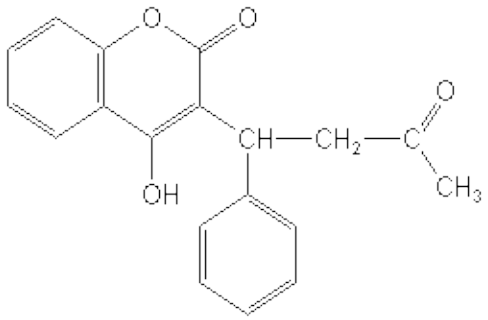
Risks of Warfarin Use

To the Federally Threatened Alameda Whipsnake (*Masticophis lateralis euryxanthus*), And the Federally Endangered Salt Marsh Harvest Mouse (*Reithrodontomys raviventris*)



Pesticide Effects Determinations
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List of Commonly Used Abbreviations and Nomenclature

µg/kg	Symbol for “micrograms per kilogram”
µg/L	Symbol for “micrograms per liter”
ºC	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
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
CDPR	California Department of Pesticide Regulation
CDPR-PUR	California Department of Pesticide Regulation Pesticide Use Reporting Database
CI	Confidence Interval
CL	Confidence Limit
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	K_{OW} (based) Aquatic BioAccumulation Model
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
SLN	Special Local Need
SMHM	Salt Marsh Harvest Mouse
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
WP	Wettable Powder
wt	Weight

1. Executive Summary

1.1. Purpose of Assessment

The purpose of this assessment is to evaluate potential direct and indirect effects on the federally threatened Alameda whipsnake (*Masticophis lateralis euryxanthus*; AW) and the federally endangered salt marsh harvest mouse (*Reithrodontomys raviventris*; SMHM) arising from FIFRA regulatory actions regarding use of the rodenticide warfarin on agricultural and non-agricultural sites. In addition, this assessment evaluates whether these actions can be expected to result in modification of designated critical habitat; of the two species assessed, critical habitat has only been designated for the AW. 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, 2004a), and consistent with a lawsuit in which warfarin was alleged to be of concern for the SMHM and AW (*Center for Biological Diversity (CBD) vs. EPA et al*. (Case No. 07-2794-JCS).)

In this assessment, direct and indirect effects to the AW and SMHM and potential modification to designated critical habitat for the AW is evaluated in accordance with the methods described in the Agency's Overview Document (USEPA, 2004a). A brief overview of each species including primary constituent elements (PCEs) is provided below:

- **Alameda Whipsnake (AW):** The AW was listed as threatened in 1997 by the USFWS. The species occurs in the Inner Coast Ranges in Contra Costa, Alameda, San Joaquin, and Santa Clara Counties in California. The PCEs for AWs are lands containing rock outcrops, talus, and small mammal burrows adjacent to woodland or annual grasslands contiguous with scrub/shrub communities with a mosaic of open and closed canopy.
- **Salt Marsh Harvest Mouse (SMHM):** The SMHM was listed by the USFWS as an endangered species in 1970. The species is found in tidal and non-tidal salt marshes along the San Francisco, San Pablo, and Suisun Bays in California. Critical habitat has not been designated for the SMHM; therefore, PCEs have not been defined.

1.2. Scope of Assessment

1.2.1. Uses Assessed

Warfarin is an anticoagulant rodenticide used for the control of nuisance mammals in areas such as buildings (in and around; indoor and outdoor), transport vehicles, agricultural fields, range and pasture, airports, golf courses, and recreational areas. Formulation types include: concentrates for mixing with feed, bait blocks, "all-in-one" bait stations, and gels. There are 19 currently registered labels that are relevant for use in California. Five of the 19 registered warfarin labels (registration numbers 3282-3, 3282-4, 3282-9, 3282-15, and 8845-39) are currently non-compliant, having failed to adopt the provisions and/or label language requirements of the 2008 risk mitigation decision (RMD). By the RMD, three points of non-conformance are shared by each of the five labels, including: unapproved formulation for homeowner, needs bait station, and missing 50 foot restriction. The target pests include: Norway rat (*Rattus norvegicus*), black

rat (*R. rattus*), house mouse (*Mus musculus*), meadow vole (*Microtus pennsylvanicus*), pine vole (*M. pinetorum*), montane vole (*M. montanus),* Eastern mole (*Scalopus aquaticus*), star-nosed mole (*Condylura cristata*), hairy-tailed mole (*Parascalops breweri*), coast mole (*Scapanus orarius*), broad-footed mole (*S. latimanus*), Townsend's mole (*S. towsendii*), and deer and white-footed mouse (*Peromyscus* spp.). Concentrations in bait are either 0.054% or 0.025% warfarin.

1.2.2. Environmental Fate Properties of Warfarin

Warfarin [3-(alpha-acetony1benzyl)-4-hydroxycoumarin] is considered moderately mobile in the environment and persistent in groundwater. The solubility in water at 20°C is 17 mg/L. The free acid [warfarin] is slightly soluble in water and has a log octanol-water partition coefficient (K_{ow}) of 2.52 at pH 3 from which one can estimate an organic carbon partition coefficient (K_{oc}) of 560 L/kg_{oc} indicating moderate adsorption to soil. Warfarin hydrolyzes very slowly in water with a half-life of 16 years at pH 7. Warfarin has a BCF of 24X if released into water.

1.3. Exposure Assessment

1.3.1. Aquatic Exposure

Aquatic exposures are not evaluated because the focus of this assessment is on two terrestrial listed species for which aquatic exposures are not relevant.

1.3.2. Terrestrial Exposure

For this assessment, it was assumed that terrestrial animals could be exposed through two different pathways. Animals may directly consume bait (primary consumption), or animals (predators/scavengers) may consume contaminated carcasses (secondary consumption). Primary consumption of warfarin was modeled using baits containing 0.025% and 0.054% warfarin. Ingestion was modeled both on a dose basis (mg a.i./kg-bw) and a dietary basis (mg a.i./kg-diet). On an oral exposure basis, doses ranged from 7.6 to 56.9 mg a.i./kg-bw and 16.5 to 122.9 mg a.i./kg-bw for 0.025% and 0.054% bait formulations (for 1000g and 10g mammals), respectively. On a dietary exposure basis, dietary concentrations were 250 mg a.i./kg-bait and 540 mg a.i./kg-bait, depending on the concentration in the bait.

Secondary consumption was modeled assuming a warfarin concentration of 2.95 mg a.i./kg-carcass, which was based on measured tissue residues from targeted monitoring studies. As with primary consumption, ingestion was modeled both on an oral dose basis (mg a.i./kg-bw) and a dietary exposure basis (mg a.i./kg-diet). For secondary exposure estimation, there was no differentiation between bait concentrations. On an oral exposure basis, doses ranged from 0.43 to 4.44 mg a.i./kg-bw/day. On a dietary exposure basis, the carcass concentration of 2.95 mg a.i./kg-carcass was used.

1.4. Toxicity Assessment

1.4.1. Terrestrial Animals

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 (for AW). Federally-designated critical habitat has been established for the AW but not for SMHM. Primary constituent elements (PCEs) were used to evaluate whether warfarin has the potential to modify designated critical habitat for AW.

The Agency evaluated registrant-submitted studies and data from the open literature to characterize warfarin 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. Consistent with the process described in the Overview Document (US EPA 2004a), this risk assessment uses a surrogate species approach in its evaluation for warfarin. Toxicological data generated from surrogate test species, which are intended to be representative of broad taxonomic groups, are used to extrapolate the potential effects on a variety of species (receptors) included under these taxonomic groupings. Based on this approach, birds serve as surrogates for reptiles and terrestrial-phase amphibians.

Warfarin is moderately toxic to birds on an acute oral (mallard duck LD_{50} =621 mg a.i./kg bw; Accession No. 00248782) and subacute dietary exposure basis (northern bobwhite LC_{50} =625 mg a.i./kg diet, MRID 00153365). No data are available to characterize chronic toxicity to birds. Warfarin is very highly to highly toxic to mammals based on the most sensitive available LD_{50} (3.0 mg a.i./kg bw for male rats, Accession No. 05002272) and LC_{50} (2.5 mg a.i./kg diet, Accession 00002461) toxicity data. No chronic toxicity data are available for mammals.

In addition to the standard (primary) toxicity studies, secondary toxicity studies were submitted to the Agency for several species of carnivorous birds and mammals. In the reviewed carnivorous bird studies, no mortality of the tested birds was observed, although some birds did show symptoms of anticoagulant poisoning, including lethargy and anorexia. In the reviewed carnivorous mammal studies, signs of anticoagulant poisoning and frank mortality were observed.

Ecological incident data documented several instances of mortality to birds presumably due to secondary toxicity through consumption of warfarin-contaminated prey. Mortalities were reported in animals expected to consume contaminated carcasses (*e.g.*, Cooper's Hawk and Bald Eagle) and those expected to consume bait directly (*e.g.*, squirrels and quail).

1.4.2. Plants

No toxicity data are available for aquatic or terrestrial plants. Due to the very low application rates (maximum of 0.003 lb a.i./placement), the mode of action, and the lack of plant incidents for warfarin, vegetative food sources are not expected to be affected by warfarin application.

1.5. Measures of Risk

Acute risk quotients (RQs) are compared to the Agency's Levels of Concern (LOCs) to identify instances where warfarin 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 warfarin use "may affect" the assessed species, and/or may cause effects to designated critical habitat, the best available additional information (*e.g.*, incidents) 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).

1.6. Summary of Risk Conclusions

Based on the best available information, the Agency makes a **May Affect, and Likely to Adversely Affect** determination for the SMHM and AW from all the uses of warfarin. Additionally, the Agency has determined that there is the potential for modification of designated critical habitat for the AW from the use of warfarin. A summary of the risk conclusions and effects determinations for each listed species assessed here and AW designated critical habitat is presented in **Table 1-1** and in **Table 1-2**. Use-specific determinations are provided in **Table 1-3**. Given the LAA determination for the SMHM and AW and potential modification of designated critical habitat for the AW, a description of the baseline status and cumulative effects for the SMHM and AW is provided in **Attachment II**.

Table 1-1. Effects Determination Summary for Effects of Warfarin on the SMHM and AW.

Species	Effects Determination	Basis for Determination
Salt marsh harvest mouse (SMHM) (*Reithrodontomys raviventris*)	May Affect, Likely to Adversely Affect (LAA)	**Potential for Direct Effects** Risk assessment indicates use of warfarin will potentially result in direct effects to the SMHM from acute and chronic toxicity. Lines of evidence include: • the acute risk to listed species LOC is exceeded for the SMHM based on consumption of bait, and chronic risk is presumed, • the likelihood of an individual effect (mortality) is approximately 1 in 1.0 based on acute oral dose and dietary exposure, • mortality through primary exposure to warfarin was documented through low acute oral LD_{50} and subacute dietary LC_{50} values obtained in toxicity studies and mortality of the rodents when fed warfarin for the secondary exposure trials, and • reported incidents of mortality of small non-target herbivorous mammals after consumption of warfarin bait. **Potential for Indirect Effects**

Table 1-1. Effects Determination Summary for Effects of Warfarin on the SMHM and AW.		
Species	**Effects Determination**	**Basis for Determination**
		Food sources Risk assessment presumes vegetative food sources are not expected to be affected by warfarin application. Due to the very low application rates (maximum of 0.003 lb a.i./placement), the mode of action, and the lack of plant incidents for warfarin, vegetative food sources are not expected to be affected by warfarin application. Risk to terrestrial invertebrate prey populations was presumed because toxicity data were not available. ***Habitat Modifications*** Adverse effects to birds and mammals may result in a reduction of abandoned bird and mammal nests, which are used as nest sites by the SMHM. Acute RQs for birds and mammals, and acute and chronic RQs for mammals, exceed LOCs. Therefore, use of warfarin may modify the habitat of the SMHM by reducing the availability of nest sites.
Alameda Whipsnake (AW) (*Masticophis lateralis euryxanthus*)	May Affect, Likely to Adversely Affect (LAA)	**Potential for Direct Effects**
		This risk assessment indicates use of warfarin will potentially result in direct effects to the AW from secondary exposure and toxicity. This is corroborated by nine reported incidents of bird mortality. Birds serve as surrogates for terrestrial phase amphibians.
		Potential for Indirect Effects
		Food sources Risk assessment indicates terrestrial vertebrate and invertebrate prey populations may be reduced. For birds and mammals consuming bait directly, acute risk LOCs are exceeded and chronic risk is presumed. It is probable that the availability of prey for AW may decrease due to reductions in the populations of birds and mammals. Reported incidents for a variety of birds and mammals also provide evidence of mortality of potential prey animals exposed to warfarin. ***Habitat Modifications*** Risk assessment indicates use of warfarin may modify the habitat of this species by reducing the availability of burrows. This conclusion is based on acute RQs for mammals that exceed the LOC and presumed chronic risk to mammals. Adverse effects to mammals may result in a reduction of available burrows, which are used as shelter by this species. In addition, the availability of prey may be reduced by the toxicity of warfarin to small birds, mammals, reptiles, and terrestrial-phase amphibians.

Table 1-2. Effects Determination Summary for the Critical Habitat Impact Analysis		
Designated Critical Habitat for:	**Effects Determination**	**Basis for Determination**
Alameda whipsnake (*Masticophis lateralis euryxanthus*)	Habitat Modification	This risk assessment indicates use of warfarin may modify the critical habitat of this species by reducing the availability of small mammal burrows. This may result in modification of PCE 3: "Lands containing rock outcrops, talus, and small mammal burrows within or adjacent to PCE 1 and or PCE 2." In addition, the availability of prey may be reduced in the critical habitat by toxicity to small birds, mammals, reptiles, and terrestrial-phase amphibians.

Table 1-3. Use Specific Summary of The Potential for Adverse Effects to Terrestrial Taxa							
Uses	**Potential for Effects to Identified Taxa Found in the Terrestrial Environment:**						
	SMHM and Small Mammals[1]		**Small Birds[2,3]**		**Invertebrates (Acute)[2]**	**Dicots**	**Monocots**
	Acute	**Chronic**	**Acute**	**Chronic**			
0.025% a.i. bait (spot treatment, bait station, gel)	Yes	Yes	Yes	Yes	Yes	No	No
0.054% a.i. bait (spot treatment, boxed bait)	Yes	Yes	Yes	Yes	Yes	No	No

[1] A yes in this column indicates a potential for direct effects to SMHM and indirect effects to SMHM and AW.
[2] A yes in this column indicates a potential for indirect effects to the SMHM and AW.
[3] Birds are considered a surrogate for terrestrial phase amphibians and reptiles including the AW.

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

When evaluating the significance of this risk assessment's direct/indirect and 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 assumptions of offsite transport in target and non-target vertebrates consuming bait, 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 SMHM and AW 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. 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 2004a) and reviewed by the U.S. Fish and Wildlife Service and National Marine Fisheries Service (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 federally threatened Alameda whipsnake (AW) and the federally endangered salt marsh harvest mouse (SMHM) arising from FIFRA regulatory actions regarding use of warfarin in buildings (in and around; indoor and outdoor), transport vehicles, agricultural fields, range and pasture, airports, golf courses, and recreational areas. This ecological risk assessment has been prepared consistent with a settlement agreement in the case *Center for Biological Diversity (CBD) vs. EPA et al*. (Case No. 07-2794-JCS).

In this assessment, direct and indirect effects to the AW and SMHM and potential modification to designated critical habitat for the AW are evaluated in accordance with the methods described in the Agency's Overview Document (USEPA 2004a). In accordance with the Overview Document, provisions of the Endangered Species Act (ESA), and the Services' *Endangered Species Consultation Handbook*, the assessment of effects associated with registrations of warfarin 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 warfarin 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 AW and SMHM and the designated critical habitat of AW 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 warfarin 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 of the AW, 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.*, gel bait, blocks, pellets and bait stations), acceptable methods of application, approved use sites, and any restrictions on how applications may be conducted. Thus, the use or potential use of warfarin in accordance with the approved product labels for California is "the action" relevant to this ecological risk assessment.

Warfarin is an anticoagulant rodenticide used for the control of nuisance mammals in areas such as buildings (in and around; indoor and outdoor), transport vehicles, agricultural fields, range and pasture, airports, golf courses, and recreational areas.

Although current registrations of warfarin allow for use nationwide, this ecological risk assessment and effects determination addresses currently registered uses of warfarin in portions of the action area that are reasonably assumed to be biologically relevant to the AW and SMHM. Further discussion of the action area for the AW and SMHM and the AW critical habitat is provided in **Section 2.5**.

