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

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Primary Authors:
Justin Housenger, Biologist
José L. Meléndez, Chemist

Secondary Review:
Gabe Rothman, Environmental Scientist
Valerie Woodard, Ph.D, Biologist
Keith Sappington, Senior Advisor

Branch Chief, Environmental Risk Assessment Branch V:
Mah T. Shamim, Ph.D.

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

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

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

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

1. 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 (AW, *Masticophis lateralis euryxanthus*), the federally endangered salt marsh harvest mouse (SMHM, *Reithrodontomys raviventris*), and the federally endangered San Joaquin kit fox (SJKF, *Vulpes macrotis mutica*) arising from FIFRA regulatory actions regarding use of difethialone 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 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, 2004), and consistent with a suit in which difethialone was alleged to be of concern to the AW, SMHM and SJKF (*Center for Biological Diversity (CBD) vs. EPA et al.* (Case No. 07-2794-JCS).

Difethialone is an anticoagulant pesticide for rodent control against commensal rats and mice. There are 25 product labels for this chemical (one of which is a Special Local Needs or SLN); however, at the time this review is prepared, seven of them do not comply with the risk mitigation decision required for ten rodenticides. All compliant and non-compliant labels were included in this assessment. [1]

The AW, a subspecies of the California whipsnake (*Masticophis lateralis*), was listed as threatened in 1997 by the USFWS. A recovery plan for this and other threatened and endangered species of the chaparral and scrub community east of San Francisco Bay, California was approved by the USFWS in 2003. Critical habitat was designated for this subspecies in 2006. 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. The subspecies occurs in the Inner Coast Ranges in Contra Costa, Alameda, San Joaquin, and Santa Clara Counties in California.

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.

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. Critical habitat has not been designated for the SJKF; therefore, PCEs have not been defined.

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[1] According to the Risk Mitigation Decision for Ten Rodenticides (EPA-HQ-OPP-2006-0955-0764), which was issued in May 2008 and revised in June 2008..."To reduce wildlife exposures and ecological risks, the Agency will require sale and distribution limits intended to prevent general consumers from purchasing residential use bait products containing four of the ten rodenticides...," which include the second generation difethialone. This is required for labels of all difethialone bait products sold after June 4, 2011. However, not all labels of difethialone rodenticide products are in compliance with this requirement at the time this assessment is completed.

1.2. Scope of Assessment

1.2.1. Uses Assessed

Difethialone is a rodenticide for control of Norway rats (*Rattus norvegicus)*, roof rats (*Rattus rattus*) and house mice (*Mus musculus*). Formulation types registered include pellets, pellet packs, blocks, mini blocks, paraffin blocks, meal, packs or pouches, paste and bait stations. Currently, labeled uses of difethialone include in and around homes, and agricultural, industrial and commercial buildings, transport vehicles and associated ports, alleys and sewers. The following uses are considered as part of the federal action evaluated in this assessment: in and around homes, and agricultural, industrial and commercial buildings, ports associated with transport vehicles (*e.g.*, trains, aircraft, ships), alleys and sewers.

Uses that occur indoors would not be expected to result in any primary exposure to the AW, SMHM, or SJKF. However, indoor use could result in secondary exposure to the AW or SJKF if a rodent ingests bait indoors and then moves outside and is consumed by the AW or SJKF. In addition, many label uses classified as "indoor" actually refer to uses that take place indoors and/or within 50 ft of residences or other structures. Therefore, all of the uses listed above (both indoor and outdoor) are considered as part of the federal action evaluated in this assessment.

1.2.2. Environmental Fate Properties of Difethialone

It appears that the primary route of dissipation/transport for difethialone might be through consumption of bait product and, because the animals do not die immediately after feeding, movement of the intact chemical in the bodies of the affected animals to distant places. Difethialone kills birds and target and possibly other non-target mammals eating bait within a period of days; therefore, movement of the chemical might be substantial during that period. Difethialone (CAS Chemical Name 3-[3-(4′-bromo[1,1′-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-2*H*-1-benzothiopyran-2-one) is a slightly soluble compound (solubility of 0.39 mg/L at 25°C) and non-volatile (vapor pressure of 5.55×10^{-7} mmHg at 25°C; calculated Henry's Law Constant of 1.00×10^{-6} atm-m^3/mole). The compound is relatively stable to hydrolysis (half-lives of 154 - 211 days at pHs 5, 7 and 9); however, the UV/ vis spectrum of the compound has a peak within the visible region, which suggests photolysis by sunlight can be important. This is confirmed by an available aqueous photolysis study in which the half-lives were nearly 1 hour at all three pHs tested (5, 7 and 9). In a sandy loam soil, difethialone degraded slowly (half-life 204 days, with no further identification data about the major degradate, which appeared to increase throughout the study). The compound was immobile in four soils tested. Based on its low vapor pressure and Henry's Law Constant, and the very low levels of volatilization observed in the aerobic soil metabolism study, this compound should not volatilize readily. Furthermore, the very short atmospheric half-lives predicted by EPIWEB v.4.0 (both, for ozone and hydroxyl radical reactions <<1 day), would indicate that atmospheric transport is not likely. Based on an estimated log K_{OW} value of 9.82 (EPIWEB v.4.0 estimate), bioconcentration in fish is anticipated should water exposure occur. The low application rates of difethialone per placement and its high tendency to be adsorbed to soils (mean $K_F \approx 8.2 \times 10^5$) indicate a low likelihood of runoff towards adjacent surface waters except when carried by eroded sediment. The applications of difethialone as bait products will not cause the chemical to drift. No monitoring data has been found for this chemical. Rodenticide test substances are not

typically considered in surface or groundwater monitoring studies. For further details about this chemical's physicochemical and fate characteristics, refer to **Tables 2-1** and **2-2**, respectively.

1.2.3. Evaluation of Degradates and Stressors of Concern

No information about possible degradates of difethialone could be derived from the available environmental fate studies. The only exception is that one degradate was observed in the aerobic soil metabolism study but it was not identified. Difethialone is relatively persistent in studies performed to assess its half-life in livers of animals. Thus, it remains intact for several months if taken at sublethal levels and it appears unlikely that major (≥10% AR) metabolites would be formed in animal tissues.

1.3. Assessment Procedures

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

1.3.1. Exposure Assessment

1.3.1.a. Aquatic Exposures

No aquatic species are relevant to the assessment of the AW and SJKF, based on their life history and feeding preferences. The aquatic plant taxon are relevant to the SMHM for the potential of indirect effects. However, difethialone has a bait formulation nature as well as low application rates. Furthermore, its strong affinity to sorb to soil suggests that it is likely to strongly sorb to bait itself; consequently, off-field runoff and exposures in aquatic environments are expected to be negligible. In conclusion, concentrations of difethialone in both freshwater and saltwater marshes are expected to be negligible and not impact aquatic vegetation. Models that estimate concentrations in surface water or calculate spray drift deposition of difethialone on aquatic habitats were therefore not applicable and not needed. No surface water monitoring data are readily available for difethialone. Based on this information, the aquatic habitat is not assessed in this document.

1.3.1.b. Terrestrial Exposures

Difethialone exposures to the AW and SJKF resulting from application of difethialone baits were evaluated by assuming the AW and SJKF consume prey species that directly consumed baits of various types (*e.g.*, pellets, blocks, paraffinized 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 above ground placements of difethialone are required to be in bait stations, which would preclude primary exposure to the SJKF. Direct effects through primary exposure would only be applicable to the SMHM via direct consumption of difethialone bait from outdoors bait applications. Indoors, applied bait is unlikely to be consumed by the SMHM.

Indirect effects to the AW and SJKF in this assessment are defined as direct effects to birds, mammals, and reptiles that directly ingest difethialone bait and serve as prey for the AW and SJKF. Since exposure occurs via consumption of organisms that have ingested baits, standard EFED models of terrestrial exposure (*e.g.*, T-REX, T-HERPS) are not considered in this assessment. The concentration of active ingredient in food was simply assumed to be the concentration of active ingredient in the bait. The Agency does not have a standard model or method of predicting secondary exposure of terrestrial animals that eat other animals which have ingested bait. As a Tier 1 (screening) risk assessment, conservative assumptions included:

1. The amount of active ingredient ingested by the AW and SJKF from secondary exposure was assumed to be equal to the amount of active ingredient that a prey item would ingest if it consumed difethialone bait at its daily ingestion rate.
2. The prey was assumed to be a house mouse, roof rat or Norway rat (the target species for which difethialone is currently registered), as well as birds and reptiles, and the amount of bait these species ingested was assumed based on their body weights. It is noted, however, that non-target animals such as other small mammals, birds, and reptiles can ingest the bait directly as well.
3. The weights of these species were assumed to be the maximums of the reported body weight ranges to maximize secondary exposure to the AW and SJKF.
4. All of the difethialone ingested by the prey was assumed to be available to and assimilated by the AW and SJKF that eats it.

Effects to terrestrial plants are not considered in this assessment due primarily to the nature of the bait placements and mode of action of difethialone. The mode of action of difethialone is to block the activity of Vitamin K epoxide reductase. This enzyme is needed for the reconstitution of Vitamin K in its cycle from Vitamin-K epoxide; therefore, difethialone steadily reduces the amount of active Vitamin K in the blood. Vitamin-K is required for the synthesis of prothrombin, which is involved in blood clotting. Since plants do not have a circulatory system, this mode of action is not relevant to plants as it is to animals. Furthermore, terrestrial plants are not expected to be exposed to difethialone given its method of application. Therefore, effects to terrestrial plants and resulting indirect effects to the assessed species are not considered in this assessment.

1.3.2. Toxicity Assessment

The assessment endpoints include direct toxic effects on survival, reproduction, and growth of individuals (through primary exposure) to the SMHM, direct toxic effects on survival, reproduction, and growth of individuals (through secondary exposure) for the AW and SJKF, and indirect effects, such as reduction of the food source and/or modification of habitat for the AW, SJKF, and SMHM. Federally-designated critical habitat has been established for the AW but not for the SMHM and SJKF. Primary constituent elements (PCEs) were used to evaluate whether difethialone has the potential to modify designated critical habitat. The Agency evaluated registrant-submitted studies and data from the open literature to characterize difethialone toxicity. The most sensitive toxicity value available from acceptable or supplemental registrant submitted studies as well as ECOTOX and OPP accepted open literature studies for each taxon relevant for estimating potential risks to the assessed species and/or their designated critical habitat was used.

