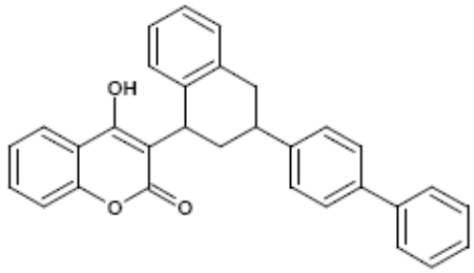
Risks of Difenacoum Use to the Federally Threatened Alameda Whipsnake (*Masticophis lateralis euryxanthus*), the Federally Endangered Salt Marsh Harvest Mouse (*Reithrodontomys raviventris*), and the Federally Endangered San Joaquin Kit Fox (*Vulpes macrotis mutica*)



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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), the federally endangered salt marsh harvest mouse (*Reithrodontomys raviventris*; SMHM), and the federally endangered San Joaquin kit fox (*Vulpes macrotis mutica*; SJKF) arising from FIFRA regulatory actions regarding use of the rodenticide difenacoum 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 three 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 difenacoum was alleged to be of concern for the AW, SMHM and SJKF (*Center for Biological Diversity (CBD) vs. EPA et al*. (Case No. 07-2794-JCS).)

In this assessment, direct and indirect effects to the AW, SMHM, and SJKF 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.
- **San Joaquin Kit Fox (SJKF):** The SJKF was listed by the USFWS as an endangered species in 1967. The species is found in a variety of habitats in the Central Valley area of California. Critical habitat has not been designated for the SJKF; therefore, PCEs have not been defined.

1.2. Scope of Assessment

1.2.1. Uses Assessed

Difenacoum is a second generation anticoagulant rodenticide used for the control of nuisance mammals in areas such as buildings (in and around; indoor and outdoor), transport vehicles, and airports. Formulation types include: bait pellets, bait blocks, and place packs. There are 3 currently registered labels that are relevant for use in California. All difenacoum end use product

formulations are bait formulations that when placed outdoors must be in bait stations and within 50 feet of a building. The target pests include: Norway rat (*Rattus norvegicus*), black rat (*R. rattus*, also known as roof rat) and house mouse (*Mus musculus*). Concentrations of all formulations are 0.005% difenacoum.

1.2.2. Environmental Fate Properties of Difenacoum

Difenacoum ($C_{31}H_{24}O_3$) has low solubility and low vapor pressure and is mobile to highly mobile in alkaline environments but immobile in acidic environments. Difenacoum is estimated to be hardly mobile in environmentally relevant conditions. Although it degrades quickly photolytically, difenacoum appears to be stable under anaerobic conditions and is very persistent to aerobic degradation. Hydrolytic degradation is pH-dependant; it is stable at low pH, very persistent at neutral pH, and more prone to hydrolysis at high pH. Thus, in the absence of direct exposure to sunlight, difenacoum may persist for long periods, and potentially accumulate in areas where there are repeated applications. However, the mobility of this chemical is limited by its low solubility, low vapor pressure, and sorption characteristics (which tend to inhibit desorption from bait formulations and leaching into groundwater). It is assumed that negligible difenacoum residue is desorbed from these formulations except under alkaline conditions in direct sunlight.

Degradation products were not identified or evaluated in studies because individual transformation products never exceeded 10% of the active substance added. Therefore, no degradate information is presented in this document.

1.3. Exposure Assessment

1.3.1. Aquatic Exposure

A previous assessment (USEPA, 2007) modeled aquatic exposure of difenacoum for this use and found that negligible concentrations of difenacoum could occur in the water column relative to the toxicity to aquatic organisms. Model results indicated a peak concentration of 0.143 parts per billion in surface waters for this use. This result is driven by difenacoum's immobility within its bait formulations. The most sensitive aquatic endpoint is an LC_{50} of 64 parts per billion for the rainbow trout (*Oncorhynchus mykiss*). This endpoint is more than two orders of magnitude greater than the EEC. Additionally, outdoor bait is placed in bait stations with a restriction of being within 50 feet from buildings and spray drift is not expected for this chemical. Given these factors, aquatic exposures are assumed to be negligible and they are not assessed.

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 exposure), or animals (predators/scavengers) may consume contaminated carcasses (secondary exposure). Primary

exposure of difenacoum was modeled using bait containing 0.005% difenacoum. Ingestion was modeled both on a dose basis (mg a.i./kg-bw) and a dietary basis (mg a.i./kg-diet; for birds only).

Secondary exposure was modeled by assuming the AW and SJKF consume prey species that directly consumed baits of various types (*e.g.*, pellets, blocks) which are defined as direct effects (through secondary exposure). This assumption was made on the basis of snakes rarely consuming anything but live prey and the fact that all placements of difenacoum are required to be in bait stations, which would preclude primary exposure to the SJKF.

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 the SMHM or the SJKF. Primary constituent elements (PCEs) were used to evaluate whether difenacoum has the potential to modify designated critical habitat for AW.

The Agency evaluated registrant-submitted studies and data from the open literature to characterize difenacoum 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 difenacoum. 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.

Primary Toxicity

Section 4 summarizes the ecotoxicity data available on difenacoum. Difenacoum is considered moderately toxic to birds based on an acute oral basis (bobwhite quail LD_{50} = 67 mg a.i./kg bw, MRID 46750922) and considered very highly toxic to birds based on a subacute dietary exposure basis (mallard LC_{50} =14.1 mg a.i./kg diet, MRID 46750926). No data are available to characterize chronic toxicity to birds. The 2007 assessment discussed an unreviewed chronic avian reproduction study (MRID 46799101) using Japanese quail that indicated no effect at any concentration tested (up to 0.1 ppm a.i.). EFED has since reviewed this study and found it to be unacceptable due to high and uneven variability, poor housing and environmental conditions of test animals, excessive control mortality and abnormal hatchling development. Japanese quail are also not a recommended species for this test guideline.

Difenacoum is very highly acutely toxic to mammals based on the most sensitive available LD_{50} (1.8 mg a.i./kg-bw for male rats, 46750935). No acceptable acute dietary or chronic mammalian data are available for difenacoum.

Secondary Toxicity

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 hemorrhaging, lethargy and anorexia. In the reviewed carnivorous mammal studies, signs of anticoagulant poisoning and mortality were observed.

1.4.2. Plants

No toxicity data are available for terrestrial plants. Due to the very low application rates (maximum of 0.00005 lb a.i./placement), and the anticoagulant mode of action, vegetative food sources are not expected to be affected by difenacoum application.

1.5. Measures of Risk

Acute risk quotients (RQs) are compared to the Agency's Levels of Concern (LOCs) to identify instances where difenacoum 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 difenacoum 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, SJKF and AW from the use of difenacoum. Additionally, the Agency has determined that there is the potential for modification of designated critical habitat for the AW from the use of difenacoum. 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, SJKF 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 Difenacoum on the SMHM and AW.

Species	Effects Determination	Basis for Determination
Salt Marsh Harvest Mouse (SMHM)	May Affect, Likely to Adversely	**Potential for Direct Effects**
		Use of difenacoum may result in direct effects to the SMHM from acute toxicity via primary exposure. Exposure estimates and acute toxicity to

Table 1-1. Effects Determination Summary for Effects of Difenacoum on the SMHM and AW.		
Species	**Effects Determination**	**Basis for Determination**
(*Reithyodontomys raviventris*)	Affect (LAA)	mammals result in acute RQs that exceed the LOCs for primary exposure to the SMHM. Primary exposure is considered the primary threat to this species. The individual probability of death is 100% for a SMHM consuming one day's food intake of difenacoum bait. Data were not available to assess chronic toxicity, however since risk is predicted for acute exposure based on mortality, and sublethal effects were observed, risk of chronic effects is also assumed. Furthermore, incidents in the United Kingdom involving small mammals have been reported in association with the use of difenacoum.
		Potential for Indirect Effects
		Terrestrial Habitat Registered uses of difenacoum may reduce SMHM rearing sites by adversely affecting small mammals that create burrows used by the SMHM. Estimated acute RQs for primary exposure to mammals exceeded acute LOCs for the small mammalian weight class considered.
Alameda Whipsnake (AW) (*Masticophis lateralis euryxanthus*)	May Affect, Likely to Adversely Affect (LAA)	**Potential for Direct Effects**
		Use of difenacoum potentially may result in direct effects to the AW from acute toxicity through secondary exposure. Dietary exposure estimates and acute toxicity to reptiles (based on acute toxicity data for birds) result in acute RQs that exceed the endangered species LOC for secondary exposure. Secondary exposure is considered the primary exposure pathway for this species. The individual probability of death is 16% and 6% for AW that ingested mice and rats, respectively who have consumed difenacoum bait for one day. Data were not available to assess chronic toxicity; however, since risk is predicted for acute exposure based on mortality, and sublethal effects were observed with sublethal acute exposure, risk of chronic effects is also assumed.
		Potential for Indirect Effects
		Food sources 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. Furthermore, incidents in the United Kingdom involving mammals and birds have been reported in association with the use of difenacoum. ***Habitat Modifications*** Use of difenacoum 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 difenacoum to small birds, mammals, and terrestrial-phase amphibians.
San Joaquin Kit Fox (SJKF) (*Vulpes macrotis mutica)*	May Affect, Likely to Adversely Affect (LAA)	**Potential for Direct Effects**
		Use of difenacoum potentially may result in direct effects to the SJKF from acute toxicity via secondary exposure. Secondary exposure is considered the primary threat to this species. The individual probability of death is 83% and 100% for a SJKF consuming a small mammal and a

Table 1-1. Effects Determination Summary for Effects of Difenacoum on the SMHM and AW.		
Species	**Effects Determination**	**Basis for Determination**
		244-g bird respectively, that have consumed difenacoum bait for one day. Data were not available to assess chronic toxicity; however, since risk is predicted for acute exposure based on mortality, and sublethal effects were observed with sublethal acute exposure and in secondary feeding studies, risk of chronic effects is also assumed. Furthermore, incidents in Europe involving mammals have been reported in association with the use of difenacoum.
		Potential for Indirect Effects
		Terrestrial prey items Use of difenacoum may reduce the abundance of terrestrial vertebrates which serve as prey for this species. Data were not available to assess chronic toxicity; however, since risk is predicted for acute exposure based on mortality, and sublethal effects were observed with sublethal acute exposure, risk of chronic effects is also assumed. Furthermore, incidents in Europe involving mammals and birds have been reported in association with the use of difenacoum.

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	Use of difenacoum 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 by Taxa.

Uses	Potential for Effects to Identified Taxa Found in the Terrestrial Environment[7]									
	SMHM and Small Mammals[1]		SJKF and Large Mammals[2]		AW and Reptiles[3]		Small Birds[4]		Terrestrial – phase Amphibians[5]	
	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic
Rodent Control	Yes	Yes[6]	Yes	Yes[6]	Yes	Yes[6]	Yes	Yes[6]	Yes	Yes[6]

1 A yes in this column indicates a potential for direct effects to the SMHM and indirect effects to the AW and SJKF.
2 A yes in this column indicates a potential for direct and indirect effects to SJKF.
3 A yes in this column indicates the potential for direct (through secondary exposure) to the AW and indirect (through prey reduction) effects to the AW.
4 A yes in this column indicates a potential for direct effects to the AW and SJKF.
5 A yes in this column indicates a potential for indirect effects to the AW.
6 Chronic toxicity data are not available to assess this species; however chronic risk is assumed based upon the high acute risks.

7 Terrestrial invertebrates and terrestrial plants, which have the potential to indirectly affect all three species were not assessed. In addition, aquatic plants which have the potential to indirectly affect the SMHM, were also not assessed because exposure is unlikely due to the use pattern..

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*, SJKF *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), the federally endangered salt marsh harvest mouse (SMHM) and the federally endangered San Joaquin Kit Fox (SJKF) arising from FIFRA regulatory actions regarding use of difenacoum 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, SMHM, and SJKF 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 difenacoum 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 difenacoum 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, SMHM, and SJKF 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 difenacoum 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 difenacoum in accordance with the approved product labels is "the action" relevant to this ecological risk assessment.

Difenacoum is an anticoagulant rodenticide used for the control of commensal rodents in non-residential buildings (in and around; indoor and outdoor within 50 feet of building) and transport vehicles.

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

2.2.1. Mechanism of Action

Difenacoum is a second-generation anticoagulant rodenticide. Anti-coagulant rodenticides act as vitamin-K antagonists that disrupt normal blood-clotting mechanisms as vitamin-K is depleted and induce capillary damage. Death results from hemorrhage, and exposed animals may exhibit increasing weakness prior to death. Behavior also may be affected (Cox and Smith, 1992).

Second-generation anticoagulants tend to be more acutely toxic than first-generation anticoagulants (*e.g.*, warfarin, chlorophacinone, diphacinone), and they are retained much longer in body tissues of primary consumers. They generally provide a lethal dose after a single feeding (first-generation anticoagulants can require multiple feedings); although death is usually delayed 5 to 10 days and animals continue feeding. Whole-body residues may increase as the chemical bioaccumulates with repeat feedings. Difenacoum is present as an unknown ratio of *cis* or *trans* isomers in typical end-use products.

2.2.2. Environmental Fate and Transport Properties

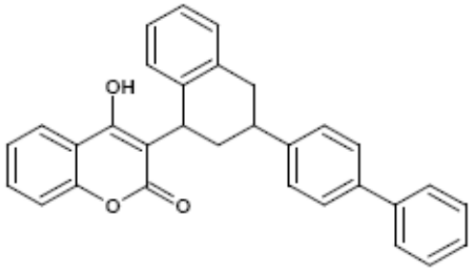
Difenacoum ($C_{31}H_{24}O_3$) has low solubility (~2 mg/L at pH 7)[1] and low vapor pressure (~2E-6 Pa m^3 mol^{-1} at pH 7)[1]. Difenacoum is mobile to highly mobile in alkaline environments (K_{OC} < 18 mL/ g o.c. at pH 8.46)[2] but immobile in acidic environments (K_{OC} > 400,000 mL/ g o.c. at pH 4.43)[2]. Difenacoum is estimated to be hardly mobile in neutral conditions (K_{OC} = 40,000 mL/ g

[1] Finnish Food Safety Authority. Difenacoum: Volume 3, Annex B: References relied on List of Tests and Studies. July 2007
[2] MRID 46750928

o.c.) [3] but increase in mobility in more alkaline conditions (K_{OC} < 17 at pH 8.46). The chemical structure of difenacoum is shown in **Fig. 2-1**.
Although difenacoum degrades quickly photolytically (aqueous photolysis half-life is about 8 hours at pH 7)[4], it appears to be stable under anaerobic conditions and is very persistent (aerobic soil half-life = 439 days)[4] to aerobic degradation. Hydrolytic degradation is pH-dependant; it is stable at low pH, very persistent ($t_{1/2}$ = 1000 days) at neutral pH, and more prone to hydrolysis at high pH ($t_{1/2}$ = 80 days)[4]. Thus, in the absence of direct exposure to sunlight, difenacoum may persist for long periods, and potentially accumulate in areas where there are repeated applications. However, the mobility of this chemical is limited by its low solubility (which hinders transport through runoff), low vapor pressure (minimizing the likelihood of aerial transport), and sorption characteristics (which tend to inhibit desorption from bait formulations and leaching into groundwater). All difenacoum end use product formulations are bait formulations. Though difenacoum may be applied in areas with high soil pH that may allow for degradation and chemical mobility, it is assumed the placement in bait stations prevents soil interaction that would cause degradation or mobility. It is assumed that negligible difenacoum residue is desorbed from these formulations.

Degradation products were not identified or evaluated in studies because individual transformation products never exceeded 10% of the active substance added. Further, the limited mobility of difenacoum in it's required bait station formulation when placed outdoors precludes exposure. Therefore, no degradate information is presented in this document.

Figure 2-1. Chemical Structure of Difenacoum



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).

[3] EpiSUITE estimate
[4] MRID 46750928

All difenacoum end use products are single active ingredient products though difenacoum residues have been found in necropsies of raptors in Britain in combination with other anti-coagulant rodenticides (Walker et al., 2010). However; this risk assessment focuses only on the toxicity of the active ingredient difenacoum since effects data are not available for the anticoagulant mixtures.

2.2.4. Summary of Previous Assessments on Difenacoum

The first and only proposed use for difenacoum in the United States was for control of Norway rats, roof rats and house mice. This use patter was considered by the Agency in 2007 (USEPA, 2007). The associated ecological risk assessment concluded that terrestrial exposure of bait pellets to non-target species and exposure of terrestrial non-target species to active ingredient in prey presents a high risk. It also concluded aquatic exposure may cause adverse effects to fish, aquatic invertebrates and aquatic plants when applied near waterbodies or in alkaline conditions, however all aquatic Risk Quotients (all RQs < 0.01) were below any Level of Concern (LOCs). EFED noted in that assessment that there was uncertainty around these RQs due to the unreviewed nature of the fate and effects studies. Finally, difenacoum may accumulate in soil with repeated applications under neutral and acidic conditons.

2.2.4.a Related Agency Actions

In 2004, an assessment of the risks of several rodenticides to terrestrial wildlife was conducted in the document, *Potential Risks of Nine Rodenticides to Birds and Nontarget Mammals: a Comparative Approach* (USEPA 2004b). This document did not evaluate difenacoum specifically, however it indicated that rodenticide baits are formulated to be lethal to small mammals, and they are not selective to nontarget species. The document stressed that all baits pose a high potential for primary risk to birds and nontarget mammals that eat bait. Avian and mammalian predators and scavengers are said to be at risk from feeding on animals poisoned with anticoagulant baits.

