. Various degradates, however, did produce developmental effects at very high concentrations as follows: > 1.25 mg/L S-methoprene acid, and ≥ 2.5 mg/L S-methoprene epoxide and 7-methoxycitronellal. No developmental effects were noted at > 30 mg/L for 7-methoxycitronellic acid. It was also concluded that field application of S-methoprene sustained – release formulations do not typically exceed 0.01 mg/l suggesting that concerns for S-methoprene mediated developmental toxicity to amphibians may be unwarranted (Degitz *et al*., 2003). According to Ankley (1998) it is unlikely that degradation products would accumulate to levels that would affect amphibian development and that the parent compound and degradates are not potential developmental toxicants to *X. laevis*. Toxicity studies on degradates are limited but a 96 hour rainbow trout study on methoprene acid produced an LC_{50} = 36.9 mg/L suggesting slight toxicity (MRID 203343). Since degradates are not found in significant amounts in the environment (< 10%) and do not appear toxic at environmental expected levels, the assessment is conducted on the parent compound.

2.2.2 Evaluation of Mixtures

The Agency does not routinely include, in its risk assessments, an evaluation of mixtures of active ingredients, either those mixtures of multiple active ingredients in product formulations or those in the applicator's tank. In the case of the product formulations of active ingredients (that is, a registered product containing more than one active ingredient), each active ingredient is subject to an individual risk assessment for regulatory decision regarding the active ingredient on a particular use site. If effects data are available for a formulated product containing more than one active ingredient, they may be used qualitatively or quantitatively in accordance with the Agency's Overview Document and the Services' Evaluation Memorandum (USEPA, 2004; USFWS/NMFS/NOAA, 2004). S-methoprene does not have registered products that contain multiple active ingredients. Therefore, 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 (USEPA, 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, and S-methoprene is also recognized by the Food and Drug Administration as a feed additive for use in cattle feeds to control horn flies (40 CFR 186.4150).

On May 30, 2007, the CBDfiled a lawsuit in the United States District Court for the Northern District of California alleging that EPA failed to comply with section 7(a)(2) of the Endangered Species Act (ESA) in regard to 47 pesticides including S-methoprene and the federally threatened California red-legged frog *(Rana aurora draytonii*) (CRLF) that is listed under the ESA (Center for Biological Diversity v. EPA, Case No. 07-2794-JCS (N.D. Cal.) (PDF)). The Agency completed an assessment that evaluated potential direct and indirect effects on the 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), (US-EPA, 2008). In addition, this assessment evaluated whether these actions can be expected to result in the destruction or adverse modification of the species' critical habitat. The conclusion was that there is a **"may affect", but "not likely to adversely affect"** determination for the CRLF from the use of S-methoprene.

2.4 Environmental Chemistry and Fate Properties

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, 2003).

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 2003). 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-dimethyl-2-nonanone (4%).	00010443, 00010441, 00010442, 05008622, 00010542, 05008610, 00010440	Acceptable
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.	CO_2 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	Acceptable

Study	Value (units)	Major Degradates *Minor Degradates*	MRID #	Study Status
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: Accumulation in edible tissues of fish and crayfish at maximum bioaccumulation factors of 457 and 75, respectively. Elimination of 93-95% of S-methoprene residues within 14 days precludes bioaccumulation in fish	Degradates not measured.	00012785	Acceptable
Molecular Weight	310.0 g/mole		Toxnet 2003	
Chemical Formula	$C_{19}H_{34}O_3$		Toxnet 2003	

Study	Value (units)	Major Degradates *Minor Degradates*	MRID #	Study Status
Density/ Relative Density/ Bulk Density	0.261 g/cm^3		Toxnet 2003	
Vapor Pressure	3.15 x 10^{-6} kPa at 20^0 C		Toxnet 2003	
Henry's Law Constant	atm-m^3/mole @ XX°C		Toxnet 2003	
Water Solubility	1.39 mg/L @ 20°C		Toxnet 2003	
Octanol – water partition coefficient (K_{OW})	5.50 @ 25°C			

2.4.1 Environmental Transport Mechanisms

The major transport mechanisms for S-methoprene exposure to aquatic ecosystems is expected to be direct application to water, either as a liquid or as a slow release formulation (i.e. granular, briquette), thus surface runoff, drift or secondary drift of volatilized or soil-bound residues leading to deposition onto nearby or distant ecosystems is an unlikely exposure scenario. Examples of such sites are: vineyards, rice fields (including wild rice), date palm orchards, fruit and nut orchards, and berry fields and bogs. Irrigated pastures may be treated after each flooding without the removal of grazing livestock. This formulation may also be applied to intermittently flooded non crop areas that include freshwater swamps and marshes, salt marshes, woodland pools and meadows, dredging spoil sites, drainage areas, waste treatment and settling ponds, ditches and other natural and manmade depressions. Unlike adulticides that are used to combat adult mosquitoes (i.e. synthetic pyrethroids), S-methoprene is not sprayed as fine droplets in order to create a mist that is intended for drift over a target site. Instead, the liquid formulation is applied directly to water as a coarse spray for maximum effectiveness.

2.4.2 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.

2.4.3 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. For the purpose of this assessment, EECs 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, briquettes, and liquid formulation) or broadcast over post or pre-flooded areas. S-methoprene exposure from indoor applications will not be included in this assessment since outdoor exposure from this use is unlikely. The use of S-methoprene in a briquette and/or granular form was identified as a potential aquatic concern in previous assessments (USEPA 2008). Since these formulations function as slow release mechanisms for delivering S-methoprene at a relatively constant concentration over a period of time (i.e. 30 – 150 days), there 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 the need for more efficient foliage penetration. The briquettes 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) are also applied directly to water. Table 2-1 shows the average amount of S-methoprene used annually in California during 2006 – 2009. The five listed categories reflect the highest S-methoprene usage through- out the state with Public Health Pest Control accounting for about 96% of the total. Table 2-2 shows the major formulations that were used (2001-2005). The sustainable release forms (briquettes, granular, pelleted/tableted, and impregnated material) accounted for about 69% of the total use.

Table 2-1 Summary of CDPR PUR Usage Data from 2006 - 2009 for S-methoprene[1]

Use	Amount of S-methoprene Used During this Time Period (lbs)
Landscape Maintance	13.89
Public Health Pest Control	8,442.76
Regulatory Pest Control	84.35
Structural Pest Control	204.20
Rights-of-Way	60.77

[1] 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.

Table 2-2 Summary of CDPR PUR Formulation Data from 2001- 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. 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, misreported uses, and misuse are not considered part of the federal action and, therefore, are not considered in this assessment. 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 cranberry bogs. The amount of S-methoprene that was expected to be released into the aquatic environment was extrapolated from label information (Appendix I) and efficacy data. These rates reflect upper bound scenarios of the expected amount of S-methoprene (see calculations Appendix I).

Table 2-3 S-Methoprene Uses and Corresponding Maximum Application Rates Considered for the Aquatic Assessment

Use	Formulation	Max. % active ingredient	Max. Rate (lbs ai/A)	Minimum Retreatment Intervals (days)	Number of applications
Woodland pools, swamps, storm drains, etc.	Briquet XR Briquet	8.62 4.24	0.014 0.0058	Every 30 days Every 150 days	As needed As needed
Woodland pools, swamps, irrigated crop lands, Rice, cranberry bogs.	Granular Sand mix	4.25 3.0	0.425 0.12	Every 7 days Every 7 days	As needed As needed
Woodland pools, berry bogs, rice fields, irrigated crop	Liquid	20	0.013	Every 7 - 10	As needed

Use	Formulation	Max. % active ingredient	Max. Rate (lbs ai/A)	Minimum Retreatment Intervals (days)	Number of applications
lands after flooding,				days	

[1]Based on data supplied by BEAD (BEAD, 4/27/2011 transmittal memo)

The values noted in Table 2-4 reflect maximum application rates forformulations that may be used in terrestrial areas where the CTS may be found. Although salamanders are not expected to directly feed on granules, the consumption of granules by small mammals could be toxic and result in secondary effects to CTS through habitat reduction (reduction in available burrows).

Table 2-4 Maximum Rate of S-Methoprene Formulations Applied to Terrestrial Site[1]

Use	Formulation	Max. % active ingredient	Max. Rate (lbs ai/A)	Minimum Retreatment Intervals (days)	Minimum Retreatment Intervals (days)
Citrus (Bait)	Granular	4.25	0.425	7	As Needed
Bushes, shrubs (Bait stations)	L	5	0.428	10	As Needed

[1]Based on data supplied by BEAD (BEAD, 4/27/2011 transmittal memo)

CDPR PUR is considered a more comprehensive source of usage data than 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. Eleven years (1999-2009) of usage data were included in this analysis. Data from CDPR PUR were obtained for every agricultural pesticide application made on every use site at the section level (approximately one square mile) of the public land survey system.[3] BEAD summarized these data to the county level by site, pesticide, and unit

[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.pestmanagement.info/nass/app_usage.cfm.

[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.

[3] Most pesticide applications to parks, golf courses, cemeteries, rangeland, pastures, and along roadside and railroad rights of way, and postharvest treatments of agricultural commodities are reported in the database. The primary exceptions to the reporting requirement are home-and-garden use and most industrial and institutional uses (http://www.cdpr.ca.gov/docs/pur/purmain.htm).

treated. Calculating county-level usage involved summarizing across all applications made within a section and then across all sections within a county for each use site and for each pesticide. The county level usage data that were calculated include: average annual pounds applied, average annual area treated, and average and maximum application rate across all nine years. The units of area treated are also provided where available.

2.5 Assessed Species

Table 2-5 provides a summary of the current distribution, habitat requirements, and life history parameters for the listed CTS. More detailed life-history and distribution information can be found in Attachment III. There are currently three CTS Distinct Population Segments (DPSs): The Sonoma County (CS) DPS, the Santa Barbara (SB) DPS, and the Central California (CC) DPS. Each DPS is considered separately in the risk assessment since they occupy different geographic areas. The main difference in the assessment will be the spatial analysis. The CTS-SB and CTS-SC were downlisted from endangered to threatened in 2004 by the USFWS, however, the downlisting was vacated by the U.S. District Court. Therefore, the Sonoma and Santa Barbara DPSs are currently listed as endangered while the CTS-CC is listed as threatened. CTS utilize vernal pools, semi-permanent ponds, and permanent ponds, and the terrestrial environment in California. The aquatic environment is essential for breeding and reproduction and mammal burrows are also important habitat for aestivation. See Figure 2-0 for California Tiger Salamander Critical Habitat and Occurrence Sections.

Table 2-5 Summary of Current Distribution, Habitat Requirements, and Life History Information for the Assessed Listed Species California Tiger Salamander (CTS) (*Ambystoma californiense*)[1]

Assessed Species	Size	Current Range	Habitat Type	Designated Critical Habitat?	Reproductive Cycle	Diet
California Tiger Salamander (CTS) (*Ambystoma californiense*)	Adult 14.2-80.5 g[2]	CTS-SC is primarily found on the Santa Rosa Plain in Sonoma County. CTS-CC occupies the Bay Area (central and southern Alameda, Santa Clara, western Stanislaus, western Merced, and the majority of San Benito Counties), Central Valley (Yolo, Sacramento, Solano, eastern Contra Costa,	Freshwater pools or ponds (natural or man-made, vernal pools, ranch stock ponds, other fishless ponds); Grassland or oak savannah communities, in low foothill regions; Small mammal burrows	Yes	Emerge from burrows and breed: fall and winter rains Eggs: laid in pond Dec. – Feb., hatch: after 10 to 14 days Larval stage: 3-6 months, until the ponds dry out, metamorphose late spring or early summer, migrate to small mammal burrows	Aquatic Phase: algae, snails, zooplankton, small crustaceans, and aquatic larvae and invertebrates, smaller tadpoles of Pacific tree frogs, CTS, toads; Terrestrial Phase: terrestrial invertebrates,

		northeast Alameda, San Joaquin, Stanislaus, Merced, and northwestern Madera Counties), southern San Joaquin Valley (portions of Madera, central Fresno, and northern Tulare and Kings Counties), and the Central Coast Range (southern Santa Cruz, Monterey, northern San Luis Obispo, and portions of western San Benito, Fresno, and Kern Counties). CTS-SB are found in Santa Barbara County.				insects, frogs, and worms

1 For more detailed information on the distribution, habitat requirements, and life history information of the assessed listed species, see Attachment II.