Section 4 summarizes the ecotoxicity data available on difethialone. Difethialone is very highly toxic to birds on an acute oral and subacute dietary exposure basis, and very highly toxic to mammals on an acute oral exposure basis. In an acute oral toxicity study with the bobwhite quail, sublethal effects that were noted included lethargy, piloerection, bloody diarrhea, limping, and bleeding from the tail feather or mouth. Statistical analysis showed significant decrease in body weight at the highest treatment group. Severe foot depressions were noted at the two highest treatment concentrations during test days 4-7 only. This study was slightly modified from the traditional avian acute oral guideline with a 14-day duration in that it had a 30-day duration due to the delayed toxicity attributed to difethialone and other second generation anticoagulants. In this study, mortality occurred up to 23 days after initial exposure to difethialone. In an acute oral toxicity study with the Norway rat, the highest treatment group (0.5 mg a.i./kg-bw) showed signs of toxicity that included bleeding from the ears, dyspnea (shortness of breath), lethargy, prostration, lacrimation, piloerection, dry feces, pale eyes, nostril discharge, tachypnea (rapid breathing), catalepsy, tremors, hyperactivity, somnolence, tow walking, and watery stool. Clinical signs of toxicity for the middle treatment group (0.45 mg a.i./kg-bw) included blood from ears, dyspnea, lethargy, dry feces, nostril discharge, piloerection, lacrimation, tremors, and tachypnea. Clinical signs of toxicity from the lowest treatment group (0.40 mg a.i./kg-bw) included blood from ears, dyspnea, lethargy, loose stool, piloerection, and tremors.

In a secondary feeding study, in which difethialone secondary hazard potential was estimated by comparing accumulated residues in rats to the dietary levels in magpies and ferrets, the magpie was the more sensitive species. Both species were assessed in a 30-35 day (depending on species used and whether the study was a range finder or definitive test) exposure that consisted of a 3 day pre-treatment period, a 5 day treatment period, and a 25-27 day post-treatment period for observations. The accumulation of residues in rat carcasses was also assessed using two different feeding scenarios, one in which animals were offered bait feed for three days and then euthanized, and one in which animals were offered bait feed until poisoning and death occurred. Rats that were allowed to consume bait until death had a slightly higher body residue count (3.1 mg a.i/kg-bw compared to 2.8 mg a.i/kg-bw for euthanized rats). There was no statistical difference in these results.

There are no available data to characterize toxicity of difethialone to the honey bee. No data are available to characterize chronic toxicity to birds and mammals.

1.3.3. Measures of Risk

Acute and chronic risk quotients (RQs) are compared to the Agency's Levels of Concern (LOCs) to identify instances where difethialone use has the potential to adversely affect the assessed species or 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 difethialone use "may affect" the assessed species, and/or may cause effects to designated critical habitat, the best available additional information is considered to refine the potential for exposure and effects, and

distinguish actions that are Not Likely to Adversely Affect (NLAA) from those that are Likely to Adversely Affect (LAA).

1.4. Summary of Conclusions

Based on the best available information, the Agency makes a May Affect, and Likely to Adversely Affect determination for AW, SMHM and SJKF from the use of difethialone. Additionally, the Agency has determined that there is the potential for modification of designated critical habitat for the AW from the use of the chemical. A summary of the risk conclusions and effects determinations for the listed species assessed here and their designated critical habitat is presented in **Table 1-1** and **Table 1-2**. Use-specific determinations are provided in **Table 1-3.** Further information on the results of the effects determination is included as part of the Risk Description in **Section 5.2**. 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 for AW, SMHM, and SJKF is provided in Attachment III.

Table 1-1. Effects Determination Summary for Effects of Difethialone on the AW, SMHM and SJKF.

Species	Effects Determination	Basis for Determination
Alameda whipsnake (AW) (*Masticophis lateralis euryxanthus*)	*May Affect* and *Likely to Adversely Affect* (LAA)	**Potential for Direct Effects**
		Risk assessment indicates use of difethialone potentially will 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 both primary and secondary exposure. Secondary exposure is considered the primary threat to 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.
		Potential for Indirect Effects
		Terrestrial prey items Risk assessment indicates use of difethialone will likely reduce the abundance of terrestrial vertebrates which serve as prey for this species. This conclusion is based on acute RQs for birds, mammals, and 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 involving small mammals have been reported in association with the use of difethialone. ***Habitat Modification*** Risk assessment indicates use of difethialone 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

Species	Effects Determination	Basis for Determination
		assumed.
Salt Marsh Harvest Mouse (SMHM) (*Reithyodontomys raviventris*)	May Affect, Likely to Adversely Affect (LAA)	**Potential for Direct Effects**
		Risk assessment indicates that use of difethialone will 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 to the SMHM. Primary exposure is considered the primary threat to 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 involving small mammals have been reported in association with the use of difethialone.
		Potential for Indirect Effects
		Terrestrial Habitat Risk assessment indicates that the registered uses of difethialone will reduce SMHM rearing sites by adversely affecting small mammals. 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**
		Risk assessment indicates use of difethialone potentially will result in direct effects to the SJKF from acute toxicity via secondary exposure. Secondary exposure is considered the primary threat to 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 involving mammals have been reported in association with the use of difethialone.
		Potential for Indirect Effects
		Terrestrial prey items Risk assessment indicates use of difethialone will likely reduce the abundance of terrestrial vertebrates which serve as prey for this species. 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. 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 involving birds and mammals have been reported in association with the use of difethialone.

Table 1-2. 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 difethialone 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, SMHM 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 to seek concurrence with the LAA determinations for AW, SMHM, and SJKF and to determine whether there are reasonable and prudent alternatives and/or measures to reduce and/or eliminate potential incidental take.

When evaluating the significance of this risk assessment's direct/indirect and habitat modification effects determinations, it is important to note that pesticide exposures and predicted risks to the 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 AW, SMHM, and SJKF 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, 2004) and reviewed by the U.S. Fish and Wildlife Service and National Marine Fisheries Service (USFWS/NMFS/NOAA, 2004).

2.1. Purpose

The purpose of this endangered species assessment is to evaluate potential direct and indirect effects on individuals of the federally threatened Alameda Whipsnake (AW), and the federally endangered Salt Marsh Harvest Mouse (SMHM) and San Joaquin Kit Fox (SJKF), arising from FIFRA regulatory actions regarding use of difethialone for rodent control. 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) entered in Federal District Court for the Northern District of California on May 17, 2010.

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, 2004).

The AW, a subspecies of the California whipsnake, was listed as threatened in 1997 by the USFWS. A recovery plan for this and other threatened and endangered species of the chaparral and scrub community east of San Francisco Bay, California was approved by the USFWS in 2003. Critical habitat was designated for this subspecies in 2006. The subspecies occurs in the Inner Coast Ranges in Contra Costa, Alameda, San Joaquin, and Santa Clara Counties in California.

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.

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.

In accordance with the Overview Document, provisions of the ESA, and the Services' *Endangered Species Consultation Handbook,* the assessment of effects associated with registrations of difethialone 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 difethialone 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 the 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 difethialone 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.*, liquid or granular), acceptable methods of application, approved use sites, and any restrictions on how applications may be conducted. Thus, the use or potential use of difethialone in accordance with the approved product labels for California is "the action" relevant to this ecological risk assessment.

Difethialone is a rodenticide (against Norway rats, roof rats and house mice) for use in and around homes, and agricultural, industrial and commercial buildings, ports associated with transport vehicles (*e.g.*, trains, aircraft, ships), alleys and sewers. There are 25 labels approved for this chemical. Formulation types registered include pellets, pellet packs, blocks, mini blocks, paraffin blocks, meal, packs or pouches, paste and bait stations.

Although current registrations of difethialone allow for use nationwide, this ecological risk assessment and effects determination addresses currently registered uses of difethialone in portions of the action area that are reasonably assumed to be biologically relevant to the AW, SMHM, and SJKF, and to the AW's designated critical habitat. Further discussion of the action area for the AW, SMHM and SJKF, and the AW's critical habitat is provided in **Section 2.7.**

2.2.1. Evaluation of Degradates

Information about environmental fate degradates of difethialone is very limited. The only exception is that one degradate was observed in one aerobic soil metabolism study; however, it was not identified. Furthermore, risk from exposure to degradation products was not considered a major concern because the majority of risk to the AW and SJKF is expected to be from acute exposure from secondary exposure from consumption of prey which feed on the intact bait. Similarly, risk from exposure to degradation products was not considered a major concern for the SMHM because the majority of the risk is expected to be from direct consumption of the bait. Contamination of soil and water from use of the bait products is expected to be minimal. Therefore, should there be formation of degradation products in soil and/ or in water, it would not be a major concern in this assessment because of minimal exposure expected from its use in bait stations. The only use of the chemical that might involve substantive exposure to water particularly if the bait is applied improperly, is application to sewers. Labels have certain requirements to prevent such events (see **Section 2.4.3).**

2.2.2. Evaluation of Mixtures

The Agency does not routinely include, in its risk assessments, an evaluation of mixtures of active ingredients, either those mixtures of multiple active ingredients in product formulations or those in the applicator's tank. In the case of the product formulations of active ingredients (that is, a registered product containing more than one active ingredient), each active ingredient is subject to an individual risk assessment for regulatory decision regarding the active ingredient on a particular use site. If effects data are available for a formulated product containing more than one active ingredient, they may be used qualitatively or quantitatively in accordance with the

Agency's Overview Document and the Services' Evaluation Memorandum (USEPA, 2004; USFWS/NMFS/NOAA, 2004).

Difethialone has one registered product that contains two active ingredients (product Reg. No. 3282-89, the other active ingredient is fipronil, at 0.0035%, intended to kill flees and other pests that infest mice and rats). There is one oral mammalian study for this product (MRID No. 47303013) in which the acute oral LD_{50} was > 5,050 mg a.i./kg-bw (male, female and combined). Fipronil is an insecticide that causes nerve excitation by targeting the γ-aminobutyric acid type A receptor system (GABA). It blocks the voltage-gated chloride channels in the neurons, resulting in removal of inhibitory mechanism and high neuronal activity. It has more binding affinity to the GABA regulated channels in the insects than in the mammals, resulting in selectivity (http://www.pw.ucr.edu/textfiles/fipronil.pdf, accessed 08/16/2011). In contrast, difethialone is an anticoagulant vitamin-K antagonist that disrupts normal blood-clotting mechanism and induces capillary damage. It would appear that no important interactions would occur between these active ingredients (*e.g.*, synergism) given that the active and inert ingredients would not be expected to have similar mechanisms of action, metabolites or toxicokinetic behavior. It appears reasonable to conclude that an assumption of dose-addition would be inappropriate; however, there is not enough data to make conclusive evaluations of mixture toxicity and it is unknown whether this formulation is reflecting an independent additive toxicity response and not an interactive effect. In the absence of detailed toxicity information, an assessment of difethialone's potential effect on the AW, SMHM, and SJKF when it is co-formulated with fipronil will be based on the toxicity of difethialone.

2.3. Previous Assessments

Reregistration Eligibility Decision

The Agency assessed the risks of rodenticide uses of several rodenticides, in the *Reregistration Eligibility Decision (RED): Rodenticide Cluster* that was published in July 1998 (USEPA, 1998b). It is noted that the document did not include difethialone; however, various mitigation measures imposed for the rodenticides involved were also required for difethialone at the time. According to the 1998 document (emphasis added):

> "In addition, outside the scope of this RED process, the Agency is requiring similar risk mitigation measures to the registrations of other rodenticide active ingredients such as zinc phosphide, warfarin and salts, ***difethialone***, cholecalciferol (vitamin D-3), and red squill and, if necessary, registrations of new rodenticide active ingredients."