Anticoagulant rodenticides are generally divided into two classes, first generation and second generation anticoagulants. Both classes have the same mode of action but second generation anticoagulants (including difenacoum) are characterized by being more acutely toxic than first generation anticoagulants (*e.g.* warfarin) and have a significantly longer liver half life. The 2004 assessment indicated that second generation anticoagulants appear not to be readily metabolized and are mostly excreted through the feces. After absorption, high concentrations circulate in the blood and are rapidly established in the liver and other tissues. Overall, the available toxicokinetic data indicated that the second-generation anticoagulants are considerably more persistent in animal tissues than are the first-generation anticoagulants, and bioaccumulation may increase whole-body residues with repeat feedings.

In November 2011, the Agency issued background papers to the FIFRA Science Advisory Panel (SAP) on a Notice of Intent to Cancel (NOIC) non-RMD compliant rodenticide products. The meeting was held in November – December, 2011. Uses of difenacoum were not specifically considered by the SAP, since all difenacoum products are RMD compliant.

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 difenacoum 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.

Difenacoum labels may be categorized into two types: labels for manufacturing uses (including technical grade difenacoum) and end-use products. While technical products, which contain difenacoum 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 difenacoum's potential use to only those sites that are specified on the labels. It is important to note that the risk assessment estimates maximum daily bait consumption by primary consumers (and therefore difenacoum intake), such that the number of applications and the application interval did not factor into the assessment.

There are currently three active national EPA registrations for difenacoum end-use products (**Appendix D**). The basic types of use patterns for difenacoum are indoor and outdoor bait stations. All of the registrations listed in **Appendix D** meet the mitigation measures set forth in the Risk Management Decision (RMD, USEPA, 2008) which was issued in May 2008 and revised in June 2008. The three labels are not approved for homeowner use, have clauses mandating the use of bait stations[5], and stipulate that baiting must occur within 50 feet of buildings. The labeled maximum amount of difenacoum per placement is 0.00005 lbs a.i. and the labels indicate that difenacoum should be reapplied daily for at least 10 days or until signs of feeding by rats has ceased. This assessment considers all difenacoum products which are registered as of 27 March 2012.

2.2.5.b. Reported Usage Data

The Agency's Biological and Economic Analysis Division (BEAD) typically provides county-level usage information using California's Department of Pesticide Regulation Pesticide Use Reporting (CDPR PUR) database[6]. CDPR PUR is considered a more comprehensive source of usage data than USDA-NASS or EPA proprietary databases. Data from CDPR PUR are typically 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.[7]

[5] The mouse and rat pellet label (EPA registration number 47629-14) includes a statement that "Tamper resistant bait stations must be used for outdoor and indoor placements of this product **if** (*emphasis added*) children, pets, non-target mammals, or birds may access bait"

[6] 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.

[7] 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

The average number of pounds of difenacoum applied in California over the three year period was 0.0067 lbs per year, (CDPR, 2009, 2010, 2011). The lack of data on industrial and institutional uses constitutes a source of uncertainty since the CDPR PUR database does not account for this usage. Given that industrial and institutional use is presumed to be a major usage scenario, the PUR data likely underestimates total usage in California because it omits the most common use patterns for difenacoum. This makes the PUR usage data of limited utility for risk characterization.

Three use site types are listed in the CDPR PUR data, *i.e.*, structural pest control, animal premise, and landscape maintenance. The reported usage was less than 0.0001 lbs per year in 2008 and 0.01 lbs in 2009 and 2010. Of the 2010 data, 0.01 lbs were used in San Fransisco County and less than 0.01 lbs were used in Kings and San Mateo Counties. In the 2009 and 2008 data, there was no usage reported in the counties that are in the current range of the AW, SMHM and SJKF.

2.3. Assessed Species

Table 2-1 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 II**. See **Figures 2-2** through **2-4** for maps of the current range for the SMHM, SJKF 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.
- **San Joaquin Kit Fox (SJKF):** The SJKF was listed as endangered in 1967 by the USFWS. The species is found in a variety of habitats in the Central Valley area of California.

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 2-1. 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

San Joaquin Kit Fox (SJKF) (*Vulpes macrotis mutica*)	Adult ~2 kg	Alameda, Contra Costa, Fresno, Kern, Kings, Madera, Merced, Monterey, San Benito, San Joaquin, San Luis Obispo, Santa Barbara, Santa Clara, Stanislaus, Tulare and Ventura counties	A variety of habitats, including grasslands, scrublands (*e.g.*, chenopod scrub and sub-shrub scrub), vernal pool areas, oak woodland, alkali meadows and playas, and an agricultural matrix of row crops, irrigated pastures, orchards, vineyards, and grazed annual grasslands. Kit foxes dig their own dens, modify and use those already constructed by other animals (ground squirrels, badgers, and coyotes), or use human-made structures (culverts, abandoned pipelines, or banks in sumps or roadbeds). They move to new dens within their home range often (likely to avoid predation by coyotes)	No, but has designated core areas	Mating and conception: late December - March. Gestation period: 48 to 52 days. Litters born: February - late March Pups emerge from their dens at about 1-month of age and may begin to disperse after 4 – 5 months usually in Aug. or Sept.	Small animals including blacktailed hares, desert cottontails, mice, kangaroo rats, squirrels, birds, lizards, insects and grass. It satisfies its moisture requirements from prey and does not depend on freshwater sources.

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

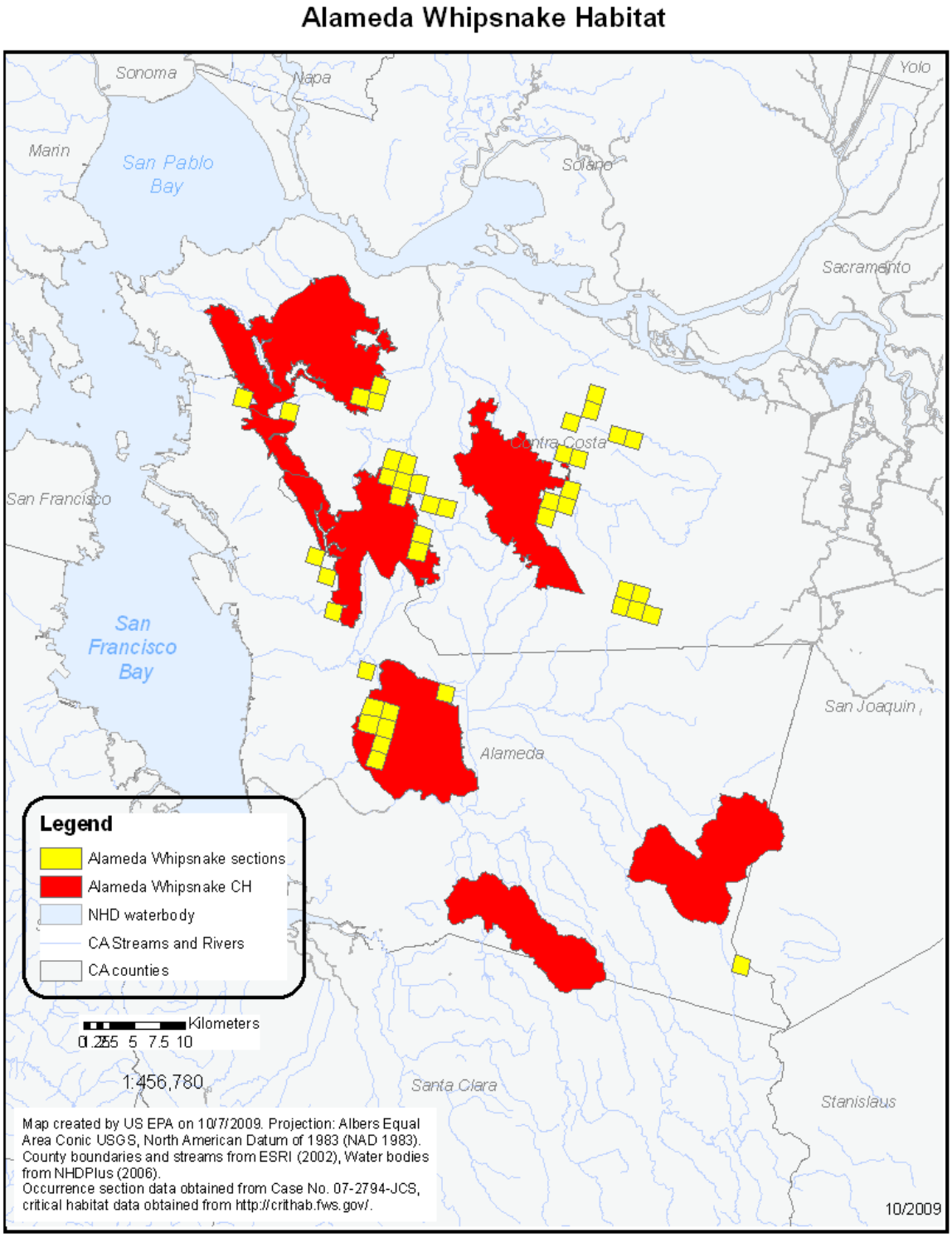


Figure 2-2. Critical habitat (CH) and occurrence sections of the Alameda Whipsnake, as identified in Case No. 07-2794-JCS

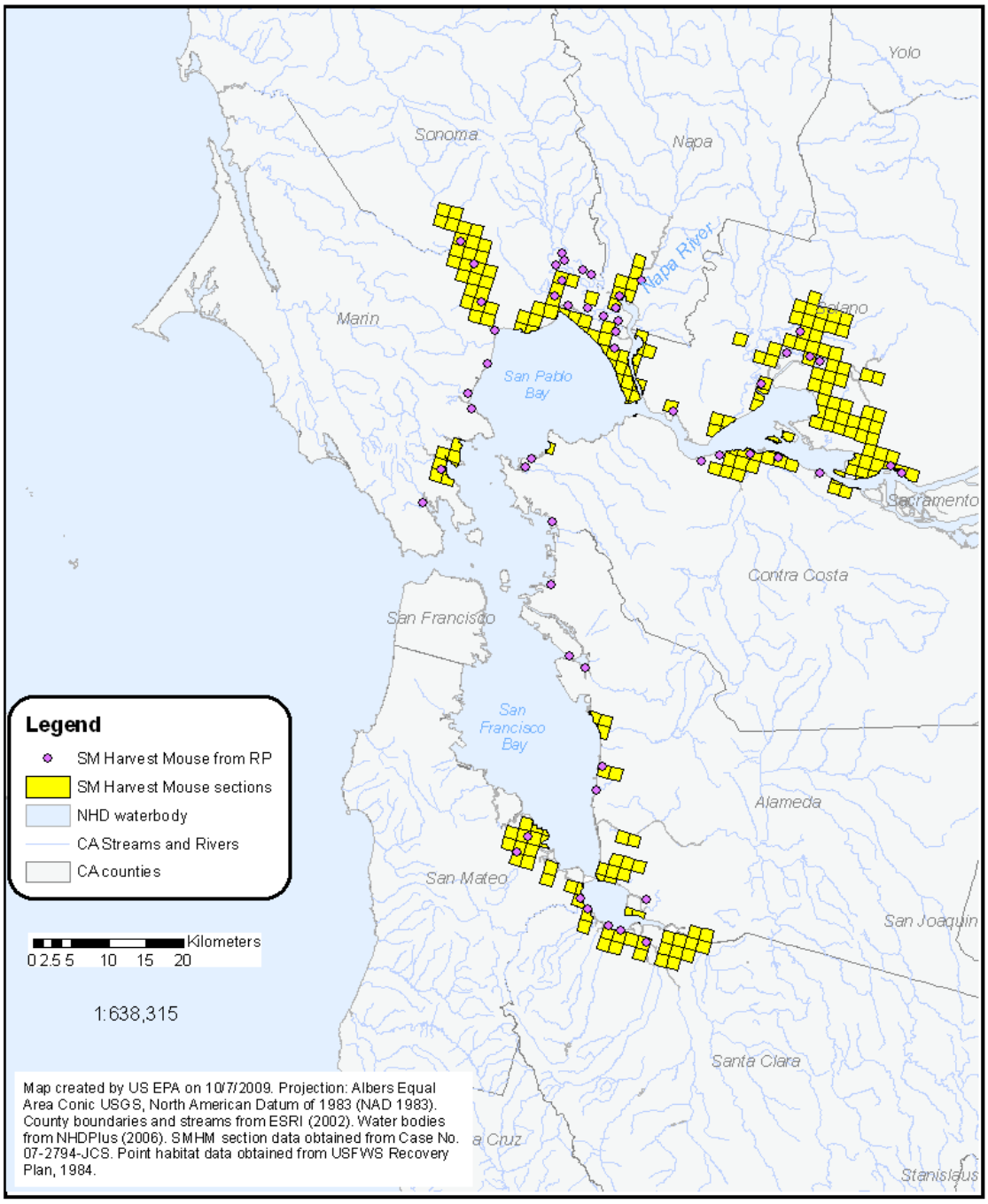


Figure 2-3. Occurrences and occurrence sections of the salt marsh harvest mouse, as identified in Case No. 07-2794-JCS. (RP is Recovery Plan)

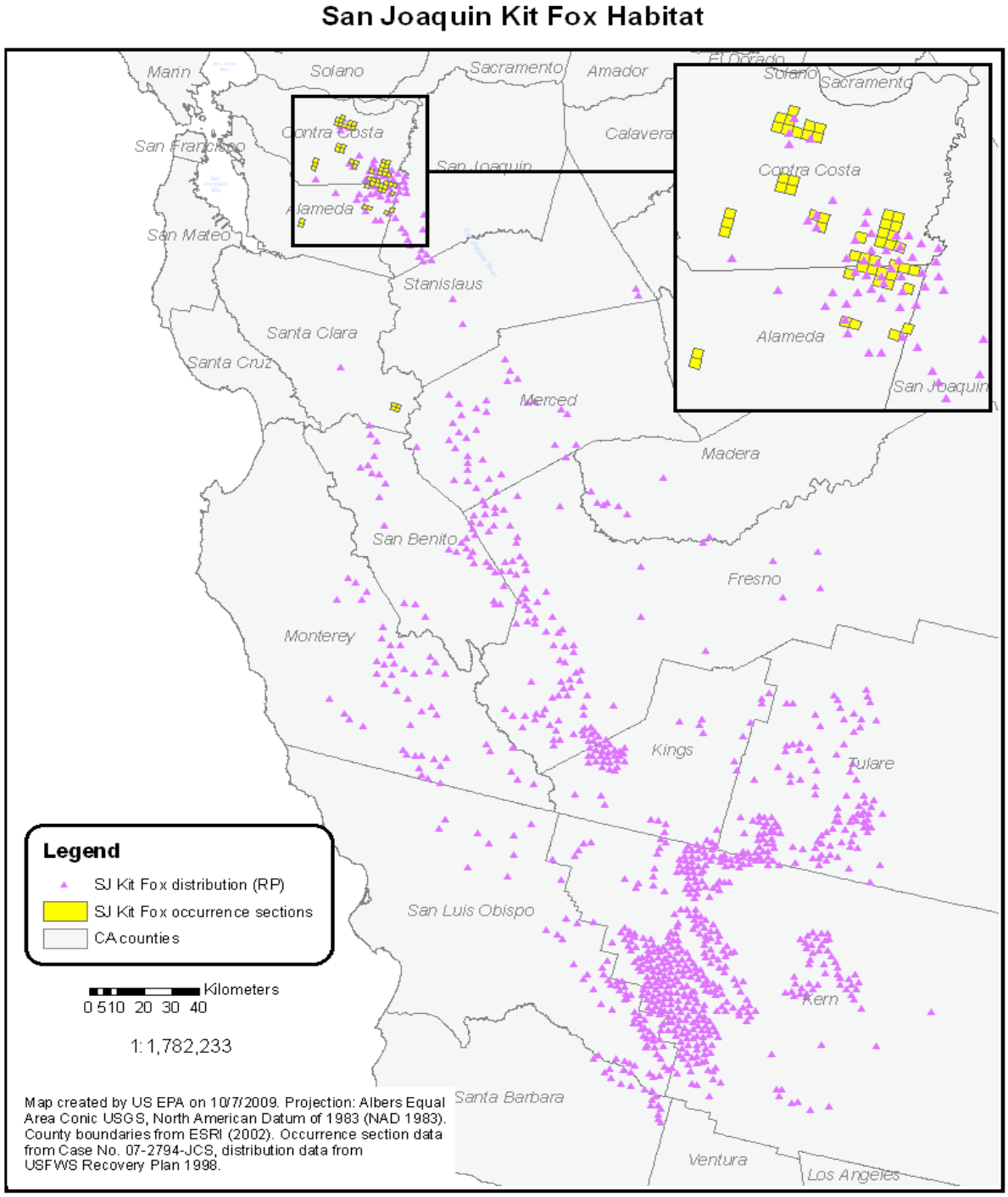


Figure 2-4. Occurrences and occurrence sections of the San Joaquin Kit Fox, as identified in Case No. 07-2794-JCS. (RP is the Recovery Plan).

2.4. Designated Critical Habitat

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

Table 2-2. 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 difenacoum 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 difenacoum is expected to directly impact living organisms within the action area, critical habitat analysis for difenacoum 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 difenacoum 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, SMHM and SJKF 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, SMHM and SJKF 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 difenacoum. An analysis of labeled uses and review of available

product labels was completed. For those uses relevant to the assessed species, the analysis indicates that, for difenacoum, 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 and in alleys.

Following a determination of the assessed uses, an evaluation of the potential "footprint" of difenacoum 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 difenacoum, these land cover types include all possible land cover types.