2 See Page 369 of Trenham *et al.* (Trenham *et al.*, 2000).

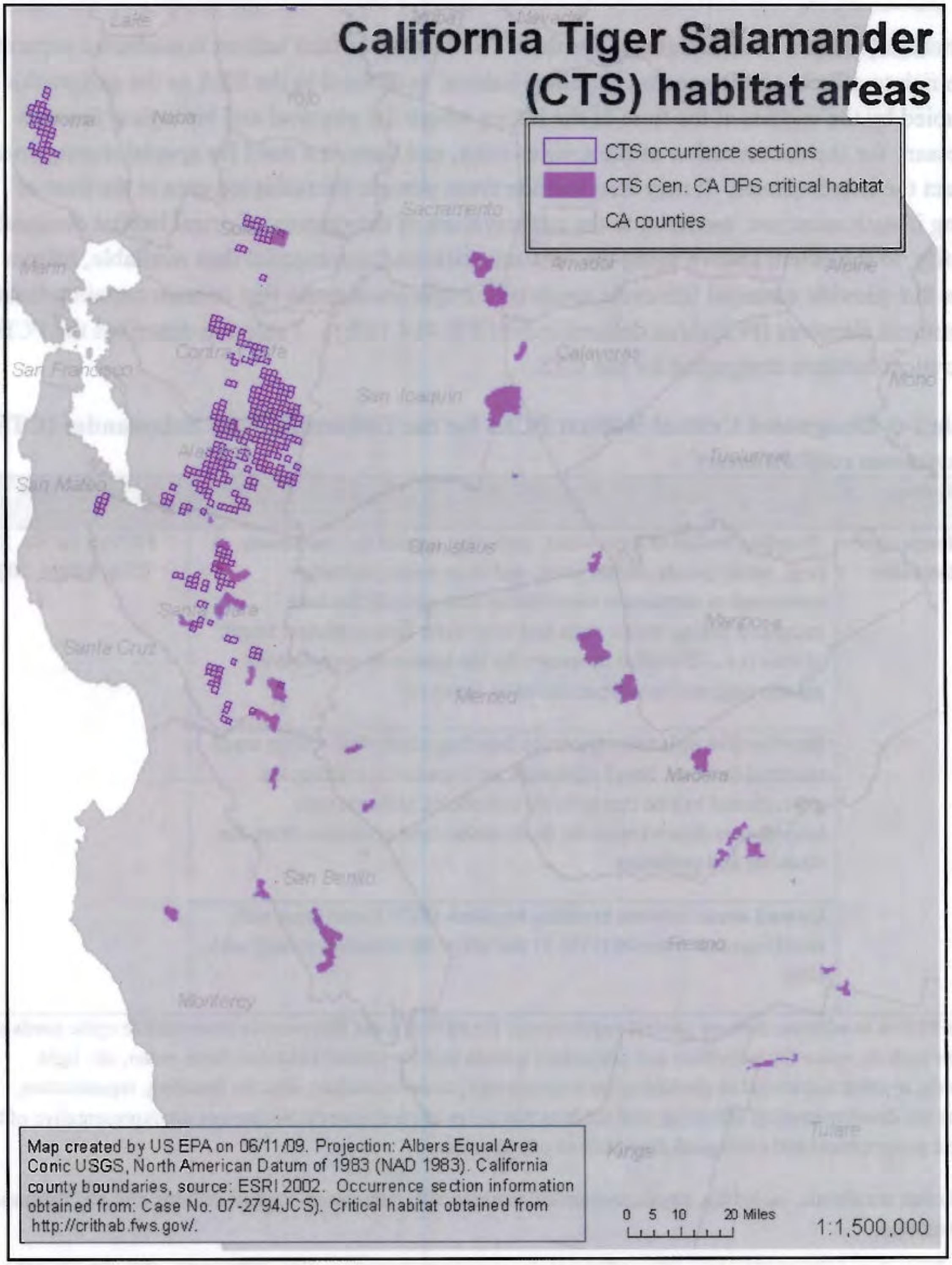


Figure 2-0. California Tiger Salamander Critical Habitat and Occurrence Sections identified in Case No. 07-2794-JCS.

2.6 Designated Critical Habitat

Critical habitat has been designated for the CTS. Risk to critical habitat is evaluated separately from risk to effects on the species. '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. 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)). Table 2-6 describes the PCEs for the critical habitats designated for the CTS.

Table 2-6 Designated Critical Habitat PCEs for the California Tiger Salamander (CTS) (*Ambystoma californiense*)[1]

Species	PCEs	Reference
California tiger salamander	Standing bodies of fresh water, including natural and man-made (*e.g.*, stock) ponds, vernal pools, and dune ponds, and other ephemeral or permanent water bodies that typically become inundated during winter rains and hold water for a sufficient length of time (*i.e.*, 12 weeks) necessary for the species to complete the aquatic (egg and larval) portion of its life cycle[2]	FR Vol. 69 No. 226 CTS, 68584, 2004
	Barrier-free uplands adjacent to breeding ponds that contain small mammal burrows. Small mammals are essential in creating the underground habitat that juvenile and adult California tiger salamanders depend upon for food, shelter, and protection from the elements and predation	
	Upland areas between breeding locations (PCE 1) and areas with small mammal burrows (PCE 2) that allow for dispersal among such sites	

[1] This PCE is in addition to more general requirements for habitat areas that provide essential life cycle needs of the species such as, 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.

[2] PCEs that are abiotic, including, physical-chemical water quality parameters such as salinity, pH, and hardness are not evaluated.

More detail on the designated critical habitat applicable to this assessment can be found in Attachment II. 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 designated critical habitat for the CTS form the basis of the critical habitat impact analysis.

As previously noted in Section 1.3, 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, 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 and LAA Effects Determination Area

2.7.1 Action Area

The action area is used to identify areas that could be affected by the Federal action. The Federal action is the authorization or registration of pesticide use or uses as described on the label(s) of pesticide products containing a particular active ingredient. The action area is defined by the Endangered Species Act as, "all areas to be affected directly or indirectly by the Federal action and not merely the immediate are involved in the action" (50 CFR §402.2). Based on an analysis of the Federal action, the action area is defined by the actual and potential use of the pesticide and areas where that use could result in effects. Specific measures of ecological effect for the assessed species that define the action area include any direct and indirect toxic effect to the assessed species and any potential modification of its critical habitat, including reduction in survival, growth, and fecundity as well as the full suite of sublethal effects available in the effects literature. 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 wetlands and aquatic areas. 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 CTS and its designated critical habitat within the state of California. For this assessment, the entire state of California is considered the action area. The purpose of defining the action area as the entire state of California is to ensure that the initial area of consideration encompasses all areas where the pesticide may be used now and in the future, including the potential for off-site transport and downstream dilution that could influence the CTS. Additionally, the concept of a state-wide action area takes into account the potential for direct and indirect effects and any potential modification to critical habitat based on ecological effect measures associated with reduction in survival, growth, and reproduction, as well as the full suite of sublethal effects available in the effects literature. It is important to note that the state-wide action area does not imply that direct and/or indirect effects and/or critical habitat modification are expected to or are likely to occur over the full extent of the action area, but rather to identify all areas that may potentially be affected by the action. The Agency uses more rigorous analysis including consideration of available land cover data, toxicity data, and exposure information to determine areas where the CTS and designated critical habitat may be affected or modified via endpoints associated with reduced survival, growth, or reproduction.

2.7.2 LAA Effects Determination Area

A stepwise approach is used to define the Likely to Adversely Affect (LAA) Effects Determination Area. An LAA effects determination applies to those areas where it is expected that the pesticide's use will directly or indirectly affect the species and/or modify its designated critical habitat using EPA's standard assessment procedures (see Attachment I) and effects endpoints related to survival, growth, and reproduction. This is the area where the "Potential Area of LAA Effects" (initial area of concern + drift distance or downstream dilution distance) overlaps with the range and/or designated critical habitat for the species being assessed. If there is no overlap between the potential area of LAA effects and the habitat or occurrence areas, a no effect determination is made. The first step in defining the LAA Effects Determination Area is to understand the federal action which is defined by the currently labeled uses for S-methoprene in California. The Agency conducted an analysis of labeled uses and a review of available product labels. Several of the currently labeled uses are special local needs (SLN) uses not specified for use in California or are restricted to specific states and are excluded from this assessment. In addition, a distinction has been made between agricultural and those that are non-food/non-agricultural uses. For those uses relevant to the assessed species, the analysis shows that, for S-methoprene, the major uses are non-food and non-agricultural (aquatic areas). Uses on agricultural lands, however, are in relation to mosquito control of flooded areas and are also be considered as part of the federal action evaluated in this assessment (Table 2-7).

Table 2-7 Summaries 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, 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), rights-of-way, agricultural and nonagricultural uncultivated areas, recreational

Use Category	Uses
	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 expected to be negligible (Table 2-8). 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 CTS 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 aquatic uses represents a 100% direct application of S-methoprene to water scenario. The maximum percent active ingredient of S-methoprene in pet shampoo is relatively low at 0.50% and assumed to be negligible in comparison to aquatic exposure modeling for this assessment based on direct application to water as an upper bound scenario.

Table 2-8 Summaries 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.

Use Category	Uses
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 CTS 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 (0.5954 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 CTS was also completed by using the maximum application rate for a liquid formulation (0.5829 lbs ai/A) to foliage (woody plants) as well as the application of granular S-methoprene (bait) 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.

2.8 Assessment Endpoints and Measures of Ecological Effect

For more information on the assessment endpoints, measures of ecological effect, see Attachment I.

2.8.1 Assessment Endpoints

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. Table 2-9 identifies the taxa used to assess the potential for direct and indirect effects from the uses of S-methoprene for the CTS. The specific assessment endpoints used to assess the potential for direct and indirect effects to each listed species are provided in Table 2-10.

Table 2-9 Taxa Used in the Analyses of Direct and Indirect Effects for the Assessed Listed Species.

Listed Species	Birds	Mammals	Terr. Plants	Terr. Inverts.	FW Fish	FW Inverts.	Estuarine /Marine Fish	Estuarine /Marine Inverts.	Aquatic Plants
California tiger salamander	Direct	Indirect (prey/ habitat)	Indirect (habitat)	Indirect (prey)	Direct Indirect (prey)	Indirect (prey)	n/a	n/a	Indirect (food/ habitat)

Abbreviations: n/a = Not applicable; Terr. = Terrestrial; Invert. = Invertebrate; FW = Freshwater
***The most sensitive fish species across freshwater environments is used to assess effects for these species because they may be found in freshwater environments.

Table 2-10 Taxa and Assessment Endpoints Used to Evaluate the Potential for Use of S-Methoprene to Result in Direct and Indirect Effects to the Assessed Listed Species or Modification of Critical Habitat.

Taxa Used to Assess Direct and Indirect Effects to Assessed Species and/or Modification to Critical Habitat or Habitat	Assessed Listed Species	Assessment Endpoints	Measures of Ecological Effects
1. Freshwater Fish and Aquatic-Phase Amphibians	Direct Effect – -CTS	Survival, growth, and reproduction of individuals via direct effects.	1a. Most sensitive fish acute LC_{50} (guideline or ECOTOX) 1b. Most sensitive fish chronic NOAEC (guideline or ECOTOX) 1c. Most sensitive fish early-life stage NOAEC
2. Freshwater Invertebrates	Indirect Effect (prey) - CTS	Survival, growth, and reproduction of individuals or modification of critical habitat/habitat via indirect effects on aquatic prey food supply (*i.e.*, freshwater invertebrates)	2a. Most sensitive freshwater invertebrate EC_{50} (guideline or ECOTOX) 2b. Most sensitive freshwater invertebrate chronic NOAEC (guideline or ECOTOX)
3. Aquatic Plants	Indirect Effect	Survival, growth, and	3a. Vascular plant acute EC_{50} (duckweed

Taxa Used to Assess Direct and Indirect Effects to Assessed Species and/or Modification to Critical Habitat or Habitat	Assessed Listed Species	Assessment Endpoints	Measures of Ecological Effects
(freshwater)	(food/habitat) -CTS	reproduction of individuals or modification of critical habitat/habitat via indirect effects on habitat, cover, food supply, and/or primary productivity (*i.e.,* aquatic plant community).	guideline test or ECOTOX vascular plant) 3b. Non-vascular plant acute EC_{50} (freshwater algae or diatom, or ECOTOX non-vascular)
4. Birds	Direct Effect -CTS (terrestrial stage)	Survival, growth, and reproduction of individuals via direct effects	4a. Most sensitive bird[a] or terrestrial-phase amphibian acute LC_{50} or LD_{50} (guideline or ECOTOX) 4b. Most sensitive bird[a] or terrestrial-phase amphibian chronic NOAEC (guideline or ECOTOX)
5. Mammals	Indirect Effect (prey/habitat from burrows/rearing sites) -CTS (terrestrial stage)	Survival, growth, and reproduction of individuals or modification of critical habitat/habitat via indirect effects on terrestrial burrows/rearing sites	5a. Most sensitive laboratory mammalian acute LC_{50} or LD_{50} (guideline or ECOTOX) 5b. Most sensitive laboratory mammalian chronic NOAEC (guideline or ECOTOX)
6. Terrestrial Invertebrates	Indirect Effect (prey) -CTS	Survival, growth, and reproduction of individuals or modification of critical habitat/habitat via indirect effects on terrestrial prey (terrestrial invertebrates)	6a. Most sensitive terrestrial invertebrate acute EC_{50} or LC_{50} (guideline or ECOTOX) 6b. Most sensitive terrestrial invertebrate chronic NOAEC (guideline or ECOTOX)

Taxa Used to Assess Direct and Indirect Effects to Assessed Species and/or Modification to Critical Habitat or Habitat	Assessed Listed Species	Assessment Endpoints	Measures of Ecological Effects
7. Terrestrial Plants	Indirect Effect (food/habitat) (non-obligate relationship) CTS	Survival, growth, and reproduction of individuals or modification of critical habitat/habitat via indirect effects on food and 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)

Abbreviations: SF=San Francisco

[a] birds are used as a surrogate for terrestrial-phase amphibians

*The most sensitive freshwater fish species is used to assess effects since CTS may be found in freshwater environments.

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 assessed species' designated critical habitat. PCEs for the assessed species were previously described in Section 2.6. Actions that may modify critical habitat are those that alter the PCEs and jeopardize the continued existence of the assessed species. 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 used to evaluate potential for direct and indirect effects are equivalent to the assessment endpoints used to evaluate potential effects to designated critical habitat. If a potential for direct or indirect effects is found, then there is also a potential for effects to critical habitat. 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.