2.2.1. Mechanism of Action

Warfarin is an anticoagulant rodenticide. Anticoagulants are vitamin-K antagonists that disrupt normal blood-clotting mechanisms and induce capillary damage. Warfarin acts by binding to the enzyme vitamin K 2,3-epoxide reductase, thereby interrupting the cellular recycling of vitamin K. Vitamin K in its hydroquinone form is an essential cofactor for the synthesis of functional prothrombin and related blood-clotting factors (Henk and Thijssen, 1995). Typically, death is delayed for four to ten or more days after a lethal dose is ingested, and animals may continue to feed and move about until shortly before death (Henk and Thijssen, 1995). Death results from hemorrhage, and exposed animals may exhibit behavior that may make them more susceptible to predation (Cox and Smith, 1992). This may result in secondary exposure to predatory animals and/or scavengers.

In contrast to the other anticoagulants, warfarin is extensively metabolized and the major route of excretion is in the urine. Meehan (1984) states that approximately half the warfarin consumed by a rat remains in the body after 6 hours, suggesting that up to 50% may be eliminated in this time period. EPA (1982) noted that only 7.6% of the warfarin consumed in bait by 11 rats remained in the carcass after a 5-day feeding period. Ford (1993; cited in Poché and Mach 2001) reported a half-life of 42 hours in the gastro-intestinal tract, which supports the conclusion that a portion of the consumed warfarin dose is rapidly eliminated from the body. However, there is evidence that warfarin residues that are not excreted though urine and fecal matter may accumulate in various body tissues, especially in the liver. Though warfarin bioaccumulates in the liver, measurable amounts have also been found in the adrenal glands, lungs, bone marrow, kidneys, and lymph nodes (Machlin 1984).

2.2.2. Environmental Fate and Transport Properties

Table 2-1 summarizes the physicochemical properties of warfarin (**Figure 2.1**) based on data available in open literature. Warfarin is considered moderately mobile in the environment (based on FAO 2000 mobility classification) and persistent in groundwater (USEPA, 2006). Volatilization from moist soil surfaces is not expected to be an important route of dissipation given an estimated Henry's Law constant of 2.8 X 10^{-9} atm-cu m/mole. If released into water, warfarin is expected to adsorb to suspended solids and sediment based upon the K_{oc}. Volatilization from water surfaces is not expected to be an important route of dissipation based upon this compound's estimated Henry's Law constant. An estimated BCF of 24x suggests a low potential for bioconcentration in aquatic organisms. With respect to abiotic routes of degradation, warfarin is stable to hydrolysis with a half-life (pH 7, 25 °C) of 16 years (Hazardous Substances Data Bank, HSDB).

Warfarin absorbs UV radiation to approximately 330 nm (Goreet *et al*., 1971) and may therefore be susceptible to direct photolysis in the environment; however, no data were found concerning the possible photolysis rate.

With respect to biotic routes of degradation, warfarin (sodium salt) was considered stable with only13% degradation over a 28-day incubation period using activated sludge (Brorson et al., 1994).

The US Geological Survey collected water samples from a network of 139 streams in 30 states during 1999 and 2000; sampling sites were biased toward streams susceptible to contamination (*i.e*., downstream of intense urbanization and livestock production). Warfarin was not detected (detection limit of 0.001 ppb) in the 84 water samples which were analyzed (Kolpin *et al*., 2002).

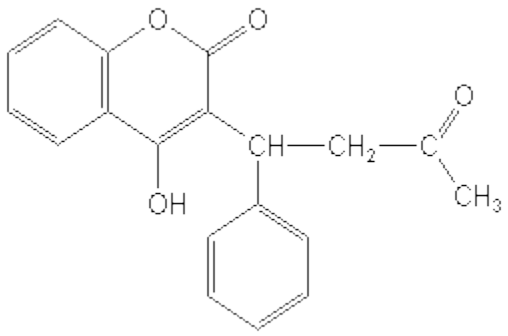

Figure 2.1. Structure of warfarin.

Table 2-1 Physical-chemical Properties of Warfarin		
Study	Value and units	**Source**
CAS Number	81-81-2	ASTER
SMILES Code	c(ccc1C(O)=C2C(-c(ccc3)cc3)CC(=O)C)cc1OC2=O	ASTER
Molecular weight	308.33	ASTER
Hydrolysis	16 years (pH 7, 25 ^{o}C)	Ellington 1989
Solubility	17 mg/L at 20 ^{o}C	Tomlin (ed.). 1997
Biodegradation	Not readily biodegradable with only a 13% degradation (COD reduction) over a 28-day incubation period. Warfarin is reduced by Nocardia and Arthrobacter sp to the alcohol.	Brorson *et al*. (1982)
Organic carbon Partition Coefficient (K_{oc})	701 L/kg_{oc} (estimated)	Lyman *et al*., 1990 Based on measured log K_{ow} of 2.70, and a regression-derived equation
Vapor pressure	$1.16X10^{-7}$ mm Hg at 21^{0}C	Hartley D, Kidd H, eds.1983
Henry's law constant	$2.8X10^{-9}$ atm-cu m/mole	Calculated
Octanol-water partition coefficient (K_{ow})	2.70	Hansch *et al*.; 1995

Warfarin has the potential to move offsite through food consumption by target species, which travel away from the application site. Other than direct consumption of bait and secondary poisoning, other routes of environmental exposure are expected to be minimal. Given the moderate adsorption to soil, movement of residues by means of leaching through the soil profile to an aquatic environment is unlikely. Further, warfarin is unlikely to volatilize, unlikely to move offsite due to method of application (mainly in bait stations), and due to the relatively low application rates (maximum of 0.003 lb a.i./placement), unlikely to runoff or leach substantially.

2.2.3. 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, 2004a; USFWS/NMFS/NOAA, 2004).

Three registered products contain multiple active ingredients (i.e., warfarin and imidacloprid, a neonicotinoid insecticide). However; this risk assessment focuses only on the toxicity of the active ingredient warfarin since effects data is not available for the warfarin-imidacloprid mixture.

2.2.4. Summary of Previous Assessments

Over the last 20 years, several ecological risk and endangered species assessments have been conducted by the Agency on warfarin. In addition, the U. S. Fish and Wildlife Service (USFWS) issued a Biological Opinion (BO) on warfarin for several listed species. These assessments are described below.

The USFWS addressed the risk of warfarin use on endangered species in a BO issued in March of 1993 in response to a 1991 request by the EPA for formal consultation on 16 registered vertebrate pest agents. Specific labels and application rates that were evaluated in the BO were not listed. The BO included an evaluation of the use of warfarin for:

- Norway rats, roof rats and house mice in and around homes and other buildings;
- agricultural, commercial, industrial, and institutional use sites;
- transportation vehicles including aircraft, ships, and railcars;

The BO concluded that labeled use of warfarin would not likely harm aquatic fauna because "its characteristic of being insoluble in water should preclude exposure" (USFWS, 1993). The BO noted concern for primary exposure "where human development has encroached on the habitat of listed rodents." Potential for secondary exposure to terrestrial animals was characterized as low. The FWS issued jeopardy calls for species, including the SMHM, listed in **Table 2-2**.

The profile of registered warfarin uses has changed since the 1993 BO was issued. For example, in 1993 at the time of the BO, many products were registered for broadcast application of bait. As of 2008, EPA requires that all rodenticide bait products marketed to general and residential consumers be sold only with bait stations; loose bait (*e.g*., pellets and meal) is prohibited from use (USEPA, 2008).

Table 2-2 Jeopardy Calls for Species Evaluated in the 1993 FWS Biological Opinion on Warfarin Use

MAMMALS			
Peromyscus polionotus ammobates Alabama beach mouse	J	*Dipodomys heermanni morroensis* Morro Bay kangaroo rat	J
Peromyscus polionotus phasma Anastasia Island beach mouse	J	*Peromyscus polionotus trissyllepsis* Perdido Key beach mouse	J
Glaucomys sabrinus coloratus Carolina northern flying squirrel	J	*Aplodontia rufa nigra* Point Arena mountain beaver	NJ
Peromyscus polionotus allophrys Choctawhatchee beach mouse	J	***Reithrodontomys raviventris*** **Salt marsh harvest mouse**	**J**
Microtus pennsylvanicus dukecampbelli Florida salt marsh vole	J	*Peromyscus polionotus niveiventris* Southeastern beach mouse	J
Dipodomys nitratoides exilis Fresno kangaroo rat	J	*Dipodomys stephensi* Stephen's kangaroo rat	NJ
Dipodomys ingens Giant kangaroo rat	NJ	*Dipodomys nitratoides nitratoides* Tipton kangaroo rat	NJ

J= Jeopardy ; NJ = No Jeopardy

The Reregistration Eligibility Decision (RED) for warfarin was published in 1991 (USEPA, 1991). For mammals, the RED found that there was a high risk of primary poisoning, but low risk of secondary poisoning because the levels of warfarin in the target animals are "generally too low to be toxic to either a predator or scavenger except under the most extreme conditions" (USEPA, 1991). Risk of primary poisoning to birds was considered to be low, based on toxicity endpoints and estimated exposure. The solubility limit of warfarin, *i.e.*, 17 mg/L at 20 ^{o}C, was treated as an exposure value to which the aquatic LC_{50} was compared for the purpose of the assessment, since no or only slight toxicity was observed up to the limit of solubility. Due to its low solubility, the very low application rate per placement (maximum of 0.003 lb a.i./placement), and general physicochemical characteristics, warfarin is not expected to result in significant aquatic exposure.

Another OPP assessment, titled "*Potential Risks of Nine Rodenticides to Birds and Non-target Mammals: a Comparative Approach*" (USEPA 2004b), evaluated primary and secondary exposure of birds and mammals to warfarin and eight other rodenticides. This document was developed to provide further guidance in developing mitigations for all rodenticides. The assessment determined that the greatest risk of warfarin use to non-target animals is via primary and secondary exposure to mammals. Risk associated with primary and secondary exposure to birds was also identified. The assessment also specified a number of factors contributing to uncertainty in assessing risks associated with anticoagulant rodenticides. Those factors that contributed the most uncertainty were: (1) missing data, including acute, chronic, and secondary toxicity as well as data regarding retention of some active ingredients in the liver, blood, and other body tissues; (2) the variable quality and quantity of existing data on metabolism and retention times in rodents and non-target species; (3) specific use information by formulation, including typical amounts applied by use site, seasonally, and annually; distances applied from buildings; amounts used in rural versus urban areas; use by Certified Applicators versus homeowners and other non-certified applicators; (4) information on the number and species of birds and non-target mammals frequenting baited areas and the likelihood of their finding and consuming bait or poisoned primary consumers in the various use areas; (5) methods to determine liver concentration(s) and total body burdens of rodenticide that would corroborate death or even if such a cause-effect relationship is appropriate (*e.g.*, the "threshold of toxicity" concentration); (6) not accounting for the impacts of sub-lethal effects on reproduction and non-target mortality (*e.g.*, clotting abnormalities, hemorrhaging, stress factors including environmental stressors, such as adverse weather conditions, food shortages, and predation); (7) not accounting for bioaccumulation of repeated sub-lethal exposures to bait or poisoned rodents utilized as food by predators and scavengers; and (8) lack of incident reporting. All of the above issues remain as uncertainties for this assessment.

EFED has completed two assessments for warfarin in the last five years. In 2008, EFED assessed Novel® Commensal Rodent Pellet #2 (DP Barcode D338364; EPA Registration No. 72500-13). Another new use application in 2008 also proposed a warfarin-imidacloprid combination product, Kaput® combo bait mini blocks for rodents and fleas (DP Barcode D347732; EPA Registration No. 72500-14), for nationwide use. Both Section 3 assessments

were for a combination product of 0.025% warfarin and 0.020% imidacloprid for the control of Norway rats, roof rats, house mice, and voles as well as their fleas. The Kaput® bait block also included prairie dogs as a target species. Combinations of multiple active ingredients had not previously been registered for any warfarin bait product. The assessments indicated that the proposed use did not differ significantly from current registered uses. Special concern was noted for two federally listed species: the Amargosa vole (*Microtus californicus scirpensis*) and the Hualapai Mexican vole (*Microtus mexicanus hualpaiensis*); however, a formal endangered species assessment was not conducted. The assessments noted that particular care should be taken so that these species are not mistaken for target species, and suggested the prohibition of baiting within their range. The Kaput® assessment also suggested that the label adopt language that would require the use of bait stations under all circumstances. It was concluded that 0.025% warfarin food baits pose low to moderate acute risk to birds but high primary and secondary risks to non-target mammals. A separate assessment was conducted for the neonicotinoid insecticide imidacloprid.

EFED is currently evaluating a Section 3 new use of warfarin for control of Black-Tailed Prairie Dogs [(*Cynonys ludovivianus*)] and White-Tailed Prairie Dogs [(*Cynomys leucurus*)] and their fleas, on rangeland and non-crop areas" in Colorado, Kansas, Montana, Nebraska, New Mexico, North Dakota, Oklahoma, South Dakota, Texas, and Wyoming. A decision has not been made regarding this new use. The use proposal will go through public process prior to registration.

A Notice of Intent to Cancel (NOIC) for five warfarin products is pending. The last day for rodenticide manufacturers to sell or distribute warfarin products that did not include the mitigation measures set forth by the RMD was June 4, 2011. Products sold by rodenticide manufacturers on or before June 4, 2011 may be sold until stocks are exhausted. Non-target exposure is expected to decrease as a consequence of the cancellation of the five non-conforming products, since warfarin products which include open-faced bait trays will no longer be sold.

2.2.5. Use Characterization

2.2.5.a. Label Summaries

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

Warfarin labels may be categorized into two types: labels for manufacturing uses (including technical grade warfarin) and end-use products. While technical products, which contain warfarin 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 pest mammals. The formulated product labels legally limit warfarin's potential use to only those sites that are specified on the labels. The uses being assessed are summarized in **Table 2-3**. It is important to note that the risk assessment estimates maximum daily bait consumption by

primary consumers (and therefore warfarin intake), such that the number of applications and the application interval did not factor into the assessment.

Table 2-3. Modeled Warfarin Uses, Scenarios, and Application Information[1]

Scenario	% a.i.	Application Rate (per application)	Maximum Number of Applications[2]	Application Interval[2]	Evaluated Exposure Pathways
Spot-bait in agriculture and non-ag	0.025	16 oz/ placement (0.003 lb a.i./placement)	NS	NS	-Terrestrial primary -Terrestrial secondary
Bait stations	0.054 0.025	16 oz/placement (0.00025 lb ai/placement)	NS	NS	-Terrestrial primary -Terrestrial secondary
Below ground (*e.g.*, burrows, runways)	0.025	3 fl oz/ 10,000 sqft (0.0002 lb ai/A)	NS	NS	-Terrestrial primary -Terrestrial secondary

1-Uses assessed based on EFED Label Data report and associated Draft Label Use Information Reports provided by BEAD.
2- NS= Not Specified. However, the risk assessment estimates maximum daily bait consumption by primary consumers (and therefore warfarin intake), such that the number of applications and the application interval did not factor into the assessment.

There are currently 19 active national EPA registrations for warfarin end-use products (**Appendix E**). The basic types of use patterns for warfarin are indoor, outdoor bait station, and outdoor spot baiting. Some of the registrations listed in Appendix E (and summarized in **Table 2-3**) do not meet the mitigation measures set forth in the RMD. The five non-conforming labels are not approved for homeowner use, do not have clauses mandating the use of bait stations, and do not stipulate that baiting must occur within 50 feet of buildings. Since the NOIC is pending, this assessment considers risk as presented from all warfarin products (including the five which are not in conformance with the RMD) which are registered as of 21 November 2011.

2.2.5.b. Reported Usage Data

The Agency's Biological and Economic Analysis Division (BEAD) provides county-level usage information using California's Department of Pesticide Regulation Pesticide Use Reporting (CDPR PUR) database[1]. 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 warfarin 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.[2] BEAD summarized these data to the county level by site, pesticide, and unit treated. Calculating county-level usage involved summarizing across all applications made within a section and then across all sections

[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.
[2] 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).

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 eleven years. The units of area treated are also provided where available.

The average number of pounds of warfarin applied in California over the eleven year period was 2.6 lbs per year. The lack of data on home and garden use constitutes a source of uncertainty since the CDPR PUR database does not account for homeowner usage[3]. Homeowner use is presumed to be a major usage scenario.

Approximately 50 use sites are listed in the CDPR PUR data. However, roughly half of all documented use in California between 1999 and 2009 were on three use site types, *i.e.*, vertebrate control, structural pest control, and landscape maintenance. The highest reported usage was 8.6 lbs in 2006, and the lowest 0.4 lb in 2008. Of the 8.6 lbs reported in 2006, 8.1 were reported by Fresno County and were categorized non-specifically as 'vertebrate pest control'.

2.3. Assessed Species

Table 2-4 provides a summary of the current distribution, habitat requirements, and life history parameters for the listed species being assessed. More detailed life-history and distribution information can be found in **Attachment III**. See **Figure 2-2 and 2-3** for maps of the current range for the SMHM and designated critical habitat for AW. A brief overview of each species is provided below:

- **Alameda Whipsnake (AW):** The AW was listed as threatened in 1997 by the USFWS. The species occurs in the Inner Coast Ranges in Contra Costa, Alameda, San Joaquin, and Santa Clara Counties in California.
- **Salt Marsh Harvest Mouse (SMHM):** The SMHM was listed by the USFWS as an endangered species in 1970. The species is found in tidal and non-tidal salt marshes along the San Francisco, San Pablo, and Suisun Bays in California.