Examples of mitigation measures imposed include the addition of an environmental hazard statement regarding contamination of water, all rodenticide products labeled for field use, except those to be applied manually against pocket gophers and moles, would be classified as restricted use, applications to control mice and rats should be labeled for applications "indoors and along the outside walls of buildings" and modified the environmental hazard statement to read "Predatory and scavenging mammals and birds might be poisoned if they feed upon animals that have eaten the bait."

This RED document included an ecological effects risk assessment that was based on environmental fate and ecotoxicological studies that had been submitted by the rodenticide registrants at that time. The assessment concluded that primary toxicity to mammals was very high for all the covered rodenticides. Furthermore, primary toxicity to birds was found to be high to very high for the single-feeding compounds (brodifacoum, bromadiolone, bromethalin). In addition, there was data on the secondary toxicity for avian and mammalian predators and/or scavengers for some (chlorophacinone and diphacinone), but not for all of the rodenticides involved. Thus, registrant-submitted secondary toxicity data was required for rodenticides used in the field, and around buildings in non-urban (*i.e.*, rural, suburban areas). Based on the environmental fate characteristics of the rodenticides involved, it was broadly concluded at the time, that they were unlikely to result in contamination of surface or ground waters. Even though they are persistent chemicals, they tend to be relatively immobile in soils and fairly insoluble in water. In the RED it was further concluded that since they are primarily applied as bait stations outdoors, their aquatic risk appeared to be negligible. Risk mitigation measures were imposed on these rodenticides in two phases: short and long term risk reduction. Certain labeling requirements were implemented, as indicated above.

Rodenticide Comparative Assessment

An assessment of the risks of difethialone to terrestrial wildlife was included in the 2004 assessment *Potential Risks of Nine Rodenticides to Birds and Nontarget Mammals: a Comparative Approach* (USEPA 2004b). Among the general conclusions in the document, it was found that difethialone was one of the two rodenticides that pose the greatest risk to non-target birds and mammals (with brodifacoum, another second generation anticoagulant rodenticide being the other). When exposure and toxicity were evaluated, difethialone was one of the three rodenticides that posed the greatest risk to birds that eat bait. Also, difethialone was one of the two rodenticides that posed the greatest potential risks to avian predators and scavengers that feed on target or nontarget animals poisoned with bait. It was 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.

The overall risk to birds from primary exposure was found to be high for difethialone and comparable to brodifacoum and zinc phosphide. For nontarget mammals, a comparative analysis found that difethialone posed less risk from primary exposure than zinc phosphide, and it was comparable to brodifacoum, bromadialone, diphacinone and warfarin.

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 difethialone) are characterized by being more acutely toxic than first generation anticoagulants (*e.g.* warfarin) and have a significantly longer liver half life.

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. Risk to wildlife from secondary exposure to

difethialone was evaluated based upon the liver retention time of the chemical. In a rat study, livers of dosed rats retained difethialone with a calculated half-life of 74 days. Overall, the available toxicokinetic data indicated that the second-generation anticoagulants like difethialone, are considerably more persistent in animal tissues than are the first-generation anticoagulants, and bioaccumulation may increase whole-body residues with repeat feedings. Difethialone was among the top two rodenticides posing the greatest secondary risk to birds and mid range for secondary risk to non-target mammals. This assessment therefore concluded that difethialone poses a higher secondary risk to wildlife than non-anticoagulant rodenticides.

2.4. Environmental Fate Properties

Table 2-1 lists the physicochemical properties of difethialone. **Table 2-2** lists the other environmental fate properties of difethialone, from the submitted environmental fate and transport studies. The structure of difethialone is provided in **Figure 2-1**.

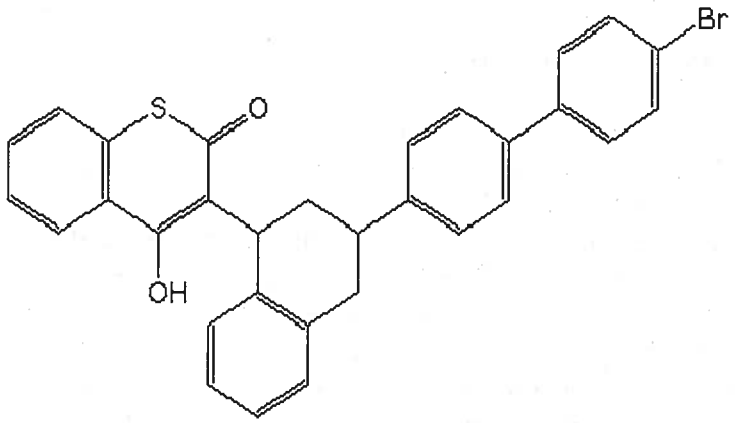

Figure 2-1. Structure of Difethialone

Table 2-1. Physicochemical Properties of Difethialone

Property	Parent Compound	
	Value and units	MRID or Source
Molecular Weight	539.495 g/mole	N/A
Chemical Formula	$C_{31}H_{23}BrO_2S$	N/A
CAS Name	3-[3-(4′-bromo[1,1′-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-2*H*-1-benzothiopyran-2-one	N/A
IUPAC Name	3-[(1*RS*,3*RS*;1*RS*,3*SR*)-3-(4′-bromobiphenyl-4-yl)-1,2,3,4-tetrahydro-1-naphthyl]-4-hydroxy-1-benzothiin-2-one where the ratios of the racemates (1*RS*,3*RS*) to (1*RS*,3*SR*) lie within the ranges 0–15 to 85–100 respectively	N/A
Bulk Density	1.3614 g/cm^3 at 25°C	MRID 42065002
Vapor Pressure	0.074 mPa = 5.55x10^{-7} mm Hg @ 25°C 'Non-volatile from a field condition'	EPIWEB v.4.0 USEPA, 2008
Henry's Law Constant	1.00 x 10^{-6} atm-m^3/mole @ 25°C	EPIWEB v.4.0 calculated from WS and VP

Property	Parent Compound	
	Value and units	MRID or Source
Water Solubility	0.4 mg/L 0.39 mg/L @ 25°C	MRID 42065002 EPIWEB v.4.0 reported value
Octanol – water partition coefficient (log K_{OW})	9.82 6.29 at pH 7.3 and RT	EPIWEB v.4.0Assessment Report [A]
Dissociation Constant (pK_a and/or pK_b)	Not available	Not available
Air-water partition coefficient (K_{AW})	$K_{AW} = C_{air}/C_{water} = HLC/RT$ $K_{AW} = 4.09 \times 10^{-5}$ 'Slightly volatile from a water surface'	Calculated Value, USEPA, 2008
Octanol-air partition coefficient (K_{OA})	$K_{OA} = \frac{K_{OW}}{K_{AW}} = \frac{K_{OW}RT}{\text{Henry's Law Constant}}$ $K_{OA} = 1.7 \times 10^{14}$	Calculated value, using EPIWEB v.4.0 value of K_{OW}
UV/visible light absorption	Three maxima at 209, 239 and 261 nm; Approximately 234, 260 and 330 nm	Reported in DER for MRID 40268902; Assessment Report [A]
Volatization Flux	Not available	Not available
$C_{water+soil}/C_{air}$	$C_{water+soil}/C_{air} = (1/K_{AW})(1/r + K_d) =$ 2.0×10^{10} 'Non-volatile from moist soil'	Assuming $K_d \approx K_F$ Calculated value USEPA, 2008

1. Assessment Report published by the EU dated 06/21/2007 (rapporteur state Norway, accessed 09/14/2011, http://circa.europa.eu/Public/irc/env/bio_reports/library?l=/assessement_directive/difethialone_210607pdf/_EN_1.0_&a=d).
2. The structure of the chemical was obtained from the difethialone data sheet available at http://www.alanwood.net/pesticides/difethialone.html (accessed 06/02/11).

One hydrolysis study was reviewed. Furthermore, three environmental fate studies were screened and cursorily reviewed only for this assessment. They included an aqueous photolysis, aerobic soil metabolism, and adsorption/ desorption (batch equilibrium) studies. Due to the lack of a full review of these studies, and no need for these studies for quantitative exposure assessment for the AW, SMHM and SJKF, the reported results from these three studies are only considered qualitatively in this assessment.

Table 2-2. Summary of Difethialone Environmental Fate Properties

Study	Value and unit	Major Degradate *Minor Degradates*	MRID No. or Citation	Study Classification, Comment
Abiotic Hydrolysis	Half-life[1] = 154-211 days, extrapolated at pH 5, 7 and 9	None identified	40268902	Acceptable
Atmospheric Degradation	Half-life[1] = 0.183 days, hydroxyl radical reaction 0.084 days, for ozone reaction	Not available	EPIWEB v.4.0 Estimate	N/A
Direct Aqueous Photolysis	Natural sunlight at RT Half-life[1] = 57.4 min, pH 5.0 61.5 min, pH 7.0 61.3 min, pH 9.0 It appeared that there was some degradation in the dark controls.	At least two metabolites, (but not identified), did not decrease in a 90 min period	42785801	Cursorily reviewed only

Study	Value and unit	Major Degradate *Minor Degradates*	MRID No. or Citation	Study Classification, Comment
Aerobic Soil Metabolism	Half-life[1] = 204 days, Sandy Loam; Non-extracted residues appeared to increase with time, with up to ~35% at 9 months; No degradation in sterile samples after 181 days; Volatiles <1%.	One was not identified and increased throughout the study	42785802	Cursorily reviewed only
Freundlich solid-water distribution coefficient (K_F)	K_F, 1/n, soil texture (series) 7.7×10^5, 1.89, MS clay (Sharkey) 5.6×10^5, 1.98, MD sand (Sassafras) 1.8×10^6, 2.30, MD sandy clay loam (Sequatchie) 1.6×10^5, 1.80, CA sandy loam (Hesperia) Mean value = 8.2×10^5	N/A	42628109	Cursorily reviewed only
Bioconcentration Factor (BCF)	Steady State BCF= log BCF = 2.74 (BCF = 555 L/Kg wet-wt) Biotransformation $t_{1/2} = 4.66 \times 10^3$ days (normalized to 10 g fish)	N/A	EPIWEB v.4.0 Estimate	N/A

Abbreviations: wt=weight

[1]Half-lives were calculated using the single-first order equation and nonlinear regression, unless otherwise specified.