2.9 Conceptual Model

2.9.1 Risk Hypotheses

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

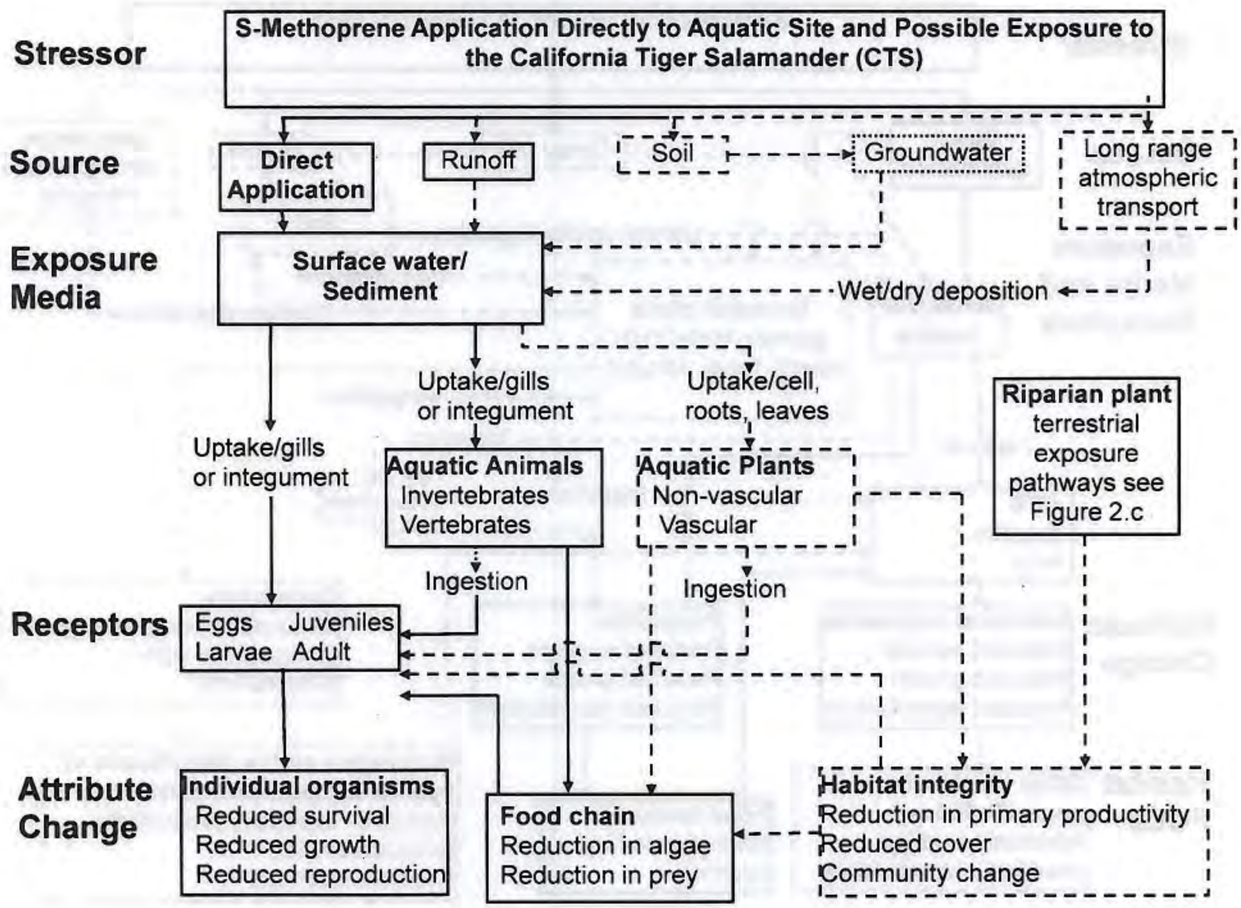
The labeled use of S-methoprene within the action area may:

- directly affect CTS by causing mortality or by adversely affecting growth or fecundity;
- indirectly affect CTS and/or modify their designated critical habitat by reducing or changing the composition of food supply;
- indirectly affect CTS and/or modify their designated critical habitat by reducing or changing the composition of the aquatic plant community in the species' current range, thus affecting primary productivity and/or cover;
- indirectly affect CTS and/or modify their designated critical habitat by reducing or changing the composition of the terrestrial plant community in the species' current range;
- indirectly affect CTS and/or modify their designated critical habitat by reducing or changing aquatic habitat in their current range (via modification of water quality parameters, habitat morphology, and/or sedimentation);

  indirectly affect CTS and/or modify their designated critical habitat by reducing or changing terrestrial habitat in their current range (via reduction in small burrowing mammals leading to reduction in underground refuge/cover).

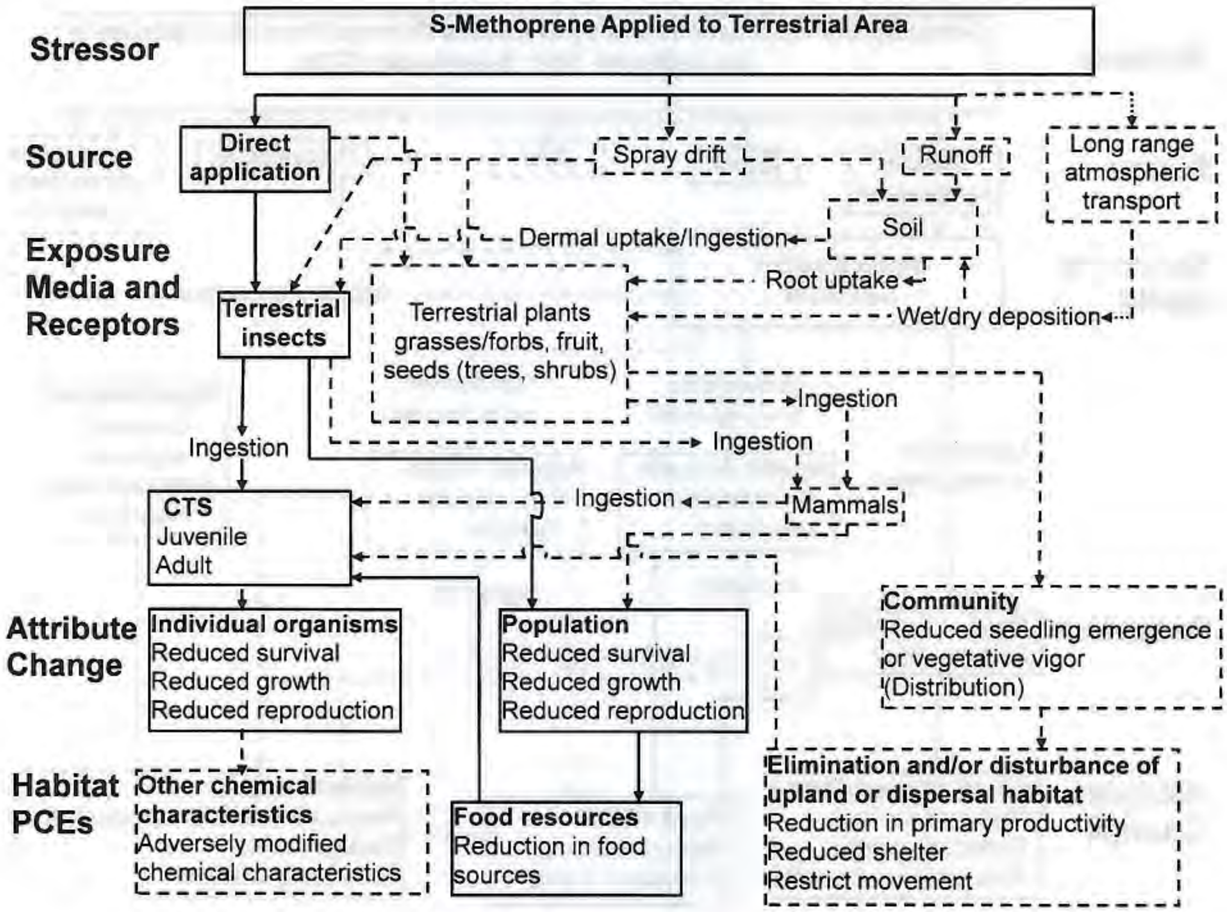
2.9.2 Diagram

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 and components of critical habitat of the CTS are shown in Figures 2-1 and 2-2. 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 CTS.



Dotted lines indicate exposure pathways that have a low likelihood of contributing to ecological risk

Figure 2-1 Conceptual Model for S-methoprene Exposure and Effects to Aquatic Phase of the California Tiger Salamander (CTS)



Dotted lines indicate exposure pathways that have a low likelihood of contributing to ecological risk

Figure 2-2 Conceptual Model for S-methoprene Exposure and Effects on Terrestrial Phase of the California Tiger Salamander (CTS)

The conceptual model is a graphic representation of the structure of the risk assessment. It specifies the S-methoprene release mechanisms, biological receptor types, and effects endpoints of potential concern. The conceptual models for CTS and the conceptual models for the aquatic and terrestrial PCE components of critical habitat are shown in Figure 2-1 and 2-2. Although the conceptual models for direct/indirect effects and modification of designated critical habitat PCEs are shown on the same diagrams, the potential for direct/indirect effects and modification of PCEs are evaluated separately in this assessment. Exposure routes shown in dashed lines are not quantitatively considered because the contribution of those potential exposure routes to potential risks to CTS and modification to designated critical habitat is expected to be negligible.

The major transport mechanisms for S-methoprene exposure to aquatic ecosystems is expected to be direct application to water, either as a liquid or as a slow release formulation (i.e. granular, briquette), thus surface runoff, drift or secondary drift of volatilized or soil-bound residues leading to deposition onto nearby or distant ecosystems is an unlikely exposure scenario. CTS aquatic life stages may be directly exposed to S-methoprene via uptake through gills/integument, as well as, indirectly affected through impact to aquatic prey items. The terrestrial use of S-methoprene as fire ant bait precludes the likelihood of exposure to aquatic areas. The exposure to small terrestrial burrow making mammals via ingestion, however, will be evaluated because of possible secondary effects to critical habitat. Adult CTS rely on underground mammal burrows for refuge/cover.

2.10 Analysis Plan

In order to address the risk hypothesis, the potential for direct and indirect effects to the assessed species, prey items, and habitat is estimated based on a taxon-level approach. 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 (USEPA, 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. Descriptions of routine procedures for evaluating risk to the San Francisco Bay Species are provided in Attachment I.

2.10.1 Measures of Exposure

The environmental fate properties of S-methoprene along with available monitoring data and use information show that direct application to water is the principle transport mechanisms for this compound to aquatic habitats. The efficacy of S-methoprene is enhanced by avoiding drift of liquid formulations (large droplet size is more efficient in penetrating foliage), and/or relying on slow release formulations (granular and briquette formulations). Terrestrial application of S-methoprene that is evaluated in this assessment is in a granular bait form. In this assessment, transport of S-methoprene through runoff and spray drift is not considered in deriving quantitative estimates of exposure to CTS, their prey and habitats. This assessment only considers exposure and toxicity of the parent compound as the identified degradates do not contain the structural moieties associated with toxicity. An estimated bioconcentration factor of 3,400 and a Koc = 2,800 suggest that the potential for S-methoprene bioconcentration in aquatic organisms is high (HSDB, 2002).

2.10.1. a Estimating Exposure in the Aquatic Environment

Measures of aquatic exposure to S-methoprene are based on a standard pond scenario of direct application to 12 inches of water using maximum labeled application rates and methods of application. Evaluation of slow release formulations included extrapolations of expected release rates and microcosm generated residues. This scenario is intended to serve as a surrogate for a wide variety of small vulnerable water bodies that occur near the top of watersheds. These include prairie potholes, swamps, bogs, and other wetlands, playa lakes, and zero and first-order streams. Larger water bodies such as second-order and higher streams and rivers, and lake reservoirs, are expected to be less vulnerable (lower EECs) than the standard pond scenario the Agency also used a modified Pesticide Root Zone Model coupled with the Exposure Analysis Model System (PRZM/EXAMS) for evaluating granular and multiple applications of the liquid to a water body.

2.10.1. b Estimating Exposure in the Terrestrial Environment

The models used to predict terrestrial EECs on potential CTS food items is the Terrestrial Residue Exposure model (T-REX). The K_{OW} (based) Aquatic BioAccumulation Model (KABAM) is used to estimate potential bioaccumulation of hydrophobic organic pesticides in freshwater aquatic food webs and subsequent risks to mammals and birds via consumption of contaminated aquatic prey. These models are parameterized using relevant reviewed registrant-submitted environmental fate data. More information on these models is available in Attachment I.

2.10.2 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 2-11). 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.

Data identified in Section 4 are used as measures of effect for direct and indirect effects and were obtained from registrant submitted studies or from literature studies identified by ECOTOX. More information on the ECOTOXicology (ECOTOX) database and how toxicological data is used in assessments is available in Attachment I.

2.10.3 Integration of Exposure and Effects

Risk characterization is the integration of exposure and ecological effects characterization to determine the potential ecological risk from uses of S-methoprene, and the likelihood of direct and indirect effects to the assessed species in aquatic and terrestrial habitats. The exposure and toxicity effects data are integrated in order to evaluate the risks of adverse ecological effects on non-target species. The risk quotient (RQ) method is used to compare exposure and measured toxicity values. EECs are divided by acute and chronic toxicity values. The resulting RQs are then compared to the Agency's levels of concern (LOCs) (USEPA, 2004) (see B). More information on standard assessment procedures is available in Attachment I.