[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).

Table 2-4. Summary of Current Distribution, Habitat Requirements, and Life History Information for the Assessed Listed Species[1]						
Assessed Species	**Size**	**Current Range**	**Habitat Type**	**Designated Critical Habitat?**	**Reproductive Cycle**	**Diet**
Salt Marsh Harvest Mouse (SMHM) (*Reithrodontomys raviventris*)	Adult 8 – 14 g	Northern subspecies can be found in Marin, Sonoma, Napa, Solano, and northern Contra Costa counties. The southern subspecies occurs in San Mateo, Alameda, and Santa Clara counties with some isolation populations in Marin and Contra Costa counties.	Dense, perennial cover with preference for habitat in the middle and upper parts of the marsh dominated by pickleweed and peripheral halophytes as well as similar vegetation in diked wetlands adjacent to the Bay	No	Breeding: March – November Gestation period: 21 – 24 days	Leaves, seeds, and plant stems; may eat insects; prefers "fresh green grasses" in the winter and pickleweed and saltgrass during the rest of the year; drinks both salt and fresh water
Alameda Whipsnake (AW) (*Masticophis lateralis euryxanthus*)	3 – 5 ft	Contra Costa and Alameda Counties in California (additional occurrences in San Joaquin and Santa Clara Counties)	Primarily, scrub and chaparral communities. Also found in grassland, oak savanna, oak-bay woodland, and riparian areas. Lands containing rock outcrops, talus, and small mammal burrows.	Yes	Emerge from hibernation and begin mating from late March through mid-June. Females lay eggs in May through July. Eggs hatch from August through November. Hibernate during the winter months.	Lizards, small mammals, nesting birds, other snakes including rattlesnakes

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

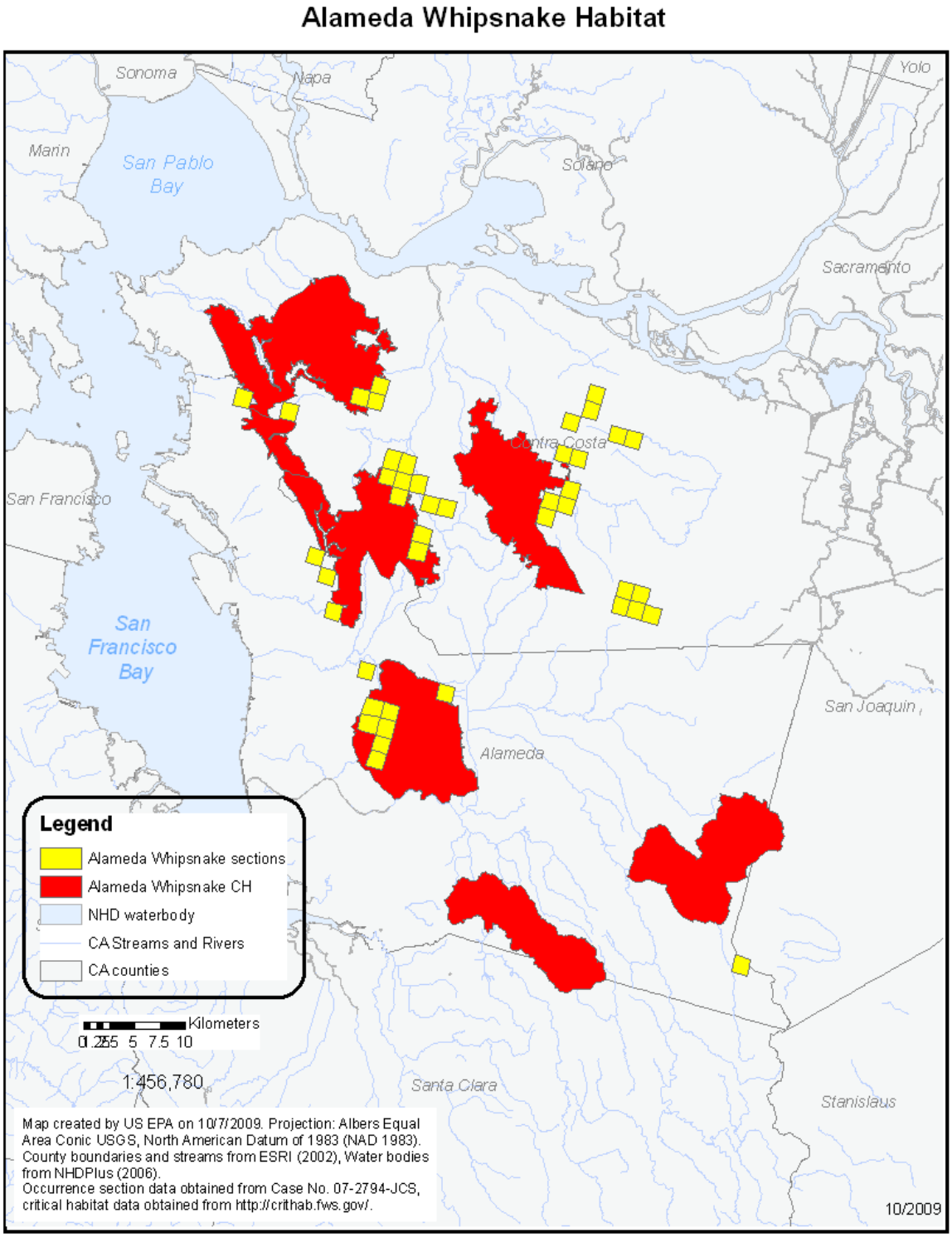

Figure 2-2. Critical habitat (CH) and occurrence sections of the Alameda Whipsnake, as identified in Case No. 07-2794-JCS

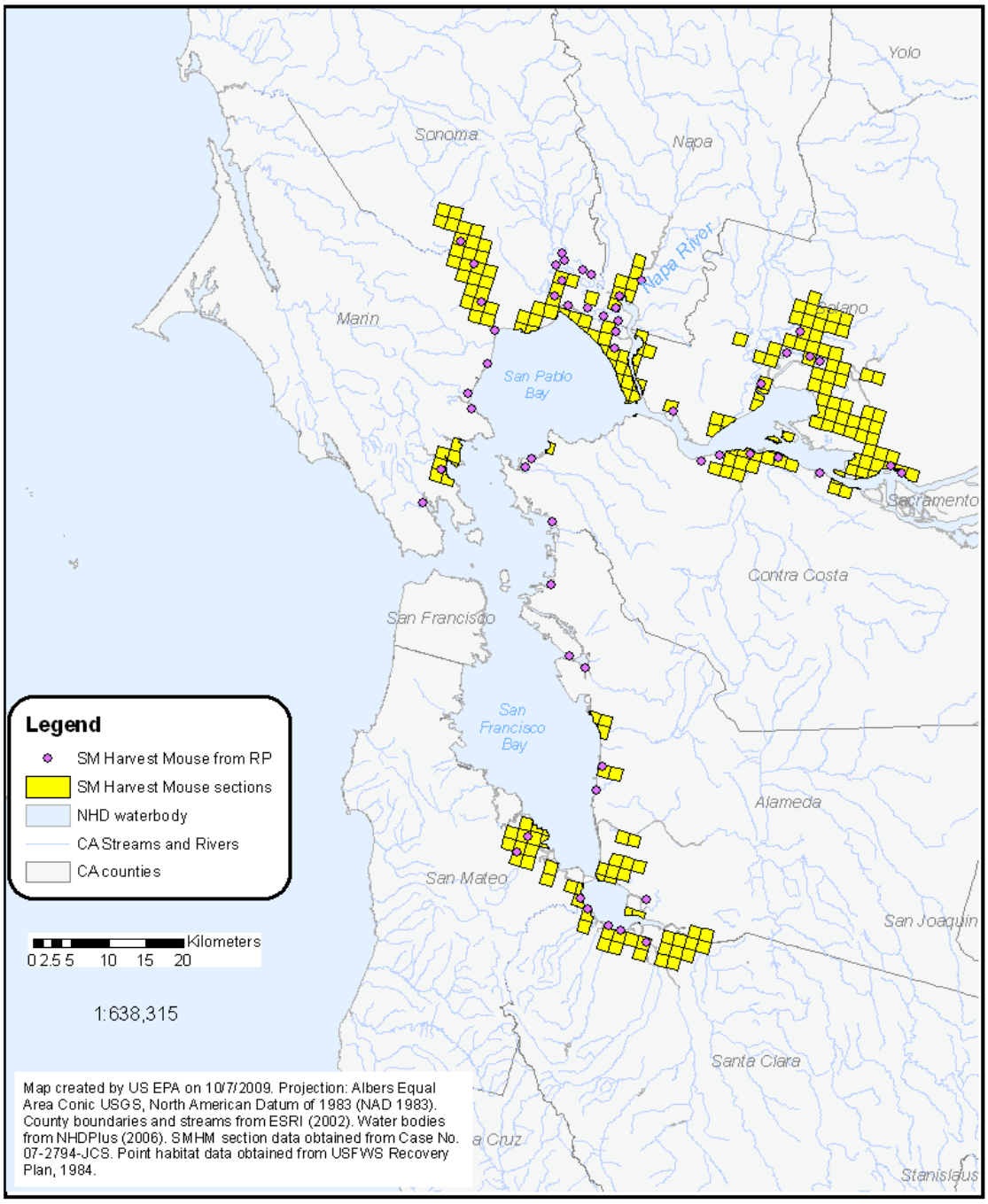

Figure 2-3. Occurrences and occurrence sections of the salt marsh harvest mouse, as identified in Case No. 07-2794-JCS. (RP is Recovery Plan)

2.4. Designated Critical Habitat

Critical habitat has been designated for the AW but not for the SMHM. 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 2-5** describes the PCEs for the critical habitats designated for the AW.

Table 2-5. Designated Critical Habitat PCEs for the Alameda whipsnake[1].

Species	PCEs	Reference
Alameda whipsnake	Scrub/shrub communities with a mosaic of open and closed canopy	71 FR 58175 58231, 2006
	Lands containing rock outcrops and talus within or adjacent to upland areas between breeding locations (PCE 1) and areas with small mammal burrows (PCE 2) that allow for dispersal among such sites	
	Woodland or annual grassland plant communities contiguous to lands containing PCE 1	

[1] These PCEs are 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.

More detail on the designated critical habitat of the AW 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 warfarin that may alter the PCEs of the designated critical habitat for the AW form the basis of the critical habitat impact analysis.

As previously noted in **Section 2.1**, the Agency believes that the analysis of direct and indirect effects to listed species provides the basis for an analysis of potential effects on the designated critical habitat. Because warfarin is expected to directly impact living organisms within the action area, critical habitat analysis for warfarin 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.

2.5. Action Area and LAA Effects Determination Area

2.5.1. 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 area 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 warfarin is likely to encompass considerable portions of the United States based on its agricultural and non-agricultural use sites. 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 AW and SMHM and the designated critical habitat of AW 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 via consumption of bait by the target species, which may move off-site and be consumed by other species, that could influence the San Francisco Bay Species. Additionally, the concept of a state-wide action area takes into account the potential for direct and indirect effects and any potential modification to AW critical habitat based on ecological effect measures associated with reduction in survival, growth, and reproduction.

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 AW and SMHM and designated AW critical habitat may be affected or modified via endpoints associated with reduced survival, growth, or reproduction.

2.5.2. 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 EFED'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 + distance over which species that feed on bait range) 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. The federal action is defined by the currently labeled uses for warfarin. An analysis of labeled uses and review of available product labels was completed. Only one special local needs (SLN) label is active, and since it is restricted to Texas, it is excluded from this assessment. For those uses relevant to the assessed

species, the analysis indicates that, for warfarin, the following agricultural uses are considered as part of the federal action evaluated in this assessment: farm premises including agricultural buildings and equipment. In addition, the following non-food and non-agricultural uses are considered: buildings (in and around, indoor and outdoor), transport vehicles, airports, golf courses, and recreational areas.

Following a determination of the assessed uses, an evaluation of the potential "footprint" of warfarin use patterns (*i.e.*, the area where pesticide application may occur) is determined. This "footprint" represents the initial area of concern, based on an analysis of available land cover data for the state of California. The initial area of concern is defined as all land cover types that represent the labeled uses described above. For warfarin, these land cover types include all possible land cover types.

An evaluation of usage information was conducted to refine the area where use of warfarin may impact the assessed species. This analysis is used to characterize where predicted exposures are most likely to occur, but does not preclude use in other portions of the action area. These data suggest that warfarin has historically been used on a wide variety of agricultural and non-agricultural sites.

Generally, the Agency conducts analysis of the spatial extent of potential LAA effects whenever LOCs are exceeded. This analysis typically is needed to determine where adverse effects may occur in relation to the treated site. This spatial analysis typically determines if the potential area of usage, and the subsequent Potential Area of LAA Effects, overlaps with areas of occurrence and/or critical habitat of the species. However, because warfarin is a vertebrate pest control that may be used in a wide variety of urban and non-urban areas, the spatial extent of warfarin cannot be limited to defined areas. The Agency assumes that warfarin potentially may be used in any area of the state. Therefore, a spatial analysis was not conducted to identify this overlap. All areas where these species occur, and all areas of the critical habitat of the AW and SMHM, are assumed to lie within the potential use area of warfarin.

2.6. Assessment Endpoints and Measures of Ecological Effect

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

2.6.1. 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 2-6** identifies the taxa used to assess the potential for direct and indirect effects from the uses of warfarin for each listed species assessed here. The specific assessment endpoints used to assess the potential for direct and indirect effects to each listed species are provided in **Table 2-7**.

Table 2-6. Taxa Used in the Analyses of Direct and Indirect Effects for the Assessed Listed Species.

Listed Species	Birds[1]	Mammals	Terr. Plants	Terr. Inverts.
Salt marsh harvest mouse	Indirect (rearing sites)	Direct Indirect (rearing sites)	Indirect (food, habitat)	Indirect (prey)
Alameda whipsnake	Direct Indirect (prey)	Indirect (prey/habitat)	Indirect (habitat)	Indirect (prey)

Abbreviations: n/a = Not applicable; Terr. = Terrestrial; Inverts. = Invertebrates
[1] Birds are considered a surrogate for terrestrial phase amphibians and reptiles including the AW.

Table 2-7. Taxa and Assessment Endpoints Used to Evaluate the Potential for Use of Warfarin 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. Birds	Direct Effect -Alameda Whipsnake	Survival, growth, and reproduction of individuals via direct effects	1a. Most sensitive bird[a] acute LC_{50} or LD_{50} (guideline or ECOTOX)
	Indirect Effect (prey/rearing sites) -Salt Marsh Harvest Mouse -Alameda Whipsnake	Survival, growth, and reproduction of individuals or modification of critical habitat/habitat via indirect effects on terrestrial prey (birds)	
2. Mammals	Direct Effect -Salt Marsh Harvest Mouse	Survival, growth, and reproduction of individuals via direct effects	2a. Most sensitive laboratory mammalian acute LC_{50} or LD_{50} (guideline or ECOTOX)
	Indirect Effect (prey/habitat from burrows/rearing sites) -Salt Marsh Harvest Mouse - Alameda Whipsnake	Survival, growth, and reproduction of individuals or modification of critical habitat/habitat via indirect effects on terrestrial prey (mammals) and/or burrows/rearing sites	
3. Terrestrial Invertebrates	Indirect Effect (prey) -Salt Marsh Harvest Mouse -Alameda Whipsnake	Survival, growth, and reproduction of individuals or modification of critical habitat/habitat via indirect effects on terrestrial prey (terrestrial invertebrates)	No data available

Table 2-7. Taxa and Assessment Endpoints Used to Evaluate the Potential for Use of Warfarin 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**
4. Terrestrial Plants	Indirect Effect (food/habitat) (non-obligate relationship) -Salt Marsh Harvest Mouse -Alameda Whipsnake	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)	No data available
[a] Birds are used as a surrogate for terrestrial-phase amphibians and reptiles.			

2.6.2. Assessment Endpoints for Designated Critical Habitat

As previously discussed, designated critical habitat is assessed to evaluate actions related to the use of warfarin that may alter the PCEs of the designated critical habitat for AW. PCEs for AW were previously described in **Section 2.4.** 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 warfarin 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.