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 difenacoum is a vertebrate pest control that may be used in a wide variety of urban and non-urban areas, the spatial extent of difenacoum cannot be limited to defined areas. The Agency assumes that difenacoum 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, SJKF and SMHM, are assumed to lie within the potential use area of difenacoum.

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 2-3** identifies the taxa used to assess the potential for direct and indirect effects from the uses of difenacoum 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 2-4**.

Table 2-3. Taxa Used in the Analyses of Direct and Indirect Effects for the Assessed Listed Species.

Listed Species	Birds[1]	Mammals	Terr. Inverts.
Salt marsh harvest mouse	Indirect (rearing sites)	Direct Indirect (rearing sites)	Indirect (prey)

Listed Species	Birds[1]	Mammals	Terr. Inverts.
Alameda whipsnake	Direct Indirect (prey)	Indirect (prey/habitat)	Indirect (prey)
San Joaquin Kit Fox	Indirect (prey)	Direct Indirect (prey)	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 2-4. Taxa and Assessment Endpoints Used to Evaluate the Potential for Use of Difenacoum 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) 1b. Most sensitive bird or terrestrial-phase amphibian or reptilian chronic NOAEC (No data available)
	Indirect Effect (prey/rearing sites) -Salt Marsh Harvest Mouse -Alameda Whipsnake -San Joaquin Kit Fox	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 -San Joaquin Kit Fox	Survival, growth, and reproduction of individuals via direct effects	2a. Most sensitive laboratory mammalian acute LC_{50} or LD_{50} (guideline or ECOTOX) 2b. Most sensitive laboratory mammalian chronic NOAEC (No data available)
	Indirect Effect (prey/habitat from burrows/rearing sites) -Salt Marsh Harvest Mouse - Alameda Whipsnake -San Joaquin Kit Fox	Survival, growth, and reproduction of individuals or modification of critical habitat/habitat via indirect effects on terrestrial prey (mammals) and/or burrows/rearing sites	
[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 difenacoum 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 difenacoum 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 1998). For this assessment, the risk is stressor-linked, where the stressor is the release of difenacoum to the environment. The following risk hypotheses are presumed in this assessment:

The labeled use of difenacoum within the action area may:

- directly affect AW, SMHM and SJKF by causing mortality or by adversely affecting growth or fecundity;
- indirectly affect AW, SJKF 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).

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 difenacoum release mechanisms, biological receptor types, and effects endpoints of potential concern. The conceptual model for AW, SJKF and SMHM and the conceptual model for the terrestrial PCE components of critical habitat for AW are shown in **Figure 2-5.** 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, SJKF 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 difenacoum 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.

Difenacoum 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 difenacoum is not sprayed directly onto plants, and because so little is expected to leach from the bait (with a maximum a.i. of 0.023 grams a.i or 0.00005 lb a.i./placement) and then be available for plant uptake, consumption of difenacoum 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 difenacoum is not volatile and the expected short dermal contact periods preclude appreciable absorption through the skin.

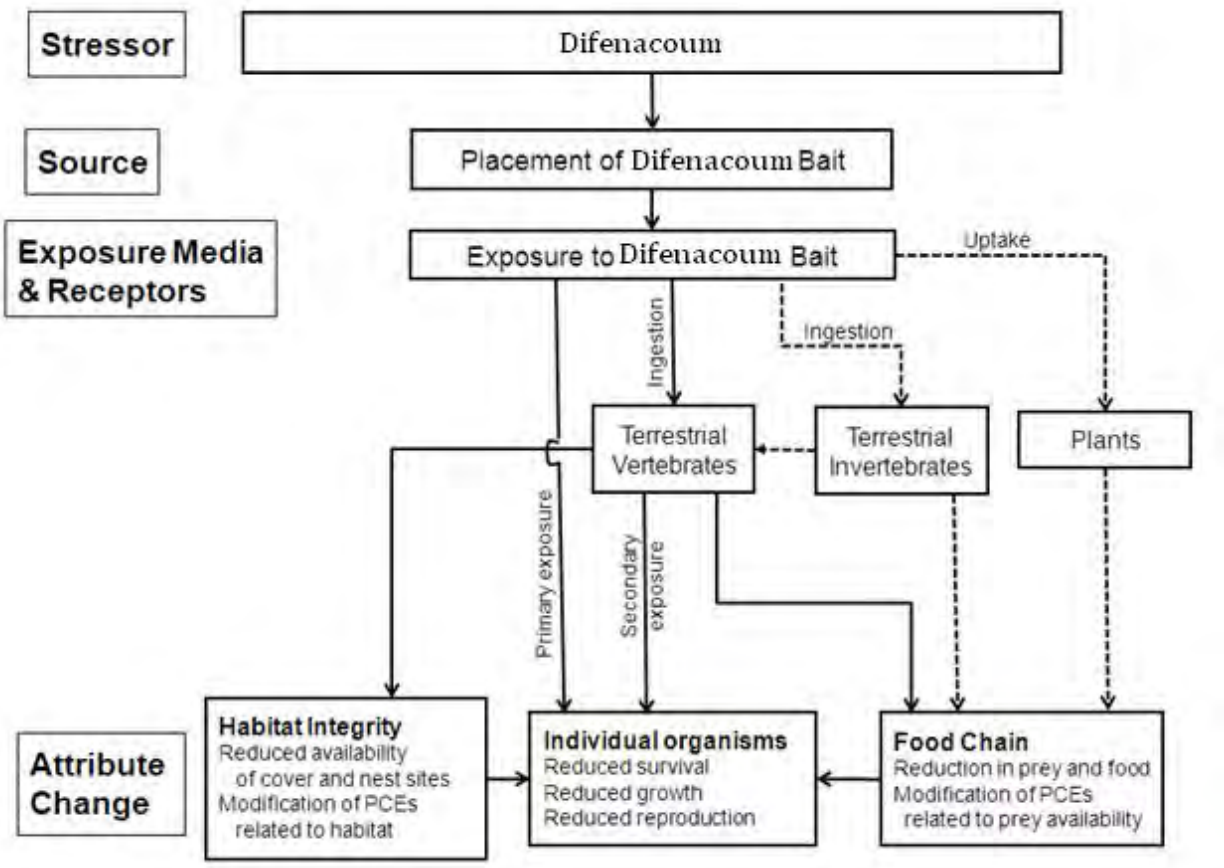


Figure 2-5. Conceptual Model Diagram of Difenacoum Exposure and Effects in Non-Target Species. Dotted lines indicate exposure pathways that have a low likelihood of contributing to ecological risk.

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 difenacoum 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 difenacoum 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 0.00005 lb a.i./placement) and that outdoor placement of bait requires bait stations, runoff appears to be a negligible 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 difenacoum 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 carcasses) 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 difenacoum are available in open literature (Gray et al., 1992, 1994). 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 difenacoum. 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 difenacoum, 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 difenacoum 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 (USEPA, 2004c). 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

The 2007 new use assessment on difenacoum listed several data gaps. Data gaps were assigned a low or high potential to affect the ecological risk assessment. 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.

Difenacoum's persistence and mobility have not been fully characterized. However, due to the limited use and the low environmental exposure of difenacoum, 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 difenacoum's persistence and mobility. Particularly, a study testing adsorption/desorption behavior in environmentally relevant conditions would be necessary.

No acceptable chronic toxicity data are available for evaluating the potential effects of difenacoum 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 difenacoum 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. In this assessment, EFED assumes chronic risk to avian organisms (and reptiles and terrestrial-phase amphibians, for which birds are used as surrogates), due to the lack of this data.

No acute toxicity data are available for a passerine species. Passerine species may metabolize difenacoum differently and may be more sensitive than quail and mallard, for which data is available. This study has **low** potential to add value to the ecological risk assessment since passerines would have to be significantly more sensitive (approximately 5 times) than bobwhite quail in order to change the risk picture. However, this study would be useful in further characterizing the potential effects to birds and reptiles.

Terrestrial plant toxicity studies are currently listed as data gaps, although difenacoum's mode of action is not expected to result in adverse effects to plants. The low application rates of difenacoum (0.00005 lb a.i./placement), with 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 difenacoum. 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.* and *,Tiers I and II (850.4400)*
- *Algal Toxicity, Tiers I and II (850.5400), three species:* Skeletonema costatum, Anabaena flos-aquae, and Navicula pelliculosa

3. Exposure Assessment

Difenacoum is formulated as bait pellets, bait blocks, and place packs sold in 8 lb or 16 lb quantities for use in and around agricultural buildings and for use by pest control operators PCOs. These baits must be placed in bait stations if they are used outdoors.

3.1. Terrestrial Animal Exposure Assessment

For assessing exposure of pesticides to terrestrial animals, the Agency typically uses T-REX (version 1.4.1) to calculate EECs for dietary exposure of terrestrial wildlife, and T-HERPS (version 1.0) 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 difenacoum 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; SJKF and 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 (USEPA 2011a, 2011b, 2012)

3.1.1. Expected Difenacoum Residues through Bait Consumption

Exposure through bait consumption is calculated using two methodologies. For the first method, difenacoum 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 generic birds and mammals and for typical weights of the SMHM and the SJKF. Food ingestion rate (FI) (dry weight) estimates were derived using allometric equations from USEPA (1993). The allometric equations for estimating FI for birds and mammals 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. Formulas for calculation of dose estimates are provided in **Table 3-1**. 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 and the SJKF 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 difenacoum (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 Difenacoum Intake based on Consumption of Bait.

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

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

Difenacoum intake (mg a.i./kg-bw/day) = FI (g dry-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 difenacoum in bait

Whereas the above primary exposure calculation method considered difenacoum consumption as a function of body weight, the second primary exposure method considers consumption independent of body weight (concentration of difenacoum as packaged in bait); namely a dietary concentration of 50 mg a.i./kg-bait. Since these EECs are not dependent on body weight or food consumption rates, they could be used for both direct effects to the SMHM and SJKF and indirect effects to all three evaluated species. However, since mammalian dietary toxicity data is not available, only indirect effects to the three evaluated species can be quantitatively evaluated and only acute RQs for birds were generated (calculated using this estimate of dietary concentration and the LC_{50} available from avian subacute dietary toxicity studies) using these exposure estimates were generated.

3.1.2. Expected Difenacoum Ingestion through Consumption of Contaminated Carcasses (Secondary Exposure)

Secondary exposure was also assessed for the AW and SJKF. These species may be exposed if they consume a vertebrate animal that has eaten difenacoum bait. Lizards in particular are believed to be the most important prey item of whipsnakes (**Attachment II**), but lizards generally feed upon insects and other terrestrial arthropods and would not likely consume rodent bait. Therefore, secondary exposure was based on consumption of small mammals, birds and other reptiles, which are likely to consume bait and are also a component of the diet of the AW and small mammals and birds for the diet of the SJKF. For assessing secondary exposure for the AW and SJKF, scenarios were considered in which both species preyed upon a house mouse or a Norway rat (representing both the low and high end of the three target organisms weights) after the prey had consumed difenacoum bait. The prey animal was assumed to have consumed a quantity of bait equal to its daily ingestion rate. Although it is possible that prey animals could

be exposed to multiple doses of difenacoum before dying, this exposure pattern was not explicitly considered in risk estimation scenarios for difenacoum. In one set of scenarios, the entire quantity of active ingredient ingested was assumed to remain in the animal at the time it was consumed. This could occur if the animal was consumed immediately after it ate the bait as the entire amount ingested would be present in the gastrointestinal tract of the prey animal. This scenario represents the high-end of possible secondary exposure. A second set of exposure scenarios was also used to represent more typical conditions. In the typical scenarios, the prey animal was assumed to be eaten 24 hours after the prey had consumed difenacoum bait. However, given the persistence of difenacoum in the liver and other tissues of organisms (Vandenbrouck et al., (2008) determined difenacoum's half life in the liver to be 61.8 days), a length of 24 hours of prey consumption after prey had consumed difenacoum bait would not likely result in degradation of difenacoum in the prey before predatory consumption.

Direct effects to the AW and SJKF (via Secondary Exposure by Consuming Mammals)

The maximum size of the prey consumed by snakes was estimated using the following allometric equation developed by King (2002).

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

In order to provide a conservative measure of exposure, the exponent used in this equation is the upper limit or the 95% confidence interval that King (2002) reported for this parameter (*i.e.*, same relationship that is assumed in the T-HERPS model). Although the weight of the AW was not available, the Agency has estimated body weight of this species from its length using the method presented in USEPA (1993). The estimated body weight of this species ranges from 2.5 to 176 g for juveniles, and 46 to 897 g for adults (USEPA 2010). 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 1450 g for adult snakes. Reported body weights of house mice and Norway rat are 18-23 g and 195-485 g, respectively (Whitaker, 1996). Therefore, the AW is predicted to be able to consume all three of these prey species, including the larger 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 and 485 g for the Norway rat).

To provide a conservative measure of exposure, the size of the AW was set at the minimum size animal that could consume prey of the size assumed for the two 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 mouse and rat was calculated to be 18.6 and 322 g, respectively. The 18.6-g snake is plausible for a juvenile AW while the 322-g snake is plausible for an adult AW.

For the SJKF, exposure through preying upon animals that consumed difenacoum bait (secondary exposure) was assessed by estimating direct effects to the prey items that were later ingested by different size classes of mammal (the SJKF) using an allometric equation based on whether the prey item was another mammal, bird, or reptiles.

The AW and SJKF are likely to be exposed to difenacoum residues from secondary exposure that occurs from consumption of prey that has consumed difenacoum bait. The AW and SJKF are capable of consuming all of the target small mammals species specified on the difenacoum bait product labels (rats and mice). Therefore, risk based on secondary exposure was conducted for AW and SJKF which feeds on a Norway rat and a house mouse. These species were assumed to have consumed difenacoum bait at their daily ingestion rate. The rat and the mouse were assumed to have consumed rodenticide bait with a difenacoum concentration of 0.005%. Assumed body weights of these prey species were 485 g for the Norway rat and 23 g for the house mouse for the AW. These represent the high end of the range for these species to represent the highest potential exposure of difenacoum from secondary exposure. The body weight of the snake was set at the weight of the minimum sized animal which would be predicted to be able to consume prey of the assumed size.

The average daily food intake rates of the two assumed prey species were estimated using the following allometric equation for mammals[8]:

$$FI = 0.621\ W^{0.564}$$

Where FI is the food intake rate in g/d, and W is the bodyweight of the rodent in grams.

The calculated FI values for the Norway rat and house mouse were 20.3, 3.64 g/d, respectively. The FI values were then converted into estimated ingested doses of difenacoum using the following equation:

$$Dose = FI \times \%AI \times (1/W) \times 10^4$$

Where:
Dose is the dose of difenacoum (mg-a.i./kg-bw)
FI is the food ingestion rate (g)
%AI is the percent active ingredient in the bait
W is the body weight (g)

To assess the maximum exposure that an AW could receive through secondary exposure, 100% of the active ingredient which was ingested by the small mammal prey was assumed to be present in the animal when it was consumed by the snake. This is plausible because a snake could prey upon the small mammal shortly after it has ingested the bait, with all of the difenacoum contamination present in the ingesta within the gastrointestinal tract of the animal, before it has had a chance to metabolize or excrete any of it. Thus, the amount of active ingredient the prey was assumed to ingest was also the dose, in mg, which the AW was assumed to ingest. This dose was then divided by the assumed body weight of the snake to convert the dose into units of mg a.i./kg bw. Finally, the acute secondary exposure risk quotients for the AW were calculated by dividing the predicted dose of difenacoum (mg-a.i./kg-bw) by the acute oral LD_{50} value for the most sensitive bird species (bobwhite quail), 66.9 mg-a.i./kg-bw, which is a surrogate value to represent the snake.

[8] T-REX (v1.4.1) User's Guide. Environmental Fate and Effects Division, Office of Pesticide Programs, US EPA. (December 11, 2008)

For mammals consuming other mammals that have ingested difenacoum bait (*i.e.*, the SJKF consuming rodents or other small mammals), a different method of estimating exposure was employed than for the AW. An equation for estimating the size of a mammal based on its prey size does not exist as it does for snakes; T-REX was used to estimate the ingestion rate for a given size mammal to consume to achieve its nutritional needs. Although there is some uncertainty in this method, specifically that this estimation is intended for insectivorous/ herbivorous mammals and not carnivores like the SJKF, there were no quantitatively acceptable residue studies available to estimate the concentration of difenacoum that a SJKF would ingest if it consumed a poisoned small mammal.

Using T-REX, the model estimates that a 2300 g mammal (average size for a SJKF) will ingest 11% of its body weight, or a 244 g small mammal. This weight represents an average sized Norway Rat since a larger Norway Rat (used for the AW) is assumed to be 485 g. This estimated body weight (244 g) was inputted into T-REX to estimate the food ingestion rate for a mammal of this size. This food ingestion rate (0.069 kg-diet/day) was multiplied by the amount of difenacoum active ingredient (50 mg a.i./kg bait) to get an estimated dose for the small mammal of 3.45 mg a.i/day. This dose, divided by the weight of the 2.3 kg SJKF yields the dose-based EEC of 1.5 mg a.i/kg/day for a SJKF ingesting a poisoned 244 g mammal.

Direct effects to the AW and SJKF (via Secondary Exposure by Consuming Birds)

The AW and SJKF are likely to be exposed to difenacoum residues from secondary exposure that occurs from consumption of prey that has consumed difenacoum bait. The AW and SJKF are capable of consuming birds as prey that may have directly ingested difenacoum bait. Therefore, risk based on secondary exposure was conducted for a snake and mammal which feeds on birds. These species were assumed to have consumed difenacoum bait at their daily ingestion rate. Birds were assumed to have consumed rodenticide bait with a difenacoum concentration of 0.005%. Assumed body weights of these prey species were 20 g for a small bird, 100 g for a medium-sized bird, and 1000 g for a large-sized bird. The body weight of the snake was set at the weight of the minimum sized animal which would be predicted to be able to consume prey of the assumed size.