2.10.4 Data Gaps

The available S-methoprene data base haves been evaluated by the Agency and found to be adequate for assessing potential risk to the CTS; therefore, there are no data gaps for this assessment.

3.0 Exposure Assessment

3.1 Label Application Rates

S-methoprene labels may be categorized into two types: labels for manufacturing uses (including technical grade S-methoprene) and end-use products. While technical products, which contain S-methoprene of high purity, are not used directly in the environment, they are used to make formulated products, which can be applied in specific areas to control mosquitoes and other pests. The formulated product labels legally limit S-methoprene's potential use to only those sites that are specified on the labels. The uses being addressed in this risk assessment are summarized in Table 3-0. All mitigation required as part of the reregistration process was completed as of November 2011 which includes Fate data (Koc).

Table 3-0 Modeled S-Methoprene Uses, Scenarios, and Maximum Application Rates[1]

Use	Formulation	Max. % active ingredient	Max. Rate (lbs ai/A)	Minimum Retreatment Intervals	Number of applications
Woodland pools, swamps, storm	Briquet XR	8.62	0.014	30 days	As needed

Use	Formulation	Max. % active ingredient	Max. Rate (lbs ai/A)	Minimum Retreatment Intervals	Number of applications
drains, etc.	Briquet	4.24	0.0058	150 days	As needed
Woodland pools, swamps, irrigated crop lands, rice, cranberry bogs.	Granular Sand mix	4.25 3.0	0.425 0.12	7 days 7 days	As needed As needed
Woodland pools, berry bogs, rice fields, irrigated crop lands after flooding,	Liquid	20	0.013	7 - 10 days	As needed
Citrus (Bait)	Granular	4.25	0.425	7 days	As needed
Shrubs, bushes	Liquid	5	0.428	10 days	As needed

[1]Based on data supplied by BEAD (BEAD, 4/27/2011 transmittal memo)

3.2 Aquatic Exposure Assessment

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 such as non agricultural and agricultural land that is flooded. Unlike adulticides that are used to combat adult mosquitoes (i.e. synthetic pyrethroids), S-methoprene is not sprayed as fine droplets in order to create a mist that is intended for drift over a target site. Instead, the liquid formulation is applied directly to water as a coarse spray for maximum effectiveness. The Agency calculated potential exposure of multiple (10) applications at seven day intervals of liquid S-methoprene (0.428 lbs ai/A) to water by modifying EXAMS (Exposure Analysis Modeling System) linked to PRZMS (Pesticide Root Zone Model) in order to evaluate the fate, transport, and exposure concentration of S-methoprene (Appendix E). This simulation of direct application to water was accomplished by modifying PRZMS to reflect 100% drift to an aquatic area. The Agency also evaluated the application of S-methoprene granular (0.425 lbs ai/A; 7 applications; 7 day intervals) to an aquatic area through PRZM/EXAMS simulation (Appendix J). The potential exposure to aquatic organisms from S-methoprene use from other sustainable release formulations (briquettes, and sand mix applications) is approached by estimating expected release rates from label information and expected efficacy (Appendix I). These extrapolated calculations do not take into consideration S-methoprene degradation (photolysis or biodegradation) or adsorption to particulate/sediment. In addition to these values, the Agency also evaluated adjusted field microcosm residue data (Appendix D). These studies represented five S-methoprene formulations (briquette, liquid, granular, sand mix, and XR Briquet) that were applied to microcosm tanks to evaluate residue concentrations present over time as required by the Agency. All applications were conducted on maximum label rates. and water samples were taken prior to application and then post application at Days

1, 2, 4, 7, 14, 21, 28, and 35. Samples were analyzed for methoprene by capillary gas chromatography. No residues were detected at or above the 10 ppb level at any sampling interval. Model generated EECs will be used to produce the RQ values for this CTS assessment and the microcosm information will serve as a supplement for comparison.

3.2.1 PRZM/EXAMS Modeling for Aquatic EECs

Liquid Larvicide: This formulation may be applied via aerial and ground equipment to irrigated croplands after flooding to control mosquito emergence. Examples of such sites are: vineyards, rice fields (including wild rice), date palm orchards, fruit and nut orchards, and berry fields and bogs. Irrigated pastures may be treated after each flooding without the removal of grazing livestock. This formulation may also be applied to intermittently flooded non crop areas. Typical sites include freshwater swamps and marshes, salt marshes, woodland pools and meadows, dredging spoil sites, drainage areas, waste water treatment and settling ponds, ditches and other natural and manmade depressions.

S-Methoprene is efficacious to Dipteran larvae; therefore, the most effective mode of application to potential breeding areas is a direct application to water for liquid formulations. In order to derive estimated environmental concentrations (EECs) of S-Methoprene to aquatic ecosystems the Agency has relied on EXAMS (Exposure Analysis Modeling System) linked to PRZMS (Pesticide Root Zone Model) in order to evaluate the fate, transport, and exposure concentration of S-Methoprene. Simulation of direct application to water was accomplished by modifying PRZMS to reflect 100% drift to an aquatic area. The EEC values that were calculated from this modeling are noted in Table 3-1.

Table 3-1 Simulated Liquid Use Pattern for Assessing Risk to CTS in the San Francisco Bay Watershed

Use	Formulation	Max. Rate (lbs ai/A	App Interval (days)	No. Applications	Application method
Bogs, swamps, marshes, ponds etc.	Liquid	0.013	7 - 10	As needed	ground/aerial[1]

[1] Application simulated assuming a course spray and no drift

Granular Formulation: The Agency also evaluated the application of S-methoprene granular (0.425 lbs ai/A; 7 applications; 7 day intervals) to an aquatic area through PRZM/EXAMS simulation. The EEC values that were calculated from this modeling are noted in Table 3-2. Direct applications were made to the USEPA/OPP standard pond and in order to facilitate the calculations, PE5 was used with a modified San Francisco area scenario. Runoff and erosion were turned off in the scenario and direct pesticide applications were applied through the spraydrift parameter. Pesticide transport by runoff and erosion were prevented by specifying that the application efficiency was zero. Direct applications to the pond were made by specifying the sparydrift parameter as 1. These two changes effectively transfer the application rate to the pond. The Base Scenario used was CAColeCropRFL_V2.text.All model inputs (liquid and granular) are given in Table 3-3.

Table 3-2 Simulated Granular Use Pattern for Assessing Risk to CTS in the San Francisco Bay Watershed

Use	Formulation	Max. Rate (lbs ai/A	App Interval (days)	No. Applications	Application method
Bogs, swamps, marshes, ponds	Granules	0.425	7 - 10	As needed	ground

3.2.1. a Chemistry Input Parameters

The chemistry input parameters used to simulate S-methoprene use are in Table 3-3 and represent the properties of the parent compound only as S-methoprene degradates are not likely to have the same efficacious activity.The solubility in water at 20^0 C is 1.39 mg/L (MRID 42290001).

Table 3-3 Chemistry Input Parameters (PRZM/EXAMS)

Property	Value	
Hydrolysis	Stable	Feb 20, 2008 RLF
Photolysis	3.1 days	Feb 20, 2008 RLF, as calculated from the information given in 3 studies in which 20% remained after 13 days, 5% remained after 7 days, and 12% remained after 7 days. Average half life is 3.1 days (of 5.6, 1.6, 2.2 days)
Aerobic Aquatic	35 days	Feb 20, 2008 RLF, 28 to 35 days
Anaerobic Aquatic	No data, Assume stable	Feb 20, 2008 RLF
Koc	2,300	Feb 20, 2008 RLF
Vapor Pressure	$2.3\text{x}10^{-5}$ torr	Feb 20, 2008 RLF
Solubility	1.4 mg/L	Feb 20, 2008 RLF
Henry Constant	$6.9\text{x}10^{-6}$ atm m^3/mole	Feb 20, 2008 RLF
Molecular Wt	310	INCHEM
Interval	7 days	
Application Rate	0.0672 kg/hA	Label 0.06 lb/acre
Max Applied Amount	0.476 kg/hA	Label: 5 to 10 lb/acre formulated = 0.2125 to 0.425 lb ai/acre; thus, Max = 0.476 kg/hA
Number of Application	7	Implied from 0.476/0.0672 = 7
Application Efficiency	0	Eliminates application to field.
Spraydrift	1.0	Forces only spraydrift applications to occur to the pond at the same rate as entered into PE5

Start of Applications	March 21	Assumed

3.2.1. b Aquatic EECs

Aquatic exposure and corresponding EECs were developed through extrapolation of label information (briquette and sand mix) and models as noted above. The Agency also evaluated and compared the results of the microcosms (i.e. granular, briquette, liquid) with the model generated results. Since the objective in applying S-methoprene to the aquatic environment is to avoid drift, the AgDRIFT and AGDISP models were not used in this assessment. Peak model generated environmental concentrations resulting from different S-methoprene uses range from 1.61 – 9.42 µg/L (Table 3-4). These estimates are supplemented with analysis of available microcosm residue studies that range from <0.20 to 4.24 ug/L (Table 3-5) for the five formulations. In general, the microcosm values were about 2X to 8X lower than the Agency calculations and model generated results. No monitoring data were reported by NAWQA for California surface waters with agricultural watersheds.

Table 3-4 EECs (ug/L) of S-Methoprene Formulations in the San Francisco Bay Watershed

Use	Formulation	% ai	App Intervals (days)	Max. Rate (lbs ai/A	Peak EEC (ug/L)	EEC at 21 days (ug/L)	EEC at 60 days (ug/L)
Woodland pools, swamps, rice fields, storm drains, etc.	Briquet[1]	4.24	150	0.0058	2.1	2.1	2.1
	Briquet XR[1]	8.62	30	0.014	5.18	5.18	5.18
	Sand mix[1]	1.3	7	0.017	6.2	6.2	6.2
Woodland pools, swamps, berry bogs, rice fields, irrigated crop lands, etc.	Granular[2]	4.25	7	0.425	9.46	7.42	5.86
Woodland pools, berry bogs, rice fields, irrigated crop lands, etc.	Liquid [2]	5 to 20	10	0.013	1.61	1.2	1.15

[1] Extrapolated values; [2] PRZM/EXAMS

The direct application of the liquid formulation (5- 20% ai) to water showed residue levels with a peak of 0.002ug/L and 0.001ug/L at 21 and 60 days. S-methoprene applied as a granular formulation (4.25 % ai) peaked at 9.42ug/L and declined to 7.42 and 5.86 ug/L at days 21 and 60, respectively. The expected residues for the Briquet (4.24 % ai), Briquet XR (8.62 % ai) and the sand mix (1.3% ai) were calculated with no consideration for degradation. The Briquet and Briquet XR had peak residues of 2.1 and 5.18ug/L, respectively and the sand mix showed and expected peak of 6.2ug/L and the same value for 21 and 60 days.

A study (Judy and Howell, 1992: MRID 428112-02) was conducted by the registrant (Zoecon Corpations) to determine the concentrations of S-methoprene in freshwater microcosms treated with sustained release and liquid formulation. Single applications of five S-methoprene formulations were applied to microcosm tanks near Oxford, Mississippi, in 1991, to evaluate S-methoprene concentrations present over time. The Liquid Larvicide (5% S-methoprene) was applied at a maximum rate of 4 fluid ounces/A, the XR Briquet (1.8% S-methoprene), and the Briquet (7.9% RS-methoprene) were applied to the microcosm at the rate of 1 briquet/113 ft^2. The pellets (4.25% S-methoprene granules) were applied to water at the maximum label rate of 10 lbs/A while the sand mix (1.3% S-methoprene) was applied at the equivalent of 20 lbs /A. Water samples were collected prior to application and post application at Days 1, 2, 4, 7, 14, 21, 28, and 35 (Table 3-5) and were analyzed for S-methoprene by capillary gas chromatography. The study results show that no S-methoprene residues were detected at or above the 10 ug/L level in any of the samples at any sampling interval (Appendix D). A summary of the findings are as follows:

- **Liquid Larvicide (5% ai)**: S-methoprene residues averaged residues were noted at 2.21ug/L on Day 1 and gradually declined to about 0.2ug/L.
- **Briquet (7.9% ai)**: Residue levels increased through Day 4 and Day 5 (4.24 ug/L) and declined from Days 14 – 35.
- **XR Briquet (1.8% ai):** The highest S-methoprene level was on Day 2 (3.37 ug/L). Throughout the remaining study the residues were lower (1.07 - 0.96 ug/L).
- **Granular (4.25% ai):** From Day 1 to Day 7 there was an increase in S-metheprene residues (0.64 – 2.1 ug/L). After Day 14 there appeared to be a steady concentration level (0.21 – 0.24 ug/L).
- **Sand Mix (1.3% ai):** These residue values were the lowest ranging from 0.35 ug/L on Day1 to about <0.20 ug/L throughout the rest of the test.

Table 3-5 Adjusted Environmental Concentrations of S-Methoprene Found in Freshwater Microcosm[1]

Use	Formulation	Peak EEC (ug/L)	4-day EEC (ug/L)	21-day EEC (ug/L)	35-day EEC (ug/L)	60-day EEC (ug/L)[1]
Woodland pools, swamps, rice fields, storm drains, etc.	Briquet	4.24	4.24	0.13	0.14	0.14
	Briquet XR	3.37	0.96	0.96	0.96	0.96
Woodland pools, swamps, berry bogs, rice fields, irrigated crop lands, etc.	Granular	2.0	1.28	0.21	<.0.20	<0.20
Woodland pools, swamps, berry bogs, rice fields, irrigated crop lands, etc.	Sand mixture	0.35	< 0.20	<0.20	< 0.20	<0.20

Use	Formulation	Peak EEC (ug/L)	4-day EEC (ug/L)	21-day EEC (ug/L)	35-day EEC (ug/L)	60-day EEC (ug/L)[1]
Woodland pools, berry bogs, rice fields, irrigated crop lands.	Liquid	2.21	0.511	0.255	0.203	0.20

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

3.2.2. Environmental Monitoring Data

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.