2.4.1. Environmental Transport Mechanisms

Potential transport mechanisms typically include pesticide surface water runoff, spray drift, and secondary drift of volatilized or soil-bound residues leading to deposition onto nearby or more distant ecosystems. However, because the only use of difethialone is in bait for rodent control, no potential for spray drift exists, and exposure from volatilization is expected to be minimal. Because difethialone bait may be used outdoors, some potential exist for residues of difethialone to leach from the bait, if exposed to rainwater or runoff. However, due to the extremely low concentration of active ingredient in the bait (0.0025%), small amount applied per placement, and the hydrophobic nature of the compound, leaching of dissolved difethialone from the bait would be so small that the potential for contaminating surface water is believed to be insignificant. Similarly, exposure of surface water via erosion of soils that sorb difethialone is also small. One possible exception is the use of the chemical in sewers, where exposure to water is possible. For the uses on sewers, one end of a wire is attached to a block or to mini-blocks while the other end is attached to a secure place in the sewer, such as the bottom step of a manhole ladder, in order to minimize removal by rats or water. The low vapor pressure for this chemical suggests volatilization from the bait is also expected to be insignificant. In addition, based on, the aerobic soil metabolism study, the concentration of volatiles was so low that no attempt was made to characterize them (<1% through one year of the analysis).

Another possible route of transport is within the bodies of animals which feed on the difethialone bait. Because poisoned animals would not be killed immediately, they would travel some

distance before dying, thereby potentially exposing other animals away from the application site. This transport within animals is an important route of exposure for the AW and the SJKF since their diet includes small mammals, birds, and reptiles, and thus they are vulnerable to secondary exposure from consuming poisoned prey.

2.4.2. Mechanism of Action

According to the 2004 Comparative Assessment (USEPA 2004b), the anticoagulant rodenticides' mode of action is as follows: "The anticoagulant rodenticides are vitamin-K antagonists that disrupt normal blood-clotting mechanisms and induce capillary damage (Pelfrene 1991). Death results from hemorrhage, and exposed animals may exhibit increasing weakness prior to death. Behavior also may be affected (Cox and Smith 1992). The anticoagulants are typically grouped into "first-generation" (warfarin, chlorophacinone, diphacinone) and "second-generation" (brodifacoum, bromadiolone, difethialone) compounds. Second-generation anticoagulants tend to be more acutely toxic than are the first-generation anticoagulants, and they are retained much longer in body tissues of primary consumers. They generally provide a lethal dose after a single feeding, although death is usually delayed 5 to 10 days and animals continue feeding. In contrast, the first-generation compounds, because they are less acutely toxic and more rapidly metabolized and/or excreted, generally must be ingested for several days to provide a dose lethal to most individuals. Diphacinone and chlorophacinone may kill some animals in a single feeding, but multiple feedings are generally needed for sufficient population control (Timm 1994)."

2.4.3. Use Characterization

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

Nationwide, difethialone is registered for use only in baits for control of three commensal rodents: the Norway rat (*Rattus norvegicus*), the roof rat (*Rattus rattus*), and the house mouse (*Mus musculus*). All three of the commensal rodent species occur in the regions of California where the AW, SMHM, and SJKF occur. Therefore, all registered products of difethialone in California could be used in the area inhabited by the assessed species. Rodent control baits containing difethialone are registered for use in and around buildings, inside transport and cargo vehicles (*e.g.*, trains, aircraft), in urban alleys, and in sewers. Difethialone products may be used in and around any type of building, including residential, industrial, and commercial structures, as well as transportation ports and terminals, and agricultural buildings. Uses that occur indoors would not be expected to result in any primary exposure to the AW, SMHM, or SJKF. However, indoor use could result in secondary exposure to the AW or SJKF if a rodent ingests bait indoors and then goes outside and is consumed by as AW or SJKF. For outdoor application, rodent control bait containing difethialone generally must be placed within 50 feet of buildings. Primary exposure of the SMHM to bait placed outdoors is likely.

Product labels for difethialone do not limit the amount of product or active ingredient that may be applied per unit area, the number of applications that can be made per unit time, or the minimum interval between applications. Labels generally state the amount of bait (*e.g.*, number of bait stations, bait blocks, or bait packages) that may be placed in one location, and the linear distance between placements. The linear distance is generally 8 to 12 feet for mice, and 15 to 30 feet for rats. The concentration of difethialone in the bait is 0.0025% for all its products (*i.e.* 25 mg a.i./kg bait). The amount of active ingredient per placement, or the amount of active ingredient per linear feet, can be calculated for most of the products. The maximum known amount of active ingredient per placement for any product is 0.000025 lb a.i. or 11.3 mg a.i. when it is used to control Norway rats or roof rats. The amount of active ingredient per placement for mice control is usually smaller. The applications of difethialone in California are generally restricted to pest control operators (PCOs), except for applications in agricultural sites. For some of the labels, however, this restriction has not been implemented at the time of this assessment.

Table 2-3 presents the uses and corresponding application rates and methods of application considered in this assessment. Difethialone has multiple use sites and application methods. In the row labeled 'use sites and application methods', sewer applications were placed separately from all other uses for the chemical. The reason is that this use has a higher potential to impact water quality than all the other uses. It is noted that the formulations for the uses in sewers include only blocks and mini-blocks and the instructions appear to minimize potential exposure to water.

According to the Risk Mitigation Decision for Ten Rodenticides (RMD) (Document ID EPA-HQ-OPP-2006-0955-0764, USEPA 2008b), which was issued in May 2008 and revised in June 2008 (emphasis added), "To reduce wildlife exposures and ecological risks, the Agency will require sale and distribution limits intended to prevent general consumers from purchasing residential use bait products containing four of the ten rodenticides that pose the greatest risk to wildlife (the second generation anticoagulants – brodifacoum, bromadiolone, difenacoum, and ***difethialone***). Moreover, bait stations will be required for all outdoor, above-ground uses of these second generation-anticoagulants." For the above mentioned rodenticide products, limits are being imposed in the package size, use sites, sales and distribution, and bait station requirements as outlined below.

1. Minimum package size: The Agency requires difethialone bait products to be sold in packages that contain ≥ 8 lb of bait for products that are labeled for use only inside of and within 50 ft of agricultural buildings and not for use in and around homes. For products intended for use by professional applicators, the minimum permissible amount of bait per package is 16 lb.
2. Use site restriction: For products in packages with at least 8 but not more than 16 lb of bait, labels are required to state that products may only be used in and around (within 50 ft) of agricultural buildings (*e.g.*, barns, hen houses), and bear the statement, "Do not use this product in homes or other human residences."
3. Sale and distribution restriction: The terms and conditions of registration for products containing difethialone are required to be amended to specify that the registrants will control distribution of the products so that they will only be distributed to or sold in

agricultural, farm and tractor stores or directly to pest control operators (PCOs) and other professional applicators, and that registrants will not sell or distribute products containing difethialone in channels of trade likely to result in retail sale in hardware and home improvement stores, grocery stores, convenience stores, drug stores, club stores, big box stores, and other general retailers.

4. Bait stations required for outdoor above ground placements: The Agency requires that all outdoor, above-ground placements of bait products containing difethialone be contained in bait stations, in order to deny non-target animals, ready access to bait. According to the RMD, most bait products are grain-based and are therefore attractive to many birds and non-target mammals; those baits with flavor enhancers (*e.g.* fish flavors) might also attract carnivores. Tamper-resistant bait stations are required if the bait placement are within reach of pets, domestic animals, non-target wildlife, or children under six years-of-age. Other types of bait stations may be constructed and used in settings, such as around livestock production buildings, where exposure to children and non-target wildlife is unlikely.

The requirements mentioned above are being imposed for labels of all difethialone rodenticide bait products sold after June 4, 2011. However, not all labels of difethialone rodenticide products are in compliance with the RMD requirements at the time this assessment is conducted. At the time of this assessment, all of the mitigation measures from the 2008 RMD have been incorporated into the labels of 18 of the 25 difethialone products. The seven non-conforming products will be included in the Rodenticides Notice of Intent to Cancel (NOIC). Prior to issuing the NOIC, the Agency is seeking independent scientific review on banning certain rat and mouse products. The seven difethialone products that do not conform to the RMD (EPA Reg. Nos. 3282-85, 3282-86, 3282-87, 3282-88, 7173-247, 7173-283 and 7173-285) may be sold after the June 4, 2011 date and will likely remain on the market until the Agency completes the NOIC process and the registrant has exhausted all of its legal appeals. Therefore, they are included in this assessment. Reasons why these products do not conform to the RMD include unapproved formulations, requirement for bait stations and/ or missing the 50-ft restriction as described in items 1 to 4, above. More information may be found at http://www.epa.gov/pesticides/mice-and-rats/consumer-prod.html and http://www.epa.gov/pesticides/mice-and-rats/ (both were accessed 09/22/2011). As per the websites mentioned above, "After June 4, 2011, any rodenticide manufacturers who distribute or sell rodenticide products that do not meet the new risk mitigation goals will face EPA actions to remove those products from the market."

Table 2-3. Summary of Difethialone Use and Label Information

Target Species	Norway rats, roof rats, and house mice	
Use Sites and Application Methods	Rodent control bait for use in and around homes and residential buildings, industrial, commercial and public buildings, food processing facilities, transport vehicles (ships, trains, aircraft) and their related ports, in and around agricultural buildings and alleys. Product should not be applied further than 50 ft from agricultural buildings. Bait stations are mandatory for outdoor above ground applications. *For some products, the mandatory language is not included.*	Sewers – One end of a wire is attached to a block or to mini-blocks while the other end is attached to a secure place such as the bottom step of a manhole ladder, in order to minimize removal by rats or water.
Bait Placement Interval	8-12 ft (mice) for 15 days or until activity ceases;	It appears to be one

	15-30 ft (rats) for 10 days or until activity ceases	placement per manhole
Formulation	Pellets, pellet packs, blocks, paraffin blocks, meal, packs or pouches, bait stations, mini-blocks, paste	
% A.I. in Bait	0.0025% (equivalent to 25 mg a.i./kg bait)	
Presentation	Varies for each product, the following examples are derived from some of the labels: Not less than 16 lb (PCOs) and 8 lb (agricultural) of bait (appropriate presentations for products conforming with the RMD; also 0.7 oz up to 50 lb; 3.8 oz up to 50 lb; 16 lb up to 50 lb, *etc.*	
App. Rate per Bait Placement	Varies for each product, maximum appears to be 16 oz (1 lb) product, equivalent to 0.000025 lb a.i. or 11.3 mg a.i./ placement. Same for sewers.	
PCO Restrictions	For some products, restricted to Pest Control Operators except for use in agricultural sites.	

The Agency's Biological and Economic Analysis Division (BEAD) provides an analysis of both national- and county-level usage information (USEPA 2011) using state-level usage data obtained from USDA-NASS[2], Doane (www.doane.com; the full dataset is not provided due to its proprietary nature) and the California's Department of Pesticide Regulation Pesticide Use Reporting (CDPR PUR) database[3]. CDPR PUR is considered a more comprehensive source of usage data than USDA-NASS or EPA proprietary databases, and thus the usage data reported for difethialone by county in this California-specific assessment were generated using CDPR PUR data. Eleven years (1999-2009) of usage data were included in this analysis. Data from CDPR PUR were obtained for every agricultural pesticide application made on every use site at the section level (approximately one square mile) of the public land survey system.[4] BEAD summarized these data to the county level by site, pesticide, and unit treated. Calculating county-level usage involved summarizing across all applications made within a section and then across all sections within a county for each use site and for each pesticide. The county level usage data that were calculated include: average annual pounds applied and number of records across all eleven years. The units of area treated are also provided where available.