The average daily food intake rates of the two assumed prey species were estimated using the following allometric equation for birds[9]:

$$FI = 0.648\ W^{0.651}$$

Where FI is the food intake rate in g/d, and W is the bodyweight of the bird in grams.

The calculated FI value for the small bird, medium bird, and large bird were 4.56, 13.00, and 58.2 g/d respectively. The FI values were then converted into estimated ingested doses of difenacoum using the following equation:

[9] T-REX (v1.4.1) User's Guide. Environmental Fate and Effects Division, Office of Pesticide Programs, US EPA. (December 11, 2008)

Dose = FI x %AI x (1/W) x 10^4

Where:
Dose is the dose of difenacoum (mg-a.i./kg-bw)
FI is the food ingestion rate (g)
%AI is the percent active ingredient in the bait
W is the body weight (g)

To assess the maximum exposure that an AW could receive through secondary exposure, 100% of the active ingredient which was ingested by the bird prey was assumed to be present in the animal when it was consumed by the snake. This is plausible because a snake could prey upon a bird shortly after it has ingested the bait, with all of the difenacoum contamination present in the ingesta within the gastrointestinal tract of the animal, before it has had a chance to metabolize or excrete any of it. Thus the amount of active ingredient the prey was assumed to ingest was also the dose, in mg, which the AW was assumed to ingest. This dose was then divided by the assumed body weight of the snake to convert the dose into units of mg a.i./kg bw. Finally, the acute secondary exposure risk quotients were calculated by dividing the predicted dose of difenacoum (mg-a.i./kg-bw) by the acute oral LD_{50} value for the most sensitive bird species (bobwhite quail), 66.9 mg-a.i./kg-bw, which is used as a surrogate for snake.

For mammals consuming birds that have ingested difenacoum bait (*i.e.*, the SJKF consuming birds), a different method of estimating exposure was employed than for the AW. As an equation for estimating the size of a mammal based on its prey size does not exist as it does for snakes, T-REX was used to estimate the ingestion rate for a given size bird to consume to achieve its nutritional needs. Although there is some uncertainty in this method, specifically that this estimation is intended for insectivorous/ herbivorous mammals and not carnivores like the SJKF, there were no quantitatively acceptable residue studies available to estimate the concentration of difenacoum that a SJKF would ingest if it consumed a poisoned small mammal.

In the previous analysis for a 2300-g SJKF consuming a mammal, T-REX estimated that the SJKF would need to eat a 244-g small mammal to fulfill its nutritional needs. This same weight can be applied for a 244-g bird. This estimated body weight (244 g) was inputted into T-REX to estimate the food ingestion rate for a bird of this size. This food ingestion rate (0.116 kg-diet/day) was multiplied by the amount of difenacoum active ingredient (50 mg a.i./kg bait) to get an estimated dose for the bird of 5.8 mg a.i/day. This dose, divided by the weight of the 2.3 kg SJKF yields the dose-based EEC of 2.52 mg a.i/kg/day for a SJKF ingesting a poisoned 244 g bird.

Direct effects to the AW and SJKF (via Secondary Exposure by Consuming Reptiles)

The AW is likely to be exposed to difenacoum residues from secondary exposure that occurs from consumption of prey that has consumed difenacoum bait. The AW is capable of consuming other reptiles such as lizards (their chief preference of food) as prey that may have directly ingested difenacoum bait. Therefore, risk based on secondary exposure was conducted for a snake which feeds on lizards. These species were assumed to have consumed difenacoum bait at their daily ingestion rate. Lizards and/or other reptiles were assumed to have consumed

rodenticide bait with a difenacoum concentration of 0.005%. Assumed body weights of these prey species were 2 g for a small reptile, 20 g for a medium-sized reptile, and 800 g for a large-sized reptile. The body weight of the snake was set at the weight of the minimum sized animal which would be able to consume prey of the assumed size.

The average daily food intake rates of the two assumed prey species were estimated using the following allometric equation for reptiles[10]:

$$FI = 0.013\ W^{0.773}$$

Where FI is the food intake rate in g/d, and W is the bodyweight of the reptile in g.

The calculated FI values for the small reptile, medium reptile, and large reptile were 0.02, 0.13, and 2.28 g, respectively. The FI values were then converted into estimated ingested doses of difenacoum using the following equation:

$$Dose = FI \times \%AI \times (1/W) \times 10^4$$

Where:
Dose is the dose of difenacoum (mg-a.i./kg-bw)
FI is the food ingestion rate (g)
%AI is the percent active ingredient in the bait
W is the body weight (g)

To assess the maximum exposure that an AW could receive through secondary exposure, 100% of the active ingredient which was ingested by the reptile prey was assumed to be present in the animal when it was consumed by the snake. This is plausible because a snake could prey upon a reptile very soon after it has ingested the bait, with all of the difenacoum contamination present in the ingesta within the gastrointestinal tract of the animal, before it has had a chance to metabolize or excrete any of it. Thus the amount of active ingredient the prey was assumed to ingest was also the dose, in mg, which the AW was assumed to ingest. This dose was then divided by the assumed body weight of the snake to convert the dose into units of mg a.i./kg BW. Finally, the acute secondary exposure risk quotients were calculated by dividing the predicted dose of difenacoum (mg-a.i./kg-bw) by the acute oral LD_{50} value for the most sensitive bird species (bobwhite quail), 66.9 mg-a.i./kg-bw, used as a surrogate to the whipsnake.

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, difenacoum data to enable the estimation of invertebrate body burden are also not available. Furthermore, although difenacoum has the propensity to bioaccumulate in the livers of terrestrial

[10] T-HERPS (v. 1.0) User's Guide. Environmental Fate and Effects Division, Office of Pesticides Programs, US EPA. September 4, 2008.

vertebrates and persist, it is unknown whether the fat body, a similar structure to the liver, is found in insects and whether difenacoum bioaccumulates in the same manner. Based on all of the available lines of evidence including mode of action, use patterns, and the dietary requirements of the assessed species, no exposure assessment was conducted for terrestrial invertebrates.

3.1.4. Exposure to Terrestrial Plants

The use of difenacoum in bait for rodent control is not expected to result in significant exposure to terrestrial plants, and therefore the risk of indirect effects to the AW mediated through modification of vegetation is expected to be negligible. Clearly, there is no spray drift exposure to plants from this use. Exposure to terrestrial plants would be limited to absorption through the roots by plants growing in soil contaminated by the bait. Only plants growing in the immediate vicinity of placed bait would be expected to be exposed to contaminated soil. Thus, the area where terrestrial plants may be exposed and potentially adversely affected is expected to be very small relative to home range of the AW, SJKF, and SMHM. Any damage that might occur to plants would not be expected to cause sufficient vegetative damage to result in deterioration of the quality of the assessed species' habitat. Additionally, the amount of residues that would leach from difenacoum bait applied at a maximum of 0.00005 lb a.i./placement is expected to be small. Furthermore, bait products used for outdoor rodent control are placed within a plastic bait station that would minimize contact with rain water. It appears unlikely that difenacoum would leach substantially from treated bait should exposure to rainwater occur.

4. Effects Assessment

This assessment evaluates the potential for difenacoum to directly or indirectly affect AW, SJKF 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 difenacoum.

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 31 August 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 difenacoum to "target" commensal rodent species (the house mouse and the Norway 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 F**. Citations of open literature papers that provide toxicological data for target rodent species are listed in **Appendix E** 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 difenacoum.

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 E**. **Appendix E** 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 F**.

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 difenacoum. A summary of the available terrestrial ecotoxicity information and the incident information for difenacoum are provided in **Sections 4.2** and **4.3**, respectively.

4.2. Toxicity of Difenacoum 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. Difenacoum is moderately toxic to birds on an acute oral exposure basis and is very highly 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. Difenacoum is very highly toxic to mammals on an acute oral exposure basis based on the rat data. No acceptable or supplemental data are available to characterize dietary or chronic or sub-lethal toxicity to mammals. Data on toxicity of difenacoum to terrestrial invertebrates and plants are not available. Additional information is provided in **Appendix C**.

Table 4-1. Terrestrial Toxicity Profile for Difenacoum

Taxa	Study Type	Species Tested	Toxicity Value and Probit Slopes Used in Risk Assessment (and their 95% confidence intervals)	Acute Classification/ Chronic Effect	Reference (MRID No.)
Bird	Avian oral toxicity	*Colinus virginianus* (Northern bobwhite)	LD_{50} = 66.9 mg/kg-bw (3.2—137.3) Slope = 1.22 (0.28—2.16)	Moderately Toxic	46750922
	Avian dietary toxicity	*Anas platyrhynchos* (Mallard duck)	LC_{50} = 14.1 mg/kg-diet (6.9—88.2) Slope = 1.13 (0.54—1.72)	Very Highly toxic	46750926
Mammal	Acute oral toxicity	*Rattus norvegicus* (Norway Rat)	LD_{50} = **1.8 mg/kg-diet (1.5-2.1) (males)** **Slope = 9.9 (3.6—16.2)** LD_{50} = 2.6 mg/kg-diet (2.3-4.0) (females) Slope = 16.0 (1.7—30.2)	Very highly toxic	46750935 46750936
	Dietary (5-day exposure)	No Data Available	N/A	N/A	N/A

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

Difenacoum is moderately to very highly toxic to birds with the most sensitive endpoints being an acute oral LD_{50} of 66.9 mg a.i./kg-bw for the bobwhite quail (*Colinus virginianus*, MRID 46750922) and a 5-day sub-acute dietary LC_{50} of 14.1 mg a.i./kg-diet for the mallard duck (*Anas platyrhynchos*, MRID 46750926). Sublethal effects noted in the acute oral study in live test birds that were euthanized and subsequently subjected to necropsy (MRID 46750922) included hemorrhaging in all birds. Post-mortem examinations indicated evidence of hemorrhaging and blood clots in the liver and other organs for birds exposed to the ≥0.75 mg/kg treatment diets in the sub-acute dietary study (MRID 46750926). No chronic toxicity data are available for birds.

4.2.1.b. Secondary Toxicity: Acute Studies

Mendenhall and Pank (1980) compared secondary hazards of 6 anticoagulant rodenticides, including difenacoum, to barn owls (*Tyto alba*). Six owls per rodenticide were exposed for either 1, 3, 6, or 10 days to rats fed with either difenacoum (50 ppm), bromadiolone (50 ppm), brodifacoum (20 ppm), or 3 first-generation anticoagulants. Exposed rats were offered free choice of bait (5 to 13 g daily) or laboratory chow for 10 days; body residues were not determined. Six of the 18 owls exposed to second-generation anticoagulants died, primarily those exposed to brodifacoum-fed rats. None of the difenacoum-exposed owls died, but the 3 owls fed for 6 or 10 days hemorrhaged, 1 severely (**Table 4-3**). None of the 6 owls offered first-generation anticoagulant-poisoned rats died nor did any exhibit signs of intoxication and residue sampling of animals was not reported .

Table 4-3. Adverse Effects of Difenacoum and Other Anticoagulants to Barn Owls Exposed To Rats Fed Bait (adapted from Mendenhall and Pank 1980)

Anticoagulant	Days exposed	No. tested/ no. dead	Sublethal effects Observed
Second-generation anticoagulants:			
Difenacoum (50 ppm)	1 3 6 10	1/0 2/0 1/0 2/0	the 3 owls offered rats for 6 or 10 days survived but all hemorrhaged (1 severely)

Wylllie (1995) and Newton et al. (1990) reported on toxic effects to barn owls fed mice exposed to difenacoum, brodifacoum, bromadiolone, or flocoumafen. The mice were fed bait (no choice) for a single day and allowed to die, which took 2 to 11 days. Residues of difenacoum in dead mice were present at greater concentrations in the liver than the rest of the carcass and were estimated to be 10-17 µg difenacoum for a whole 35g mouse. Dead mice were then offered to

the owls in 3 phases, each phase separated by a recovery period lasting at least 75 days. In phase I, each owl (6 per rodenticide) was offered 3 mice for 1 day only. Surviving owls were offered 6 mice each during a 3-day period in phase II and 12 mice each during a 6-day period in phase III. Mortality, evidence of external bleeding, and blood-coagulation times were monitored. The 6 owls fed difenacoum-dosed mice survived with no evidence of external bleeding, and blood-coagulation times returned to normal within 5 to 23 days. None of the owls exposed to bromadiolone-poisoned mice died or exhibited signs of hemorrhaging, and blood coagulation times returned to normal 4 to 6 days after treatment. In contrast, 4 of the 6 owls fed brodifacoum-exposed mice died from phase I treatment. During phases II and III, both survivors bled from the mouth, feet, and newly-grown feathers for up to 30 days, and blood-coagulation times did not reach normal until 16 to 78 days after treatment.

The registrant also supplied a report by Gray et al. (1992), similar to the published paper by Gray et al. (1994) cited in EFED's comparative risk assessment (USEPA, 2004b). In this study, the authors examined effects in barn owls fed mice that were exposed to difenacoum, brodifacoum, and flocoumafen. Batches of mice (6 per rodenticide) were fed 50 ppm wax block bait either in weighed amounts of 1.0 g – 5.0 g for 23 hours or *ad libitum* for 24 or 48 hours. Representative mice from each batch were selected and analyzed for residues, and mice contained 0.95 – 5.20 mg/kg of residue depending on the rodenticide (specific data were not provided). Four owls per rodenticide were fed treated mice for 15 days, and the amount of rodenticide fed to the owls was varied by feeding mice that fed on more or less bait or by feeding them more or fewer mice. Details of the feeding scheme for each owl are not provided. After the 15-day treatment period the owls were fed untreated mice and observed for another 15 days or until death. One owl fed difenacoum died, while one owl fed flocoumafen and two fed brodifacoum died. Owls fed difenacoum ate 36 – 128 µg difenacoum per day, and the owl that died ate 128 µg/day. This owl ate the second highest dose of the four, but had the highest liver residue (**Table 4-4**). No other effects were described in the report. Susceptibility and possibly absorption and elimination varied between the owls, and the sample size was very small. The authors concluded that although the toxicity of difenacoum and the other rodenticides was high, the risk resulting from a baiting program was low due to the low incidence of treated mice likely to be present in a pulse-baiting program using a retrievable wax block bait available intermittently. These conclusions do not necessarily translate directly to the proposed use of difenacoum, however, since the pelleted end-use product is not retrievable and can be used in permanent bait stations.

Table 4-4: Secondary Toxicity in Barn Owls (from Gray, 1992, 1994)

			Residue Concentration (mg/kg)		
Survival Status	Anticoagulant consumed (µg/day)	Cumulative consumption (mg/kg owl bw)	Liver	Fat	Muscle
Difenacoum (50 ppm):					
Survived (n=3)	36 – 128	1.6 – 5.5	0.06 – 0.14	<0.01	0.01
Died (n=1)	101	3.7	0.25	0.01	0.01

4.2.2. Toxicity to Mammals

4.2.2.a. Primary Toxicity: Acute and Chronic Studies

Several studies were submitted on difenacoum's (or its *cis* or trans isomers) effect on a number of mammalian species including the rat (*Rattus norvegicus*), house mouse (*Mus musculus*), rabbit (*Oryctolagus cuniculus*), domestic dog (*Canis lupus familiaris*), domestic cat (*Felis sylvestris cattus*), sheep (*Ovis aries*), domestic pig (*Suc scrofa*), guinea pig (*Cavia porcellus*) and hamster (*Mesocricetus auratus*). However, of the mammalian acute oral studies, only the rat studies were determined to be acceptable.

Difenacoum is very highly toxic to mammals on an acute oral exposure basis based on the rat data (male acute oral LD_{50} = 1.8 mg/kg-bw, MRID 46750935). Additionally, MRID 46766206 shows that the *cis* isomer may be more toxic than the *trans* isomer and the house mouse may be more sensitive than the rat with male mice exposed to the *cis* isomer having an LD_{50} of 0.45 mg a.i./kg-bw (female mice exposed to the *cis* isomer in the same study had an LD_{50} of 1.0 mg a.i./kg-bw). EFED typically averages the male and female LD_{50} values if a difference exists between them. The male rat LD_{50} value will be used to calculate RQs and the male mouse data with the *cis* isomer will be used for risk characterization. This approach may be the best compromise for representing a more sensitive species and maintaining the use of a reliable LD_{50} estimate. No acceptable chronic mammalian data is available.

4.2.2.b. Secondary Toxicity

Shore et al. (2003) present data on residues in polecats (*Mustela putorius*) accidentally killed on roads in England. Livers were analyzed for residues of second-generation anticoagulant rodenticides, including difenacoum, bromadiolone, flocoumafen, and brodifacoum. This work was an extension of that presented in Shore et al. (1996) and Shore et al. (1999), which were discussed in EFED's comparative risk assessment (USEPA, 2004b). Of 100 carcasses collected for all three studies between 1992 and 1999, 31 contained residues of at least one second-generation anticoagulant. Difenacoum was the most prevalent compound detected in 26 animals. Four polecats contained residues of multiple anticoagulants: two contained residues of difenacoum and bromadiolone, and the other two contained residues of difenacoum, bromadiolone, and brodifacoum. Among these studies, the authors concluded that the residues resulted from hunting activities of the polecats on nearby farms in winter and that they were probably killed when they dispersed into the landscape at a later time. Since the terminal phase liver elimination half-life of most second-generation anticoagulants in mammals can exceed 100 days, difenacoum and the other compounds can be detected for months after ingestion. Although the sampling scheme was unlikely to permit the collection of fatally poisoned animals, the maximum liver residue of difenacoum (0.917 ug/g) and bromadiolone (0.23 ug/g) approached that of a polecat that was poisoned by secondary exposure (Birks 1998, as cited in Shore et al. 2003) and a stoat that died after feeding on contaminated voles (Grolleau et al. 1989, as cited in Shore et al. 2003), respectively.