3.3. Measure of Terrestrial Exposure

3.3.1 Terrestrial Exposure Modeling

For assessing exposure of pesticides to terrestrial animals, the Agency typically uses T-REX to calculate EECs for dietary exposure of terrestrial wildlife, and KABAM to calculate bioaccumulation (Koc = 2,300; Kow = 5.50; estimated bioconcentration factor of 3,400). The EEC values used for terrestrial animal exposure (T-REX) 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 (laboratory 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. Terrestrial EECs are based on the maximum application rate of 0.428 lbs ai/A to be applied to agricultural crop soil and woody plants (Table 3-6) and assumed this to be an upper bound scenario. Using the T-REX model (version 1.3.1, December 22, 2006) with maximum input values (4 applications; 0.428 lb a.i./A; 10-day application interval) the Agency was able to estimate terrestrial exposure for avian and mammalian species. Table 3-7 shows Upper Bound EECs that range from 11.41 – 182.59 ppm.

The values noted in Table 3-6 reflect maximum application rates for formulations that can be used in terrestrial areas where the CTS may be found. Although salamanders are not expected to directly feed on granules, the consumption of granules by small mammals could be toxic and result in secondary effects to CTS through habitat reduction (reduction in available burrows).

Table 3-6. Maximum Rate of S-Methoprene Formulations Applied to Terrestrial Site[1]

Use	Formulation	Max. % active ingredient	Max. Rate (lbs ai/A)	Minimum Retreatment Intervals (days)	Number of Applications
Citrus (Bait)	Granular	4.25	0.3	30 days	As Needed
Agricultural crop soil	Liquid	5	0.428	10 days	As Needed

[1]Based on data supplied by BEAD (BEAD, 4/27/2011 transmittal memo)

Table 3-7. Dose and Dietary-based EECs for Avians (T-REX Output)

	Avian Class and Body Weight	
Dose-based EECs (mg/kg-bw)	20g	100g
Short Grass	182.59	104.12
Tall Grass	83.69	47.72
Broadleaf Plants/Small Insects	102.71	58.57
Fruits/Pods/Seeds/Large Insects	11.41	6.51
Dietary-based EECs (ppm)	**Residues (ppm)**	
Short Grass	112.38	
Tall Grass	51.51	
Broadleaf Plants/Small Insects	63.21	
Fruits/Pods/Seeds/Large Insects	7.02	

Table 3.8 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 salamanders do not directly feed on granular particles, this scenario was included as an upper bound scenario to evaluate secondary effects to the CTS via habitat reduction (e.g. toxic effects to burrowing mammals). The values noted in Table 3-8 reflect maximum application of the granular formulation to terrestrial areas where the CTS may be found. A complete description of the input parameters and output is contained in Appendix H.

Table 3-8. Characterization of S-Methoprene Granular Application Representing a Maximum of 0.3 lbs ai/A Using T-REX for a 20 g bird (Granular Weight = 0.425 mg, 30 day exposure)

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 LD_{50})	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

Since S-methoprene is a non-ionic, organic compound with a log Kow of 5.50 and a Koc of 2,800, there is a potential for bioaccumulation. This concern is also reinforced by an estimated bioconcentration factor of 3,400 that suggests that the potential for bioconcentration in aquatic organisms is very high (HSDB, 2002). The Agency evaluated bioaccumulation of S-methoprene and the possible toxic risk to mammals and birds that might consume aquatic organisms that may have high tissue levels of the compound, through the Kow (based) Aquatic BioAccumumaltion Model (KABAM). This model incorporates seven trophic levels and calculates pesticide tissue levels residues for different levels of an aquatic food web. The model then uses pesticide tissue concentrations in aquatic animals to estimate dose- and dietary-based exposure and associated risks to mammals and birds consuming aquatic organisms (USEPA 2008). The possible impact to burrow making mammals is the focus of this portion of the analysis. If small mammals are at toxic risk from S-methoprene bioaccumulation, there could be secondary effects to habitat that is critical to CTS survival. Table 3-9 shows EECs for birds and mammals that could consume fish contaminated by S-methoprene. Mammalian dietary based EECs ranged from 5.26 – 31.16 ppm and the range for avian species ranged from 6.14 – 31.16 ppm.

Table 3-9 EECs for Mammals and Birds Consuming Fish Contaminated by S-methoprene.

Wildlife Species	Biological Parameters				EECs (pesticide intake)	
	Body Weight (kg)	Dry Food Ingestion Rate (kg-dry food/kg-bw/day)	Wet Food Ingestion Rate (kg-wet food/kg-bw/day)	Drinking Water Intake (L/d)	Dose Based (mg/kg-bw/d)	Dietary Based (ppm)
Mammals						
fog/water shrew	0.02	0.140	0.585	0.003	3.080	5.26
rice rat/star-nosed mole	0.1	0.107	0.484	0.011	2.950	6.10
small mink	0.5	0.079	0.293	0.048	4.264	14.54
large mink	1.8	0.062	0.229	0.168	3.331	14.54
small river otter	5.0	0.052	0.191	0.421	2.777	14.54
large river otter	15.0	0.042	0.157	1.133	4.896	31.16
Avian						
sandpipers	0.0	0.228	1.034	0.004	6.3480	6.14
cranes	6.7	0.030	0.136	0.211	1.0624	7.82

rails	0.1	0.147	0.577	0.010	4.2911	7.43
herons	2.9	0.040	0.157	0.120	1.5582	9.90
small osprey	1.3	0.054	0.199	0.069	2.8987	14.54
white pelican	7.5	0.029	0.107	0.228	3.3249	31.16

4. 0 Effects Assessment

This assessment evaluates the potential for S-methoprene to directly or indirectly affect CTS or modify their designated critical habitat. Assessment endpoints for the effects determination for CTS include direct toxic effects on the survival, reproduction, and growth, as well as, indirect effects, such as reduction in prey base or modification of its habitat. In addition, potential modification of critical habitat is assessed by evaluating effects to the PCEs, which are components of the critical habitat areas that provide essential life cycle needs for CTS. Direct effects to the aquatic-phase CTS are based on toxicity information for freshwater fish, while terrestrial-phase amphibian effects are based on avian toxicity data, given that birds are generally used as surrogate for terrestrial-phase amphibians.

As described in the Agency's Overview document (USEPA, 2004a), the most sensitive endpoint for each taxon is used for risk estimation. For this assessment, evaluated taxa include freshwater fish and invertebrates, aquatic plants, birds, mammals, and terrestrial invertebrates and plants. Acute (short-term) and chronic (long-term) toxicity information is characterized based on registrant-submitted studies and a comprehensive review of the open literature.

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 than aquatic organisms to S-methoprene exposure. Avian and mammal species show practically no acute toxic effects after exposure to S-methoprene and there is no phytotoxicity. There were no avian reproductive effects at the levels tested. 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 Summaries 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} = 760 ug/L (240-120 ug/L)	Mortality
	Chronic		

TAXA	MEASURE OF EFFECT		
Survival, growth and/ or reproduction of:	**Species**	**Toxicity**	**Endpoint**
	Fathead minnow *(Pimephales promelas)*	NOAEC = 480 ug/L LOAEC = 840 ug/L	Growth effected
Freshwater Invertebrates	***Acute***		
	Daphnia magna	EC_{50} = 330 ug/L (110 – 520 ug/L)	Mortality
	Chronic		
	Daphnia magna	NOAEC = 10 ug/L LOAEC = 51 ug/L	Mortality
Birds	***Acute***		
	Mallard duck (*Anas platyrhynchos*)	LC_{50}>10,000 ppm	Mortality
	Mallard duck (*Anas platyrhynchos*)	LD_{50}>2,000 mg ai/kg	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 Plants	***Acute***		
	Blue Green Algae	LOAEL = 500 mg/L	Mortality
	Rice (*Oryza sativa*)	NOAEL = 2000 ug/L	

4.1 Ecotoxicity Study Data Sources

Toxicity endpoints are established based ondata generated from guideline studies submitted by the registrant, and from open literature studies that meet the criteria for inclusion into the ECOTOX datebase maintained by EPA/Office of Research and Development (ORD) (USEPA, 2004a). Open literature data presented in this assessment were obtained from previous risk assessments as well as ECOTOX information obtained on November 2005. In order to be included in the ECOTOX database, papers must meet the following criteria:

1. The toxic effects are related to single chemical exposure;
2. The toxic effects are on aquatic or terrestrial plant or animal species;

3. There is a biological effect on live, whole organisms;
4. A concurrent environmental chemical concentration/dose or application rate is reported; and
5. There is an explicit duration of exposure.

Open literature toxicity data on the effects of S-methoprene to "target" insects (mosquitoes and other Dipterian species), which include efficacy studies were not considered in deriving the most sensitive endpoint for terrestrial and aquatic invertebrates. The registrant has submitted acute invertebrate studies that will be used in this assessment. Therefore, toxicological data on target species of insects were not included in the ECOTOX open literature search that was conducted by the Agency, and are not included in the summary table provided in Appendix J.

Data that pass the ECOTOX screen are evaluated along with the registrant-submitted data, and may be incorporated qualitatively or quantitaivelyinto this endangered species assessment. In general, effects data in the open literature that are more conservative than the registrant-submitted data are considered. The degree to which open literature data are quantitatively or qualitatively characterized for the effects determination is dependent on whether the information is relevant to the assessment endpoints (i.e., survival, reproduction, and growth) identified in Section 2.8. For example, endpoints such as behavior modifications are likely to be qualitatively evaluated, because quantitative relationships between modifications and reduction in species survival, reproduction, and/or growth are not available. Although the effects determination relies on endpoints that are relevant to the assessment endpoints of survival, growth, and reproduction, it is important to note that the full suite of sublethal endpoints potentially available in the effects literature (regardless of their significance to the assessment endpoints) are considered, as they are relevant to the understanding of the area with potential effects, as defined for the action area.

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

In addition to registrant-submitted and open literature toxicity information, other sources of information, including use of the acute probit dose response relationsip to establish the probability of an individual effect and reviews of ecological incident data, are considered to further refine the characterization of potential ecological effects associated with exposure to S-methoprene. A summary of available terrestrial and aquatic ecotoxicity information and the incident information for S-methoprene are provided in Section 4.1 and 4.2.

4.2 Evaluation of Terrestrial Ecotoxicity Studies

4.2.1 Terrestrial Plant Toxicity/Exposure

Applicators apply S-methoprene liquid formulations to terrestrial or aquatic areas use a coarse spray (aerial and ground application) for more efficient penetration of the surrounding plant canopy. S-methoprene structure and mode of action as an insect growth regulator does not appear to have phytotoxicty characteristics. The compound breaks down through ester hydrolysis O-demethylation and oxidative cleavage of the C-4 double bond (FAO, 2005). Further metabolism in plants results in the corporation of metheprene-derived fragments ino natural products. Plant toxicity test, however, are very limited in the open literature and are used as a qualitative assessment of S-methoprene phytotoxicity. Some studies showed an evaluation of the toxic effect of S-methioprene to a particular insect pest and noted that the plants that the pest resided on were not harmed. Athanassioa *et al.*, (2010) studing the control of *psocids* on maize, rice, and wheat noted that plants were exposued to 1, 5, and 10 mg/L of methoprene for 40 days without any phytotoxicity. Parrella (1983) in evaluating efficacy of methoprene to *Liriomyza trifolii* found no effects to chrysanthemums. Parrella and Rob (1982) screening several bedding plants for toxic effects of methoprene found no phytotoxicity to the tested plants which included Antirrhinum, Impatiens, Verbena, Zinnia, broccoli, courgettes, peas, and tomatoes.

4.2.2 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 4-1. 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 values range from LD_{50} >2,000 mg/kg to LC_{50} = 10,000 ppm. The reproductive study on Bobwhite quail showed no reproductive effects at two levels tested, 3 and 30 ppm (Table 4.1).

Table 4-1 Summary of Avian Toxicity for S-Methoprene

Test Species	% ai	Endpoint	MRID No. Author/Year	Status/Comments
Acute Toxicity				
Mallard duck (*Anas platyrhynchos*)	Tech.	LD_{50}>2,000 mg ai/kg	003202508 Fink/1972	Acceptable, practically non-toxic
Bobwhite quail (*Colinus virginianus*)	Tech	LC_{50} > 10,000 ppm	003202509 Fink/1972	Acceptable, practically non-toxic
Mallard duck (*Anas platyrhynchos*)	Tech.	LC_{50}>10,000 ppm	003202509 Fink/1972	Acceptable, practically non-toxic
Chronic Toxicity				
Bobwhite quail (*Colinus virginianus*)	Tech.	No reproductive effects at 3 and 30 ppm.	003202511 Fink&Reno/ 1973	Acceptable, effect level not determined

4.2.3 Mammal Toxicity 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 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 (RS 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 shows 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	Status/Comments
Rat *(Rattus norvegicus)*	RS-methoprene tech	14-day	LD_{50} >10,000 mg/kg	00024607 Hallesy *et al.*/1972	Acceptable, practically nontoxic
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

4.2.4 Insect Toxicity Studies

Although S-methoprene is most toxic to Dipterans (*e.g.*, mosquitoes, midges), it is also toxic to non-target species that include Hemiptera, Lepidoptera, and Coleopteran. Mosquitoes are very sensitive to methoprene exposure at about 1.0 ug/L (Lawler, 2000). Ali *et al.,* (2008) showed that Chironomidae (*Glypotendipes carus* and *paripes*) had an LC_{90} = 55.0 and 82.0 ug/L, respectively (Table 4-3).