A summary of difethialone usage for California is provided below in **Table 2-4.** CDPR PUR data show that difethialone is used in all of the counties in California where the AW may occur (four rows shaded blue in **Table 2-4**. Due to its use as vertebrate control bait products, the pattern of use of difethialone is characterized as numerous applications of small amounts of active ingredient. The average annual use per county was no more than 0.3 pounds for any county in California. Unlike conventional agricultural pesticides, the area treated was generally not reported in this database, and therefore the average application rate (*i.e.*, expressed in units lb a.i./A) could not be calculated.

[2] United States Department of Agriculture (USDA), National Agricultural Statistics Service (NASS) Chemical Use Reports provide summary pesticide usage statistics for select agricultural use sites by chemical, crop and state. See http://www.pestmanagement.info/nass/app_usage.cfm.

[3] 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 .

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

Table 2-4. Summary of California Department of Pesticide Registration (CDPR) Pesticide Use Reporting (PUR) Data from 1999 to 2009 for Current Difethialone Uses [A]

County	Average Annual Pounds Applied [B]	Number of Records
Alameda	0.099534	820
Amador	0.000591	73
Butte	0.002240	141
Calaveras	0.000232	21
Colusa	0.000853	46
Contra Costa	0.077029	968
Del Norte	0.000034	13
El Dorado	0.002012	186
Fresno	0.022079	610
Glenn	0.000264	20
Humboldt	0.000030	7
Imperial	0.010123	172
Inyo	0.000765	62
Kern	0.010625	350
Kings	0.003287	179
Lake	0.000842	72
Los Angeles	0.281839	6377
Madera	0.008366	468
Marin	0.037651	399
Mariposa	0.000023	8
Mendocino	0.001343	65
Merced	0.010637	326
Mono	0.000125	11
Monterey	0.006323	411
Napa	0.008089	312
Nevada	0.000679	43
Orange	0.159396	2008
Placer	0.009337	335
Riverside	0.133894	3124
Sacramento	0.017105	454
San Benito	0.001894	179
San Bernadino	0.040946	1956
San Diego	0.211038	1064
San Francisco	0.063180	743
San Joaquin	0.005752	303
San Luis Obispo	0.007016	581
San Mateo	0.064307	734
Santa Barbara	0.035656	714
Santa Clara	0.075794	774
Santa Cruz	0.006495	311
Shasta	0.000865	137
Siskiyou	0.000021	12
Solano	0.008063	463
Sonoma	0.007132	387
Stanislaus	0.018431	316

County	Average Annual Pounds Applied [B]	Number of Records
Sutter	0.011804	189
Tehama	0.001019	136
Trinity	0.000068	2
Tulare	0.006275	242
Tuolumne	0.000214	21
Ventura	0.079834	1395
Yolo	0.007794	389
Yuba	0.003661	133

A. Table is based on data supplied by BEAD (USEPA, 2011). All counties are included in this table; however, rows shaded blue are counties where the AW, SMHM and SJKF are known to inhabit.
B. Results were rounded to six figures after the decimal for illustration purposes.

Use sites listed in the database in BEAD's report, for difethialone in California counties include airports, almond, animal premise, unspecified bean, commodity fumigation, forest/ timberland, fumigation (other), industrial site, landscape maintenance, greenhouse flower, greenhouse plants in containers, outdoor plants in containers, poultry, public health, regulatory pest control, research commodity, rights-of-way, structural pest control, uncultivated agricultural, and vertebrate control. EFED notes that difethialone apparently is not used as a fumigant, on almonds or on beans. It appears that these categories of site names were used to generally describe sites where rodenticide fumigant products may also be used or described unregistered use sites. As noted above, this database does not include residential uses of difethialone. However, it is noted that the average pounds of active ingredient per county is relatively small, compared to broadcast applications of agricultural pesticides. **Table 2-5** shows a summary of the average (of eleven years) of pounds of active ingredient used per year for each site name. The use sites with the highest average pounds active ingredient per year were structural pest control and landscape maintenance.

Table 2-5. Average pounds of difethialone per year for all use sites [A]

Site Name	Average pounds of active ingredient/Year
Almond	8.1×10^{-5}
Animal premise	5.1×10^{-3}
Beans, unspecified	4.5×10^{-5}
Commodity fumigation	2.0×10^{-3}
Forest, timberland	6.8×10^{-6}
Fumigation, other	1.8×10^{-4}
Industrial site	1.0×10^{-4}
Landscape maintenance	0.105
Greenhouse flowers	4.5×10^{-5}
Greenhouse plants	1.7×10^{-4}
Outdoor plants	9.8×10^{-5}
Poultry	5.5×10^{-6}
Public health	7.2×10^{-5}
Regulatory pest control	7.8×10^{-6}
Research commodity	3.2×10^{-6}
Rights-of-way	4.0×10^{-3}
Structural pest control	1.44
Uncultivated agricultural	2.3×10^{-4}
Vertebrate control	1.2×10^{-2}

A. All averages are stated with two significant figures except for the two highest values, which were stated with three significant figures.

2.5. Assessed Species

Table 2-5 provides a summary of the current distribution, habitat requirements, and life history parameters for the listed species being assessed. More detailed life-history and distribution information can be found in Attachment III. See **Figures 2-2** through **2-4** for maps of the current range and designated critical habitat, if applicable, of the AW, SMHM, and SJKF, respectively.

The AW, a subspecies of the California whipsnake, was listed as threatened in 1997 by the USFWS. A recovery plan for this and other threatened and endangered species of the chaparral and scrub community east of San Francisco Bay, California was approved by the USFWS in 2003. Critical habitat was designated for this subspecies in 2006. The subspecies occurs in the Inner Coast Ranges in Contra Costa, Alameda, San Joaquin, and possibly Santa Clara Counties.[5]

For the SJKF, prior to 1930, kit foxes inhabited most of the San Joaquin Valley from southern Kern County north to eastern Contra Costa County and eastern Stanislaus County. Although no reason was given for the decline, it was believed that by 1930 the kit fox range had been reduced by more than half, with the largest remaining portion being in the western and southern portions of the Valley.

Although no extensive survey has been conducted of the historical range, kit foxes are thought to inhabit suitable habitat on the San Joaquin Valley floor and in the surrounding foothills of the coastal ranges, Sierra Nevada, and Tehachapi mountains. Kit foxes have been found on all the larger, scattered islands of natural land on the Valley floor in Kern, Tulare, Kings, Fresno, Madera, San Benito, Merced, Stanislaus, San Joaquin, Alameda, and Contra Costa counties. They also occur in the interior basins and ranges in Monterey, San Benito, San Luis Obispo, and, possibly, Santa Clara counties; and in the upper Cuyama River watershed in northern Ventura and Santa Barbara counties and southeastern San Luis Obispo County.[6]

The salt marsh harvest mouse is found in the marshes of Corte Madera, Richmond, and South San Francisco Bay. This species is generally restricted to saline (salty) or brackish (somewhat salty) marsh habitats around the San Francisco Bay Estuary, and is found in mixed saline/brackish areas in the Suisun Bay area and has been found in one brackish area in the southern South San Francisco Bay.[7]

[5] http://www.epa.gov/espp/factsheets/alameda-whipsnake.pdf
[6] http://esrp.csustan.edu/speciesprofiles/profile.php?sp=vuma
[7] http://ecos.fws.gov/docs/five_year_review/doc3221.pdf

Table 2-6. Summary of Current Distribution, Habitat Requirements, and Life History Information for the Assessed Listed Species [A]

Assessed Species	Size	Current Range	Habitat Type	Designated Critical Habitat?	Reproductive Cycle	Diet
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, terrestrial invertebrates, terrestrial-phase amphibians, 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).	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.

Assessed Species	Size	Current Range	Habitat Type	Designated Critical Habitat?	Reproductive Cycle	Diet
			They move to new dens within their home range often (likely to avoid predation by coyotes)			
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

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

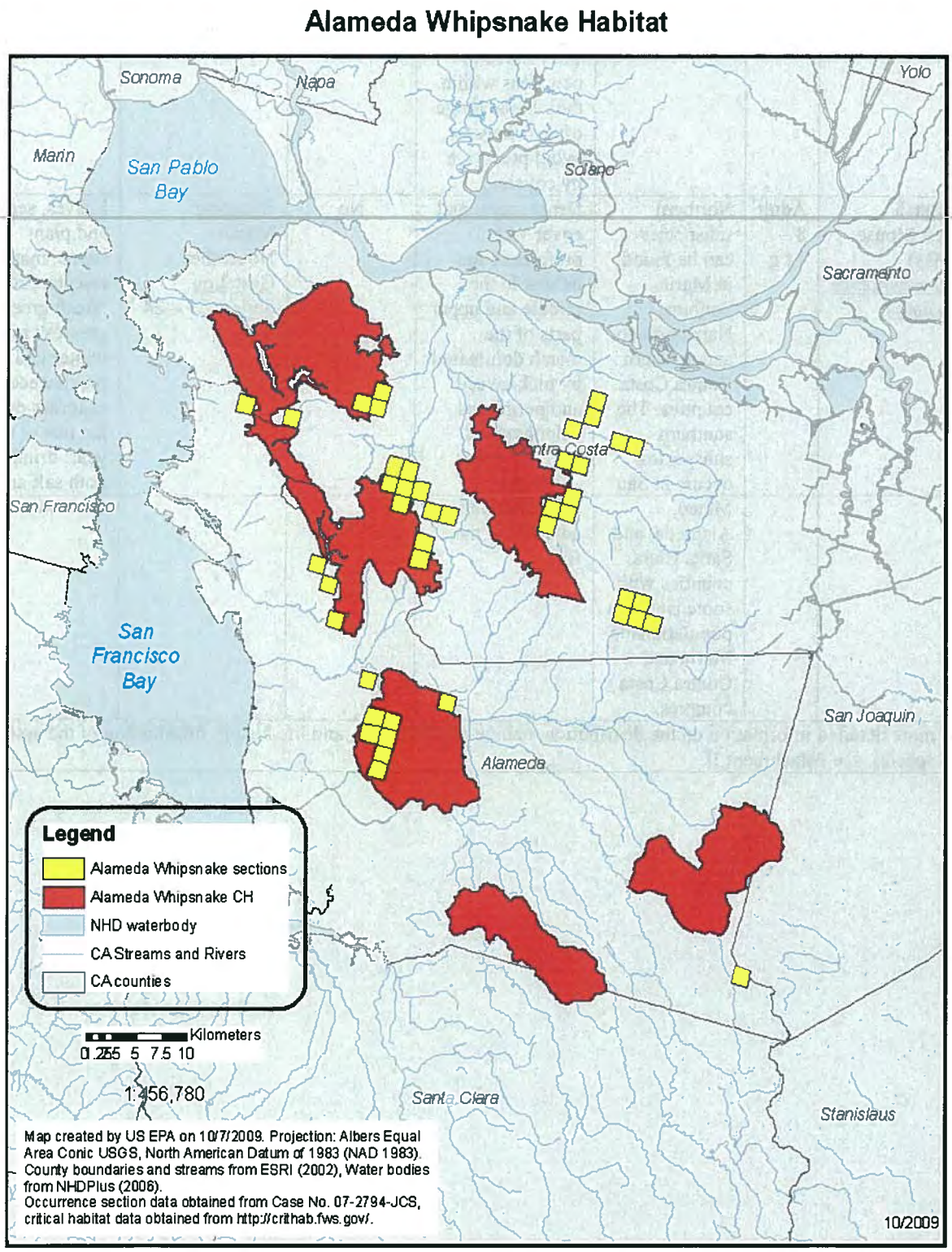

Figure 2-2. Alameda Whipsnake Critical Habitat and Occurrence Sections Identified in Case No. 07-2794-JCS