2.7. Conceptual Model

2.7.1. 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 1998a). For this assessment, the risk is stressor-linked, where the stressor is the release of warfarin to the environment. The following risk hypotheses are presumed in this assessment:

The labeled use of warfarin within the action area may:

- directly affect AW and SMHM by causing mortality or by adversely affecting growth or fecundity;
- indirectly affect AW and SMHM and/or modify AW designated critical habitat by reducing or changing the composition of food supply;
- indirectly affect AW and SMHM and/or modify AW designated critical habitat by reducing or changing the composition of the terrestrial plant community in the species' current range;
- indirectly affect SMHM and AW and/or modify AW designated critical habitat by reducing or changing terrestrial habitat in their current range (via reduction in small burrowing mammals leading to reduction in underground refugia/cover).

2.7.2. Risk Hypothesis Description and Conceptual Diagram

The conceptual model is a graphic representation of the structure of the risk assessment. It specifies the warfarin release mechanisms, biological receptor types, and effects endpoints of potential concern. The conceptual model for AW and SMHM and the conceptual model for the terrestrial PCE components of critical habitat for AW are shown in **Figure 2-4.** 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 will be 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 AW and SMHM and modification to designated critical habitat for AW is expected to be negligible. As previously discussed in Section 1.3.1, aquatic exposure is not relevant to this assessment.

The conceptual model assumes that warfarin will be available to non-target organisms, and as toxic food bait, it will adversely affect terrestrial species. The major sources of exposure of non-target terrestrial animals are expected to be ingestion of the formulated food bait and consumption of vertebrate body tissues or invertebrates that have eaten the food bait (USEPA 2004b). Exposure via these routes is expected primarily for birds and mammals, though it is likely that other terrestrial animals such as reptiles, terrestrial amphibians, and terrestrial invertebrates may be at risk if they consume invertebrates or tissues of vertebrates that have eaten bait.

Warfarin is applied as a bait, it tends to bind to organic matter, and little is expected to partition into drinking water sources (*e.g.* puddles) compared to that which is available for direct consumption on the bait itself; therefore, this route of exposure was not assessed. Since warfarin is not sprayed directly onto plants, and because so little is expected to leach from the bait (with a maximum a.i. of 0.003 lb a.i./placement) and then be available for plant uptake, consumption of warfarin on plants is not considered as a route of exposure. Dermal and inhalation routes of exposure are not expected to be important routes of exposure for grain-based, rodenticide food bait because warfarin is not volatile and the expected short dermal contact periods preclude appreciable absorption through the skin.

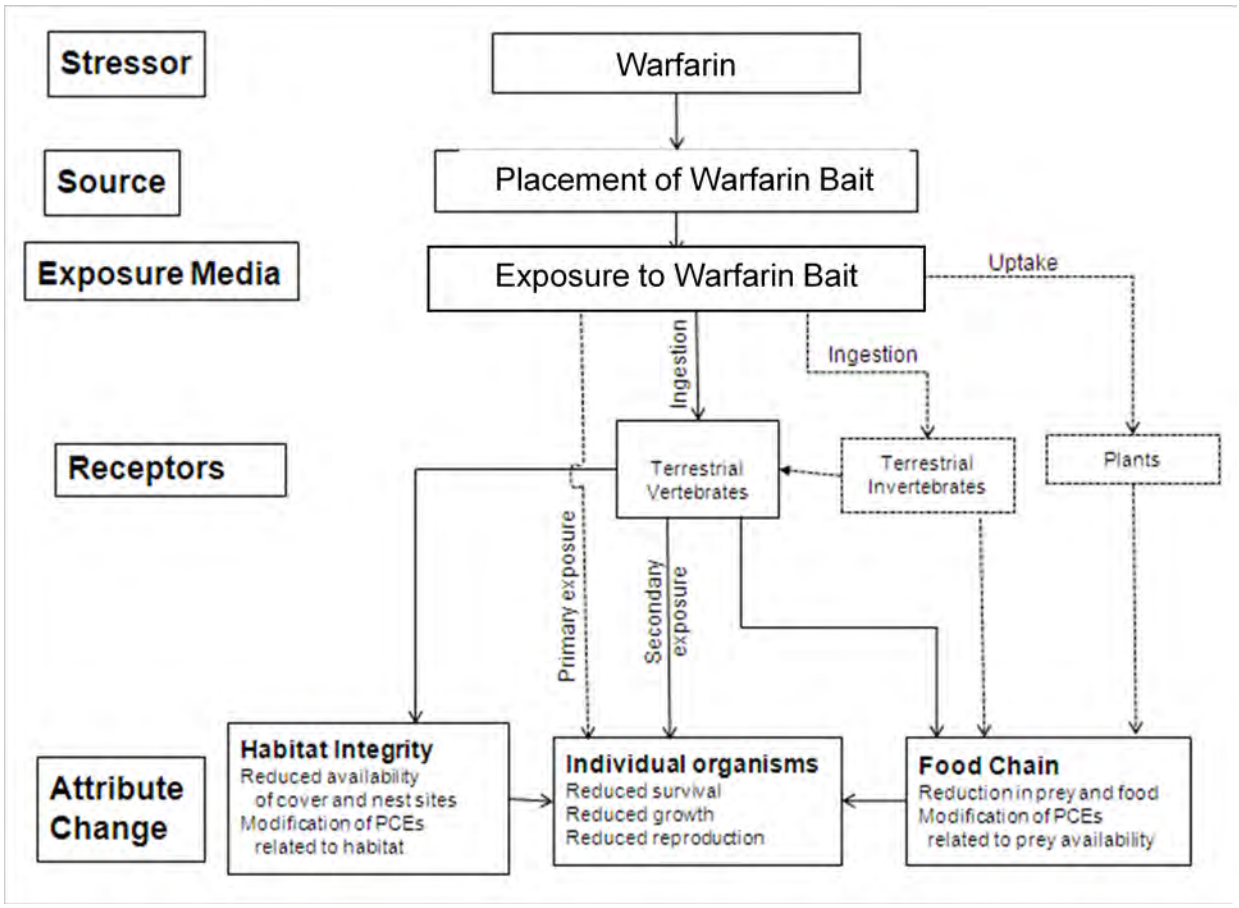

Figure 2-4. Conceptual Model Diagram of Warfarin Exposure and Effects in Non-Target Species. Dotted lines indicate exposure pathways that have a low likelihood of contributing to ecological risk.

2.8. 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 warfarin 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 2004a), the likelihood of effects to individual organisms from particular uses of warfarin 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**.

2.8.1. Measures of Exposure

Due to the very low application rate per placement (of a maximum of 0.003 lb a.i./placement) and that oftentimes the bait is applied in bait stations, runoff appears to be a minor route of offsite transport compared to other routes such as dispersal of contaminated target species. No degradates of concern were identified.

2.8.1.a. Estimating Primary Terrestrial Exposure

EFED's exposure assessment for the rodenticides differs from that for most conventional pesticides. For a rodenticide, the bait itself is the potential food item of concern due to primary consumption. Thus, the amount of active ingredient in the formulated bait is used as an EEC. This information is used to estimate the amount of bait that non-target birds and mammals of various sizes need to consume to obtain a dose expected to be lethal to 50% of the individuals in the population (*i.e.*, LD_{50} dose). Estimates of food-ingestion rates (g dry matter per day) are determined from established allometric equations presented in the Wildlife Exposure Factors Handbook (USEPA 1993). The concentration of warfarin in bait is also used to estimate initial dietary exposure (mg a.i. per kg in bait) which in turn is used to calculate avian and mammalian dietary RQs.

2.8.1.b. Estimating Secondary Terrestrial Exposure

Secondary exposure analysis (from carcass) requires consideration of residues in tissues of target and non-target organisms that are commonly consumed by predators and scavengers. Moreover, it is important to know how long the residue persists in body tissues. Tissue residue concentrations for non-target animals fed warfarin were submitted by the registrant and are available in open literature. Additionally, a number of laboratory tests using avian and mammalian predators and scavengers are available to assess mortality from secondary exposure resulting from consumption of prey animals that have been exposed to warfarin. Design and methodology vary among studies, adding variability to the results and analysis; however, the

variability could not be quantified at this time. Until standard methods and testing requirements for such studies are developed, these tests provide the best data available.

2.8.2. Measures of Effect

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

2.8.2.a. Integration of Exposure and Effects

Risk characterization is the integration of exposure and ecological effects characterization to determine the potential ecological risk from agricultural and non-agricultural uses of warfarin, and the likelihood of direct and indirect effects to the assessed species in aquatic and terrestrial habitats. The exposure and toxicity data are integrated in order to evaluate the likelihood of adverse ecological effects, *i.e*, risk, 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 2004a) (see **Appendix B**). More information on standard assessment procedures is available in **Attachment I**.

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

As part of the risk characterization, an interpretation of acute RQs for listed species is discussed. This interpretation is presented in terms of the chance of an individual event (*i.e*., mortality or immobilization) should exposure at the EEC actually occur for a species with sensitivity to warfarin on par with the acute toxicity endpoint selected for RQ calculation. To accomplish this interpretation, the Agency uses the slope of the dose-response relationship available from the toxicity study used to establish the acute toxicity measures of effect for each taxonomic group that is relevant to this assessment. The individual effects probability associated with the acute RQ is based on the mean estimate of the slope and an assumption of a probit dose-response relationship. In addition to a single effects probability estimate based on the mean, upper and lower, estimates of the effects probability are also provided to account for variance in the slope, if available.

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

2.8.4. Data Gaps

Data gaps were assigned a low or high potential to affect the ecological risk assessment. Studies were requested for the data gaps listed below as part of an EFED risk assessment conducted for a new use of warfarin to control prairie dogs (DP barcode not yet assigned). While still considered data gaps according to 40 CFR Part 158, studies with a low potential to add value are unlikely to influence the effect determinations because alternate methods and weights of evidence (*i.e.,* acute-to-chronic ratio, scaling factors, or consideration of environmentally relevant concentrations relative to effects thresholds) lead the Agency to certain conclusions in the absence of study data. When alternative methods and weights of evidence cannot be used, relevant studies are identified as having a high likelihood of influencing the effect determination and are requested to better characterize potential risks to both non-listed and listed species. It is important to note that a study that is currently assigned a low potential to add value could be changed to high potential based on future proposed uses, submitted data, and/or incidents.

Since the data used in this assessment were derived from open literature and were not based on USEPA-guideline studies, warfarin's persistence and mobility have not been fully characterized. However, due to the limited use and the low environmental exposure of warfarin, no data gaps have been identified at this time. Should the use rate increase significantly or if a new use is proposed in the future, guideline studies may be necessary for a better understanding of warfarin's persistence and mobility.

No chronic toxicity data are available for evaluating the potential effects of warfarin on birds or reptiles. A data gap is identified for the *Avian Reproduction Study* (850.2300). Both the waterfowl and upland game bird reproduction studies have **high** potential to add value to the ecological risk assessment. This study would provide valuable information on sublethal effects caused by warfarin, such as, hemorrhaging in adults, viability of offspring, and behavior modifications that may make birds more susceptible to predation. This study could also provide information on the potential for recovery and time to recovery for poisoned birds.

The current 40 CFR Part 158 specifies testing of a passerine species. Information regarding relative toxicity to passerines, which may metabolize warfarin differently, would be useful in further characterizing the potential effects to birds and reptiles.

Terrestrial plant toxicity studies are currently listed as data gaps, although warfarin's mode of action is not expected to result in adverse effects to plants. The low application rates of warfarin (0.003 lb a.i./placement), with many treatments occurring indoors or in bait stations, precludes considerable exposure to plants. These studies have **low** potential to add value to the ecological risk assessment as low risks were noted in the current assessment.

Several aquatic toxicity studies are currently listed as data gaps. These studies have **low** potential to add value to the ecological risk assessment as low aquatic exposure and consequently low risks are anticipated from the current uses of warfarin. These studies are:

- *Freshwater Fish Early Life-Stage Toxicity Test (850.1400)*
- *Freshwater Invertebrate Life Cycle Toxicity Test (850.1300)*

- *Estuarine/Marine Fish Acute Toxicity Test (850.1075*)
- *Estuarine/Marine Fish Early-life Stage Toxicity Test (850.1400)*
- *Aquatic Plant Toxicity Test Using* Lemna *spp.,Tiers I and II (850.4400)*
- *Algal Toxicity, Tiers I and II (850.5400), four species*

3. Exposure Assessment

Warfarin is formulated as encapsulated grain baits, or as granules, gels, blocks, and mini-block baits. These baits may be sold in bait stations, placed into pre-existing bait stations, injected into burrows, or be used for spot-baiting.

3.1. Terrestrial Animal Exposure Assessment

For assessing exposure of pesticides to terrestrial animals, the Agency typically uses T-REX to calculate EECs for dietary exposure of terrestrial wildlife, and T-HERPS to calculate refined EECs for dietary exposure to reptiles and amphibians. However, these models only calculate EECs (and risk quotient based on the EECs) for natural wildlife food items such as plants, seeds, and insects that are exposed from foliar application of pesticides. These models are not appropriate for calculating EECs for animals that directly consume bait products, or that consume other animals which consume the bait products. In lieu of these models, the Agency used alternative approaches as described below.

For this assessment, it was assumed that terrestrial animals could be exposed to warfarin through two different pathways. Animals may directly consume bait (primary consumption; SMHM), or animals may consume contaminated carcasses either killed or scavenged by the consumer (secondary consumption; AW). Both approaches and the expected exposure levels are detailed below. This approach is consistent with that of the other San Francisco Bay listed species rodenticide assessments.

3.1.1. Expected Warfarin Residues through Bait Consumption

Exposure through bait consumption is calculated using two methodologies. For the first method, warfarin exposure is calculated on a mg a.i./kg-bw basis, where kg-bw is the body weight in kilograms for the consuming individual for three standard weight classes of birds and mammals, which are potential prey items for the AW and SMHM, and typical weights of the SMHM to represent primary consumption of warfarin bait. Food ingestion rate (FI) (dry weight) estimates were derived using allometric equations from USEPA (1993). The allometric equations for estimating FI for birds (passerines) and mammals (rodents) were used as these would best approximate those individuals with high potential for consuming bait and they would give the most conservative (highest) exposure estimates. Food dry weight was converted to wet weight assuming the bait contained 10% water, similar to the assumption that seeds for wildlife consumption contain 10% water (USEPA 1993). Formulas for calculation of dose estimates are provided in **Table 3-1**, and warfarin exposure estimates (on a dose basis) are provided in **Table 3-2**. The default weight classes of birds are small (20 g), medium (100 g), and large (1000 g), and the default weight classes for mammals are small (15 g), medium (35 g), and large (1000 g).

EECs for direct effects to the SMHM are calculated based on estimated average body weights for the species. EECs for indirect effects of reduction in prey (birds, reptiles, terrestrial amphibians, and mammals) and habitat (*e.g.*, use of nests and burrows by the listed species) are also calculated. RQs are generated by dividing these exposure estimates of warfarin (mg a.i./kg-bw) for a given weight class by the most conservative toxicity endpoint for the relevant taxa adjusted for the default body weights. RQs using these exposure estimates were generated for acute bird and mammal (using LD_{50} data).

Table 3-1 Formulas for Calculation of Warfarin Intake based on Consumption of Bait.

Passeriform bird food intake (g, dry weight): FI (g dry-wt/day) = $0.398 * Wt(g)^{0.850}$

**Rodent mammal food intake (g, dry weight*): FI (g dry-wt/day) = $0.621 * Wt(g)^{0.564}$

Food intake (g, wet weight): FI (g wet-wt/day) = FI (g dry-wt/day) / 0.90

Warfarin intake (mg a.i./kg-bw/day) = FI (g wet-wt/day) * **C** mg a.i./kg-bait / Wt(g)

Where: Wt(g) = weight (in grams) of the bird or mammal consumer
C(mg a.i./kg-bait) = concentration of warfarin in bait

*Equation for SMHM and generic bait-consuming rodents

Table 3-2 Expected Warfarin Intake for SMHM and Generic Bird and Mammal Weights based on Consumption of Bait.

Species or Taxa	% a.i. in bait	Weight (g)	Food intake (g dry-wt/day)	Food intake (g wet-wt/day)	Warfarin intake (mg a.i./kg-bw/day)
SMHM	0.025	10	2.3	2.5	56.9
	0.054	10	2.3	2.5	122.9
Passeriform Birds*	0.025	20	5.1	5.6	63.5
		100	19.9	22.2	49.9
		1000	141.2	156.9	35.3
	0.054	20	5.1	5.6	137.1
		100	19.9	22.2	107.7
		1000	141.2	156.9	76.3
Rodent Mammals	0.025	15	2.9	3.2	47.7
		35	4.6	5.1	32.9
		1000	30.6	34.0	7.6
	0.054	15	2.9	3.2	103.0
		35	4.6	5.1	71.2
		1000	30.6	34.0	16.5

* Birds also serve as surrogate for reptiles and terrestrial-phase amphibians

Whereas the above primary exposure calculation method considered warfarin consumption as a function of body weight, the second primary exposure method considers consumption independent of body weight (concentration of warfarin as packaged in bait); namely dietary concentrations of 250 mg a.i./kg-bait and 540 mg a.i./kg-bait. Since these EECs are not dependent on body weight or food consumption rates, they can be used for both direct effects to the SMHM and indirect effects to the two evaluated species. RQs are calculated using this estimate of dietary concentration and the LC_{50} (mg a.i./kg-diet) available from subacute dietary toxicity studies. Acute RQs using these exposure estimates were generated for birds and mammals (using LC_{50} data).