McDonald et al. (1998) tested for anticoagulant residues in stoats (*Mustela erminea*) and weasels (*Mustela nivalis*) shot or trapped on game farms in England. Nine out of 40 stoats and three out of 10 weasels contained residues of at least one rodenticide. Difenacoum was not found in any of the animals tested; however, in the area of England in which the study was conducted, coumatetralyl is used more widely, and was the most frequently detected anticoagulant. According to the authors, difenacoum and bromadiolone were the most widely used rodenticides in the rest of England, and these are more toxic than coumatetralyl. Thus, the authors concluded that the hazards of secondary poisoning may be more pronounced where difenacoum and bromadiolone anticoagulants are used. This conclusion is supported by the results of Shore et al. (2003).

These studies indicate the widespread occurrence of secondary exposure resulting from rodent control activities. All three species are exclusively carnivorous and would have been very unlikely to consume bait directly. Polecats are known to prey on animals that are targets of rodent control programs. However, weasels and stoats feed on these animals much less often, so predators do not necessarily have to forage around buildings in order to be secondarily exposed (McDonald et al. 1998).

Residue Studies: Difenacoum resistance has occurred in some areas of Europe. Atterby et al. (2005) used difenacoum-resistant and -susceptible laboratory rats to determine whole-body residues in target animals resulting from feeding on 25 ppm difenacoum bait for 5, 10, or 20 days. Susceptible rats were fed for 5 days, moderately-resistant rats were fed for 5 or 10 days, and highly reisistant rats were fed for 5, 10, and 20 days. Moderately- and highly-resistant rats that fed for 5 days contained higher residues (0.63 and 0.68 mg/kg bw, respectively) than susceptible rats, largely because the susceptible rats stopped feeding after Day 2. Highly resistant rats fed for 20 days and contained residues of 0.74 mg/kg bodyweight of parent difenacoum. Susceptible rats that fed for 5 days contained residues of 0.52 mg/kg bodyweight. From these data, the authors determined that the whole-body half-life for difenacoum was 12 hours, although previous work by the authors suggested 36 hours. Based on modeling from these data, the authors predicted that a resistant rat would achieve a maximum whole-body reside of 0.95 mg/kg bodyweight, reaching an asymptote after approximately 5 days. Previous work by the authors suggested that the half-life was 36 hours, the susceptible rats would have reached a whole-body residue of 2.99 mg/kg bw, and the resistant rats would have reached a whole-body residue of 4.95 mg/kg bw. Both predictions indicate the potential for residues to remain in target animals after they feed on difenacoum bait

4.3. Incident Database Review

A review of the Ecological Incident Information System (EIIS, version 2.1.1) and 'Aggregate Incident Reports' (v. 1.0) for ecological incidents involving difenacoum was completed on March 1, 2012 In addition, the Avian Monitoring Information System (AIMS) database was reviewed on March 1, 2012. . No incidents involving difenacoum were recorded in either the Aggregate Incident Reports or AIMS databases and no incidents involving difenacoum use in the United States were reported in EIIS. However, EIIS did have incident data for two incidents of undetermined legality in the U.K involving difenacoum.

The Scottish Agricultural Science Agency analyzed the livers of nine red kites found in Scotland between 1997 and 1999. Two of the nine samples tested were positive for difenacoum. In I016488-005 a female two year old kite was found dead with trace residues of brodifacoum and difenacoum (and also some unquantified residues of aldicarb). The certainty that difenacoum contributed to the incident was considered possible in this incident. In I016488-009, a second two year old female kite was found dead with 0.04 ppm brodifacoum and 0.2 ppm difenacoum residues in the liver. Hemorrhaging in the brain was considered to be caused by rodenticide poisoning. The certainty that difenacoum contributed to this incident was considered probable.

Some incident data are available from the United Kingdom's Wildlife Incident Investigation Scheme (WIIS). This program is used to monitor the effects of pesticides on wildlife after they are approved for use, relying on reports by the public and interested organizations to find affected wildlife. Annual reports of incidents are available for incidents recorded from 1998 to 2006, which are available at http://www.pesticides.gov.uk/environment.asp?id=58. These incidents have been tabulated in **Table 4-5** below. Many of these incidents involve predatory and scavenging birds or mammals, indicating the prevalence of secondary and possibly tertiary exposure among the incidents. The exposure of granivorous and omnivorous birds also indicates the occurrence of primary exposure. At attempt to examine the exact differences between registered uses in the U.S. and U.K. was made, but whether significant differences in the use patterns and rates between registered products in the United Kingdom and U.S. exist are currently unknown.

Table 4-5. Wildlife incidents involving difenacoum recorded in the UK's Wildlife Incident Investigation Scheme.

Year	No. Incidents[1]	Species Involved[2]
2006	36[3]	cat, stoat, weasel, dog, buzzard, kestrel, barn owl, red kite, fox, ferel pigeons, peacock
2005	15	buzzard, kestrel, red kite, badger, fox, grey squirrel, rat, bantam chicken, cat, dog, goose
2004	20	blackbird, buzzard, crow, house sparrow, red kite, sparrowhawk, badger, fox, pony, cat, dog
2003	11	crow, dove, red kite, badger, rabbit, cat, dog
2002	24	buzzard, feral pigeon, red kite, fox, cat, dog
2001	8	buzzard, red kite, badger, pine marten
2000	15	buzzard, red kite, badger, fox, cat, dog
1999	19	buzzard, house sparrow, red kite, tawny owl, fox, cat, dog
1998	9	buzzard, pheasant, dog, cat

[1]Some cases were attributed to abuse, a few "incidents" involve reports of bait only (e.g., a member of the public reporting unprotected rodenticide baits in areas accessible to the public). Several additional incidents involved rodenticides in each year, but residues were present at "sub-lethal"levels and were not included in the table. The number involving difenacoum were not specified; the numbers of these cases were: 33 in 2005, 38 in 2004, 10 in 2003, 18 in 2002, and were unspecified in 2001 and years prior.
[2]Scientific names are not provided in the reports. Common names are listed as given in the reports.

[3]Appears inflated over other years because "sub-lethal" exposures were not separated from the count in the preliminary quarterly reports for 2006.

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, SJKF and AW or for modification to AW designated critical habitat from the use of difenacoum 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 listed 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 difenacoum (**Section 3.1**) and the appropriate toxicity endpoints from **Table 4-1**.

5.1.1. Exposures in the Terrestrial Habitat:

5.1.1.a. Direct Effects to the SMHM (via primary exposure) and to the AW and SJKF (via secondary exposure)

As previously discussed in **Section 2**, potential direct effects to terrestrial species are based on bait applications of difenacoum for use in and around non-residential buildings including industrial, commercial and public buildings, food processing facilities, transport vehicles (ships, trains, aircraft) and their related ports, in and around agricultural buildings.

Potential risks to birds, mammals, reptiles, and terrestrial-phase amphibians were evaluated for both primary and secondary exposure. Primary exposure was based on the animal directly consuming the difenacoum bait (which was assumed for the SMHM) and has indirect ramifications for the AW and SJKF as through reduction in prey. This section focuses on direct effects to the SMHM through primary exposure (direct consumption of difenacoum bait) and to the AW and SJKF through secondary exposure, that is, consuming a bird, mammal, or reptile that has directly ingested difenacoum bait. Although not confirmed by research, the probability of the AW and SJKF, which typically eat live prey, consuming difenacoum bait is believed to be low; therefore, this route of exposure was not estimated for the AW and SJKF. As the exposure

information from the literature and the ecological incidents from Europe in this assessment have shown, difenacoum maintains a high level of lethality in a primary consumer's tissues that are available for secondary consumers like the AW and SJKF.

Direct effects to the SMHM (through Primary Exposure)

As discussed in **Section 3.1.1**, the SMHM is likely to be exposed to difenacoum residues from primary exposure that occurs from direct ingestion of difenacoum bait. Bait with one concentration of difenacoum was modeled, 0.005%.

Acute dose-based risk quotients were calculated by dividing the expected dose of difenacoum (mg-a.i./kg-bw) by the acute oral LD_{50} value for the male rat, 1.8 mg-a.i./kg-bw (**Table 4-1**). Results of these calculations are presented in **Table 5-1**.

Dose-based RQs for acute effects to mammals are presented in **Table 5-1**. These RQs represent risk of direct effects to the SMHM mediated through effects on small mammals.

Table 5-1. Dose-Based Acute RQs for Effects to the SMHM from Consumption of Difenacoum Bait

Mammal (bodyweight)	Bait Type	%AI	FI[1] (g/d)	Dose (mg/kg-bw)	Acute RQ[2]
SMHM (10 g)	Rodent Control	0.005	2.3	11.4	**6.3**

LOC exceedances (acute RQ > 0.1) are bolded.
[1] Daily food ingestion rate (dry weight)
[2] Based on the FI and the male rat acute oral LD_{50} of 1.8 mg/kg-bw.

Since the acute RQs for the SMHM exceeded the LOC (RQ of **6.3**) for listed and non-listed species, use of difenacoum has the potential to cause direct effects to the SMHM. There were no subacute dietary or chronic mammalian risk quotients that could be calculated because toxicity data on the subacute dietary or chronic effects of difenacoum to mammals are not available

Based on the RQs and the LD_{50} in the most sensitive acute oral toxicity study to mammals and using a slope of 9.9 (95% CI: 3.6—16.2), calculations were made to determine the probability that a particular individual would be killed by the dose predicted for a SMHM consuming difenacoum bait. The individual probability of death is 100% or 1 in 1 animal (95% CI: 100%--100%) for SMHM that ingested difenacoum bait.

Direct effects to the AW and SJKF (through Secondary Exposure Consuming Mammals)

As discussed in **Section 3.1.2**, the AW and SJKF are likely to be exposed to difenacoum residues from secondary exposure that occurs from consumption of prey that has consumed difenacoum bait. The AW and SJKF are capable of consuming the target small mammals species specified on the difenacoum bait product labels (rats and mice). Assumed body weights of these prey species were 485 g for the Norway rat and 23 g for the house mouse for the AW.

The amount of active ingredient the prey was assumed to ingest was also the dose, in mg, which the AW was assumed to ingest. This dose was then divided by the assumed body weight of the snake to convert the dose into units of mg a.i./kg bw. Finally, the acute secondary exposure risk quotients for the AW were calculated by dividing the predicted dose of difenacoum (mg-a.i./kg-bw) by the acute oral LD_{50} value for the most sensitive bird species, 66.9 mg-a.i./kg-bw, which is a surrogate value to represent the snake. Results of these calculations are presented in **Table 5-2.**

Table 5-2. Dose-based RQs for Acute Effects to the AW from Consumption of Mammals which Ingested Difenacoum Bait

Bait Type	Prey Species	Assumed weight of AW	%AI in Bait	Prey FI[1] (mg/d)	Dose (mg a.i./kg BW)	Acute RQ[2]
Rodent control	Norway rat (485 g)	322 g	0.005	20.3	3.15	0.05
	House mouse (23 g)	18.6 g	0.005	3.64	9.78	**0.15**

LOC exceedances (acute RQ > 0.1) are bolded.
[1] Daily food ingestion rate.
[2] Based on the dose in the ingested prey and the avian acute oral LD_{50} of 66.9 mg/kg-bw

Because the acute RQs for secondary exposure exceed 0.1 for the smaller AW consuming small rodents (RQ = **0.15**), the LOC for acute effects to listed species, use of difenacoum has the potential to directly affect the AW that feeds upon small mammals which ingested difenacoum bait, by way of secondary exposure.

Based on the RQs and the LD_{50} in the most sensitive acute oral toxicity study to birds and using a probit slope of 1.22 (95% CI: 0.28--2.16), calculations were made to determine the probability that a particular individual would be killed by the dose predicted for a AW consuming rats and mice that have consumed difenacoum bait. The individual probability of death is 16% or 1 in 6.35 animals (95% CI: 4%--41%) and 6% or 1 in 17.8 animals (95% CI: 0.2%--36%) for AW that ingested mice and rats, respectively who have consumed difenacoum bait for one day.

For mammals consuming other mammals that have ingested difenacoum bait (*i.e.*, the SJKF consuming rodents or other small mammals), a different method of estimating exposure was employed than for the AW. As an equation for estimating the size of a mammal based on its prey size does not exist as it does for snakes, T-REX was used to estimate the ingestion rate for a given size mammal to consume to achieve its nutritional needs as discussed in **Section 3.1.2**.

When the dose based EEC (as derived in **Section 3.1.2**) is divided by the LD_{50} (adjusted for weight of the 2300-g SJKF) of 1.2 mg a.i/kg, the resulting RQ is **1.25** (**Table 5-3**).

Table 5-3. Dose-based RQs for Acute Effects to the SJKF from Consumption of Mammals which Ingested Brodifacoum Bait

Bait Type	Prey Species	Assumed weight of	%AI in Bait	Dose received by	Dose-based EEC for	Adjusted LD_{50}	Acute RQ[2]

		SJKF		prey FI[1] (mg a.i./d)	SJKF (mg a.i./kg BW)	(mg a.i./kg BW)	
Rodent control	Small mammal (244 g)	2300 g	0.005	1.72	1.5	1.2	**1.25**

LOC exceedances (acute RQ > 0.1) are bolded.

[1] As estimated by the product of the ingestion rate of a 244 g small mammal and the mg a.i. of difenacoum in the bait (50 mg a.i.)

[2] Based on the dose-based EEC in the ingested prey and the adjusted LD_{50} of a 2300-g mammal of 1.2 mg/kg-bw

Since the acute RQ for secondary exposure exceeds 0.1, the LOC for acute effects to listed species, use of difenacoum has the potential to directly affect the SJKF that feeds upon small mammals which ingested difenacoum bait, by way of secondary exposure.

Based on the RQs and the LD_{50} in the most sensitive acute oral toxicity study to mammals and using a probit slope of 9.9 (95% CI: 3.6--16.2), calculations were made to determine the probability that a particular individual would be killed by the dose predicted for a SJKF consuming a small mammal. The individual probability of death is 83% or 1 in 1.2 animals (95% CI: 64%--94%) for a SJKF consuming a small mammal that ingested difenacoum bait.

Direct effects to the AW and SJKF (through Secondary Exposure Consuming Birds)

The AW and SJKF are likely to be exposed to difenacoum residues from secondary exposure that occurs from consumption of prey that has consumed difenacoum bait. The AW and SJKF are capable of consuming birds as prey that may have directly ingested difenacoum bait. Therefore, risk based on secondary exposure was conducted for a snake and mammal which feeds on birds, as discussed in **Section 3.1.2**.

To assess the maximum exposure that an AW could receive through secondary exposure, 100% of the active ingredient which was ingested by the bird prey was assumed to be present in the animal when it was consumed by the snake. This dose was then divided by the assumed body weight of the snake to convert the dose into units of mg a.i./kg bw. Finally, the acute secondary exposure risk quotients were calculated by dividing the predicted dose of difenacoum (mg-a.i./kg-bw) by the acute oral LD_{50} value for the most sensitive bird species, 66.9 mg-a.i./kg-bw, which is used as a surrogate for snake. Results of these calculations are presented in **Table 5-4.**

Table 5-4. RQs for Acute Effects to the AW from Consumption of Birds which Ingested Difenacoum Bait

Bait Type	Prey Species	Assumed weight of AW	%AI in Bait	Prey FI[1] (mg/d)	Dose (mg a.i./kg BW)	Acute RQ[2]
Rodent control	Small bird (20 g)	16.2	0.0050	4.55	14.04	**0.21**
	Medium Bird (100 g)	74.1	0.0050	13.00	8.77	**0.13**
	Large Bird (1000 g)	631	0.0050	58.1	4.60	0.07

LOC exceedances (acute RQ > 0.1) are bolded.
[1] Daily food ingestion rate.
[2] Based on the dose in the ingested prey and the avian acute oral LD_{50} of 66.9 mg/kg-bw

Because the acute RQs for secondary exposure for small and medium birds exceed 0.1 (RQs ranged from 0.07 **– 0.21**), the LOC for acute effects to listed species, use of difenacoum has the potential to directly affect the AW that feed upon birds which ingested difenacoum bait by way of secondary exposure.

Based on the RQs and the LD_{50} in the most sensitive acute oral toxicity study to birds using a probit slope of 1.22 (95% CI: 0.28—2.16) , calculations were made to determine the probability that a particular individual would be killed by the dose predicted for an AW consuming a contaminated bird. The individual probability of death is 20% or 1 in 4.9 animals (95% CI: 7%--43%) for an AW consuming a small bird, 14% or 1 in 7.15 animals (95% CI: 3%--40%) for an AW consuming a medium bird, or 8% or 1 in 12.6 animals (95% CI: 1%--37%) for an AW consuming a large bird that ingested difenacoum bait.

For mammals consuming birds that have ingested difenacoum bait (*i.e.*, the SJKF consuming birds), a different method of estimating exposure was employed than for the AW. As an equation for estimating the size of a mammal based on its prey size does not exist as it does for snakes, T-REX was used to estimate the ingestion rate for a given size bird to consume to achieve its nutritional needs as discussed in **Section 3.1.2**.

When the dose-based EEC (as derived in **Section 3.1.2**) is divided by the adjusted LD_{50} of 1.2 mg a.i/kg for the 2300-g SJKF, the resulting RQ is **2.1** (**Table 5-5**). This RQ exceeds the acute endangered species, acute restricted use and acute non-listed species LOCs and therefore there is risk to the SJKF that have consumed birds which have ingested difenacoum bait.