Table 4-3 Toxicity of Methoprene to Insects

Species	RS-methoprene % ai	Results	Reference
Water boatman (*Corisella decolor sp.*)	10	96 hr LC_{50} = 1,650 ug/L	Miura, 1973
Backswimmers (*N. unifasciata*)	10	24 hr LC_{50} = 1,200 ug/L	Miura, 1973
Honey bee (Apis mellifera L.)	10	31 day feeding. No effect at 1,000 ug/L methoprene	Barker and Waller, 1978
Diving Beetles (*Laccophilus sp.)*	10	72 hr LC_{50} = 2.000 ug/L	Miura, 1973
Mosquitoes larvae (*Ochlerotatus nigromaculis*)	10	Toxic effect at 1.0 ug/L	Lawler, 2000
Midge (*Glypotendipes carus*)	Tech S-methoprene	LC_{90} = 55.0 ug/L	Ali *et al.*, 2008
Midge (*G. paripes*)	Tech S-methoprene	LC_{90} = 82.0 ug/L	Ali *et al.*, 2008

Toxicity to terrestrial animals is categorized using the system shown in Table 4-4 (USEPA, 2004a). Toxicity categories for terrestrial plants have not been defined.

Table 4-4 Categories of Acute Toxicity for Avian and Mammalian Studies

Toxicity Category	Oral LD_{50}	Dietary LC_{50}
Very highly toxic	< 10 mg/kg	< 50 mg/kg-diet
Highly toxic	10 – 50 mg/kg	50 – 500 mg/kg-diet
Moderately toxic	51 – 500 mg/kg	501 – 1000 mg/kg-diet
Slightly toxic	501 – 2000 mg/kg	1001 – 5000 mg/kg-diet
Practically non toxic	>2000 mg/kg	>5000 mg/kg-diet

4.3. Evaluation of Aquatic Ecotoxicity Studies

4.3.1. Aquatic Plant Assessment

ECOTOX produced two studies that included a study by Wurtsbaugh and Apperson (1978) that showed that Blue-green algae exposed to methoprene produced a LOAEL = 500 ug/L, suggesting that the compound is practically non toxic to nonvascular plants and a study on rice (vascular plant) showing a NOAEL = 2,000 ug/L (Table 4.5).

Table 4.5 Toxicity of S-Methoprene to Aquatic Plants

Test Species		Endpoint	Source/Year	Status/Comments

	% ai			
Aquatic PlantsToxicity (Nonvascular)				
Blue-Green Algae	Tech.	LOAEL=500 ug/L	Wurtsbaugh, and Apperson, 1978	Acceptable/Practically non-toxic
Aquatic Plant Toxicity (Vascular)				
Rice *(Oryza sativa)*	Tech.	NOAEL = 2,000 ug/L	Steelman *et al.*, 1975	Acceptable/Practically non-toxic

4.3.2 Fish Toxicity Studies

The Agency has summarized the 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 4-6).

Table 4-6 Summaries of Fish Toxicity Studies for S-methoprene (Parent Compound, Metabolites, and Formulation)

Species	(% ai)	Results	MRID	Classification/Comments
Bluegill sunfish (*Lepomis macrochirus*)	Tech.	96hr LC_{50} =1.52 mg/L	00010388	Acceptable, moderately toxic
Rainbow trout (*Oncorhynchus mykiss*)	Tech.	96hr LC_{50}=1.6 mg/L	2033423	Acceptable, moderately toxic
	Tech	96hr LC_{50} = 4.39 mg/L	2033423	Acceptable, moderately toxic
	Tech	96hr LC_{50} > 50 mg/L	00010643	Acceptable, slightly toxic
	Tech.	96hr LC_{50} =106 mg/L	2033423	Acceptable, practically nontoxic
	Tech	96 hr LC_{50} = 0.76 mg/L (0.24 – 1.2 mg/L)	43351901	Acceptable//Highly toxic
	10	96hr LC_{50} = 28.4 mg/L	2033423	Supplemental, moderately

Species	(% ai)	Results	MRID	Classification/Comments
				toxic
	4E	96hr LC_{50} = 6.0 mg/L	2033423	Supplemental, slightly toxic
	Metabolite ZR-1946 (methoprene acid)	96hr LC_{50} = 36.9 mg/L	2033423	Supplemental, slightly toxic
	10	96hr LC_{50} =52.7 mg/L	2033423	Supplemental, slightly toxic
	10	96hr LC_{50}=1.55 mg/L; (1.10-2.41 mg/L)	2033423	Supplemental, moderately toxic
	68.9	24hr LC_{50}>10.0 mg/L	2033423	Supplemental, slightly toxic
	68.9	24hr LC_{50}=80.0 mg/L (80 -100 mg/L)	2033423	Supplemental, slightly toxic

A chronic toxicity study on the fathead minnow produced a NOAEC value of 0.048 mg/L and a LOAEC of 0.084 mg/L. The effects that were noted included decreased weight and length of juveniles. No other effects were noted (Table 4-7).

Table 4-7 Freshwater Fish: Chronic Exposure (Growth/Reproduction) Studies

Species	% ai	Effect	MRID	Status
Fathead minnow *(Pimephales promelas)*	Tech	NOAEC = 0.048 mg/L LOAEC = 0.084 mg/L Growth effects	42811201	Acceptable

4.3.3 Aquatic Freshwater Invertebrates Toxicity Studies

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 4-8).

Table 4-8 Freshwater Invertebrates Acute Toxicity Studies with S-Methoprene

Species	% ai	Effect	MRID	Status/Comments
Acute Toxicity				
Daphnia magna	Tech.	48 hr. $EC_{50} = 0.36$ mg/L (0.21-0.55 mg/L)	43163301	Supplemental, highly toxic
Daphnia magna	Tech.	48hr. $EC_{50} = 0.33$ mg/L (0.11 – 0.52 mg/L)	003203609	Acceptable, highly toxic
Chronic Toxicity				
Daphnia magna	Tech.	LOAEC = 0.051 mg/L; NOAEC = 0.01 mg/L	2033145	Supplemental,

Toxicity to fish and aquatic invertebrates is categorized using the system shown in Table 4-9 (USEPA, 2004a). Toxicity categories for aquatic plants have not been defined.

Table 4-9 Categories of Acute Toxicity for Fish and Aquatic Invertebrates

LC_{50} (mg/L)	Toxicity Category
<0.1	Very highly toxic
>0.1 - 1	Highly toxic
> 1 - 10	Moderately toxic
> 10 - 100	Slightly toxic
> 100	Practically non toxic

4.3.4 Field Studies: Non-Target Organisms

A comprehensive study on the effects of S-methoprene to zooplankton, insects and breeding birds was conducted in Minnesota by the Metropolitan Mosquito Control District (Hershey *et al.*, 1998; Niemi *et al.*, 1999). Wetlands in Wright County were sampled for three years (1988-1990) in order to evaluate natural variability in 179 genera of insects. 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. Examination of the reproductive success and clutch size of red-winged blackbirds (*Agelaius phoeniceus*) during this study period showed that S-methoprene exposure (through effects to food sources) had no adverse impact on this species or the bird community inhabiting the wetlands. Macroinvertebrate sampling was repeated by Drs. M.Balcer and K. Schmude of Lake Superior Research Institute, Superior, WI, in 1997 and 1998. These sampling cofirmed generally high numbers of insects and other invertebrates in all the sites. The only difference between treated and untreated sites was lower populations of some groups within the Chironomidae in treated sites, but other Chironomid groups were higher, resulting in no difference in Chironomid numbers or biomass as a whole (Schmude and Balcer 1997, Balcer *et al.* 1999).

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 (prevent emergence of adults) 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 4-10).

Table 4-10 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 affected by inhibiting adult emergence.
	0.015 kg a.i./ha; EC	No effects
Schmude and Balcer (1997) Balcer *et al.* (1999).	0.275 – 0.32 lbs ai/A	Minn. wetlands: 7th and 8th year high number of insects and macroinvertebrates at all sites. No difference in chironomid numbers or biomass.
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.	Minn. wetlands: 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 ai/A); EC	Caged mayflies. Substantial decrease in emergence.

4.4 Incident Data Base Review

A review of the Ecological Incident Information System (EIIS, version 2.1), the 'Aggregate Incident Reports' (v. 1.0) database, and the Avian Monitoring Information System (AIMS) for ecological incidents involving S-methoprene was completed on December 1, 2011. No terrestrial or plant incidents were reported.

There is one incident that submitted by Wellmark International that discusses an alledge link between S-methoprene and oyster mortality in Austrialia in 2006 (I017246-001). S-methoprene was applied at 15g ai/ha of a partially micro-encapsulated formulation and one oyster bed of spat was effected (mortality). Since this applies to estuarine/marine organisms it is not relevant to the CTS assessment.

5.0 Risk Characterization

5.1 Risk Estimation

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 CTS 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) (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

Risk	Description	RQ	Taxa
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 Exposures in the Aquatic Habitat

Based on surrogate freshwater toxicity data and extrapolated and model generated EECs, the Agency has calculated RQ values to reflect a wide range of uncertainty. The extrapolated values (Briquet and sand mix) are theoretical expected environmental water concentration calculated from label information regarding weight of briquete, 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 model generated (PRZM/EXAMS) EECs reflect liquid and granular formulations based on 1 in 10 year peak EECs in a standard pond. 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, cranberry bogs). Residue data from the controlled freshwater microcosm studies will be used as supplemental information.

The model generated and extrapolated EEC values in Table 5-1 produced low RQs for the five S-methoprene formulations that are registered for use on a wide range of aquatic areas that support mosquito populations (marshes, swamps, culverts, wetlands, flooded orchards, flooded agricultural fields, old tires, etc). The slow release formulations are efficacious over a period of time (*e.g.*, 150 days), while the liquid formulation was applied at 10 day intervals. Using the acute and chronic fish data as a surrogate for the aquatic-phase of the CTS, the RQ values (acute RQs < 0.01 – 0.01; chronic RQs < 0.1 – 0.01) prove to be less than the endangered species LOC (acute LOC > 0.05; chronic LOC ≥ 1.0), suggesting a "no effect" to these organisms. An evaluation of S-methoprene effects to aquatic invertebrates by using *Daphnia magna* as a surrogate species shows that acute (< 0.01 - 0.03) and chronic (0.12 – 0.74) RQs are below the LOC that defines risk to these organisms (acute LOC> 0.1 - 0.5; chronic LOC ≥ 1.0). Aquatic plant data was used to evaluate S-methoprene affects on a potential CTS food item and/or habitat component. Blue green algae represented non-vascular aquatic plants while rice was used for vascular aquatic plants. The calculated RQs for aquatic plants were very low (< 0.01 – 0.02) which does not exceed the LOC =1.

Based on the preceeding analysis, S-methoprene does not have the potential to directly affect the aquatic phase of the CTS. Additionally, since the acute and chronic RQs are not exceeded, there is not a potential for indirect effects to the CTS that rely on fish and/or aquatic invertebrates as food during some portion of their life-stage or the use of plants as food/habitat.

Table 5-1 Acute and Chronic RQs for Aquatic Organisms Based on EECs from Extrapolated S-Methoprene Release Rates for Briquets, and Model Generated EECs for Granular and Liquid Formulations Applied to a Shallow (1 ft) 1 Acre Body of Water

Freshwater Fish							
Use Site	**Formulation**	**Species and Toxicity Endpoint**	**Peak EEC (ug/L)**	**Acute RQs**	**Species and Toxicity Endpoint**	**EEC at 21 days (ug/L)**	**Chronic RQs**
Woodland pools, swamps, rice fields, storm drains, etc.	Briquet[1]	Rainbow trout (760 ug/L)	2.1	**< 0.01**	Fathead minnow NOEC (480 ug/L)	2.1	**< 0.01**
	Briquet XR[1]		5.18	**0.01**		5.18	**0.01**
	Sand mix[1]		6.2	**0.01**		6.2	**0.01**
Woodland pools, swamps, cranberry bogs, rice fields, irrigated crop lands, etc.	Granular[2]	Rainbow trout (760 ug/L)	9.46	**0.01**	Fathead minnow NOEC (480 ug/L)	7.42	**0.01**
Woodland pools, cranberry bogs, rice fields, irrigated crop lands, etc.	Liquid [3]	Rainbow trout (760 ug/L)	1.61	**< 0.01**	Fathead minnow NOEC (480 ug/L)	1.2	**< 0.01**
Freshwater Invertebrates							
Use Site	**Formulation**	**Species and Toxicity Endpoint**	**Peak EEC (ug/L)**	**Acute RQs**	**Species and Toxicity Endpoint**	**EEC at 21 days (ug/L)**	**Chronic RQs**
Woodland pools, swamps, rice fields, storm drains, etc.	Briquet[1]	Daphnia magna (EC_{50} = 330 ug/L	2.1	**0.01**	*Daphnia magna* (NOAEC 10.0 ug/L)	2.1	**0.21**
	Briquet XR[1]		5.18	**0.01**		5.18	**0.52**
	Sand mix[1]		6.2	**0.02**		6.2	**0.12**
Woodland pools, swamps, cranberry bogs, rice fields, irrigated crop lands, etc.	Granular[2]	Daphnia magna (EC50 = 330 ug/L	9.46	**0.03**	*Daphnia magna* (NOAEC 10.0 ug/L)	7.42	**0.74**

Woodland pools, cranberry and, rice fields, irrigated crop lands, etc.	Liquid [3]	Daphnia magna (EC50 = 330 ug/L	1.61	**< 0.01**	*Daphnia magna* (NOAEC 10.0 ug/L)	1.2	**0.02**
Freshwater Plants							
Use Site	**Formulation**	**Species and Toxicity Endpoint**	**Peak EEC (ug/L)**	**Acute RQs**	**Species and Toxicity Endpoint**	**Peak EEC (ug/L)**	**Acute RQs**
Woodland pools, swamps, rice fields, storm drains, etc.	Briquet[1] Briquet XR[1] Sand mix[1]	Blue-Green Algae LOEC = 500 ug/L	2.1 5.18 6.2	**< 0.01** **0.01** **0.01**	Rice *(Oryza sativa)* LOEC = 2,000 ug/L	2.1 5.18 6.2	**< 0.01** **< 0.01** **< 0.01**
Woodland pools, swamps, cranberry bogs, rice fields, irrigated crop lands, etc.	Granular[2]	Blue-Green Algae LOEC = 500 ug/L	9.46	**0.02**	Rice *(Oryza sativa)* LOEC = 2,000 ug/L	9.46	**< 0.01**
Woodland pools, cranberry and, rice fields, irrigated crop lands, etc.	Liquid [3]	Blue-Green Algae LOAEC = 500 ug/L	1.61	**< 0.01**	Rice *(Oryza sativa)* LOEC = 2,000 ug/L	1.61	**< 0.01**

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).