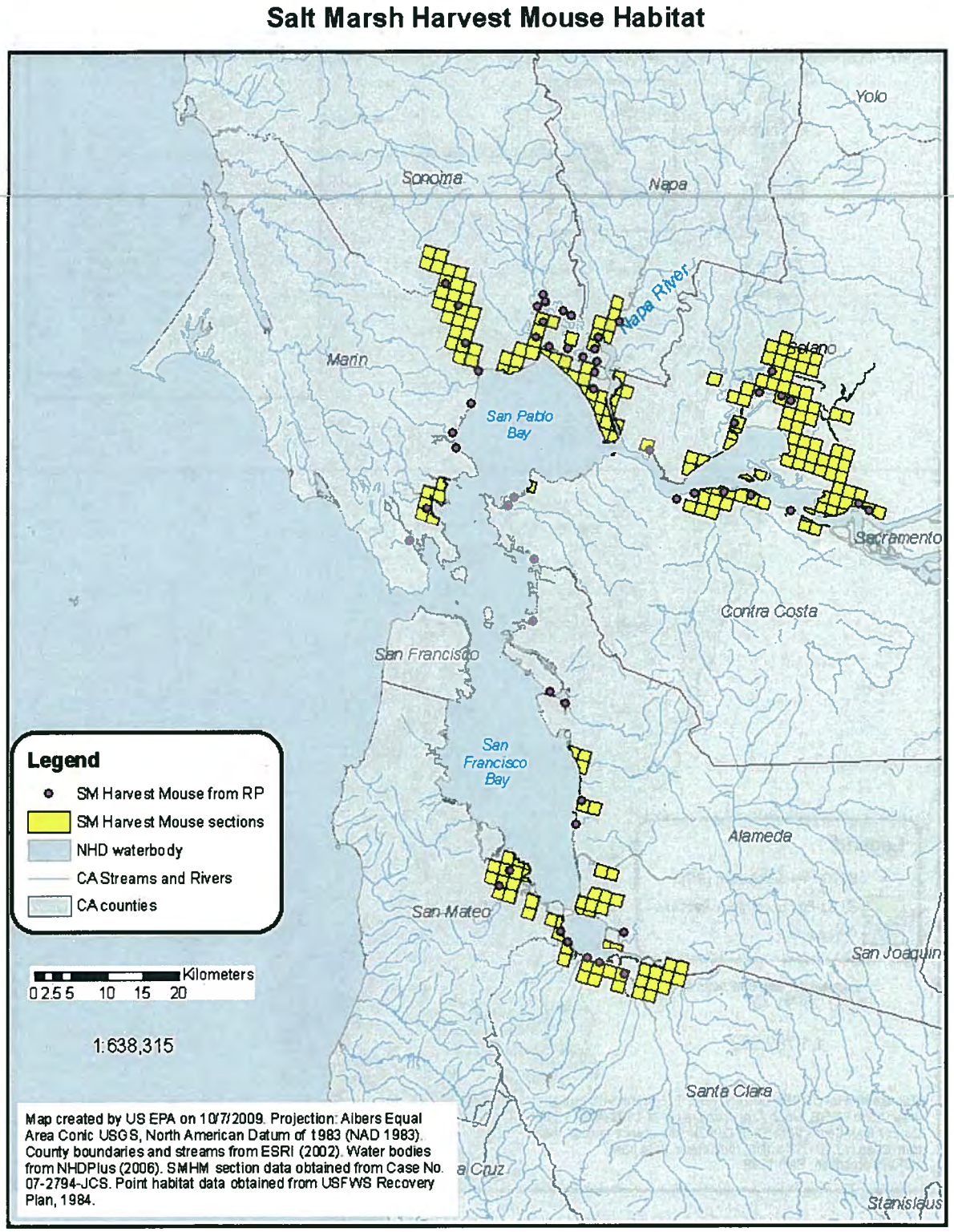

Figure 2-3. Salt Marsh Harvest Mouse Habitat and Occurrence Sections Identified in Case No. 07-2794-JCS

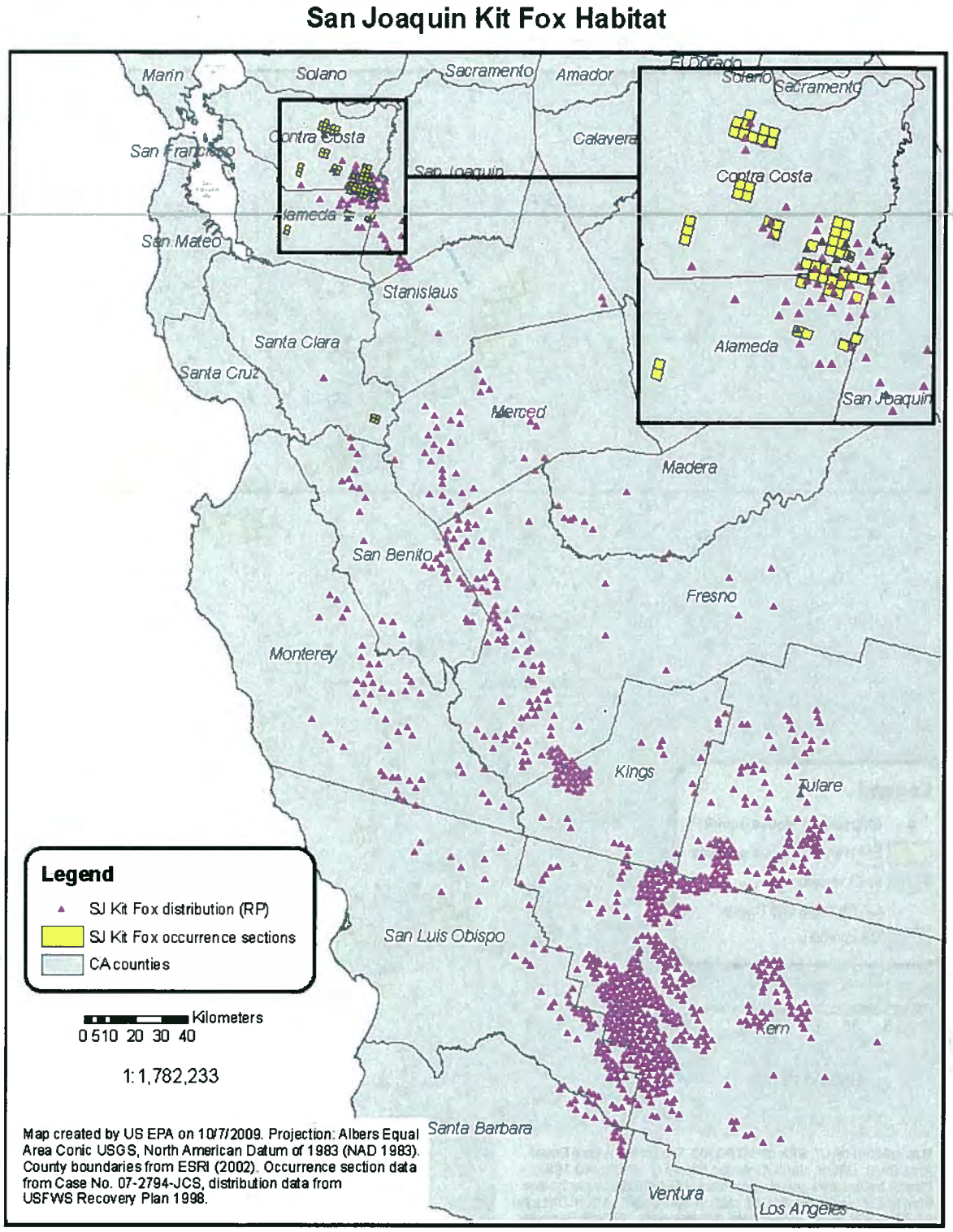

Figure 2-4. San Joaquin Kit Fox Habitat and Occurrence Sections Identified in Case No. 07-2794-JCS

2.6. Designated Critical Habitat

Critical habitat has been designated for the AW. 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 2-7** describes the PCEs for the critical habitats designated for the AW.

Table 2-7. Designated Critical Habitat PCEs for the Alameda Whipsnake (AW)[A]

Species	PCEs	Reference
Alameda whipsnake	PCE 1: Scrub/shrub communities with a mosaic of open and closed canopy	71 FR 58175 58231, 2006
	PCE 2: Woodland or annual grassland plant communities contiguous to lands containing PCE 1	
	PCE 3: Lands containing rock outcrops, talus, and small mammal burrows within or adjacent to PCE 1 and or PCE 2	

[A] 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 applicable to this assessment can be found in **Attachment II**. Activities that may destroy or modify critical habitat are those that alter the PCEs and jeopardize the continued existence of the species. Evaluation of actions related to use of difethialone 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 difethialone is expected to directly impact living organisms within the action area, critical habitat analysis for difethialone is limited in a practical sense to those PCEs of critical habitat that are biological or that can be reasonably linked to biologically mediated processes.

2.7. Action Area and LAA Effects Determination Area

2.7.1. Action Area

The action area is used to identify areas that could be affected by the Federal action. The Federal action is the authorization or registration of pesticide use or uses as described on the label(s) of pesticide products containing a particular active ingredient. The action area is defined by the Endangered Species Act as, "all areas to be affected directly or indirectly by the Federal action

and not merely the immediate are involved in the action" (50 CFR §402.2). Based on an analysis of the Federal action, the action area is defined by the actual and potential use of the pesticide and areas where that use could result in effects. Specific measures of ecological effect for the assessed species that define the action area include any direct and indirect toxic effect to the assessed species and any potential modification of its critical habitat, including reduction in survival, growth, and fecundity as well as the full suite of sublethal effects available in the effects literature. It is recognized that the overall action area for the national registration of difethialone is likely to encompass considerable portions of the United States based on its widespread use for rodent control. 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 designated critical habitat for the 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 spray drift and downstream dilution 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 critical habitat based on ecological effect measures associated with reduction in survival, growth, and reproduction, as well as the full suite of sublethal effects available in the effects literature.