3.1.2. Expected Warfarin Ingestion through Consumption of Contaminated Carcasses

Exposed AW, mammals, birds, terresstrial-phase amphibians and reptiles have the potential to consume prey that has consumed warfarin bait. The determination of warfarin intake by individuals consuming warfarin poisoned animals or carcasses is calculated in a manner similar to the approach for individuals consuming bait (**Section 3.3.1**). Empirical residue data are used instead of bait concentration of warfarin (**Table 3-4**). Warfarin body burdens in carcasses collected from field and laboratory studies were determined in mammals, after exposure to warfarin bait. Data are available for a variety of small- and medium-sized mammalian granivores and omnivores. No data were identified for other exposed taxonomic groups (*e.g.*, birds and reptiles). For all studies, it was assumed the concentrations were reported using wet weights of the mammals.

In laboratory studies, mean warfarin residues in mammalian carcass ranged from 0.038 to 104 mg a.i./kg-bw. The relatively high mean residue values are most likely a result of the study design where Aulerich *et al*. (1987) fed rabbits low doses of warfarin bait for an extended period of time. This study indicates that mammals consuming small amounts of warfarin bait over an extended period are able to survive poisoning, perhaps because they are capable of building a tolerance to warfarin in this situation, or because they become more effective at metabolizing the compound. This study also suggests that these mammals have the potential to accumulate large amounts of warfarin residue (104 and 82 mg a.i./kg-bw, **Table 3-3**) which may make them significantly more toxic to secondary consumers than a mammal that consumed a single lethal dose. Because of the lack of data on warfarin residues in birds, terrestrial-phase amphibians and reptiles, it was assumed the mammalian body burden data would be relevant for all the evaluated taxonomic groups. Since the registered bait blocks contain 0.025% or 0.054% a.i. (250 or 540 mg a.i/kg-bait), the low dose values found in the majority of the residue studies, aside from the Aulerich data, likely do not reflect true field conditions either. For this assessment, the body residue level of 2.95 mg a.i./kg-carcass (Townsend *et al*. 1984) is used because the bait concentration used in the Towsend et al. study (200 mg a.i./kg bait) is the closest approximation of the concentration found in the registered warfarin baits.

Table 3-3 Warfarin Residue Levels in Mammalian Primary Consumers.						
mg ai/kg bait	**Target species**	**Site**	**Sample size**	**Days exposed**	**Mean whole-carcass residue (mg a.i./kg-bw)**	**Reference**
200[a]	Mouse	Laboratory	17	3	2.95 ± 0.26 (SE)	Townsend *et a*l. 1984
67[a]	Rabbit	Laboratory	Not reported	35	104	Aulerich *et al*. 1987
50[a]	Mouse	Laboratory	62	3	1.63 ± 0.1 (SE)	Townsend *et a*l. 1981
50[a]	Mouse	Laboratory	18	3	1.58 ± 0.1 (SE)	Townsend *et a*l. 1984
25[a]	Rabbit	Laboratory	Not reported	35	82	Aulerich *et a*l. 1987
10[a]	Mouse	Laboratory	15	3	0.42 ±0.04 (SE)	Townsend *et al*. 1984

[a] warfarin baits registered in the U.S. are 0.025% and 0.054% a.i.

Warfarin exposure through carcass consumption is calculated as mg a.i./kg-bw, where kg-bw is body weight in kilograms of the consuming individual for three weight classes of birds and mammals. EECs for direct effects to the AW are calculated based on estimated average body weights for the AW. For this analysis, weight classes of 50, 1000, and 2000 g individuals were used to better represent the larger size carnivores and scavengers relative to the full range of bird and mammal weights. Food ingestion, based on food dry weight consumption, estimates were derived using the generic bird and mammal allometric equations from Wildlife Exposure Factors Handbook (EPA 1993). Food dry weight was converted to wet weight assuming the consumed prey/carcass contained 68% water (Wildlife Exposure Factors Handbook, EPA 1993). Formulas for calculation of dose estimates are provided in **Table 3-4**, and warfarin exposure estimates on a dose basis are provided in **Table 3-5**.

Secondary exposure was also evaluated for assessing risk to the AW. This species may be exposed if it consumes a vertebrate animal that has eaten warfarin bait. Lizards in particular are believed to be the most important prey item of whipsnakes (USFWS 2005), but lizards generally feed upon insects and other terrestrial arthropods and would not likely consume rodent bait. It is noted that secondary exposure to lizards may occur through consumption of invertebrates that fed on warfarin-killed rodents, another potential exposure route to the AW via lizard prey. However, secondary exposure was based only on consumption of small mammals, which are also a component of the diet of the AW, since no data were available to quantitatively estimate warfarin body burden in lizards that consumed invertebrates having residues of warfarin. For assessing secondary exposure for the AW, scenarios were considered in which a snake preyed upon a house mouse, a broad-footed mole, or a Norway rat after they prey had consumed warfarin bait. The maximum size of the prey consumed by snakes may be estimated using the following allometric equation developed by King (2002).

$$\text{Prey Size (g)} = \text{Snake body weight (g)}^{1.071}$$

To make this assessment protective, the exponent used in this equation is the upper limit of the 95% confidence interval that King (2002) reported for this parameter. The weight of the AW was not available, but the Agency has estimated body weight of this species from its length using the method presented in USEPA (1993). The body weights of this species were estimated to range from 2.5 to 176 g for juveniles and 46 to 897 g for adults (USEPA 2010a). Using the upper bounds of these ranges, and the allometric equation given above, the maximum prey size for the AW was estimated to be 254 g for juvenile snakes and 1454 g for adult snakes. Reported body weights of house mice, Eastern mole (similar to the broad-footed mole), and Norway rat are 18-23 g, 82-140 g, and 195-485 g, respectively (Whitaker, 1996). Therefore, the AW is expected to be able to consume all three of these prey species, including the Norway rat. In this assessment, the upper limit of the reported ranges was used for the body weight of each prey (23 g for the house mouse, 140 g for the broad-footed mole, and 485 g for the Norway rat.)

The size of the AW was set at the minimum size animal that could consume prey of the size assumed for the three prey species. This was done by setting the prey size in the allometric equation for maximum prey size, given above, and solving for snake body weight. The minimum snake size to consume the maximum sized mouse, mole, and rat was calculated to be 18.6, 101, and 322 g, respectively. The 18.6-g snake is plausible for a juvenile AW, the 322-g snake is plausible for an adult AW, and the 101-g snake is plausible for either an adult or large juvenile AW.

RQs are generated by dividing exposure estimates of warfarin (mg a.i./kg-bw) for a given weight class by the most conservative toxicity endpoint for the relevant taxa adjusted for the default body weights. RQs using these exposure estimates were generated for acute bird and mammal (using LD_{50} data).

Table 3-4 Formulas for Calculation of Warfarin Intake based on Consumption of Contaminated Carcasses.

Bird food intake (g, dry weight): FI (g dry-wt/day) = $0.648 * Wt(g)^{0.651}$

**Mammal food intake (g, dry weight*): FI (g dry-wt/day) = $0.235 * Wt(g)^{0.822}$

***Snake food intake (g, wet weight):* FI (g wet-wt/day) = $Wt(g)^{1.071}$

Food intake (g, wet weight): FI (g wet-wt/day) = FI (g dry-wt/day) / 0.32

Warfarin intake (mg a.i./kg-bw/day) = FI (g wet-wt/day) * 2.95 mg a.i./kg-carcass / Wt(g)

Where: Wt(g) = weight (in grams) of the bird or mammal consumer

*Equation for generic carnivorous mammals
**Equation for AW

Table 3-5 Expected Warfarin Intakes for AW and Carnivorous Birds and Mammals based on Consumption of Contaminated Carcasses				
Species or Taxa	**Weight (g)**	**Food intake (g dry-wt/day)**	**Food intake (g wet-wt/day)**	**Warfarin intake (mg a.i./kg-bw/day)[1]**
AW	18.6	-	23	3.63
	101	-	140	4.09
	322	-	485	4.44
Birds*	50	8.3	25.8	1.53
	1000	58.2	181.7	0.54
	2000	91.3	285.4	0.42
Mammals	50	5.9	18.3	1.08
	1000	68.7	214.7	0.63
*surrogate for reptiles and terrestrial-phase amphibians [1] See Table 3-8 for derivation.				

The second exposure method for consumption of contaminated carcasses results in a carcass concentration of 2.95 mg a.i./kg-carcass (approximate concentration of registered warfarin baits, most conservative data taken from the available literature, Townsend *et al*., 1984). RQs are calculated using this estimate of dietary concentration and the LC_{50} (mg a.i./kg-diet) available from dietary toxicity studies. RQs using these exposure estimates were generated for acute bird and mammal (using LC_{50} data).

3.1.3. Exposure to Terrestrial Invertebrates

Exposure to invertebrates can occur through consumption and contact with bait. These individuals could then either be negatively affected or be available to be consumed by any of the two evaluated species as well as other animals. Methodology for estimation of terrestrial invertebrate exposure through contact with treated bait is currently not available. In addition, warfarin data to enable the estimation of invertebrate body burden are also not available. Therefore, for this assessment, it was assumed that dietary warfarin exposure through the consumption of contaminated invertebrates was comparable to consumption of contaminated mammals (**Section 3.4.2**).

4. Effects Assessment

This assessment evaluates the potential for warfarin to directly or indirectly affect AW and SMHM or modify designated critical habitat of the AW. Assessment endpoints for the effects determination for each assessed species include direct toxic effects on the survival, reproduction, and growth, as well as indirect effects, such as reduction of the prey base or modification of its habitat. In addition, potential modification of AW critical habitat is assessed by evaluating effects to the PCEs, which are components of the critical habitat areas that provide essential life cycle needs of each assessed species. Direct effects to reptiles (AW) are based on avian toxicity data, given that birds are generally used as a surrogate for terrestrial-phase amphibians and reptiles. For this assessment, only secondary exposure is considered for the AW since it is highly unlikely that the AW would consume bait.

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 birds (used as a surrogate for terrestrial-phase amphibians and reptiles) and mammals. Acute (short-term) toxicity information is characterized based on registrant-submitted studies and a comprehensive review of the open literature on warfarin.

4.1. Ecotoxicity Study Data Sources

Toxicity endpoints are established based on data generated from guideline studies submitted by the registrant, and from open literature studies that meet the criteria for inclusion into the ECOTOX database maintained by EPA/Office of Research and Development (ORD) (USEPA, 2004a). Open literature data presented in this assessment were obtained from previous risk assessments as well as ECOTOX information obtained on 26 May 2011. In order to be included in the ECOTOX database, papers must meet the following minimum criteria:

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

Open literature toxicity data on the effects of warfarin to "target" commensal rodent species (the house mouse, the Norway rat, and the wood rat), which include efficacy studies, were not considered in deriving the most sensitive endpoint for terrestrial mammals. In the case of rodenticides, adequate data on the toxicity to rats and mice are already provided by acute mammalian toxicity studies that the rodenticide registrants are required to submit for product registration. Therefore, toxicological data on target species of rats and mice were not included in the ECOTOX open literature search that the Agency conducted, and are not included in the summary table provided in **Appendix G**. Citations of open literature papers that provide toxicological data for target rodent species are listed in **Appendix F** with the code "TARGET" given after the citation. While toxicological findings were not included in the summary of acute toxicity endpoints in this document, some of these papers which were deemed useful were obtained and used to provide supplemental information for characterizing the toxicity of warfarin.

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

is important to note that the full suite of sublethal endpoints potentially available in the effects literature (regardless of their significance to the assessment endpoints) are considered, as they are relevant to the understanding of the area with potential effects, as defined for the action area.

Citations 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.*, the endpoint is less sensitive) are included in **Appendix F**. **Appendix F** 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 is presented in **Appendix G**.

In addition to registrant-submitted and open literature toxicity information, other sources of information, including use of the acute probit dose-response relationship to establish the probability of an individual effect and reviews of ecological incident data, are considered to further refine the characterization of potential ecological effects associated with exposure to warfarin. A summary of the available terrestrial ecotoxicity information and the incident information for warfarin are provided in **Sections 4.2** and **4.3**, respectively.

EFED has received an additional registrant submitted data package (MRID 48528600) for warfarin in support of a pending Section 3 New Use registration. This submission is currently in review. However, cursory review indicates that the data, which include efficacy analyses and toxicity comparisons among rodenticides, are unlikely to alter the risk conclusions of the warfarin litigation assessment conducted for San Francisco Bay area listed species.

4.2. Toxicity of Warfarin to Terrestrial Organisms

Table 4-1 summarizes the most sensitive terrestrial toxicity endpoints, based on an evaluation of both the registrant-submitted studies and the open literature. Warfarin is slightly toxic to birds on an acute oral exposure basis and is moderately toxic to birds on a subacute dietary exposure basis. No acceptable or supplemental data are available to characterize chronic or sub-lethal toxicity to birds. Warfarin is very highly to highly toxic to mammals on an acute oral exposure basis. No acceptable or supplemental data are available to characterize chronic or sub-lethal toxicity to mammals. Data on toxicity of warfarin to terrestrial invertebrates and plants are not available. Additional information is provided in **Appendix C**.

Table 4-1. Terrestrial Toxicity Profile for Warfarin					
Taxa	**Study Type**	**Species Tested**	**Toxicity Value Used in Risk Assessment**	**Acute Classification/ Chronic Effect**	**Reference (Accession No.)**
Bird	Avian oral toxicity	*Anas platyrhynchos* (Mallard)	LD_{50} = 621 mg/kg-bw	Slightly toxic	00248782
	Avian dietary toxicity	*Colinus virginianus* (Northern bobwhite)	LC_{50} = 625 mg/kg-diet	Moderately toxic	00153365
Mammal	Acute oral toxicity	*Rattus norvegicus* (Norway Rat)	LD_{50} = 3.0 mg/kg-diet	Very highly toxic	05002272
	Dietary (5-day exposure)	*Rattus norvegicus* (Norway Rat)	LC_{50} = 4.41 ppm	Very highly toxic	Teeters 1981 (TMN 126)[a]

[a] Teeters, W.R (1981) Warfarin technical: Toxicity to Laboratory Rat: Test No. 126. (U.S. Environmental Protection Agency, pesticides Regulation Div., Agricultural Research Center, Animal Biology Laboratory, unpublished report)

Acute toxicity to terrestrial animals is categorized using the classification system shown in **Table 4-2** (USEPA, 2004a). Toxicity categories for terrestrial plants have not been defined.

Table 4-2. 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

4.2.1. Toxicity to Birds

4.2.1.a. Primary Toxicity: Acute and Chronic Studies

Warfarin is slightly to moderately toxic to birds with the most sensitive endpoints being an acute oral LD_{50} of 621 mg a.i./kg-bw for the mallard duck (*Anas platyrhynchos*) *and* a 5-day sub-acute dietary LC_{50} of 625 mg a.i./kg-diet for the bobwhite quail (*Colinus virginianus*) (Accession No. 00248782 and 00153365). Sublethal effects noted in the acute oral study in live test birds that were euthanized and subsequently subjected to necropsy (MRID 00248782) included hemorrhaging. Sublethal effects noted in the subacute dietary study in all test groups (Accession No. 00153365) included lethargy and anorexia. Effects noted during the necropsy of birds that died during the subacute dietary study included subdermal hematomas, presence of significant amounts of clotted blood in the visceral cavity, and pale diffuse coloration of the liver. No chronic toxicity data are available for birds.

4.2.1.b. Secondary Toxicity: Acute Studies

The effects of warfarin poisoned carcasses on predatory and scavenging birds have been evaluated in the studies summarized in **Table 4-3**. Though no mortalities were reported, warfarin residues were detected in the four tissues examined in tawny owls (*Strix aluco*) and there was a decrease in prothrombin levels, a blood plasma protein that plays a critical role in the final stages of blood coagulation. No other signs of toxicity were reported. There were no overt signs of anticoagulant toxicity for black-billed magpies (*Pica pica*) which are avian scavengers. Though the study on owls does not allow for quantification of effect, it confirms that secondary exposure can result in effect.