Table 5-5. RQs for Acute Effects to the SJKF from Consumption of Birds which Ingested Difenacoum Bait

Bait Type	Prey Species	Assumed weight of SJKF	%AI in Bait	Dose received by prey [1] (mg a.i./d)	Dose-based EEC for SJKF (mg a.i./kg BW)	Adjusted LD_{50}	Acute RQ[2]

Rodent control	Bird (244-g)	2300 g	0.005	5.8	2.52	1.2	**2.1**

LOC exceedances (acute RQ > 0.1) are bolded.
[1] As estimated by the product of the ingestion rate of a 244 g bird and the mg a.i. of difenacoum in the bait (50 mg a.i.)
[2] Based on the dose-based EEC in the ingested prey and the adjusted LD_{50} of a 2300-g mammal of 1.2 mg/kg-bw

Based on the RQs and the LD_{50} in the most sensitive acute oral toxicity study to mammals and the probit slope of 9.9 (95% CI: 3.6--16.2) was used per the study results and subsequent analysis with Toxanal), calculations were made to determine the probability that a particular individual would be killed by the dose predicted for a SJKF consuming a contaminated bird. The individual probability of death is 100% or 1 in 1 animal (95% CI: 88%--100%) for an SJKF consuming a 244-g bird that ingested difenacoum bait.

Direct effects to the AW (through Secondary Exposure Consuming Reptiles)

The AW is likely to be exposed to difenacoum residues from secondary exposure that occurs from consumption of prey that has consumed difenacoum bait. The AW is capable of consuming other reptiles such as lizards (their preferred food) as prey that may have directly ingested difenacoum bait. Therefore, risk based on secondary exposure was conducted for a snake which feeds on lizards. The body weight of the snake was set at the weight of the minimum sized animal which would be able to consume prey of the assumed size, as described in **Section 3.1.2.**

To assess the maximum exposure that an AW could receive through secondary exposure, 100% of the active ingredient which was ingested by the reptile prey was assumed to be present in the animal when it was consumed by the snake. This dose was then divided by the assumed body weight of the snake to convert the dose into units of mg a.i./kg bw. Finally, the acute secondary exposure risk quotients were calculated by dividing the predicted dose of difenacoum (mg-a.i./kg-bw) by the acute oral LD_{50} value for the most sensitive bird species, 66.9 mg-a.i./kg-bw, used as a surrogate for the whipsnake. Results of these calculations are presented in **Table 5-6.**

Table 5-6. RQs for Acute Effects to the AW from Consumption of Reptiles which Ingested Difenacoum Bait

Bait Type	Prey Species	Assumed weight of AW	%AI in Bait	Prey FI[1] (mg/d)	Dose (mg a.i./kg bw)	Acute RQ[2]
Rodent control	Small reptile (2 g)	1.91	0.0050	0.02	0.52	< 0.01
	Medium reptile (20 g)	16.2	0.0050	0.13	0.40	< 0.01
	Large reptile (800 g)	513	0.0050	2.28	0.22	< 0.01

LOC exceedances (acute RQ > 0.1) are bolded.
[1] Daily food ingestion rate.
[2] Based on the dose in the ingested prey and the avian acute oral LD_{50} of 0.26 mg/kg-bw

Because the acute RQs for secondary exposure do not exceed any acute LOCs, direct risk to the AW by way of secondary exposure is not indicated from consumption of reptiles that have ingested difenacoum.

5.1.1.b. Indirect Effects to the AW, and SJKF (via direct effects to prey items leading to prey reduction) and to the SMHM (through reduction of rearing sites).

Reptiles

Direct acute effects from primary exposure to prey items for the AW and SJKF (mammals, birds, and reptiles) ingesting difenacoum bait were evaluated by assuming an individual directly consumes a bait product containing difenacoum at its daily ingestion rate. As all difenacoum products are the same percent a.i., 0.005%, only one concentration of difenacoum was assessed. The average daily food intake rate was estimated using the allometric equation for insectivorous reptiles (Nagy, 1987 as cited in USEPA, 1993) as discussed in **Section 3.1.1**.

Acute risk quotients were calculated by dividing the expected dose of difenacoum (mg-a.i./kg-bw) by the acute oral LD_{50} value for the northern bobwhite, 66.9 mg-a.i./kg-bw (surrogate for reptiles). RQs for acute toxicity to reptiles are given in **Table 5-7**. These RQs represent risk of direct effects to the reptiles from direct consumption of difenacoum bait. They are also applicable to indirect effects to this species mediated through adverse effects to reptiles and terrestrial-phase amphibians which serve as prey for the AW. The Agency's level of concern for risk to the three size classes of AW (LOC = 0.1) from direct consumption of difenacoum bait is not exceeded with RQs < 0.01 for all size classes.

Table 5-7. Acute RQs for to Reptiles that Consume Difenacoum Bait

Size (bodyweight)	%AI	FI[1] (g/d)	Dose (mg ai/kg-BW)	Acute Dose-Based RQ[2]
Small (2 g)	0.005	0.022	0.55	< 0.01
Medium (20 g)	0.005	0.13	0.32	< 0.01
Large (800 g)	0.005	2.3	0.14	< 0.01

LOC exceedances (acute RQ > 0.1) are bolded.
[1] Daily food ingestion rate based on an allometric equation for insectivorous reptiles.
[2] Based on the FI and the northern bobwhite oral LD_{50} of 66.9 mg/kg-bw

Birds

Indirect risk posed to the AW and SJKF mediated by toxic effects to birds was assessed using an approach similar to that used for reptiles, except the allometric equation for food ingestion rate (FI) was for birds rather than for reptiles. Risk was again assessed for bait with a difenacoum concentration of 0.005%. The average daily food intake rate was estimated using the following allometric equation for birds (Nagy, 1987 as cited in USEPA, 1993) as discussed in **Section 3.1.1.**

Acute RQs were calculated by dividing the expected dose of difenacoum (mg-a.i./kg-bw) by the acute oral LD_{50} value for the northern bobwhite, 66.9 mg-a.i./kg-bw (**Table 5-8**). Because the AW preys upon birds, they may be indirectly affected by adverse effects on bird populations. Dietary-based RQs for birds which consume difenacoum bait were also calculated by simply dividing the concentration of difenacoum in the bait by the subacute dietary LC_{50} value for the mallard duck (14.1 mg a.i./kg-diet). The difenacoum concentrations in the bait, when expressed as parts-per-million (mg-a.i./kg), is 50 for rodenticide bait. Therefore, the dietary RQs for indirect effects resulting from toxicity to birds is **3.5** for rodenticide bait.

Table 5-8. Acute RQs for Birds that Consume Difenacoum Bait

Size (bodyweight)	%AI	FI[1] (g/d)	Dose (mg/kg-a.i.)	Dose-based Acute RQ[2]	Diet-based Acute RQ[3]
Small (20 g)	0.005	4.56	11.4	**0.17**	**3.5**
Medium (100 g)	0.005	13.0	6.5	**0.10**	**3.5**
Large (1000 g)	0.005	58.2	2.91	0.04	**3.5**

LOC exceedances (acute RQ > 0.1) are bolded.
[1] Daily food ingestion rate.
[2] Based on the FI and the northern bobwhite oral LD_{50} of 66.9 mg/kg-bw.
[3] Based on the %AI of the bait and the mallard duck dietary LC_{50} of 14.1 mg/kg-diet.

There were no chronic avian risk quotients that could be calculated because toxicity data on the chronic effects of difenacoum to birds are not available.

Because the acute risk quotient for reptiles exceed the Agency's listed species LOCs for small and medium birds (dose-based acute RQs ranged from 0.04 **– 0.17**, and diet-based acute RQs were **3.5**), use of difenacoum has the potential to cause indirect effects to the AW.

Mammals

Risk quotients were calculated to assess risk to small and medium sized mammals which directly consume difenacoum bait and which may serve as prey for the AW and SJKF. These also apply to effects to small mammals that may provide rearing sites for the SMHM. Bait with one concentration of difenacoum was modeled, 0.005%. The small mammals were assumed to consume their average daily food intake in the form of the bait as discussed in **Section 3.1.1**.

Acute dose-based risk quotients were calculated by dividing the expected dose of difenacoum (mg-a.i./kg-bw) by the acute oral LD_{50} value for male rates, 1.8 mg-a.i./kg-bw (**Table 4-1**). Results of these calculations for acute effects to mammals are presented in **Table 5-9**. These RQs represent risk of indirect effects to the AW and SJKF mediated through effects on small mammals.

Table 5-9. Dose-Based RQs for Acute Effects to Prey Mammals from Consumption of Difenacoum Bait

Mammal (bodyweight)	Bait Type	%AI	FI[1] (g/d)	Dose (mg/kg-bw)	Acute RQ[2]
House mouse (23g)	Rodent control	0.005	3.64	7.91	**4.4**
Norway rat (485 g)	Rodent Control	0.005	20.3	2.09	**1.2**

LOC exceedances (acute RQ > 0.1) are bolded.
[1] Daily food ingestion rate.
[2] Based on the FI and the male rat acute oral LD_{50} of 1.8 mg/kg-bw

Since the acute RQs for a 23-g mammal and a 485 g mammal exceeded the LOCs (RQs ranged from **1.2—4.4**) for listed and non-listed species, use of difenacoum has the potential to cause indirect effects to the AW and SJKF mediated through effects on small mammals which serve as prey for the AW and SJKF. Additionally, direct effects to these small mammals indirectly affect the SMHM by reducing the number of rearing sites.

Dose –based RQs for small and medium mammals (presented in **Table 5-10**) which consume difenacoum bait were also calculated. Dose-based RQs ranged from **3.6 – 6.3**. Dietary RQs could not be calculated.

Table 5-10. Dosed-Based RQs for Acute Effects to Mammals from Consumption of Difenacoum Bait

Size (bodyweight)	%AI	FI[1] (g/d)	Dose (mg/kg-a.i.)	Dose-based Acute RQ[2]
Small (20 g)	0.005	4.56	11.4	**6.3**
Medium (100 g)	0.005	13.0	6.5	**3.6**

LOC exceedances (acute RQ > 0.1) are bolded.
[1] Daily food ingestion rate.
[2] Based on the FI and the male rat LD_{50} of 1.8 mg/kg-bw.

Based on the RQs and the LD_{50} in the most sensitive acute oral toxicity study to mammals (a a slope of 9.9 was used) Calculations were made to determine the probability that a particular individual would be killed by the dose predicted for a SJKF consuming a contaminated small or medium mammal. The individual probability of death is 100% (1 in 1) for an SJKF consuming a small or medium mammal that ingested difenacoum bait. Similarly, the probability of chance of death from a small, medium, or large mammal consuming difenacoum bait would be 1 in 1 (100%) using a slope of 9.9.

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 difenacoum, the small application rate per placement (0.00005 lb a.i./placement), and the lack of any reported incidents over several decades of use. Further, since difenacoum 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 as food and/or habitat during at least some portion of their life-cycle (*i.e.* SMHM, SJKF and the AW) is considered low.

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

For difenacoum 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 difenacoum'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 difenacoum. A preliminary effects determination of "may effect" was made for both SMHM, SJKF 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 Difenacoum - Direct and Indirect Effects

Taxa	LOC Exceedances (Yes/No)	Description of Results of Risk Estimation	Assessed Species Potentially Affected
Birds, Reptiles, and Terrestrial-Phase Amphibians	Non-listed Species (Yes)	Risk of acute toxic effects to SMHM and AW prey (birds that feed on any difenacoum bait, and to terrestrial-phase amphibians which feed on bait used for rodent control). Chronic risk presumed for primary (direct bait	Indirect Effects: SMHM and AW

Taxa	LOC Exceedances (Yes/No)	Description of Results of Risk Estimation	Assessed Species Potentially Affected
		consumption) and secondary (contaminated carcass consumption) toxicity.	
	Listed Species (Yes)	AW: Risk of acute secondary poisoning to snakes feeding on prey which ingested difenacoum bait. Chronic risk presumed for consumption of contaminated prey.	Direct Effects: AW
Mammals	Non-listed Species (Yes)	Risk of acute effects to small mammals that feed on difenacoum bait. Risk of acute toxic effects to mammals that serve as prey that feed on difenacoum bait. Chronic risk presumed for primary and secondary toxicity	Indirect Effects: SMHM and AW
	Listed Species (Yes)	SMHM: Acute LOCs exceeded and chronic risk presumed for primary toxicity (direct bait consumption). SJKF: Acute LOCs exceeded and chronic risk presumed for secondary toxicity (contaminated carcass consumption)	Direct Effects: SMHM and SJKF

Table 5-12. Risk Estimation Summary for Difenacoum– 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)	Risk of acute toxic effects to birds, reptiles and terrestrial-phase amphibians that feed on difenacoum bait that may be consumed by the AW.	AW
Mammals	Non-listed Species (Yes)	Risk of acute effects to small mammals that feed on difenacoum bait that serve as prey for the AW. Risk of acute effects to small mammals that feed on difenacoum bait that create burrows for the AW.	AW

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 **5.2.4**. 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 difenacoum. 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 primary risk of direct effects of brodifacoum bait on the SMHM is believed to be direct consumption of difenacoum bait. The SMHM diet mainly includes the pickleweed plant but the small pellet like difenacoum bait could be ingested.

Although extensive use of difenacoum bait products is not believed to currently occur in the region where the SMHM occurs, it is possible that future use will increase in those counties where SMHM occurs. These counties (Marin, Sonoma, Napa, Solano, Contra Costa, San Mateo,

Alameda, and Santa Clara counties) include many highly developed and densely populated areas. Placement of difenacoum rodenticide bait around farm and industrial buildings in these counties would provide widespread opportunities for small mammals like the SMHM to encounter difenacoum bait. Since rodenticide bait would most likely be used in areas where high rodent populations exist, this might further increase the direct impacts to the SMHM.

The extent to which the restriction of the placement of the rodenticide bait to within 50 ft of exterior walls of buildings could reduce primary exposure of the SMHM to difenacoum bait is uncertain. Having an average weight of 8-14 g, the SMHM could easily be exposed to difenacoum in tamper resistant bait stations and consume difenacoum bait.

Since the dose-based acute RQs for the SMHM exceeded the LOC (RQ of **6.3**) for listed and non-listed species, use of difenacoum has the potential to cause direct effects to the SMHM. Additionally, there is evidence that mice may be more sensitive to difenacoum than rats based on the differential toxicity of difenacoum's *cis* isomer to lab rats and house mice (MRID 46766206) Dietary-based RQs could not be calculated.

Based on the RQs and the LD_{50} in the most sensitive acute oral toxicity study to mammals (a slope of 9.9 was determined from the study report), calculations were made to determine the probability that a particular individual would be killed by the dose predicted for a SMHM consuming bait. The individual probability of death is 100% (1 in 1) for a SMHM that ingested difenacoum bait.

5.2.1.b. Indirect Effects

Difenacoum use may result in indirect effects to the SMHM due to a reduction in rearing sites.

Potential effects of difenacoum to small mammals that provide rearing sites for the SMHM were evaluated (which would constitute indirect effects to the SMHM). For small mammals that consume bait directly, dose-based acute RQs exceeded Acute Endangered Species, Acute Restricted Use and Acute LOCs (**Table 5-1**). In addition, the probability of an individual mortality occurrence is 100% (1 in 1) for small mammals ingesting brodifacoum bait directly. Therefore, there is a high likelihood that the availability of rearing sites for SMHM use may decrease due to reductions in populations of small mammals.

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 difenacoum, the very low application rates (maximum of 0.00005 lb a.i./placement), and the lack of any reported incidents. Since difenacoum 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 difenacoum is a vertebrate pest control agent that may be used in a wide variety of urban and non-urban areas, the spatial extent of difenacoum cannot be limited to defined areas. The Agency assumes that difenacoum 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 difenacoum.

An alternative type of spatial analysis was conducted to characterize the potential use of difenacoum bait products within the region where the assessed species may occur. Since outdoor use of difenacoum bait for rodent control should be within 50 ft of the wall of a building, the extent of this use is expected to be highly correlated with human development. Therefore, spatial analyses were conducted in which the occurrence locations of the SMHM were overlaid with a representation of human development. The "Developed" land cover classes of the NLCD were used to represent the intensity of human development. These land cover classes were displayed with gray shading, with darker grays representing areas of more intense development. This layer was overlaid on segments and points that represent the location of the assessed species. The results of these spatial analyses are shown in **Figure 5-1**.