It is important to note that the state-wide action area does not imply that direct and/or indirect effects and/or critical habitat modification are expected to or are likely to occur over the full extent of the action area, but rather to identify all areas that may potentially be affected by the action. The Agency uses more rigorous analysis including consideration of available land cover data, toxicity data, and exposure information to determine areas where AW and designated critical habitat may be affected or modified via endpoints associated with reduced survival, growth, or reproduction.

2.7.2. LAA Effects Determination Area

Typically, when assessing the potential for use of a pesticide to affect threatened or endangered species, the Agency determines a Likely to Adversely Affect (LAA) Effects Determination Area. This is the area where the pesticide's use is expected to directly or indirectly affect the species and/ or modify its designated critical habitat, as determined by applying EFED's standard assessment procedures (see Attachment I) based on effects endpoints related to survival, growth, and reproduction. The LAA Effects Determination Area is typically designated as the area where the land use corresponds with land use on which the pesticide is likely to be used (*e.g.* row crops or orchards), plus the area outside this use area which could receive exposure via spray drift and/ or downstream transport at levels that are potentially toxic for the species of concern.

Difethialone is used in and around any type of building, including residential, commercial, industrial, and commercial structures, as well as transportation ports and terminals. For these uses, land cover classes include, developed high intensity, developed low intensity, developed medium intensity and possibly developed open space. Because difethialone may be used in and around agricultural buildings, there are several land cover categories identified for this use too, including cultivated crops, orchards/ vineyards, pasture/ hay. A multiplicity of land cover types

apply to difethialone. In the case of this assessment, the area of potential use of difethialone does not appear to be restricted spatially. Considering the wide use pattern of rodent control baits, difethialone potentially could be used in most terrestrial land use type. Thus, any area of the state of California is considered an area of potential use of difethialone bait, and thus the assessed species potentially could be exposed to difethialone wherever they occur.

2.8. Assessment Endpoints and Measures of Ecological Effect

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

2.8.1. Assessment Endpoints

A complete discussion of all the toxicity data available for this risk assessment, including resulting measures of ecological effect selected for each taxonomic group of concern, is included in **Section 4** of this document. **Table 2-8** identifies the taxa used to assess the potential for direct and indirect effects from the uses of difethialone 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 2-9**.

Table 2-8. Taxa Used in the Analyses of Direct and Indirect Effects for the Assessed Listed Species.

Listed Species	Birds	Mammals	Terrestrial Invertebrates	Reptiles
Alameda Whipsnake	Indirect (prey)	Indirect (prey/habitat)	Indirect (prey)	Indirect (Prey)
Salt Marsh Harvest Mouse	N/A	Direct Indirect (rearing sites)	Indirect (prey)	N/A
San Joaquin Kit Fox	Indirect (prey)	Direct (indirect (prey)	Indirect (prey)	Indirect (prey)

N/A = Not applicable to the assessed species.

Table 2-9. Taxa and Assessment Endpoints Used to Evaluate the Potential for Use of Difethialone 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
Birds*	Direct Effect - Alameda Whipsnake	Survival, growth, and reproduction of individuals via direct effects (secondary exposure i.e. consuming prey that have ingested difethialone bait)	Acute: Most sensitive bird or terrestrial-phase amphibian acute LC_{50} or LD_{50} Chronic: Most sensitive bird or terrestrial-phase amphibian chronic NOAEC (No data available)

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
	Indirect Effect (prey) - Alameda Whipsnake	Survival, growth, and reproduction of individuals or modification of critical habitat/habitat via indirect effects on terrestrial prey	
Mammals	Direct Effect - Salt Marsh Harvest Mouse - San Joaquin Kit Fox Indirect Effect (prey/habitat from burrows) - Alameda Whipsnake - Salt Marsh Harvest Mouse (from rearing sites) - 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	Acute: Most sensitive laboratory mammalian acute LC_{50} or LD_{50} Chronic: Most sensitive laboratory mammalian chronic NOAEC (No data available)

* Birds are used as a surrogate for terrestrial-phase amphibians and reptiles.

2.8.2. Assessment Endpoints for Designated Critical Habitat

As previously discussed, designated critical habitat is assessed to evaluate actions related to the use of difethialone that may alter the PCEs of the assessed species' designated critical habitat. PCEs for the assessed species were previously described in **Section 2.6**. Actions that may modify critical habitat are those that alter the PCEs and jeopardize the continued existence of the assessed species. Therefore, these actions are identified as assessment endpoints. It should be noted that evaluation of PCEs as assessment endpoints is limited to those of a biological nature (*i.e.*, the biological resource requirements for the listed species associated with the critical habitat) and those for which difethialone effects data are available.

Assessment endpoints used to evaluate potential for direct and indirect effects are equivalent to the assessment endpoints used to evaluate potential effects to designated critical habitat. If a potential for direct or indirect effects is found, then there is also a potential for effects to critical habitat. Some components of these PCEs are associated with physical abiotic features (*e.g.*, presence and/or depth of a water body, or distance between two sites), which are not expected to be measurably altered by use of pesticides.

2.9. Conceptual Model

2.9.1. Risk Hypotheses

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

The labeled use of difethialone within the action area may:

- directly affect the AW and SJKF by causing mortality or by adversely affecting growth or fecundity via secondary poisoning from consumption of contaminated prey;
- directly affect the SMHM by causing mortality or by adversely affecting growth or fecundity via primary poisoning by direct consumption of difethialone bait
- indirectly affect AW and SJKF and/or modify the AW designated critical habitat by reducing or changing the composition of food supply;
- indirectly affect the SMHM by reducing the number of small mammalian rearing sites by direct effects on small mammals
- indirectly affect AW and/or modify their designated critical habitat by reducing or changing terrestrial habitat in their current range (via reduction in small burrowing mammals leading to reduction in underground refugia/cover).

2.9.2. Diagram

The conceptual model is a graphic representation of the structure of the risk assessment. It specifies the difethialone release mechanisms, biological receptor types, and effects endpoints of potential concern. Typically, a separate diagram is created for terrestrial and aquatic exposure and effects. For this assessment, however, only a single diagram of terrestrial exposure and effects is depicted (**Figure 2-2**) because use of difethialone is not expected to result in significant exposure or effects to the AW, SMHM, and SJKF through aquatic pathways. Although the conceptual models for direct/indirect effects and modification of designated critical habitat PCEs are shown on the same diagram, the potential for direct/indirect effects and modification of PCEs are evaluated separately in this assessment. Exposure routes shown in dashed lines are not quantitatively considered because the contribution of those exposure routes to potential risks to AW, SMHM, and SJKF and modification to designated critical habitat is expected to be negligible.

As shown in the diagram, consumption of prey (small mammals, birds, and other reptiles) that have ingested difethialone from intact bait is considered to be the primary route of exposure to the AW and SJKF. Furthermore, small mammals, birds, and lizards are potential prey items for the AW, while small mammals and birds are potential prey items for the SJKF. For the SMHM, direct consumption of difethialone bait is considered as the primary route of exposure. The quantitative risk assessment therefore focuses on both the primary and secondary routes of exposure. Although terrestrial invertebrates are a potential prey item of the AW, SJKF, and the SMHM, exposure and indirect effects to these species are assumed to have a negligible contribution to overall risk, based on use practices and the specific mode of action of difethialone. Terrestrial plants, which may provide habitat for the assessed species, are also

assumed to represent a negligible component of overall risk, given the same rationale. The presumed negligible exposure routes and indirect effects include:

- Consumption of terrestrial invertebrates which consume intact bait.
- Consumption of terrestrial invertebrates which ingest soil contaminated by dislodgement of difethialone from the intact bait.
- Consumption of plants which have taken up difethialone from residues dislodged from the bait into the soil.
- Indirect food chain effects resulting from difethialone reducing the abundance of plants and terrestrial invertebrates.
- Potential indirect effects to the SMHM based on direct effects to terrestrial and aquatic plants as habitat

Exposure routes through water and aquatic organisms are not considered in the assessment given the use pattern of difethialone

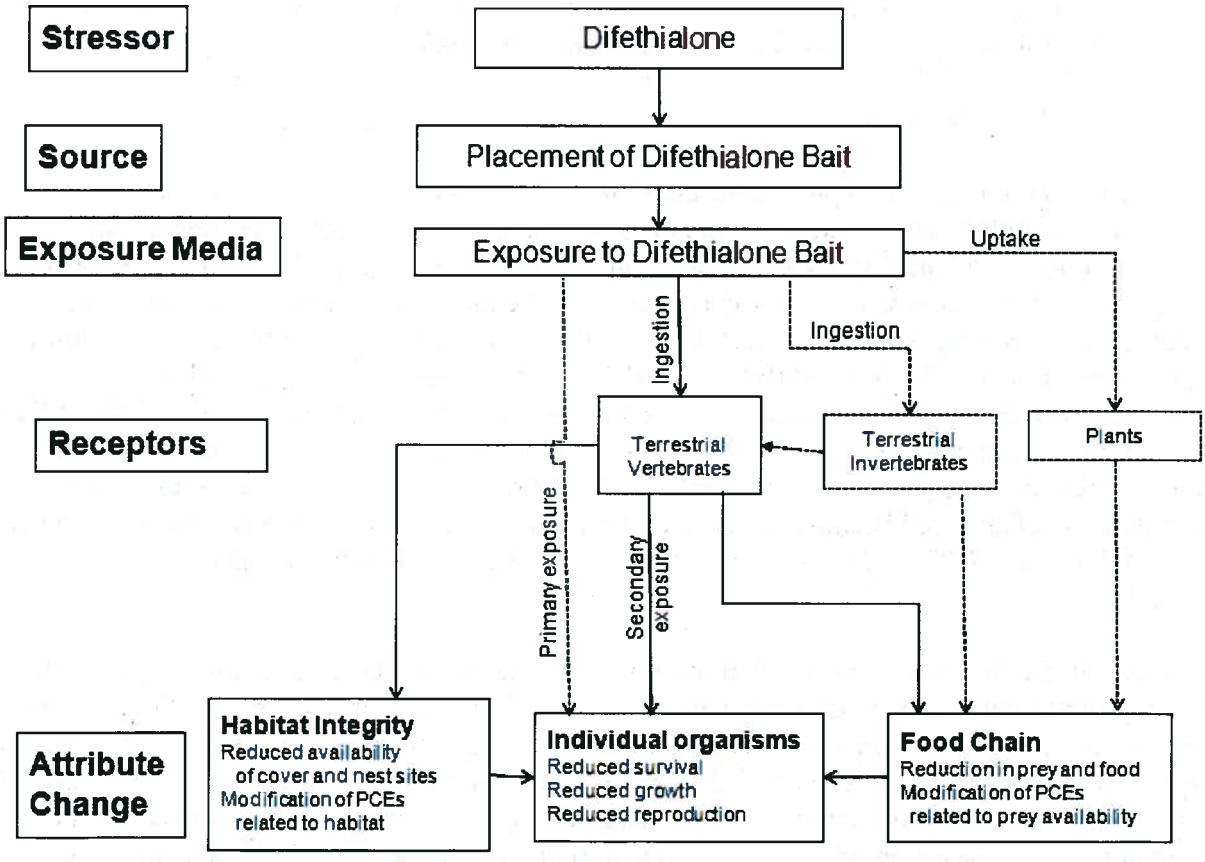

Figure 2-5. Conceptual model depicting stressors, exposure pathways, and potential effects to terrestrial organisms from the use of difethialone
Dotted lines indicate exposure pathways that are not assessed.