Table 4-3 Secondary Hazards of Warfarin to Birds in Laboratory Studies

Predator/ scavenger species	Prey offered to birds	No. prey offered daily per bird	No. days Birds exposed	Mortality outcome	Signs of toxicity	Reference
Strix aluco (Tawny owl)	Phase 1- mice fed sodium warfarin treated wheat for 3 days Phase 2- mice orally dosed with 0.54 mg or 0.27 mg sodium warfarin per 0.1 mL of water	1-2	Phase 1- 4 owls were fed warfarin-treated mice on alternate days for 3 months Recovery Period- 3 weeks Phase 2- 2 owls were fed treated mice with 10-20 times higher warfarin levels for 28 days	No mortalities out of the 4 tawny owls exposed	Decrease in prothrombin levels	J004518 Townsend et al. 1981
Pica pica (Black-billed magpie)	Rats fed warfarin bait at 0.05%[a] bait for a maximum of 7 days	One rat offered day 1 and again as necessary	Fed treated rats for 5 days, birds observed for a total of 22 days	No mortalities out of 16 magpies exposed	No signs of anticoagulant poisoning	MRID 449950-04 (Supplemental)

[a] rat and mouse baits registered in the U.S. are 0.025% ai and 0.054%

4.2.2. Toxicity to Mammals

4.2.2.a. Primary Toxicity: Acute and Chronic Studies

Warfarin is very highly toxic to mammals on an acute exposure basis with the most sensitive endpoint being an acute oral LD_{50} of 3.0 mg a.i./kg-bw (Accession No. 05002272). No chronic data are available for the effects of long-term warfarin exposure to mammals.

4.2.2.b. Secondary Toxicity: Acute Studies

The effects of warfarin poisoned carcasses on predatory and scavenging animals have been evaluated in the studies summarized in **Table 4-4**. Of the 7 available studies, one study exposing the least weasel (*Mustela nivalis*) to warfarin-treated mice resulted in 3 mortalities and signs of toxicity (increased clotting time) in the 3 survivors. The remaining studies did not report any mortalities or signs of toxicity. Though there were no mortalities or signs of toxicity described in the majority of studies reported in **Table 4-4**, warfarin's acute primary toxicity and persistence in liver tissue are likely to result in an increased likelihood of secondary exposure to predatory animals. In addition, exposed animals may exhibit behavior that may make them more susceptible to predation (Cox and Smith, 1992). This may further increase exposure to predatory animals.

Table 4-4 Secondary Hazards of Warfarin to Mammals in Laboratory Studies

Predator/ scavenger species	Prey offered to mammal	No. prey offered daily per mammal	No. days Mammals exposed	Mortality outcome	Signs of toxicity	Reference
Mink	New Zealand white rabbits minus the contents of the digestive track. Rabbits were exposed to either 25 or 67 ppm warfarin bait. Rabbit carcasses and cleaned digestive tracts were ground and mixed with other dietary ingredients to yield nominal concentrations of 2.2, 3.9, 7.0, 12.5, and 22.5 mg/kg	*Ad libitum*	28 days	No mortalities out of the 4 mink exposed	No signs of toxicity were reported	E039689 Aulerich *et al*. 1987
Least weasel	Mice fed 0.001% bait, 0.005% bait, Or 0.02% bait for 3 days	*Ad libitum*	90 29-90 12-57	0 1 2	2 (ct) 1 (ct) No survivors	Townsend *et al*. 1984
European ferret	Prairie dogs fed 0.05% bait for 15 days	1	7	0	0	Carlet and Mach 1997
European ferret	Prairie dogs fed 0.05% bait for 15 days	*Ad libitum*	5	0	0	Mach 1998
Racoon	Rats fed 0.025% bait for 5 days	1 3	5 5	0 0	0 0	EPA 1982

[a] ct = increased blood coagulation time

4.3. Incident Database Review

A review of the Ecological Incident Information System (EIIS, version 2.1) and 'Aggregate Incident Reports' (v. 1.0) for ecological incidents involving warfarin was completed on April 27, 2011. In addition, the Avian Monitoring Information System (AIMS) database was reviewed on May 12, 2011. The results of this review for terrestrial incidents are in **Table 4-5**. No plant or aquatic incidents were reported.

Of the 10 reported terrestrial incidents, nine were considered to be probable or highly probable for death due to warfarin. Incidents were reported from a combination of both agricultural and residential uses. Mortalities included non-target species that were likely primary consumers of the bait (*e.g.,* grey squirrel and bobwhite quail) as well as carnivorous species (*e.g.,* peregrine falcon, bald eagle, and great horned owl) likely to have preyed upon the granivorous individuals and experienced secondary toxicity through consumption of contaminated prey. Residue levels detected in several of the incident reports confirm exposure to warfarin. The incident data indicate that non-target granivores are likely to be exposed to and consume treated bait and that exposure results in toxic effects. The incident reports contained within the EIIS precede label changes that have subsequently restricted use of the product to bait stations. Whether the label restrictions have resulted in a decrease in adverse primary and secondary impacts to non-target species is uncertain; however, the absence of more recent incident reports cannot be construed as the absence of incidents.

Table 4-5 Reported EIIS Incident Summaries for Warfarin

Incident ID	Year	Legality	Certainty*	State	Land Use	Generic Species and (Number Affected)	Comments
I013810-014	1971	Undetermined	4	NY	Unknown	Squirrel (1)	No comments
I004169-001	1981	Registered	3	VA	Agricultural	Bobwhite Quail (3)	Gross pathology found lesions in the birds consistent with warfarin poisoning; no analytical data were included in the report.
R000-02-054	1981	Undetermined	3	NY	Unknown	Squirrel (1)	Warfarin detected in the liver at 0.22 ug/g. Gross pathology-pallor of muscle and/or internal organs, inter- and intramuscular hemorrhages, hemorrhage and/or serum in pericardial sac.
I006306-016	1986	Undetermined	3	NJ	Unknown	Peregrine Falcon (1)	Warfarin detected in the liver at 1.48 ppm
I003350-001	1995	Undetermined	4	NY	Residential	Bald Eagle (1)	Warfarin detected in liver at 1.4 ppm. Second year male diagnosed with warfarin intoxication. Gross pathology- hemorrhage in alimentary canal.
I003909-008	1995	Undetermined	4	NY	Residential	Bald Eagle (1)	Immature female was found dead with signs of hemorrhage in the intestines. Chemical analysis found warfarin residues; residue amounts were not reported.
I008146-001	1998	Registered Use	3	NY	Unknown	Great Horned Owl (1)	Warfarin detected in liver at 0.73 ug/g
I011130-001	2000	Undetermined	3	GA	Agricultural	Bald Eagle (1)	Chemical residues were found in the gut contents, or bait material and biological or biochemical evidence
I017045-005	2001	Registered Use	2	OR	Unknown	Turtle (1)	No comments
CEE-TV # 61108	1998	Undetermined	3	NY	Unknown	Cooper's Hawk (1)	Warfarin detected in liver at 0.73 ug/g

* Certainty code: 0 = Unrelated, 1 = Unlikely, 2 = Possible, 3 = Probable, 4 = Highly Probable

No warfarin incidents have been recorded in the EFED Aggregate Incident database.

5. Risk Characterization

Risk characterization is the integration of the exposure and effects characterizations. Risk characterization is used to determine the potential for direct and/or indirect effects to the SMHM and AW or for modification to AW designated critical habitat from the use of warfarin in CA. The risk characterization provides an estimation (**Section 5.1**) and a description (**Section 5.2**) of the likelihood of adverse effects; articulates risk assessment assumptions, limitations, and uncertainties; and synthesizes an overall conclusion regarding the likelihood of adverse effects to the assessed species or their designated critical habitat (*i.e.*, "no effect," "likely to adversely affect," or "may affect, but not likely to adversely affect"). In the risk estimation section, risk quotients are calculated using EFED procedures and models that have been modified to assess rodenticide risk. In the risk description section, additional analyses may be conducted to help characterize the potential for risk.

5.1. Risk Estimation

Risk is estimated by calculating the ratio of exposure to toxicity. This ratio is the risk quotient (RQ), which is then compared to pre-established acute and chronic levels of concern (LOCs) for each category evaluated (**Appendix B**). For acute exposures to the birds (and, thus, reptiles and terrestrial-phase amphibians) and mammals, the acute risk LOC is 0.1.

Acute risks to terrestrial animals are estimated based on primary and secondary exposures resulting from application of warfarin (**Section 3.4**) and the appropriate toxicity endpoints from **Table 4-1**.

5.1.1. Exposures in the Terrestrial Habitat: Birds (surrogate for Reptiles)

Potential direct acute effects to the AW are evaluated by considering dose- and dietary-based EECs through the consumption of warfarin-contaminated prey (**Sections 5.1.2.c and 5.1.2.d**).

Potential for indirect effects to the AW may result from direct acute effects to birds, small mammals and/or terrestrial-phase amphibians due to a reduction in prey. Potential indirect effects to the SMHM may result from direct acute effects to birds due to a reduction in bird nests which the mouse uses as rearing sites. RQs for indirect effects are calculated from dose- and dietary-based EECs based on the consumption of bait (**Sections 5.1.2.a and 5.1.2.b**) and warfarin-contaminated prey (**Sections 5.1.2.c and 5.1.2.d**). These RQs are calculated for a range of avian body weights.

5.1.1.a. Risks from Direct Bait Consumption (based on acute oral toxicity)

In the case of primary exposure, it is assumed the bait containing warfarin is ingested by non-target animals and results in a toxic response. For acute oral dosing, exposure is measured as mg a.i./kg-bw (**Section 3.1.1** and **Table 3-2**). Toxicity is measured by the LD_{50} obtained from the

single acute oral exposure studies for birds. The LD_{50} values are adjusted for the weight of the assessed animals (birds: 20, 100, 1000 g, using the default scaling factor from Mineau *et al*., 1996.) (**Table 5-1**). Avian RQs based on acute single-dose oral studies are provided in **Table 5-2**; the acute risk to listed species LOC of 0.1 is exceeded for all size classes of birds consuming 540 mg a.i. warfarin/kg-bait. The acute risk to listed species LOC is exceeded for small and medium-sized birds consuming 250 mg a.i. warfarin/kg-bait.

Table 5-1 Formulas for Calculation of Weight-adjusted Avian Warfarin LD_{50}s.

Adjusted avian LD_{50}: $Adj.LD_{50} = LD_{50}\left(\frac{AW}{TW}\right)^{(x-1)}$ where:

Adj. LD_{50} = adjusted LD_{50} (mg/kg-bw)
LD_{50} = endpoint reported from bird study (621 mg/kg-bw)
TW = body weight of tested animal (1580g mallard)
AW = body weight of assessed animal (20g, 100g, and 1000g)
x = Mineau scaling factor for birds; EFED default 1.15

Table 5-2 Bird Acute RQs based on a Single-dose of Warfarin through consumption of bait

	Warfarin concentration in bait (mg a.i./kg-bait)	Weight (g)	Warfarin intake (mg a.i./kg-bw/day)[1]	Adjusted LD_{50} (mg a.i./kg-bw)[2]	RQ[3]
Passeriform Birds*	250	20	63.5	322	**0.20**
		100	49.9	410	**0.12**
		1000	35.3	579	0.06
	540	20	137.1	322	**0.43**
		100	107.7	410	**0.26**
		1000	76.3	579	**0.13**

*surrogate for reptiles and terrestrial-phase amphibians
[1] See **Table 3-2** (**Table 3-1** for derivation).
[2] See **Table 5-1** for derivation.
[3] Bolded RQs exceed listed species LOCs.

5.1.1.b. Risks from Direct Bait Consumption (based on dietary toxicity)

For toxic response elicited from the dietary exposure route extended over several days, exposure is measured as mg a.i./kg-bait (**Section 3.3.1**). Toxicity is measured by the subacute dietary LC_{50} (5 days on treated diet) for birds. Avian dietary-based RQs are provided in **Table 5-3**; the risk to listed species and non-listed species LOCs were exceeded for the 0.054% a.i. bait formulation. The acute risk to listed species LOC is exceeded for the 0.025% a.i. bait formulation.

Table 5-3 Bird Acute RQs based on a 5-day Exposure to Warfarin in the Diet (consumption of bait)

	Warfarin concentration in bait (mg a.i./kg-bait)	LC_{50} (mg a.i./kg-diet)	RQ[1]
Birds*	250	625	**0.40****
	540	625	**0.86*****

*surrogate for reptiles and terrestrial-phase amphibians
[1] Bolded (**) RQs exceed listed species LOCs.
Bolded (***) RQs exceed listed species and non-listed species LOCs.

Avian chronic exposure could not be calculated because no chronic data are available. In the absence of data to the contrary, risk is presumed.

5.1.1.c. Risks from Consumption of Warfarin-contaminated Carcasses (based on acute oral toxicity)

To determine secondary exposure EECs, available literature concerning carcass residue concentrations were considered. A previous assessment (USEPA 2004*b*) compiled available mammalian residue data. No bird or reptile residue data for primary consumption of warfarin were located. Thus, for this assessment, a mammal carcass concentration of 2.95 mg a.i./kg-carcass was used to estimate exposure. Expected warfarin intake (mg a.i./kg-bw/day) was calculated in **Table 3-6.** As in the direct consumption risk estimation, the LD_{50}'s are adjusted for the weight of the assessed birds (50, 1000, 2000 g). These heavier weights are used to better represent carnivores and scavengers which tend to be larger individuals. Acute risk LOCs for listed and non-listed species are not exceeded for the AW or generic weight classes of birds consuming contaminated carcasses using dose-based RQ values **(Table 5-4).**

Table 5-4 AW and Carnivorous/Scavenger Bird Acute RQs Based on a Single Oral Dose of Warfarin, consumption of contaminated carcasses

Species or Taxa	Weight (g)	Warfarin intake (mg a.i./kg-bw/day)[1]	Adjusted LD_{50} (mg a.i./kg-bw)[2]	RQ[3]
AW	18.6	3.63	319	0.01
	101	4.09	411	0.01
	322	4.44	489	0.01
Birds*	50	1.53	370	<0.01
	1000	0.54	580	<0.01
	2000	0.42	643	<0.01

*surrogate for reptiles and terrestrial-phase amphibians
[1] See **Tables 3.8** and **3.9** for derivation.
[2] See **Table 5-1** for derivation with assessed body weights used in this table.
[3] Bolded (***) RQs exceed listed species and non-listed species LOCs.

5.1.1.d. Risks from Consumption of Warfarin-contaminated Carcasses (based on dietary toxicity)

For toxic effects elicited from the dietary exposure route extended over several days, exposure is measured as mg a.i./kg-carcass. Toxicity is measured by the LC_{50} obtained from the subacute dietary studies (5 days on treated diet) for birds and mammals. Avian RQs based on dietary studies are provided in **Table 5-5**; carnivore/scavenger bird LOCs were not exceeded.

Table 5-5 AW and Bird Acute RQs based on a 5-day Exposure to Warfarin in the Diet, consumption of contaminated carcasses

Species or Taxa	Warfarin concentration in carcass (mg a.i./kg-carcass)	LC_{50} (mg a.i./kg-diet)	RQ[1]
AW	2.95	625	<0.01
Birds*	2.95	625	<0.01

*surrogate for reptiles and terrestrial-phase amphibians
[1] Bolded (***) RQs exceed Listed Species, Restricted Use, and Acute Risk LOCs.

Based on the preceding analysis (Sections 5.1.1.a to 5.1.1.d), warfarin does not have the potential to directly affect the AW (**Tables 5-4 and 5-5**) although it may indirectly affect the AW through prey base reduction (**Table 5-3**). Since the acute risk LOCs are exceeded, with chronic risk presumed due to lacking chronic toxicity data, there is a potential for indirect effects to those listed species that rely on birds (and, thus, reptiles) during at least some portion of their life-cycle. As noted previously, the SMHM depends on birds for nesting sites and the AW depends on birds and reptiles, *e.g.*, lizards, as a source of food.

5.1.2. Exposures in the Terrestrial Habitat: Mammals

Potential direct acute effects to the SMHM are evaluated by considering dose- and dietary-based EECs based on the consumption of warfarin-treated bait (**Sections 5.1.3.a and 5.1.3.b**).

Potential for indirect effects to the AW may result from direct acute effects to mammals due to a reduction in prey. Potential indirect effects to the SMHM due to a reduction in rearing sites may result from direct acute effects to mammals. RQs for indirect effects are calculated from dose- and dietary-based EECs based on the direct toxicity through ingestion of bait (**Sections 5.1.3.a and 5.1.3.b**) and via secondary toxicity through ingestion of warfarin-contaminated prey (**Sections 5.1.3.c and 5.1.3.d**). These RQs are calculated for a range of mammal body weights.

5.1.2.a. Risks from Direct Bait Consumption (based on acute oral dose toxicity)

In the case of primary exposure, it is assumed the bait containing warfarin is ingested by non-target animals and evokes a toxic response. For adverse effects elicited from acute oral dose exposure route, exposure is measured as mg a.i./kg-bw (**Section 3.3.1** and **Table 3-3**). Toxicity is measured by the LD_{50} obtained from the single oral dose studies for mammals. The LD_{50}s are adjusted for the weight of the assessed mammals (15, 35, 1000 g) (**Table 5-6**). Mammalian dose-based RQs are provided in **Table 5-7** and exceed the acute risk to listed and non-listed species LOCs for all species and weight classes evaluated.