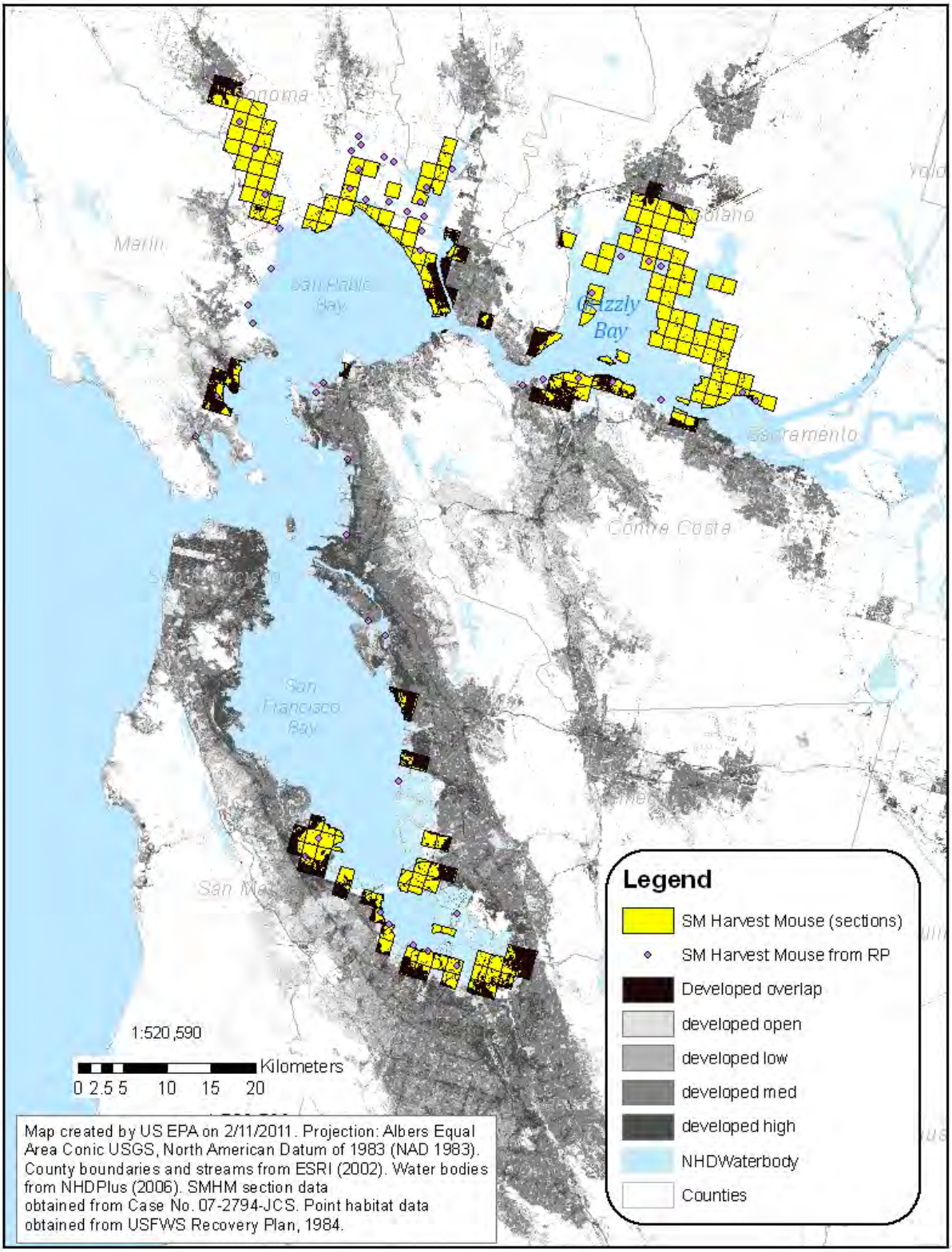


Figure 5-1. Map showing the occurrence of the salt marsh harvest mouse in relation to the intensity of human development.

5.2.1.d. Effects Determination

The results of this risk assessment indicates that use of difenacoum 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 difenacoum bait placed for control of a variety of rodent species. Ingestion of a single application of bait is likely to be lethal to the SMHM and multiple feedings are possible since difenacoum may be rebaited for at least ten days. Even if the ingested dose of difenacoum 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 difenacoum 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 primary risk of direct effect of difenacoum bait on the AW is believed to be secondary poisoning. Secondary poisoning may occur if a whipsnake consumes small mammals, birds, or reptiles that feed directly on the bait. The AW diet includes small mammals, birds, and reptiles, and it may consume any of the target species that difenacoum bait products are meant to control (rats and mice). Whether a whipsnake would scavenge upon a dead mammal that was killed by difenacoum bait is unknown, but since rats and mice poisoned by difenacoum may not die for several days, considerable opportunity would exist for the snake to prey on a poisoned small mammal before it dies. Furthermore, small mammals partially incapacitated by difenacoum exposure would likely be attractive prey to the snakes. Sublethal effects of difenacoum include lethargy and tremors. These sublethal symptoms likely would make poisoned rodents easier to catch.

Extensive use of difenacoum bait products is believed to be possible in the region where the AW occurs. The counties where the AW occurs (Contra Costa, Alameda, San Joaquin and Santa Clara Counties) include many highly developed and densely populated areas. Placement of difenacoum rodenticide bait around residential homes, farm buildings, commercial buildings, and recreation buildings in these counties would provide widespread opportunities for the snake to encounter prey poisoned by brodifacoum bait. The snakes may occur in close proximity to these buildings, for example by living in the crawlspace underneath a home, or in or under a utility shed or agricultural building. Since rodenticide bait would most likely be used in areas where high rodent populations exist, the dense abundance of rodents in these areas may attract the snake.

The restriction of the placement of the rodenticide bait to within 50 ft of exterior walls of buildings is not expected to protect the AW exposure to difenacoum. There is no reason to believe that this snake would not venture near buildings, especially when one considers the term "buildings" includes buildings of all types, not just homes (*e.g.*, besides residential, includes

commercial and industrial buildings/ structures, as well as transportation ports and terminals, and agricultural buildings). Furthermore, the acute rat toxicity studies showed that small mammals which feed on the brodifacoum bait may not die for several days and additionally surviving mammals may feed on bait on multiple succeeding days. Poisoned small mammals may also travel considerable distance away from the buildings and bait stations during that time. Therefore, the AW may be exposed through secondary exposure even if they do not forage near buildings.

Risk quotients for secondary poisoning show that the amount of active ingredient that a mouse ingests would pose a risk of acute toxicity to a whipsnake that feeds on it (acute RQ for AW feeding on mice: **0.15**). Although risk quotients for seconday poisoning indicate that the amount of active ingredient that a rat ingests from a single feeding of bait would not pose a risk of acute toxicity to a whipsnake (acute RQ for AW feeding on rats: 0.05), a rat ingesting contaminated bait on multiple days may pose risk to the AW. Risk quotients for secondary poisoning also show that the amount of active ingredient that a small or medium bird ingests would pose a risk of acute toxicity to an AW that feeds on it (acute RQs for AW feeding on birds range from 0.07—**0.21**)

Although the RQs for secondary exposure were based on a single feeding of a single rodent, bird, or reptile, it is worth noting that the AW could feed upon multiple contaminated rodents, birds, or reptiles. While its feeding on multiple poisoned animals may be infrequent, the tendency of difenacoum to persist in the liver and accumulate over time with repeated exposure is worth noting and could result in even higher RQs than those estimated previously.

Risk quotients indicate that direct consumption of difenacoum bait by the AW would not pose an acute risk to the AW. Acute RQs for a reptile that directly ingested the bait were all less than 0.01**.** It is also uncertain if the AW would feed directly on the baits. The pellets or blocks of rodenticide baits would not be attractive food to an AW. It seems unlikely that a snake would be attracted to this bait since it does not provide the movement, odor, or heat cues that snakes normally use to identify prey.

In conclusion, the weight of evidence justifies the conclusion that the labeled uses of difenacoum are *Likely to Adversely Affect* the survival of the AW. This conclusion is based primarily on risk from direct effects of the snake exposure to difenacoum, in particular from secondary exposure that may occur from consumption of poisoned prey particularly small mammals and small and medium birds.

5.2.2.b. Indirect Effects

The risk assessment also identified the potential for difenacoum use to cause indirect effects on the AW. These indirect effects would be mediated through direct toxic effects on birds, small mammals, terrestrial-phase amphibians, and other reptiles, causing reduction in their abundance. Reduced abundance of these species would indirectly affect the AW by reducing the availability of prey, thereby possibly jeopardizing the ability of the species to meet its energy demands for survival and reproduction. Furthermore, since the AW uses small mammal burrows for cover and foraging (**Attachment II**), reduced small mammal abundance may affect the habitat of the

AW by reducing the abundance of these burrows. These indirect effects, however, are expected to have less impact on the success of this species than the direct toxicity effects.

Mortality caused by the use of these products may be great enough to cause significant declines in the populations of small mammal species. However, effects on abundance would likely be localized to areas around buildings where bait stations are placed for rodent control. This limited area of use would make widespread effects on small mammal populations unlikely. Lizards in particular are believed to be the most important prey item of whipsnakes (**Attachment II**). Lizards generally feed upon insects and other terrestrial arthropods and would not likely consume rodent bait. Additionally, although the AW diet may include terrestrial invertebrates, as mentioned previously, these organisms are not likely to consume rodent bait.

Based on the RQs and the LD_{50} in the most sensitive acute oral toxicity study to birds (probit slope of 1.22), calculations were made to determine the probability that a particular individual would be killed by the dose predicted for a AW consuming rats and mice that have consumed difenacoum bait. The individual probability of death is 16% (1 in 6.35) and 6% (1 in 17.8) for AW that ingested mice and rats, respectively who have consumed difenacoum bait for one day. Similarly, the individual probability of death is 20% (1 in 4.9) for an AW consuming a small bird, 14% (1 in 7.15) for an AW consuming a medium bird, or 8% (1 in 12.6) for an AW consuming a large bird that ingested difenacoum bait.

5.2.2.c. Spatial Extent of Potential Effects

Similar to the analysis for the SMHM, it is assumed that difenacoum 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.

As previously discussed, an alternative type of spatial analysis was conducted to characterize the potential use of difenacoum bait products within the region where the assessed species may occur. Since outdoor use of difenacoum bait for rodent control should be within 50 ft of the wall of a building, the extent of this use is expected to be highly correlated with human development. Therefore, spatial analyses were conducted in which the occurrence locations of the AW were overlaid with a representation of human development. The “Developed” land cover classes of the NLCD were used to represent the intensity of human development. These land cover classes were displayed with gray shading, with darker grays representing areas of more intense development. This layer was overlaid on segments and points that represent the location of the assessed species, and for the AW, the location of its critical habitat. The results of these spatial analyses are shown in **Figure 5-2**.

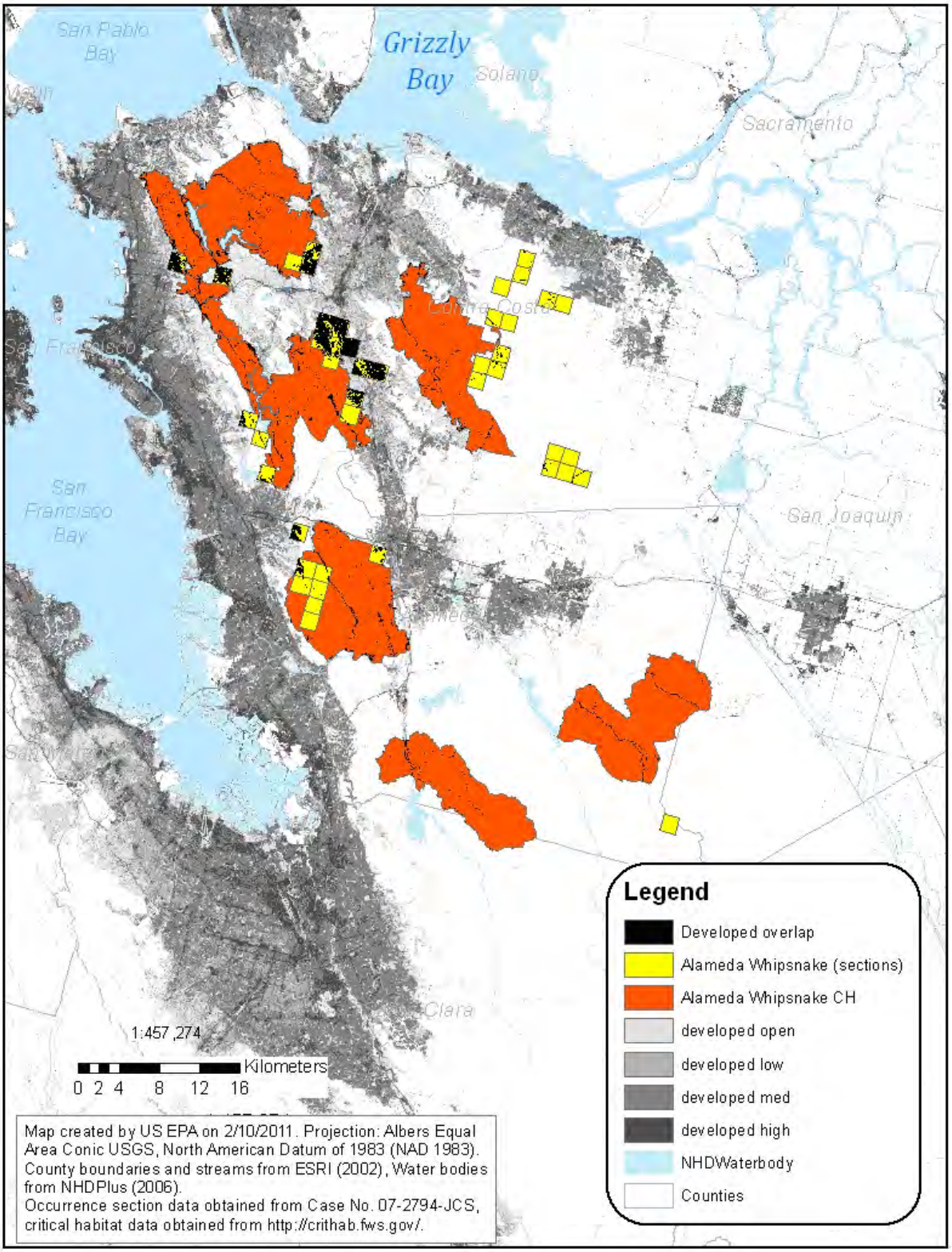


Figure 5-2. Map showing the occurrence of Alameda whipsnake, and its critical habitat, in relation to the intensity of human development.

5.2.2.d. Modification of designated critical habitat

Critical habitat has been defined for the AW. As discussed above, the potential for difenacoum 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 difenacoum in bait products to is not expected to result in significant effects on plants. Due to the mode of action of difenacoum, effects to plants are not expected.

5.2.2.e. Effects Determination

The results of this risk assessment indicates that use of difenacoum 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 difenacoum. 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 San Joaquin Kit Fox

5.2.3.a Direct Effects

Direct effects to the SJKF are possible, based on the registered uses of difenacoum. The dose-based acute RQs calculated in the risk estimation for secondary exposure of the SJKF to difenacoum exceeded the Acute Endangered Species LOC, acute restricted use, and acute LOC (**Tables 5-3** and **5-5**). Therefore, there is potential for mortality to the SJKF through consumption of prey that ingested difenacoum bait. Chronic studies for the toxicity of difenacoum to mammals were not available but due to the high acute toxicity and effects of difenacoum to mammals; therefore, chronic risk is also assumed.

For individual chance of effect calculations, dose-based acute RQs for secondary of the SJKF were used. The probability of an individual effect to the SJKF using the dose-based RQ was calculated using a slope estimate of 9.9 from the male rat acute oral LD50 study. The RQ for a SJKF ingesting a 244-g mammal is **1.25 (Table 5-3)** The individual probability of death is 83% (1 in 1.2) for a SJKF consuming a small mammal that ingested difenacoum bait.
The RQ for a SJKF ingesting a 244 g bird is **2.1** (**Table 5-5**). The estimated chance of an individual acute mortality of the SJKF ingesting a difenacoum poisoned bird is 100% (1 in 1). These results indicate that the probability of an individual mortality occurrence is high and that difenacoum has the potential to directly affect the SJKF via secondary exposure.

5.2.3.b Indirect Effects

Indirect effects to the SJKF may occur through the potential for difenacoum to adversely affect the abundance and quality of available mammalian, avian and reptilian prey items.

For nonlisted mammalian prey consuming bait directly, acute dose-based RQs exceeded Acute LOCs (RQs ranged from **1.2 – 4.4**) (**Table 5-9**). Based on the RQs and the LD_{50} in the most sensitive acute oral toxicity study to mammals (probit slope of 9.9), calculations were made to determine the probability that a particular individual would be killed by the dose predicted for a mammal ingesting difenacoum bait. The individual probability of death is 100% (1 in 1) for a Norway rat or house mouse that ingested difenacoum bait.

For non-listed avian prey consuming bait directly, the acute RQs for primary exposure exceed 0.1 for small and medium birds (acute oral dose based RQs ranged from (0.04 **-0.17** and the acute dietary RQ was **3.5**), the LOC for acute effects to listed species, use of difenacoum has the potential to indirectly affect the SJKF by affecting the abundance of avian prey items.

Based on the RQs and the LD_{50} in the most sensitive acute oral toxicity study to birds (slope of 1.22 for the acute oral study and 1.13 for the acute dietary study), calculations were made to determine the probability that a particular individual would be killed by the dose predicted for a bird consuming difenacoum bait. According to the acute oral data, the individual probability of death is 17% (1 in 5.75) for a small bird consuming difenacoum bait, 11% (1 in 8.99) for a medium bird consuming difenacoum bait or 4% (1 in 22.7) for a large bird consuming difenacoum bait. However, according to the avian acute dietary data, the individual probability of death is 73% (1 in 1.37) for a bird of any size consuming difenacoum bait.

For non-listed reptilian prey consuming bait directly, the acute RQs for primary exposure all were less than 0.01, use of difenacoum is unlikely to affect the SJKF by affecting the abundance of reptilian prey items.

5.2.3.c Spatial Extent of Potential Effects

Similar to the analysis for the SMHM and AW, it is assumed that difenacoum 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.

As previously discussed, an alternative type of spatial analysis was conducted to characterize the potential use of difenacoum bait products within the region where the assessed species may occur. Since outdoor use of difenacoum bait for rodent control should be within 50 ft of the wall of a building, the extent of this use is expected to be highly correlated with human development. Therefore, spatial analyses were conducted in which the occurrence locations of the SJKF were overlaid with a representation of human development. The "Developed" land cover classes of the NLCD were used to represent the intensity of human development. These land cover classes were displayed with gray shading, with darker grays representing areas of more intense development. This layer was overlaid on segments and points that represent the location of the assessed species. The results of these spatial analyses are shown in **Figure 5-3**.