The RQs generated from the upper bound values from this field data also suggest no direct acute or indirect risk from S-methoprene exposure to the aquatic-phase of the CTS (fish acute RQs < 0.01, fish chronic RQs < 0.01 – 0.02; aquatic ingvertebrate acute RQs = 0.14 – 0.96, chronic RQs 0.01 – 0.02; plant acute RQs < 0.01 – 0.01). All RQs were below the Agency LOC values (acute LOC < 0.05; chronic LOC = 1).

Table 5-2 Acute and Chronic RQs for Aquatic Organisms Based on Maximum Adjusted EECs from Microcosm Treated With S-Methoprene Granular, Briquets, and Liquid Formulations

Formulation	Peak EEC	Acute RQs LC_{50} = 760 ug/L	35 day EEC (ug/L)	Chronic RQs (NOAEC = 48 ug/L)

	(ug/L)			
Freshwater Fish				
Briquets	4.24	< 0.01	0.14	< 0.01
Briquet XR	3.37	< 0.01	0.96	0.02
Granular	2.10	< 0.01	0.21	< 0.01
Liquid	2.21	< 0.01	0.20	< 0.01
Formulation	**Peak EEC (ug/L)**	**Acute RQs LC_{50} = 330 ug/L**	**35 day EEC (ug/L)**	**Chronic RQs (NOAEC = 10 ug/L)**
Freshwater Invertebrates				
Briquets	4.24	0.01	0.14	0.01
Briquet XR	3.37	0.01	0.96	0.01
Granular	2.10	0.01	0.21	0.02
Liquid	2.21	0.01	0.20	0.02
Formulation	**Peak EEC (ug/L)**	**Acute RQs LOEC = 500 ug/L**	**Peak EEC (ug/L)**	**Acute RQs LOEC = 2,000 ug/L**
Freshwater Blue Green Algae			**Vascular Plant (rice)**	
Briquets	4.24	0.01	4.24	< 0.01
Briquet XR	3.37	0.01	3.37	< 0.01
Granular	2.10	< 0.01	2.10	< 0.01
Liquid	2.21	< 0.01	2.21	< 0.01

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 1×10^{-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 /L) and the aquatic invertebrate (Daphnia EC_{50} = 330 ug/L) were 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 CTS is ~ 1 in 418,000,000 suggesting "no effect" of S-methoprene direct exposure to the aquatic-phase CTS (Table 5-3).

Table 5-3 Chance of Individual Acute Effects to Aquatic-Phase CTS Using Surrogate Freshwater Fish and Daphnia 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 Exposures in the Terrestrial Habitat

Potential direct acute effects to the CTS are evaluated by considering dose- and dietary-based EECs based on the consumption of S-methoprene-contaminated food items. Based on surrogate avian toxicity data, the maximum allowable Liquid S-methoprene application rate (0.428 lbs a.i./acre, 4 applications, 10-day interval) and upper bound Kenaga values from T-REX, there is no potential for direct adverse effects on terrestrial-phase CTS individuals from S-methoprene use in California (Appendix H). Using calculations based on bird ingestion rates, dietary, and weight categories, Table 5-4 shows RQ values for acute avian dose-base risk at < 0.01 – 0.10 for 20g and 100g birds that consume small/large insects. The acute avian dietary-based RQs ranged from < 0.01 – 0.02 suggesting no direct effect to the CTS with no exceedence of the endangered species LOC > 0.1.

Potential for indirect effects to the CTS may result from direct acute effects to terrestrial organisms due to a reduction in prey or the habitat that they produce (small mammal burrows). Mammalian dose- and dietary-based RQs, however, do not exceed the LOC > 0.1 suggesting that that there is no potential for indirect effects to the CTS from toxicity to small mammals. Acute exposure dietary-based RQ ranged from 0.0 1– 0.08 while dose-based RQs ranged < 0.01 – 0.01. These RQ values do not exceed the Agency's LOC for avian and mammalian endangered species concerns (acute LOC > 0.1).

Table 5-4 Acute and Chronic RQs for Terrestrial-Phase CTS (Based on Upper Bound Kenaga Values from T-REX).

DIETARY CATEGORY	Acute Avian RQ: Dose-Based		Acute Avian RQ Dietary-Based	Acute Mammalian RQ Dietary Based
	20 g	100 g		
Short grass	0.18	0.08	0.02	0.08
Tall grass	0.08	0.04	0.01	0.04
Broadleaf plants/small insects	0.10	0.04	0.01	0.05
Fruits/pods/seeds/large insects	0.01	< 0.01	< 0.01	0.01
DIETARY CATEGORY	**Mammalian RQ: Dose Based 15 g mammal**		**Mammalian RQ: Dose Based 35 g mammal**	

	Acute	Chronic	Acute	Chronic
Short grass	0.01	0.56	0.01	0.48
Tall grass	< 0.01	0.26	< 0.01	0.22
Broadleaf plants/small insects	< 0.01	0.31	< 0.01	0.27
Fruits/pods/seeds/large insects	< 0.01	0.01	< 0.01	0.01

Bolded RQs exceed the Agency's endangered species LOC

Additionally, the granular LD_{50}/square foot results that were generated in T-REX also suggest that the broadcast application of S-methoprene granulars (0.03 lbs ai/A) should not create an acute toxicity concern for the terrestrial-phase of the CTS or to birds and mammals in general. The number of granules needed to achieve the adjusted LD_{50} is about 114,984 suggesting an unlikely event (Appendix H) Table 5.5).

Table 5.5 T-Rex Printout for Granular Application Representing a Maximum of 0.03 lbs ai/A Broadcast to an Open Field (size of the granule is 0.425 mg, 30 day exposure in the environment)

Estimation of the number of granules needed to achieve toxicity thresholds	
No. of granules needed to achieve adjusted LD_{50}	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

5.2.2. a Bioaccumulation of S-methoprene (Terrestrial Organisms)

Since S-methoprene is a non-ionic, organic compound with a log Kow of 5.50 and a Koc of 2,800, there is a potential for bioaccumulation by animals consuming fish contaminated with S-methoprene. This concern is also reinforced by an estimated bioconcentration factor of 3,400 that suggests that the potential for bioconcentration in aquatic organisms is very high (HSDB, 2002). The Agency evaluated (Table 5-5) bioaccumulation of S-methoprene and the possible toxic risk to mammals and birds that might consume aquatic organisms that may have high tissue levels of the compound, through the Kow (based) Aquatic BioAccumumaltion Model (KABAM). This model incorporates seven trophic levels and calculates pesticide tissue levels residues for different levels of an aquatic food web. The model then uses pesticide tissue concentrations in aquatic animals to estimate dose- and dietary- based exposure and associated risks to mammals and birds consuming aquatic organisms (USEPA 2008). The possible impact to burrow making mammals (mink, water shrew, and mole) is the focus of this portion of the analysis. If small mammals are at toxic risk from S-methoprene bioaccumulation, there could be secondary effects to habitat that is critical to CTS survival. Table 5-6 shows RQ values for birds and mammals that could consume fish contaminated by S-methoprene. Mammalian acute dose- and dietary-based RQs ranged from < 0.001 – 0.00, while chronic RQs are 0.001 – 0.05. Avian Dose-and dietary-based RQs are 0.001 – 0.006.

The calculated RQs show that there is no potential for indirect secondary effect (habitat) to the terrestrial phase of CTS from toxic effects to small mammals that consume S-methoprene contaminated prey items.

Table 5-5 RQ Values for Mammals and Birds Consuming Fish Contaminated by S-methoprene (KABAM Output)

Wildlife Species	Acute RQs		Chronic RQs	
	Dose Based	Dietary Based	Dose Based	Dietary Based
Mammal				
fog/water shrew	< 0.001	N/A	0.006	0.001
rice rat/star-nosed mole	< 0.001	N/A	0.008	0.001
small mink	< 0.001	N/A	0.018	0.003
large mink	0.001	N/A	0.020	0.003
small river otter	0.001	N/A	0.022	0.003
large river otter	0.001	N/A	0.050	0.006
Avian				
sandpipers	0.006	0.001	N/A	0.205
cranes	0.000	0.001	N/A	0.261
rails	0.003	0.001	N/A	0.248
herons	0.001	0.001	N/A	0.330
small osprey	0.002	0.001	N/A	0.485

5.3 Primary Constituent Elements of Designated Critical Habitat

5.3.1 Aquatic-Phase of CTS

For S-methoprene use, the assessment endpoints for designated critical habitat PCEs involve the same endpoints as those assessed relative for direct and indirect effects to the CTS assessed here. Therefore, the effects determinations for direct and indirect effects are used as the basis of the effects determination for potential modification to designated critical habitat.

Aquatic-phase CTS are known to eat diatoms, algae, and detritus (larvae CTS) and aquatic and terrestrial invertebrates (juvenile CTS). Although dipterians are not a major food resource for the CTS a reduction in one Dipterian populations can be offset by the increase in another. Schmude and Balcer 1997, Balcer *et al.* 1999 found that the only difference between S-methoprene treated and untreated sites was lower populations of some groups within the Chironomidae treated sites, but other Chironomid groups were higher, resulting in no difference in Chironomid numbers or biomass as a whole.

S-methoprene is not toxic to aquatic plants (Table 5-1 RQs < 0.01 - 0.01) and the RQs generated through extropolation and aquatic modeling suggest no direct acute or indirect risk from S-methoprene exposure to the aquatic-phase of the CTS (fish acute RQs < 0.01, fish chronic RQs < 0.01 – 0.02; aquatic invertebrate acute RQs < 0.01 – 0.03, chronic RQs 0.01 – 0.02; plant acute RQs < 0.01 – 0.01). All RQs were below the Agency LOC values (acute LOC < 0.05; chronic LOC = 1). As a supplement to these findings the Agency also evaluated calculated exposure values with adjusted microcosm field values (Table 5-2) and did not find any exceedences (RQs < 0.01 – 0.02) to the endangered LOC = 0.05. CTS

The Agency also calculated the chance of individual fish and 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 S-methoprene exposure is about 1 in 418,000,000 (Table 5-3), which may be considered as a highly unlikely event. Therefore, the Agency concludes a **"may affect" but "not likely to adversely affect"** for the assessment point regarding the survival, growth, and reproduction of CTS individuals via effects to aquatic food supply.

5.3.2 Terrestrial-Phase of CTS

Adult and juvenile CTSs forage in aquatic and terrestrial habitats. The main food sources for juvenile terrestrial-phase CTSs 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 CTS. Therefore, there should not be an indirect affect to the CTS from S-methoprene exposure to potential terrestrial food. Although, S-methoprene is efficacious to Dipterian larvae, these organisms are not major components of the CTSs diet. Since, salamanders appear to be opportunistic feeders, a decline in adult mosquitoes and black flies should not influence terrestrial feeding habits. Indirect effects on the CTS are not expected from toxicity to aquatic organisms that are prey items, from a decline in terrestrial and aquatic plants (no phytotoxicity issues), or from any toxic impact to small mammals that provide burrows that CTS use. For birds or mammals consuming S-methoprene bait, acute and chronic LOCs were not exceeded.

There were no endangered species exceedances for maximum application of liquid or granular formulations (baits). This suggests that there is very low potential for direct adverse effects to terrestrial-phase CTS from S-methoprene use in California. The slope for the LC_{50} of the most sensitive avian species (mallard duck LD_{50} >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 CTS

After running KABAM, the calculated RQ values for mammals consuming fish contaminated with S-methoprene showed no concern from an acute dose based levels (RQ = 0.00) and no concern from a chronic dietary bases (RQ = 0.002 – 0.012) (Appendix F; Table 3.5).