Table 5-6 Formula for Calculation of Weight-adjusted Mammalian Warfarin LD_{50}s

Adjusted mammalian LD_{50}: $Adj.LD_{50} = LD_{50}\left(\frac{TW}{AW}\right)^{(0.25)}$ where:

Adj. LD_{50} = adjusted LD_{50} (mg/kg-bw)
LD_{50} = endpoint reported from mammal study (3 mg/kg-bw)
TW = body weight of tested animal (175g laboratory rat)
AW = body weight of assessed animal (15g, 35g, 1000g)

Table 5-7 SMHM and Mammalian Acute based on a Single-dose of Warfarin through consumption of bait					
Species or Taxa	**Warfarin concentration in bait (mg a.i./kg-bait)**	**Weight (g)**	**Warfarin intake (mg a.i./kg-bw/day)[1]**	**Adjusted LD_{50} (mg a.i./kg-bw)[2]**	**Acute Dose-based RQ[3]**
SMHM	250	10	56.9	6.1	**9.33**
	540	10	122.9	6.1	**20.2**
Rodent mammals	250	15	47.7	5.5	**8.67**
		35	32.9	4.5	**7.31**
		1000	7.6	1.9	**4.00**
	540	15	103.0	5.5	**18.7**
		35	71.2	4.5	**15.9**
		1000	16.5	1.9	**8.68**

[1] See **Table 3-2** and **3-3** for derivation.
[2] See **Table 5-6** for derivation.
[3] Bolded RQs exceed listed species and non-listed species LOCs.

5.1.2.b. Risks from Direct Bait Consumption (based on dietary toxicity)

For toxic response elicited from the dietary exposure route extended over several days, exposure is measured as mg a.i./kg-bait (**Section 3.3.1**). Toxicity is measured by the LC_{50} obtained from the subacute dietary toxicity studies (5 days on treated diet) for mammals. Mammalian dietary-based RQs are provided in **Table 5-8**; acute risk to listed and non-listed species LOCs are exceeded for both bait concentrations.

Table 5-8 Mammalian Acute RQs based on a 5-day Exposure to Warfarin in the Diet (consumption of bait)			
	Warfarin concentration in bait (mg a.i./kg-bait)	**LC_{50} (mg a.i./kg-diet)**	**Acute Dietary-based RQ[1]**
Mammals	250	4.41	**56.7*** **
	540	4.41	**123*** **

[1] Bolded (***) RQs exceed listed species and non-listed species LOCs.

5.1.2.c. Risks from Consumption of Warfarin-contaminated Carcasses (based on acute oral dose toxicity)

To determine secondary exposure EECs, available literature concerning carcass residue concentrations were considered. A previous assessment (USEPA 2004b) compiled available mammalian residue data. No bird or reptile residue data for primary consumption of warfarin were located. For this assessment, a carcass concentration of 2.95 mg a.i./kg-carcass (Townsend *et al*., 1984) was used to estimate exposure. Expected warfarin intake (mg a.i./kg-bw/day) was calculated in **Table 3-6**. As in the direct consumption risk estimation, the LD_{50}'s are adjusted for the weight of the assessed mammals (50 and 1000 g). These heavier weights are used to better represent carnivores and scavengers which tend to be larger individuals. The acute risk to listed species LOC is exceeded for all mammal weight classes evaluated (**Table 5-9**).

Table 5-9 Carnivorous/Scavenger Mammal Acute Dose-based RQs based on a Single-dose acute oral exposure to Warfarin, consumption of contaminated carcasses				
Species or Taxa	**Weight (g)**	**Warfarin intake (mg a.i./kg-bw/day)[1]**	**Adjusted LD_{50} (mg a.i./kg-bw)[2]**	**Acute Dose-based RQ[3]**
Mammals	50	1.08	4.10	**0.26****
	1000	0.63	1.94	**0.33****
[1] See **Table 3-5** and **3-6** for derivation. [2] See **Table 5-7** for derivation. [3] Bolded (**) RQs exceed listed species LOCs.				

5.1.2.d. Risks from Consumption of Warfarin-contaminated Carcasses (based on dietary toxicity)

For toxic response elicited from the dietary exposure route extended over several days, exposure is measured as mg a.i./kg-carcass. Toxicity is measured by the LC_{50} obtained from the subacute dietary studies (5 days on treated diet) for birds and mammals. Mammalian RQs based on dietary studies are provided in **Table 5-10**; acute risk to listed and non-listed species LOCs are exceeded.

Table 5-10 Mammal Acute Dietary-based RQs based on a 5-day Exposure to Warfarin in the Diet, consumption of contaminated carcasses			
Species or Taxa	**Warfarin concentration in carcass (mg a.i./kg-carcass)**	**LC_{50} (mg a.i./kg-diet)**	**Dietary-based RQ[1]**
Mammals	3.0	4.41	**0.68*****
[1] Bolded (***) RQs exceed listed species and non-listed species LOCs.			

Based on the preceding analysis (Sections 5.1.2.a to 5.1.2.d), warfarin has the potential to directly affect the SMHM. Additionally, since the acute risk LOCs are exceeded, there is a potential for indirect effects to those listed species that rely on mammals during at least some portion of their life-cycle (*i.e.*, SMHM and AW).

5.1.4. Exposures in the Terrestrial Habitat: Terrestrial Plants

RQs were not calculated for terrestrial plants as toxicity data were not available. However, presumed direct risks to terrestrial plants are assumed to be negligible, given the mode of action and method of application for warfarin, the small application rate per placement (0.003 lb a.i./placement), and the lack of any reported incidents over several decades of use. Further, since warfarin is not sprayed directly onto plants, and because so little is expected to leach from the bait and then be available for plant uptake, exposure is expected to be minimal.

Based on this analysis, the potential for indirect effects to those listed species that rely on terrestrial plants during at least some portion of their life-cycle (*i.e.* SMHM and the AW) is considered low.

5.1.5. Primary Constituent Elements of Designated Critical Habitat for the AW

For warfarin use, the assessment endpoints for AW designated critical habitat PCEs involve the same endpoints as those being assessed relative to the potential for direct and indirect effects to the listed species 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.

5.2. 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 spatial extent of potential effects is discussed for each of the listed species including any potential overlap between areas where potential usage may result in LAA effects and areas where species are expected to occur (including any designated critical habitat). The final No Effect/May Affect determination is made after the spatial analysis is completed at the end of the risk description for each species. 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 warfarin'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 for AW, the Agency concludes a preliminary "may affect" determination for the FIFRA regulatory action regarding warfarin. A preliminary effects determination of "may effect" was made for both SMHM and AW and for the critical habitats of the AW. A summary of the risk estimation results are provided in **Table 5-11** for direct and indirect effects to the listed species assessed here and in **Table 5-12** for the PCEs of their designated critical habitat.

Table 5-11. Risk Estimation Summary for Warfarin - Direct and Indirect Effects

Taxa	LOC Exceedances (Yes/No)	Description of Results of Risk Estimation	Assessed Species Potentially Affected
Birds and Reptiles	Non-listed Species (Yes)	Acute LOCs exceeded and chronic risk presumed for primary toxicity (direct bait consumption). Chronic risk presumed for secondary toxicity (contaminated carcass consumption).	Indirect Effects: SMHM and AW
	Listed Species (Yes)	AW: Chronic risk presumed for consumption of contaminated prey	Direct Effects: AW
Mammals	Non-listed Species (Yes)	Acute LOCs exceeded and chronic risk presumed for primary toxicity (direct bait consumption). Acute LOCs exceeded and	Indirect Effects: SMHM and AW

Table 5-11. Risk Estimation Summary for Warfarin - Direct and Indirect Effects			
Taxa	**LOC Exceedances (Yes/No)**	**Description of Results of Risk Estimation**	**Assessed Species Potentially Affected**
		chronic risk presumed for secondary toxicity (contaminated carcass consumption).	
	Listed Species (Yes)	SMHM: Acute LOCs exceeded and chronic risk presumed for primary toxicity (direct bait consumption).	Direct Effects: SMHM
Terrestrial Invertebrates	Listed Species (Yes)	RQs not calculated; risk presumed	Direct/Indirect Effects: SMHM and AW
Terrestrial Plants – Monocots	Non-listed Species (No)	RQs not calculated, minimal risk based on mode of action, method of application, and lack of incidents	Indirect Effects: SMHM and AW
Terrestrial Plants - Dicots	Non-listed Species (No)	RQs not calculated, minimal risk based on mode of action, method of application, and lack of incidents	Indirect Effects: SMHM and AW

Table 5-12. Risk Estimation Summary for Warfarin – 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**
Birds, Reptiles, and Terrestrial-Phase Amphibians	Non-listed Species (Yes)	Acute LOCs exceeded and chronic risk presumed for primary toxicity (direct bait consumption). Chronic risk presumed for secondary toxicity (contaminated carcass consumption).	AW
	Listed Species (Yes)	AW: Chronic risk presumed for consumption of contaminated prey	
Mammals	Non-listed Species (Yes)	Acute LOCs exceeded and chronic risk presumed for primary toxicity (direct bait consumption). Acute LOCs exceeded and chronic risk presumed for secondary toxicity (contaminated carcass consumption).	AW
Terrestrial Invertebrates	Listed Species (Yes)	RQs not calculated; risk presumed	AW
Terrestrial Plants - Monocots	Non-listed Species[1] (No)	RQs not calculated, minimal risk based on mode of action, method of application, and lack of incidents	AW
Terrestrial Plants - Dicots	Non-listed Species[1] (No)	RQs not calculated, minimal risk based on mode of action, method of application, and lack of incidents	AW

Table 5-12. Risk Estimation Summary for Warfarin – 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**
[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.

A description of the risk and effects determination for each of the established assessment endpoints for the assessed species and their designated critical habitat is provided in **Sections 5.2.1** through **5.2.3**. The effects determination section for each listed species assessed will follow a similar pattern. Each will start with a discussion of the potential for direct effects, followed by a discussion of the potential for indirect effects. These discussions do not consider the spatial analysis. For those listed species that have designated critical habitat, the section will end with a discussion on the potential for modification to the critical habitat from the use of warfarin. Finally, a discussion of any potential overlap between areas of concern and the species (including any designated critical habitat) is presented. 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.

5.2.1. Salt-Marsh Harvest Mouse

5.2.1.a. Direct Effects

The SMHM is at risk from uses of warfarin. The labels include directions for spot-baiting; therefore, treated bait may be easily accessible and attractive to non-target mammals, including the SMHM, for consumption. Based on bait concentrations of 250 mg a.i./kg-bait and 540 mg a.i./kg-bait, acute dose-based RQs (9.33 to 20.2) for SMHM consuming a single-dose of warfarin in bait (**Table 5-7**) and the acute dietary-based RQ (56.7 to 123) for SMHM based on a 5-day dietary toxicity study (**Table 5-8**), the acute risk to listed species (LOC (0.1)) is exceeded. Therefore, there is potential for direct acute mortality to the SMHM through consumption of bait for a single day or over a several day period.

Incidents were reported in the United States (**Table 4-5**) of non-carnivorous/non-scavenger mammals that died from warfarin poisoning. Squirrels are the only documented rodent species having an incident report, and a liver tissue residue analysis detected warfarin at 0.22 µg/g. Confirmed incidents of warfarin poisoning under the currently registered labels corroborates the conclusions of the risk estimation process that identified primary and secondary toxicity risks based on the current label rates.

The Agency uses the probit dose-response relationship as a tool for providing additional information on the potential for acute direct effects to individual listed species and aquatic animals that may indirectly affect the listed species of concern (USEPA 2004a). For these calculations, the highest RQs for SMHM from the primary exposure to bait (direct consumption) were used. The probability of an individual effect to SMHM was calculated based on an acute oral study with a default slope of 4.5 and a subacute dietary toxicity study with a default slope of 4.5.

For the acute oral exposure and baits containing 250 mg a.i./kg-bait, the corresponding estimated chance of an individual acute mortality to the SMHM at an RQ of 9.33 is 1 in 1.0 (**Table 5-13**). For the acute oral exposure and baits containing 540 mg a.i./kg-bait, the corresponding estimated chance of an individual acute mortality to the SMHM at an RQ of 20.2 is 1 in 1.0 (**Table 5-13**).

For the dietary exposure and baits containing 250 mg a.i./kg-bait, the corresponding estimated chance of an individual acute mortality to the SMHM at an RQ of 56.7 is 1 in 1.0 (**Table 5-13**). For the dietary exposure and baits containing 540 mg a.i./kg-bait, the corresponding estimated chance of an individual acute mortality to the SMHM at an RQ of 123 is 1 in 1.0 (**Table 5-13**).

Using a weight-of-evidence approach, the following information was considered to conclude that there **is** a potential for direct effects to the SMHM from the registered uses of warfarin:

- the acute risk to listed species LOCs are exceeded for the SMHM based on consumption of bait (both concentrations of 250 mg a.i./kg-bait and 540 mg a.i./kg-bait); since no chronic data are available to discount risk, chronic risk is presumed;
- the likelihood of an individual effect (mortality) is approximately 1 in 1.0 using acute dose- and dietary-based RQ values,

- mortality through primary exposure to warfarin was documented through low LD_{50} and LC_{50} values obtained in toxicity studies and mortality of the rodents when fed warfarin for the secondary exposure trials, and
- reported incidents for small non-target herbivorous mammals in the EIIS database.

5.2.1.b. Indirect Effects

Indirect effects on the SMHM may occur through the potential for warfarin impacts on birds and mammals that provide rearing sites and terrestrial invertebrates that are prey of the SMHM. Indirect effects on the SMHM are not expected from terrestrial plants that provide food and habitat and aquatic plants that provide habitat, as risks to these taxa from warfarin are considered low.

For birds and mammals consuming bait directly and/or consuming warfarin-contaminated carcasses, acute LOCs are exceeded and chronic risk is presumed. With the exception of an acute avian oral dose exposure, the probability of an individual mortality occurrence is very high for both birds and mammals (**Tables 5-13 and 5-14**), thus it is considered likely that the availability of burrows for SMHM use may decrease due to reductions in the populations of other mammals and birds. Reported incidents for a variety of birds and mammals also provide evidence of the likelihood of mortality for animals providing burrows and rearing sites for the SMHM.

Table 5-13 Probit Dose-Response Analysis for Mammals

Warfarin (250 mg a.i./kg-bait exposure)		
Taxa (study type)[1]	**Acute Effect Slope (95% C.I.)**	**Chance of Individual Effect at Derived Acute RQ (95% C.I.)**
Mammal oral dose (max RQ = 9.33)	Mortality Slope = 4.5 (default)	1 in 1
Mammal dietary (max RQ = 56.7)	Mortality Slope = 4.5 (default)	1 in 1
Warfarin (540 mg a.i./kg-bait exposure)		
Taxa (study type)[1]	**Acute Effect**	**Chance of Individual Effect at Derived Acute RQ (95% C.I.)**
Mammal oral dose (max RQ = 20.2)	Mortality Slope = 4.5 (default)	1 in 1
Mammal dietary (max RQ = 123)	Mortality Slope = 4.5 (default)	1 in 1

[1] Reported RQs are the maximum values based on primary exposure (bait consumption).

Table 5-14 Probit Dose-Response Analysis for Birds		
Warfarin (Secondary toxicity; single oral dose)		
Taxa (study type)	**Acute Effect Slope (95% C.I.)**	**Chance of Individual Effect at Derived Acute RQ (95% C.I.)**
Bird oral dose (max RQ = 9.33)	Mortality Slope = 4.5 (default)	1 in 1
Warfarin (Secondary toxicity; 5-day exposure to warfarin in the diet)		
Taxa (study type)	**Acute Effect**	**Chance of Individual Effect at Derived Acute RQ (95% C.I.)**
Bird dietary (max RQ = 123)	Mortality Slope = 0.811 (-0.06,1.68)	1 in 19 (1 in 1.83, 1 in 2,570)

RQs were not calculated for terrestrial and aquatic plants as toxicity data were not available. EFED presumed direct risks were low to terrestrial and aquatic plants given the mode of action of warfarin, the very low application rates (maximum of 0.003 a.i./placement), and the lack of any reported incidents. Since warfarin is not sprayed directly onto plants, and because so little is expected to leach from the bait and then be available for plant uptake, exposure is expected to be minimal. Based on this analysis, the potential for indirect effects to the SMHM due to impacts to terrestrial and aquatic plants is considered low.