It is noted that the map depicted in **Figure 5-3** appears quite different from **Figures 5-1** and **Figures 5-2**, showing the overlap of the SMHM and AW, respectively. There are two points of explanation for this. The first reason is the overall scale of the map in **Figure 5-3** as compared to **Figures 5-1** and **5-2**. When one notices the zoom scale on **Figure 5-3**, it is noted that the zoomed out level is approximately 3 times that of **Figures 5-1** and **5-2**. This is primarily because the SJKF is much more widely distributed than the AW and the SMHM and so to account for all areas of occurrence, the map is further zoomed out. The second reason is that the grayscale used to show low to high areas of development as well as the black areas that are used to show overlap in **Figures 5-1** and **5-2** are not visible on the further zoomed out **Figure 5-3**. Attempts were made to include these areas but the areas of overlap and occurrence blended into one another and precluded the ability to tell a difference between these areas. During consultation with the Services, the Agency will develop further refinement of the spatial overlap between land cover and SJKF habitat.

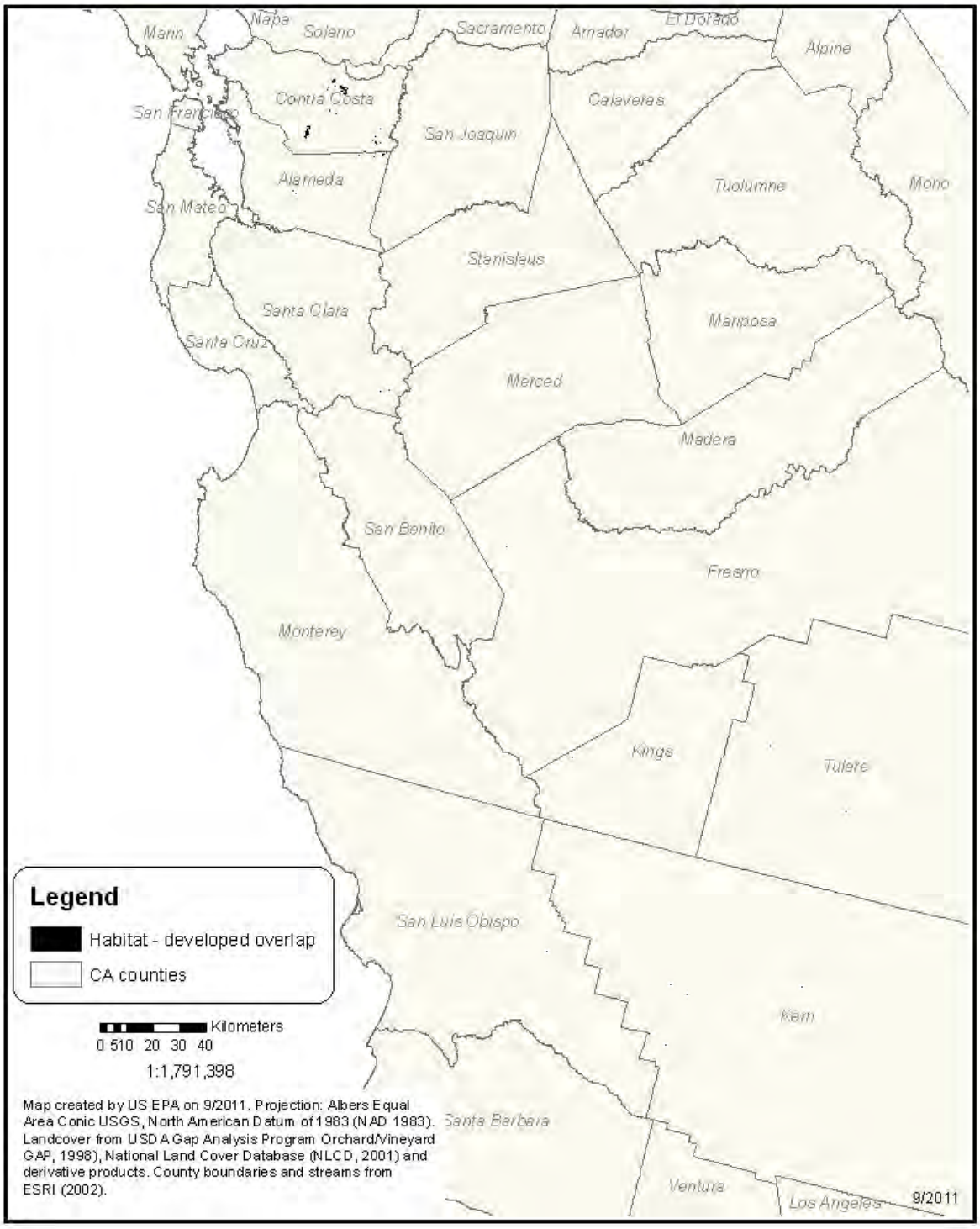


Figure 5 3. Map showing the occurrence of the San Joaquin Kit Fox in relation to the intensity of human development.

5.2.3.d Effects Determination for San Joaquin Kit Fox (SJKF)

The results of this risk assessment indicates that use of difenacoum in baits for vertebrate pest control poses a high risk of acute toxicity to the SJKF resulting from secondary exposure. Secondary poisoning may occur when the SJKF preys upon small mammals, birds, reptiles or other vertebrate prey species which have ingested difenacoum bait. Further risk to this species would be posed by sublethal behavioral and neurological effects which may result from acute exposure to difenacoum. Finally, indirect effects are also possible from use of this product reducing the abundance of vertebrate prey.

Therefore, the Agency makes **"may affect"** and **"likely to adversely affect**" determination for the SJKF, based on the potential for direct and indirect effects.

5.2.4 Addressing the Risk Hypotheses

In order to conclude this risk assessment, it is necessary to address the risk hypotheses defined in **Section 2.2.2**. 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 difenacoum on the AW, SJKF and SMHM and the designated critical habitat of the AW.

- directly affect the AW and SJKF by causing mortality or by adversely affecting growth or fecundity via secondary exposure by consuming prey that have ingested difenacoum bait;
- directly affect the SMHM by causing mortality or by adversely affecting growth or fecundity via primary poisoning by direct consumption of difenacoum bait;
- indirectly affect the SMHM by reducing the number of small mammalian rearing sites by direct effects on mammals;
- indirectly affect the AW and/or modify the designated critical habitat of the AW by reducing or changing the composition of food supply; and
- indirectly affect the AW and/or modify its designated critical habitat of the AW by reducing or changing terrestrial habitat in their current range via reduction in availability of small burrowing mammals burrows used by the AW for cover.

Indirect effects mediated through effects on terrestrial and aquatic plant communities were judged to be discountable.

6. Uncertainties

6.1 Fate and Transport Properties

Uncertainty in the exposure assessment stems mainly from assumption made in the assessment related to the consumption of difenacoum bait by various types of animals. Animals were

assumed to consume an amount of bait equal to their predicted daily food ingestion rate. Ingestion of bait is most certain for omnivorous small mammals because the bait is designed to be attractive to rodents. However, small mammals could eat less bait than their average daily ingestion rate, either because they are also feeding on other food sources, or because they exhibit bait shyness. Alternatively, if other food is scarce and they find the bait to be very attractive, then they could exhibit gorging behavior, consuming bait in excess of their average daily intake rate or have additional difenacoum in their system after consuming bait for multiple days. The consumption of difenacoum bait by animals other than small mammals is less certain. No incidents or field studies have shown that species other than small mammals consume the bait. Animals which feed predominantly on live prey, including the AW and SJKF, may not consume the bait.

The food intake rate was estimated from the body weights of the animals using allometric equations. How well the generic allometric equations used predict the specific food intake rate of the assessed species introduces further uncertainty. For example, the relationship for the AW was based on an equation developed for insectivores, whereas the AW consumes a wide variety of vertebrate prey in addition to terrestrial invertebrates.

The assessment of secondary exposure to the AW and SJKF involves additional uncertainties. A conservative assumption was made that the entire amount of active ingredient consumed by the prey is present in the prey animal when it is consumed by the snake. In reality, the amount of active ingredient in the prey may decrease between the time the prey consumes the bait and the time the prey is consumed by the snake as the result of elimination and detoxification. However, difenacoum has been found to have a long half life both in the liver tissue and in blood plasma (61.8 days in the liver and 20.4 in plasma, Vanenbrouck et al., 2008).

The dose of difenacoum from secondary exposure is dependent on the size of the prey. The size of prey that the AW was predicted to be able to consume is uncertain. As described in **Section 3.1.2**, the body weight of the AW was estimated from an equation based on its length, and this body weight as then used in a second equation to predict the maximum size of the prey. Because the AW is a slender snake, these equations may overestimate both the body weight of this snake, and the maximum size prey which it may consume. Specifically, it is uncertain if an adult AW would be able to ingest a large Norway rat, even though these equations predict that it would.

Uncertainties that apply to most assessments completed for the San Francisco Bay Species Litigation are discussed in **Attachment I**. This section describes additional uncertainties specific to this assessment.

6.2 Effects Assessment Uncertainties

6.2.1 Data Gaps and Uncertainties

The lack of research that directly measures the secondary poisoning hazard of difenacoum in terrestrial animals brings uncertainty in the conclusions of the secondary poisoning assessment. Secondary poisoning studies, in which animals are fed prey which have been allowed to feed on the bait, would reduce the uncertainties in the conclusion of the secondary poisoning risk of difenacoum.

Finally, acceptable avian reproduction data is not available for difenacoum. This increases the uncertainty of the risk assessment for the AW because birds are used as surrogates for reptiles in toxicity testing. Without avian reproduction data, chronic risks to the AW could not be assessed. Additionally, there were no mammalian reproduction data available for the analysis of direct and indirect effects to the SMHM, and indirect effects to the AW and SJKF.

An additional level of uncertainty are the uses of estimating exposure of doses of difenacoum and the amount a prey item would carry over to an AW or a SJKF. The equations used were for insectivorous/herbivorous reptiles and mammals as equations for carnivorous reptiles were not available from the *Wildlife Exposure Handbook* were not available.

6.2.2 Use of Surrogate Species Effects Data

While the available toxicity data provides fairly certain information on the acute toxicity of difenacoum to small mammals and birds (including the SMHM), extrapolation of these species to the AW (reptile) and SJKF (large mammal) is uncertain. Extrapolation to potential toxic effects to reptile and amphibian prey of the AW and SJKF is also uncertain.

6.2.3 Sublethal Effects

When assessing acute risk, the screening-level 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.

Difenacoum is an anticoagulant pesticide. It has been shown to cause numerous adverse behavioral and neuromuscular effects at sublethal levels. The possible impact of these sublethal effects on the survival and reproduction of the assessed species was only qualitatively characterized. To the extent to which sublethal effects are not considered in the quantitative risk assessment, the potential direct and indirect effects of difenacoum on listed species may be underestimated.

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 difenacoum to AW, SJKF and SMHM and the designated critical habitat for AW.

Based on the best available information, the Agency makes a ***May Affect*** and a ***Likely to Adversely Affect*** (**LAA**) determination for the use of difenacoum relative to the AW, SMHM

and SJKF. Additionally, the Agency has determined use of difenacoum has the potential to cause modification of the designated critical habitat of the AW from the use of the chemical. Given the LAA determination for the AW, SMHM, and SJKF, and potential modification of designated critical habitat for the AW, a description of the baseline status and cumulative effects is provided in **Attachment III**.

A summary of the risk conclusions and effects determinations for the AW, SMHM and SJKF and the AW's critical habitat, given the uncertainties discussed in **Section 6** and **Attachment I**, is presented in **Table 7-1** and **Table 7-2**. and Use specific effects determinations are provided in **Table 7-3.**

Table 7-1. Effects Determination Summary for Effects of Difenacoum on the Alameda Whipsnake, Salt Marsh Harvest Mouse and San Joaquin Kit Fox

Species	Effects Determination	Basis for Determination
Alameda whipsnake (AW) (*Masticophis lateralis euryxanthus*)	*May Affect* and *Likely to Adversely Affect* (LAA)	**Potential for Direct Effects**
		Use of difenacoum potentially may result in direct effects to the AW from acute toxicity through secondary exposure. Dietary exposure estimates and acute toxicity to reptiles (based on acute toxicity data for birds) result in acute RQs that exceed the LOC for secondary exposure. Secondary exposure is considered the primary threat to this species. The individual probability of death is 16% and 6% for AW that ingested mice and rats, respectively who have consumed difenacoum bait for one day. Data were not available to assess chronic toxicity; however, since risk is predicted for acute exposure based on mortality, and sublethal effects were observed with sublethal acute exposure, risk of chronic effects is also assumed.
		Potential for Indirect Effects
		Terrestrial prey items Use of difenacoum may reduce the abundance of terrestrial vertebrates which serve as prey for this species. This conclusion is based on acute RQs for birds and mammals (though not for reptiles) which exceed the LOCs. Data were not available to assess chronic toxicity; however, since risk is predicted for acute exposure based on mortality, and sublethal effects were observed with sublethal acute exposure, risk of chronic effects is also assumed. Furthermore, incidents in the United Kingdom involving mammals and birds have been reported in association with the use of difenacoum. ***Habitat Modification*** Use of difenacoum may modify the habitat of this species by reducing the availability of small mammal burrows. This conclusion is based on acute RQs for mammals that exceed the LOCs. Data were not available to assess chronic toxicity; however, since risk is predicted for acute exposure based on mortality, and sublethal effects were observed with sublethal acute exposure, risk of chronic effects is also assumed.
Salt Marsh Harvest Mouse (SMHM) (*Reithyodontomys*	May Affect, Likely to Adversely Affect (LAA)	**Potential for Direct Effects**
		Use of difenacoum may result in direct effects to the SMHM from acute toxicity via primary exposure. Exposure estimates and acute toxicity to mammals result in acute RQs that exceed the LOCs for primary exposure

Species	Effects Determination	Basis for Determination
raviventris)		to the SMHM. Primary exposure is considered the primary threat to this species. The individual probability of death is 100% for a SMHM consuming one day's food intake of difenacoum bait. Data were not available to assess chronic toxicity, however since risk is predicted for acute exposure based on mortality, and sublethal effects were observed with sublethal acute exposure, risk of chronic effects is also assumed. Furthermore, incidents in the United Kingdom involving small mammals have been reported in association with the use of difenacoum.
		Potential for Indirect Effects
		Terrestrial Habitat Registered uses of difenacoum may reduce SMHM rearing sites by adversely affecting small mammals that create burrows used by the SMHM. Estimated acute RQs for primary exposure to mammals exceeded acute LOCs for the small mammalian weight class considered.
San Joaquin Kit Fox (SJKF) (*Vulpes macrotis mutica)*	May Affect, Likely to Adversely Affect (LAA)	**Potential for Direct Effects**
		Use of difenacoum potentially may result in direct effects to the SJKF from acute toxicity via secondary exposure. Secondary exposure is considered the primary threat to this species. The individual probability of death is 83% and 100% for a SJKF consuming a small mammal and a 244-g bird respectively, that have consumed difenacoum bait for one day. Data were not available to assess chronic toxicity; however, since risk is predicted for acute exposure based on mortality, and sublethal effects were observed with sublethal acute exposure, risk of chronic effects is also assumed. Furthermore, incidents in the United Kingdom involving mammals have been reported in association with the use of difenacoum.
		Potential for Indirect Effects
		Terrestrial prey items Use of difenacoum may reduce the abundance of terrestrial vertebrates which serve as prey for this species. Data were not available to assess chronic toxicity; however, since risk is predicted for acute exposure based on mortality, and sublethal effects were observed with sublethal acute exposure, risk of chronic effects is also assumed. Furthermore, incidents in the United Kingdom involving mammals and birds have been reported in association with the use of difenacoum.

Table 7-2. Effects Determination Summary for the Critical Habitat Impact Analysis

Designated Critical Habitat for:	Effects Determination	Basis for Determination
Alameda whipsnake (AW) (*Masticophis lateralis euryxanthus*)	Habitat Modification	Risk assessment indicates use of difenacoum 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, and terrestrial-phase amphibians.

Table 7-3. Use Specific Summary of the Potential for Adverse Effects by Taxa

Uses	Potential for Effects to Identified Taxa Found in the Terrestrial Environment[7]									
	SMHM and Small Mammals[1]		SJKF and Large Mammals[2]		AW and Reptiles[3]		Small Birds[4]		Terrestrial – phase Amphibians[5]	
	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic
Rodent Control	Yes	Yes[6]	Yes	Yes[6]	Yes	Yes[6]	Yes	Yes[6]	Yes	Yes[6]

1 A yes in this column indicates a potential for direct effects to the SMHM and indirect effects to the AW and SJKF.
2 A yes in this column indicates a potential for direct and indirect effects to SJKF.
3 A yes in this column indicates the potential for direct (through secondary exposure) to the AW and indirect (through prey reduction) effects to the AW.
4 A yes in this column indicates a potential for direct effects to the AW and SJKF.
5 A yes in this column indicates a potential for indirect effects to the AW.
6 Chronic toxicity data are not available to assess this species; however chronic risk is assumed based upon the high acute risks.
7 Terrestrial invertebrates and terrestrial plants, which have the potential to indirectly affect all three species were not assessed. In addition, aquatic plants, which have the potential to indirectly affect the SMHM, were also not assessed.

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, SJKF 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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MRID	Citation Reference
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MRID	Citation Reference
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MRID	Citation Reference
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