5.3 Risk Description

The risk description synthesizes overall conclusions regarding the likelihood of adverse impacts leading to a preliminary effects determination (*i.e.*, "no effect," "may affect, but not likely to adversely affect," or "likely to adversely affect") for the assessed species and the potential for modification of their designated critical habitat based on analysis of risk quotients and a comparison to the Level of Concern. The final No Effect/May Affect determination is made after the spatial analysis is completed at the end of the risk description. If there is no overlap of the species habitat and occurrence sections with the Potential Area of LAA Effects a No Effect determination is made.

If the RQs presented in the Risk Estimation (Section 5.1) show no direct or indirect effects for the assessed species, and no modification to PCEs of the designated critical habitat, a preliminary "no effect" determination is made, based on S-methoprene's use within the action area. However, if LOCs for direct or indirect effect are exceeded or effects may modify the PCEs of the critical habitat, the Agency concludes a preliminary "may affect" determination for the FIFRA regulatory action regarding S-methoprene. A preliminary "may effect but not adversely effect" was made for the CTS and for its critical habitat. A summary of the risk estimation results are provided in Table 5-6 for direct and indirect effects to the CTS assessed here and in Table 5-7 for the PCEs of the designated critical habitat.

Table 5-6 Risk Estimation Summary for S-methoprene - Direct and Indirect Effects

Taxa	LOC Exceedances (Yes/No)	Description of Results of Risk Estimation	Assessed Species Potentially Affected
Freshwater Fish and Aquatic-phase Amphibians	Non-listed Species (No)	No LOCs exceeded	Indirect Effects: CTS
	Listed Species (No)	No LOCs exceeded	Direct Effects: CTS
Freshwater Invertebrates	Non-listed Species (No)	No LOC exceeded	Indirect Effects:CTS
Vascular Aquatic Plants	Non-listed Species (No)	No LOC exceeded	Indirect Effects: CTS
Non-Vascular Aquatic Plants	Non-listed Species (No)	No LOC exceeded.	Direct Effects: CTS
Birds, Reptiles, and Terrestrial-Phase Amphibians	Non-listed Species (No)	No LOC exceeded	Indirect Effects: CTS
	Listed Species (No)	No LOC exceeded	Direct Effects: CTS
Mammals	Non-listed Species (No)	No LOC exceeded	Indirect Effects: CTS
Terrestrial Invertebrates	Listed Species (No)	No LOC exceeded	Direct/Indirect Effects: CTS
Terrestrial Plants - Monocots	Non-listed Species (No)	RQ not calculated . Compound is not phytotoxic based on mode of action and lack of incidents.	Indirect Effects: CTS
Terrestrial Plants - Dicots	Non-listed Species (No)	RQ not calculated . Compound is not phytotoxic based on mode of action and lack of	Indirect Effects: CTS

Taxa	LOC Exceedances (Yes/No)	Description of Results of Risk Estimation	Assessed Species Potentially Affected
		incidents.	

Table 5-7 Risk Estimation Summary for S-methoprene – Effects to Designated Critical Habitat. (PCEs)

Taxa	LOC Exceedances (Yes/No	Description of Results of Risk Estimation	Species Associated with a Designated Critical Habitat that May Be Modified by the Assessed Action
Freshwater Fish and Aquatic-phase Amphibians	Non-listed Species (No)	No LOC exceeded	CTS
	Listed Species (No)	No LOC exceeded	
Freshwater Invertebrates	Non-listed Species (No)	No LOC exceeded	CTS
Vascular Aquatic Plants	Non-listed Species (No)	NO LOC exceeded	CTS
Non-Vascular Aquatic Plants	Non-listed Species (No)	No LOC exceeded	CTS
Birds, Reptiles, and Terrestrial-Phase Amphibians	Non-listed Species (No)	No LOC exceeded	CTS
	Listed Species (No)	No LOC exceeded	
Mammals	Non-listed Species (No)	No LOC exceeded	CTS
Terrestrial Invertebrates	Non-listed Species (No)	No LOC exceeded	CTS
Terrestrial Plants - Monocots	Non-listed Species[1] (No)	RQ not calculated minimal risk. Compound is not phytotoxic based on mode of action and lack of incidents.	CTS
Terrestrial Plants - Dicots	Non-listed Species[1] (No)	RQ not calculated minimal risk. Compound is not phytotoxic based on mode of action and lack of incidents.	CTS

1- Only non-listed LOCs were evaluated because none of the assessed species have an obligate relationship with terrestrial monocots and dicots.

Following a preliminary "may affect" determination, additional information is considered to refine the potential for exposure at the predicted levels based on the life history characteristics (*i.e.*, habitat range, feeding preferences, *etc.*) of the assessed species. Based on the best available information, the Agency uses the refined evaluation to distinguish those actions that "may affect, but are not likely to adversely affect" from those actions that are "likely to adversely affect" the assessed species and its designated critical habitat. The criteria used to make determinations that the effects of an action are "not likely to adversely affect" the assessed species or modify its designated critical habitat include the following:

- Significance of Effect: Insignificant effects are those that cannot be meaningfully measured, detected, or evaluated in the context of a level of effect where "take" occurs for even a single individual. "Take" in this context means to harass or harm, defined as the following:
  - Harm includes significant habitat modification or degradation that results in death or injury to listed species by significantly impairing behavioral patterns such as breeding, feeding, or sheltering.
  - Harass is defined as actions that create the likelihood of injury to listed species to such an extent as to significantly disrupt normal behavior patterns which include, but are not limited to, breeding, feeding, or sheltering.
- Likelihood of the Effect Occurring: Discountable effects are those that are extremely unlikely to occur.
- Adverse Nature of Effect: Effects that are wholly beneficial without any adverse effects are not considered adverse.

5.4.1 California Tiger Salamander

5.4.1a Potential for Direct Adverse Effects

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 CTSs, and/or a reduction and/or modification of food sources and burrows 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 CTS, 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 CTS from S-methoprene exposure. The conclusion is that there is a **"may affect", but "not likely to adversely affect"** determination for the CTS 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 CTS individuals via effects to food supply (*i.e.* freshwater fish and invertebrates);

Although there was no 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 direct effects from exposure (aquatic system using fish and Dapnia as surrogates) is about 1 in 418,000,000 which is 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 model derived exposure values) that did not exceed LOCs for direct or indirect effects to the aquatic-phase CTS. Therefore, the Agency concludes a **"may affect" but "not likely to adversely affect"** reading for this assessment point.

5.4.1. b Indirect Effects - Designated Critical Habitat PCEs

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 on the CTSs food preferences in Section 2.5.3, these insects are not included in their diet. The Agency assumes that the CTS is an opportunistic feeder and will supplement its diet with available invertebrates and small animals. S-methoprene is non toxic to terrestrial and aquatic plants as noted in Section 4.2.1 and Table 4-5 and non toxic to small mammals (CTS uses the burrows made by small mammals). Therefore, the Agency concluded "no habitat modification" from S-methoprene use. Although there is widespread overlap of potential S-methoprene with watersheds of the CTS the Agency has also determined that there is no potential for modification of CTS 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 that is phytotoxic. Since CTS relys on the use of small mammal burrows for shelter, the Agency has found that S-methoprene exposure to these animals should not exceed the LOC for mammalian toxicity. Critical habitat as defined for CTS will not be modified or reduced from S-methoprene use. Further information on the results of the effects determination are included as part of the Risk Description in Section 5.2.

5.4.2 Addressing the Risk Hypotheses

In order to conclude this risk assessment, it is necessary to address the risk hypotheses defined in Section 2.9.1. Based on the conclusions of this assessment, **the hypotheses can be rejected**, meaning that the stated hypotheses do not represent concerns in terms of direct and indirect effects of S-methoprene on CTS and its designated critical habitat.

The labeled use of S-methoprene within the action area is not likely to:

- directly affect CTS by causing mortality or by adversely affecting growth or fecundity;
- indirectly affect CTS and/or modify their designated critical habitat by reducing or changing the composition of food supply;

- indirectly affect CTS and/or modify their designated critical habitat by reducing or changing the composition of the aquatic plant community in the species' current range, thus affecting primary productivity and/or cover;
- indirectly affect CTS and/or modify their designated critical habitat by reducing or changing the composition of the terrestrial plant community in the species' current range;
- indirectly affect CTS and/or modify their designated critical habitat by reducing or changing aquatic habitat in their current range (via modification of water quality parameters, habitat morphology, and/or sedimentation);
- Indirectly affect CTS and/or modify their designated critical habitat by reducing or changing terrestrial habitat in their current range (via reduction in small burrowing mammals leading to reduction in underground refuge/cover).

6.0 Uncertainties

6.1 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 (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 CTS.

6.2 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.

6.3 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.

6.4 Effects Assessment Uncertainties

6.4.1 Use of Surrogate Species Effects Data

Guideline toxicity tests and open literature data on S-methoprene are not available for aquatic-phase amphibian; therefore, freshwater fish are used as surrogate species for aquatic-phase amphibians and birds are used for the terrestrial-phase CTS. Endpoints based on freshwater fish ecotoxicity data are assumed to be protective of potential direct effects to aquatic-phase amphibians including the CTS, and extrapolation of the risk conclusions from the most sensitive tested species to the aquatic-phase CTS is likely to overestimate the potential risks to those species. Efforts are made to select the organisms most likely to be affected by the type of compound and usage pattern; however, there is an inherent uncertainty in extrapolating across phyla. In addition, the Agency's LOCs are intentionally set very low, and conservative estimates are made in the screening level risk assessment to account for these uncertainties.

6.4.2 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 CTS.

6.4.3 Impact of Multiple Stressors on the Effects Determination

The influence of length of exposure and concurrent environmental stressors to the CTS (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.

6.4.4 Potential Exposure to Pesticide Mixtures

As discussed in Section 2.2, this assessment evaluates potential effects resulting from exposure to S-methoprene and 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.

6.5 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 (2006 – 2009) 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.

6.6 Uncertainty Regarding S-Methoprene Affects

6.6.1 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).

6.6.2 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 al.*, 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 CTS 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 CTS.

7.0 Risk Conclusion

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 CTS and its designated critical habitat.

Based on the best available information, the Agency makes a "**may affect", but "not likely to adversely affect"** determination for the CTS from the use of S-methoprene.

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 CTSs diet in Section 2.5.3, these insects are not a major component of their diet. The CTS 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 CTS occurrence, the Agency has also determined that there is no potential for modification of CTS 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. Since CTS relies on small mammal burrows for shelter, the Agency evaluated the toxicity of S-methoprene to small mammals and concluded that LOCs were not exceeded relative to current use rates and that this compound is non toxic to these animals (burrows will not be affected). Further information on the results of the effects determination are included as part of the Risk Description in Section 5.2 (refer to Tables 7-0 and 7-1).

Table 7-0 Effects Determination Summary for Direct and Indirect Effects of S-methoprene on the California Tiger Salamander

Assessment Endpoint	Effects Determination	Basis
Aquatic-Phase (Eggs, Larvae, Adults)		
1. Survival, growth, and reproduction of CTS 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 CTS.
2. Survival, growth, and reproduction of CTS 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 CTS.
3. Survival, growth, and reproduction of CTS individuals via indirect effects on habitat, cover, and/or primary	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

Assessment Endpoint	Effects Determination	Basis
productivity (*i.e.*, aquatic plant community)		risk.
4. Survival, growth, and reproduction of CTS 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 CTS 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 LC_{50} 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 CTS 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 CTS and low magnitude of anticipated individual effects to potential prey items, S-methoprene is not expected to indirectly affect the terrestrial form of the CTS. 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 CTSs diet.
7. Survival, growth, and reproduction of CTS individuals via indirect effects on habitat (*i.e.*, riparian vegetation, mammalian burrows)	Not Likely to Adversely Affect	S-methoprene is not toxic to plants, plants are not at risk. S-methoprene is not toxic to small mammals.

Table 7-1 Effects Determination Summary for S-Methoprene Exposure to the California Tiger Salamander Critical Habitat

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

Table 7-1 Effects Determination Summary for S-Methoprene Exposure to the California Tiger Salamander 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 CTSs.	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 CTSs 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 CTSs and their food source.	No Habitat Modifications	S-methoprene does not affect plant life and aquatic chemical (DO) components that are necessary for aquatic CTS 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 CTSs: 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 CTS shelter (mammalian burrows), 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). Compounds is non toxic to small mammals so burrows will not be affected.
Elimination and/or disturbance of dispersal habitat: Upland or riparian dispersal habitat within designated units	No Habitat Modifications	Given that S-methoprene is not intended to control plants on the application sites, there is no potential for impacts to

Table 7-1 Effects Determination Summary for S-Methoprene Exposure to the California Tiger Salamander Critical Habitat

Assessment Endpoint	Effects Determination	Basis
Aquatic Phase PCEs (Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)		
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		terrestrial plants that comprise these habitats. Compound is non toxic to small mammals so burrows will not be affected.
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 CTS. Salamanders are opportunistic feeders and should supplement their diet with other terrestrial organisms. Dipterans are not listed as a component of the CTSs diet.
Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CTSs and their food source.	No Habitat Modifications	Although S-methoprene is toxic to Dipterian insects this does not pose acute risk to the CTS. Salamanders are opportunistic feeders and should supplement their diet with other terrestrial organisms. Dipterans are not listed as a component of the CTSs 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.

Based on the **may affect", but "not likely to adversely affect"** conclusions of this assessment, formal consultation with the U. S. Fish and Wildlife Service under Section 7 of the Endangered Species Act should be initiated.

When evaluating the significance of this risk assessment's direct/indirect and 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 CTS 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 salamanders. 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 salamanders and potential adverse modification to critical habitat.

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