5.2.1.c. Spatial Extent of Potential Effects

Generally, the Agency conducts analysis of the spatial extent of potential LAA effects whenever LOCs are exceeded. This analysis typically is needed to determine where adverse effects may occur in relation to the treated site. This spatial analysis typically determines if the potential area of usage, and the subsequent Potential Area of LAA Effects, overlaps with areas of occurrence and/or critical habitat of the species. However, because warfarin is a vertebrate pest control agent that may be used in a wide variety of urban and non-urban areas, the spatial extent of warfarin cannot be limited to defined areas. The Agency assumes that warfarin potentially may be used in any area of the state and that that use may occur in any of the land use categories that are identified in the National Land Cover Database (NLCD). Therefore, a spatial analysis was not conducted to identify this overlap. All areas where the SMHM occurs are assumed to lie within the potential use area of warfarin.

5.2.1.d. Effects Determination

The results of this risk assessment indicates that use of warfarin in baits for vertebrate pest control poses a risk of direct effects to the SMHM resulting from acute and presumed chronic toxicity. This species has the potential to come into contact with warfarin bait placed for control of a variety of rodent species. Ingestion of this bait is likely to be lethal to the SMHM. Even if the ingested dose of warfarin is not lethal, sublethal behavioral and neurological effects may adversely affect the survival of individuals of this species. Finally, some risk of indirect effects is possible because use of warfarin may reduce the abundance of small mammals and birds, which may reduce the availability of nest sites. Therefore, the Agency makes **"may affect"** and **"likely to adversely affect**" determinations for the SMHM based on the potential for direct and indirect effects to this species.

5.2.2. Alameda Whipsnake

5.2.2.a. Direct Effects

The AW is at risk from uses of warfarin. The AW can be secondarily exposed to warfarin through consumption of warfarin-contaminated prey. There were no acute risk to listed species LOC exceedances (see Tables 5-4 and 5-5; acute RQs ranged from <0.01 to 0.01). The LD_{50} and LC_{50} used to calculate the RQs were based on granivorous species. These species prefer to eat a relatively consistent quantity of food on a daily basis. Carnivores and scavengers may consume a large meal (an entire carcass) once every several days. Given this feeding strategy, the AW may get a larger single-dose exposure of warfarin than is currently estimated in the risk quotient methodology. Chronic risk of direct effects from uses of warfarin to the AW is presumed due to lack of chronic toxicity data for warfarin.

In addition to the avian toxicity data used to calculate RQs, data were available from two studies in which carnivorous birds (surrogates for reptiles) were fed prey that had been poisoned with warfarin (**Table 4-3**). Though no mortalities were reported, warfarin residues were detected in the four tissues examined in tawny owls and there was a decrease in plasma prothrombin levels, a blood plasma protein that plays a critical role in the final stages of blood coagulation. No other signs of toxicity were reported. If these birds were exposed to warfarin under natural conditions, these signs of reduced fitness combined with natural stressors could potentially reduce fitness of the affected individuals and, thus, make them more prone to mortality/predation.

The Agency uses the probit dose-response relationship as a tool for providing additional information on the potential for acute direct effects to individual listed species and aquatic animals that may indirectly affect the listed species of concern (USEPA 2004a). For these calculations, the highest RQs for AW from the secondary exposure to warfarin (carcass consumption) were used. The probability of an individual effect to AW from consumption of contaminated carcasses was calculated based on an acute oral study with a default slope of 4.5 and a dietary study with a probit slope of 0.811 with 95% confidence bounds of (-0.06, 1.68) (**Table 5-14**). For the acute oral exposure, the corresponding estimated chance of an individual acute mortality to the AW at an RQ of 0.01 is 1 in 8.86E+18. For the dietary exposure, the corresponding estimated chance of an individual acute mortality to the AW at an RQ of 0.01 is roughly 1 in 19 (with respective upper and lower bounds of 1 in 1.83 to 1 in 2,570). It is noted that the probit slope value used to calculate probabilities for dietary exposure to birds is low, which is indicative of a weak dose-response relationship.

Using a weight-of-evidence approach, the following pieces of evidence were weighed:

- Chronic risk to the AW through consumption of warfarin-contaminated prey is presumed due to the absence of data,
- the likelihood of an individual effect (mortality) resulting from secondary exposure is not probable (based on acute oral and dietary secondary toxicity studies), with probabilities of 1 in 8.86E+18 and 1 in 19, respectively, and
- incidents involving predatory birds (surrogates for reptiles) demonstrate that secondary toxicity is possible.

From these lines of evidence, there **is** a potential for direct effects to the AW from the registered uses of warfarin.

5.2.2.b. Indirect Effects

Indirect effects on the AW may occur through the potential for warfarin impacts on birds (which are surrogates for reptiles), mammals, reptiles, terrestrial-phase amphibians, and terrestrial invertebrates that are prey of the AW. Mammals also provide habitat (burrows). The likelihood of indirect effects on the AW from adverse effects on terrestrial plants that provide habitat is considered low.

For organisms consuming bait directly (SMHM) or consuming warfarin-contaminated carcasses (AW), acute risk LOCs are exceeded and chronic risk is presumed. With the exception of avian acute oral exposure, the probability of an individual mortality occurrence exceeds 30% for both birds and mammals (**Table 5-13**), thus there is a high likelihood that the availability of prey for AW use may decrease due to reductions in the populations of other reptiles and mammals. Reported incidents for a variety of birds and mammals also provide evidence to the likelihood of mortality for animals providing food sources for the AW.

5.2.2.c. Spatial Extent of Potential Effects

Generally, the Agency conducts analysis of the spatial extent of potential LAA effects whenever LOCs are exceeded. This analysis typically is needed to determine where adverse effects may occur in relation to the treated site. This spatial analysis typically determines if the potential area of usage, and the subsequent Potential Area of LAA Effects, overlaps with areas of occurrence and/or critical habitat of the species. However, because warfarin is a vertebrate pest control agent that may be used in a wide variety of urban and non-urban areas, the spatial extent of warfarin cannot be limited to defined areas. The Agency assumes that warfarin potentially may be used in any area of the state and that that use may occur in any of the land use categories that are identified in the NLCD. Therefore, a spatial analysis was not conducted to identify this overlap. All areas where the AW occurs are assumed to lie within the potential use area of warfarin.

5.2.2.d. Modification of designated critical habitat

Critical habitat has been defined for the AW. As discussed above, the potential for warfarin use to adversely modify the critical habitat of the AW stems primarily from reduction of prey species and potential reduction of small mammal burrows. Use of rodenticide bait has the potential to adversely affect the abundance of small mammals and other vertebrates within the critical habitat. Since the AW prey on small mammals (along with other types of terrestrial vertebrates and invertebrates), adverse effects on these communities could adversely affect the habitat by reducing the abundance of prey. Birds, reptiles, terrestrial-phase amphibians, and terrestrial arthropods are also prey of the AW. Reductions in the abundance of these types of prey are also possible, although less certain because the likelihood that these types of animals would consume bait designed for rodents and moles is uncertain. In addition to prey effects, the AW makes use of small mammal burrows for refuge and foraging. Therefore, reduction of small mammal

abundance could adversely affect the critical habitat by reducing the availability of this important habitat resource.

As discussed in **Section 5.1.5**, use of warfarin in bait products to is not expected to result in significant effects on plants. Due to the mode of action of warfarin, effects to plants are not expected.

5.2.2.e. Effects Determination

The results of this risk assessment indicates that use of warfarin in baits for vertebrate pest control poses a risk of chronic toxicity to the AW resulting from secondary exposure. Further risk to this species would be posed by sublethal behavioral and neurological effects which may result from acute or chronic exposure to warfarin. Finally, indirect effects are also possible from use of this product reducing the abundance of vertebrate and invertebrate prey, and possibly reducing the availability of small mammal burrows. Therefore, the Agency makes **"may affect"** and **"likely to adversely affect**" determinations for the AW, and a **habitat modification determination** for its designated critical habitat, based on the potential for direct and indirect effects and effects to the PCEs of critical habitat.

5.2.3. Addressing the Risk Hypotheses

In order to conclude this risk assessment, it is necessary to address the risk hypotheses defined in **Section 2.5.1**. Based on the conclusions of this assessment, many of the hypotheses cannot be rejected, meaning that the stated hypotheses represent concerns in terms of direct and indirect effects of warfarin on the AW and SMHM and the designated critical habitat of the AW.

The labeled use of warfarin within the action area may:

- directly affect AW and SMHM by causing mortality or by adversely affecting growth or fecundity;
- indirectly affect AW and SMHM and/or modify AW designated critical habitat by reducing or changing the composition of food supply;
- indirectly affect SMHM and AW and/or modify AW designated critical habitat by reducing or changing terrestrial habitat in their current range (via reduction in small burrowing mammals leading to reduction in underground refugia/cover).

The risk assessment did indicate that three of the risk hypothesis may be rejected. The hypotheses which were rejected are that use of warfarin may:

- indirectly affect SMHM 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 SMHM by reducing or changing aquatic habitat in their current range (via modification of water quality parameters, habitat morphology, and/or sedimentation);
- indirectly affect AW and SMHM and/or modify AW designated critical habitat by reducing or changing the composition of the terrestrial plant community in the species' current range.

6. Uncertainties

6.1. Fate and Transport Properties

Since the data used in this assessment were derived from open literature and were not based on USEPA-guideline studies, warfarin's persistence and mobility have not been fully characterized. However, due to the limited use and the low environmental exposure of warfarin, no data gaps have been identified at this time. Should the use rate increase significantly or if a new use is proposed in the future, guideline studies may be necessary for a better understanding of warfarin's persistence and mobility.

6.2. Effects

No data are currently available to assess the reproductive/chronic toxicity of warfarin to birds and mammals.

Assumptions used in this assessment which create or address effects uncertainties are listed below:

- The assumption of 10% water is a source of uncertainty regarding the amount of food consumed,
- the assumption that the body burden residue levels are the same for birds, terrestrial phase amphibians and other reptiles as determined in mice by Townsend *et al.* (i.e., warfarin is metabolized similarly across taxa) is an uncertainty, and
- because of the lack of data on warfarin residues in birds, terrestrial-phase amphibians, and reptiles, it was assumed the mammalian body burden data would be relevant for all the evaluated taxonomic groups.

6.2.1. Use of Surrogate Species Effects Data

Guideline toxicity tests and open literature data on warfarin are not available for terrestrial-phase amphibians or reptiles; therefore, birds are used as surrogate species for assessing effects to the AW. Endpoints based on avian ecotoxicity data are assumed to be protective of potential direct effects to reptiles including the AW, and extrapolation of the risk conclusions from the most sensitive tested species to the AW may overestimate the potential risks to that 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.

6.2.2. Sublethal Effects

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

effect (sublethal endpoint) and the assessment endpoints. However, the full suite of sublethal effects from valid open literature studies is considered for the characterization purposes. While no chronic toxicity data are available for birds and mammals, these taxa exhibited a range of sublethal effects including subdermal hematomas (hemorrhage), clotting abnormalities, and liver discoloration discovered during necropsy in the acute and sub-acute data submitted to the Agency.

6.3. Data Gaps

Data gaps were assigned a low or high potential to add value to the ecological risk assessment. High potential studies will allow for better characterization of potential risks by eliminating uncertainties for both non-listed and listed species that cannot be accounted for using alternate methods or weights of evidence (*i.e.*, acute-to-chronic ratio, scaling factors, or consideration of environmentally relevant concentrations relative to effects thresholds). Low potential studies are unlikely to change risk determinations because alternate methods and weights of evidence may possibly be used in the absence of data.

Although the data used in this assessment were derived from open literature, due to the limited use and the low environmental exposure of warfarin, no data gaps have been identified at this time. However, should the use rate increase significantly or if a new use is proposed, guideline studies may be necessary for a better understanding of warfarin's persistence and mobility.

Data gaps are listed below; details for each data gap are discussed in **Section 2.9.4.** Studies with a **high potential** to add value to the risk assessment are:

- *Avian Reproduction Study (850.2300)*
- *Avian Acute Oral Toxicity Test (850.2100), one passerine species.*

7. Risk Conclusions

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

Based on the best available information, the Agency makes a **May Affect, and Likely to Adversely Affect** determination for the SMHM and AW from all uses of warfarin. Additionally, the Agency has determined that there is the potential for modification of designated critical habitat for the AW from the use of warfarin. A summary of the risk conclusions and effects determinations for each listed species assessed here and their designated critical habitat is presented in **Table 7-1** and in **Table 7-2**. Use-specific determinations are provided in **Table 7-3**. Given the LAA determination for the SMHM and AW and potential modification of designated critical habitat for the AW, a description of the baseline status and cumulative effects for the SMHM and AW is provided in **Attachment II**.

Table 7-1. Effects Determination Summary for Effects of Warfarin on the SMHM and AW.		
Species	**Effects Determination**	**Basis for Determination**
Salt marsh harvest mouse (SMHM) (*Reithrodontomys raviventris*)	May Affect, Likely to Adversely Affect (LAA)	**Potential for Direct Effects** Risk assessment indicates use of warfarin will potentially result in direct effects to the SMHM from acute and chronic toxicity. Lines of evidence include: • the listed species LOCs are exceeded, and chronic risk is presumed, for the SMHM based on consumption of bait , • the likelihood of an individual effect (mortality) is approximately 1 in 1.0 based on acute oral and dietary exposure, • mortality through primary exposure to warfarin was documented through low LD_{50} and LC_{50} values obtained in toxicity studies and mortality of the rodents when fed warfarin for the secondary exposure trials, and • reported incidents of mortality of small non-target herbivorous mammals after consumption of warfarin bait. **Potential for Indirect Effects** ***Food sources*** Risk assessment presumes vegetative food sources are not expected to be affected by warfarin application. Due to the very low application rates (maximum of 0.003 lb a.i./placement), the mode of action, and the lack of plant incidents for warfarin, vegetative food sources are not expected to be affected by warfarin application. Risk to terrestrial invertebrate prey populations was presumed because toxicity data were not available. ***Habitat Modifications*** Adverse effects to birds and mammals may result in a reduction of abandoned bird and mammal nests, which are used as nest sites by the SMHM. Acute RQs for birds and mammals, and acute and chronic RQs for mammals, exceed LOCs. Therefore, use of warfarin may modify the habitat of the SMHM by reducing the availability of nest sites.
Alameda Whipsnake (AW) (*Masticophis lateralis euryxanthus*)	May Affect, Likely to Adversely Affect (LAA)	**Potential for Direct Effects** This risk assessment indicates use of warfarin will potentially result in direct effects to the AW from secondary exposure and toxicity. This is corroborated by nine reported incidents of bird mortality. Birds serve as surrogates of terrestrial phase amphibians. **Potential for Indirect Effects** ***Food sources*** Risk assessment indicates terrestrial vertebrate and invertebrate prey populations may be reduced. For birds and mammals consuming bait directly, acute risk LOCs are exceeded and chronic risk is presumed. It is probable that the availability of prey for AW may decrease due to reductions in the populations of birds and mammals. Reported incidents for a variety of birds and mammals also provide evidence of mortality of potential prey animals exposed to warfarin. ***Habitat Modifications*** Risk assessment indicates use of warfarin may modify the habitat of this species by reducing the availability of burrows. This conclusion is based on acute RQs for mammals that exceed the LOC. Adverse effects to mammals may result in a reduction of available burrows, which are used as shelters by this species.

Table 7-2. Effects Determination Summary for the Critical Habitat Impact Analysis		
Designated Critical Habitat for:	**Effects Determination**	**Basis for Determination**
Alameda whipsnake (*Masticophis lateralis euryxanthus*)	Habitat Modification	This risk assessment indicates use of warfarin may modify the critical habitat of this species by reducing the availability of small mammal burrows. This may result in modification of PCE 3: "Lands containing rock outcrops, talus, and small mammal burrows within or adjacent to PCE 1 and or PCE 2." In addition, the availability of prey may be reduced in the critical habitat by toxicity to small birds, mammals, reptiles, and terrestrial-phase amphibians.

Table 7-3. Use Specific Summary of The Potential for Adverse Effects to Terrestrial Taxa							
Uses	**Potential for Effects to Identified Taxa Found in the Terrestrial Environment:**						
	SMHM and Small Mammals[1]		**Small Birds[2,3]**		**Invertebrates (Acute)[4]**	**Dicots[5]**	**Monocots[5]**
	Acute	**Chronic**	**Acute**	**Chronic**			
0.025% a.i. bait (spot treatment, bait station, gel)	Yes	Yes	Yes	Yes	Yes	No	No
0.054% a.i. bait (spot treatment, boxed bait)	Yes	Yes	Yes	Yes	Yes	No	No

[1] A yes in this column indicates a potential for direct effects to SMHM and indirect effects to SMHM and AW.
[2] A yes in this column indicates a potential for indirect effects to the SMHM and AW.
[3] Birds are considered a surrogate for terrestrial phase amphibians and reptiles including the AW.
[4] A yes in this column indicates a potential for indirect effects to SMHM and AW.
[5] A yes in this column indicates a potential for indirect effects to SMHM.

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

When evaluating the significance of this risk assessment's direct/indirect and 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 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 SMHM and AW 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.

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