2.10. Analysis Plan

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

Descriptions of routine procedures for evaluating risk to the San Francisco Bay Species are provided in **Attachment I**.

2.10.1. Measures of Exposure

In this assessment, transport of difethialone through runoff and spray drift are not considered in deriving quantitative estimates of difethialone exposure to AW, SMHM, and SJKF, their prey and habitats because significant contributions of difethialone in water due to runoff are unlikely. Difethialone is applied as baits and spray drift does not occur. Based on the conceptual model, exposure from direct consumption of bait is considered for the SMHM. Consumption of the bait via secondary exposure is considered for the AW and SJKF (in which these species consume prey that has previously ingested difethialone bait). These exposures constitute direct effects. Indirect effects include reduction of prey for the SJKF and SMHM, and a reduction in the potential rearing sites for the SMHM based on a reduction in other small mammals capable of building burrows. Because the aquatic exposure is not relevant to the AW and SJKF, and is believed to be negligible to the SMHM, it is also not included in this assessment. For difethialone, the vapor pressure and Henry's Law Constant are relatively low (1.00×10^{-6} mmHg and 5.55×10^{-7} atm-m^3/mole, respectively). Furthermore, its estimated atmospheric half-life is short (0.2 days for hydroxyl radical reaction and 0.1 days for ozone reaction, EPIWEB v.4.0). Thus, atmospheric transport appears to be unlikely.

A high K_{OW} value (log K_{OW} 9.82, EPIWEB v.4.0 estimate) suggests a potential for high bioaccumulation in aquatic and terrestrial receptors. Difethialone is relatively stable to hydrolysis at all pHs tested and the half-life in rat liver tissue was 108 days (Lechevin *et al.* 1988).

There has also been literature suggesting that difethialone residues are prevalent and widespread throughout wildlife. In an article by Lima and Salmon (2010), environmental impacts to raptor populations were evaluated based on anticoagulant rodenticide use in California. In this study, 53 birds from San Diego County were recovered and tested for the presence of four first generation anticoagulants (chlorophacinone, diphacinone, coumachlor, and warfarin) and three second generation anticoagulants (difethialone, brodifacoum, and bromadiolone). The results of the analysis indicated that difethialone was present in 5 of the 53 carcasses (9.4%).

Albert *et al.* (2009) presented rodenticide residue information on three species of owls in Western Canada from the time period of 1988 – 2003. Barn owls (*Tyto alba*), barred owls, (*Strix varia*), and great horned owls (*Bulbo virginianus*) were collected during this time period in the provinces of British Columbia and the Yukon Territory. The livers of the birds were analyzed for brodifacoum, bromadiolone, chlorophacinone, diphacinone, difethialone, and warfarin. The results indicated that 10 out of 78 (13%) barred owls, 1 out of 25 barn owls (4%), and 3 out of 61 (5%) of great horned owls had detectable levels of difethialone.

Riley *et al.* (2007) conducted a monitoring study in which bobcats and mountains lions in the Southern California were tested for anticoagulant exposure and the correlation of exposure to the parasitic disease mange. A total of 39 bobcats and 4 mountain lions were tested. Out of the 39 bobcats tested, 10 (26%) had trace amounts of difethialone present in their livers. Trace amounts were positive in the assay to detect but defined as being below the minimum detectable level (mdl) which for difethialone was 0.25 ppm. The primary attributed cause of death for these bobcats was either being struck by a car or mange and not difethialone poisoning. Difethialone was also detected at 0.66 ppm in two of the four (50%) mountain lions that were part of this study. Based on the above mentioned lines-of-evidence, bioaccumulation and the presence of sublethal levels of difethialone in wildlife appear likely.

For conventional pesticides, standard measures of exposure are based on aquatic and terrestrial models that predict estimated environmental concentrations (EECs) using maximum labeled application rates and methods of application as specified on the label. These models are parameterized using relevant reviewed registrant-submitted environmental fate data. More information on these models is available in **Attachment I**. However, for this assessment, the primary pathway of difethialone exposure for terrestrial animals through consumption of another animal that has directly ingested the bait.

With the persistence of difethialone in the liver and the propensity of the chemical to bioaccumulate, there exists the chance for multiple feedings on multiple prey that have been poisoned by difethialone. This assessment however, assumes that a single day's exposure is most likely for the AW. Snakes in general, the AW included, generally consume large meals (often more than its own body weight) and do not eat again for several days, possibly weeks. This assessment considers single and multiple days of exposure for the SJKF consuming poisoned birds, but only a single day's exposure for the SJKF and the SMHM consuming poisoned mammals. These assumptions are based on available dietary studies for mammals.

2.10.1.a. Estimating Exposure in the Terrestrial Environment

Primary avian and mammalian consumers (pigeons, squirrels, and any other avian and mammalian organism that is not carnivorous) may consume difethialone bait if they encounter it. Therefore, terrestrial exposure for birds and mammals was based on dietary exposure to the bait itself. The concentration of difethialone in the diet was assumed to be equal to the maximum concentration of bait in products registered for rodent control uses (0.0025 mg a.i./kg). Indirect risks to AW and SJKF (through prey reduction) and SMHM (through decreased small mammal rearing sites) were assessed by assuming that mammals, birds, reptiles, and terrestrial-phase

amphibians may directly consume the bait. Direct risk to the SMHM was also assessed by assuming direct consumption of difethialone bait itself. However, for the AW and SJKF, which are carnivorous species,[8] the primary route of exposure was assumed to be from secondary poisoning, that is from consumption of prey which fed directly on difethialone bait. For the SMHM, the primary route of exposure was from consumption of difethialone bait. The prey species were assumed to be one of the target species in the label including the Norway rat, roof rat and the house mouse, but also could include other mammals, birds, reptiles, and terrestrial-phase amphibians. The residues of difethialone in the prey were assumed to be the amount that the prey would consume in one day if it fed on the bait. All of the residues consumed by the prey were assumed to be available and assimilated by the AW and SJKF when it consumed the prey. All these assumptions are considered conservative.

2.10.2. Measures of Effect

Data identified in **Section 2.8** are used as measures of effect for direct and indirect effects. Data were obtained from registrant submitted studies or from literature studies identified by ECOTOX. More information on the ECOTOXicology (ECOTOX) database and how toxicological data is used in assessments is available in **Attachment I.**

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

2.10.3. Data Gaps

From the ecological effects side of the assessment, there are no studies submitted to characterize chronic toxicity to avian species (avian reproduction studies) and mammalian species (2-generation mammalian reproduction). An acute contact toxicity study with the honey bee is also not available for this assessment. From the environmental fate perspective, required studies are available; however, some of them were only cursorily reviewed for this assessment.

3. Exposure Assessment

Formulation types registered for difethialone include pellets, pellet packs, blocks, mini blocks, paraffin blocks, meal, packs or pouches, paste and bait stations. There is no application equipment described in the label. Bait is applied by hand to specified places.

[8] http://www.fws.gov/desfbay/Archives/Whip/Whip.htm

Since there is no potential for spray drift and low potential for aquatic exposure, only terrestrial exposure is evaluated in this assessment.

3.1. Label Application Rates and Intervals

Difethialone labels may be categorized into two types: labels for manufacturing uses (including technical grade difethialone and its formulated products) and end-use products. While technical products, which contain difethialone 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 commensal rodents like Norway rats, roof rats and house mice. The formulated product labels legally limit difethialone's potential use to only those sites that are specified on the labels.

In May 2008 a Risk Mitigation Decision for Ten Rodenticides (RMD) was issued; this was followed by a revision in June 2008 (USEPA 2008b). According to the RMD, effective June 4, 2011, the Agency requires sale and distribution limits intended to prevent general consumers from purchasing residential use bait products containing difethialone. Moreover, bait stations are required for all outdoor, above ground uses of difethialone. However, not all labels of difethialone rodenticide products are in compliance with this requirement at the time this assessment is conducted. For the purposes of this assessment, all compliant and non-compliant uses of difethialone are assessed according to existing labels.

The RMD entails primarily two important provisions that relate to difethialone. First, for all second generation anticoagulants (the family to which difethialone belongs), application will be restricted to tamper resistant bait boxes for all above ground uses. Second, package size restrictions on these second generation anticoagulants prevent the average consumer from purchasing any package size less than 16 lbs. Prior to the RMD, consumers (particularly homeowners looking for readily accessible rodent control options) were able to purchase difethialone-containing products in small packaging in grocery stores, warehouses and many other commercials establishments.

It is expected that the movement of difethialone to tamper resistant bait boxes will reduce primary exposure of difethialone to non-target organisms, but the potential for secondary exposure will still exist as difethialone (as will be discussed) is very acutely toxic to birds and mammals and has a long persistence in body tissues, making it available to secondary consumers, like the AW and SJKF. Even with labels not in full conformance with the RMD (*i.e.*, those not in bait stations), the RMD is not expected to have an impact of the primary or secondary exposure to difethialone to the AW and SJKF. The reason for this is that the AW and SJKF is not expected to directly consume difethialone bait, whether it is in a bait station or not, and the risk for secondary exposure likely remains the same as prey that are available to the AW and SJKF will still be at risk for exposure to difethialone. There is the possibility that the removal of difethialone from the homeowner use market may reduce the total number of pounds of difethialone that is available for exposure but this type of analysis is beyond the scope of this assessment.

The movement of difethialone to tamper resistant bait boxes could reduce primary exposure of difethialone to the SMHM but this is uncertain. For larger mammals or birds that have the potential to consume loose difethialone bait not in stations, the risk of primary exposure would be expected to be reduced by the movement into tamper resistant bait stations. For very small mammals, such as the SMHM, that would still be able to get into the bait boxes, as they are designed for small rodents, the risk of primary exposure would still remain.

Currently registered urban and rural uses of difethialone within California include (a) bait for rat and mice control in and around buildings, transportation vehicles and associated ports, *etc.*, and (b) rat and mice control in sewers. The uses being assessed are summarized in **Table** 3-1.

Table 3-1. Difethialone Uses and Application Information

Use (App. Method)	Formulation	% AI in Bait	Maximum App. Rate per Bait Placement (mg a.i./placement)	Bait Placement Interval and Restrictions
Bait for rat and mouse control in and around buildings, transportation vehicles, ports, *etc.*	Pellets, pellet packs, blocks, paraffin blocks, mini blocks, meal, packs or pouches, bait stations, paste	0.0025	11.3	8-12 ft (mice) 15-30 ft (rats) Bait generally should be placed within 50 ft of a building
Rodent control bait for use in sewers	Blocks, paraffin blocks, mini blocks			NS

Abbreviations: App. = application, NS = not stated, ft = feet.
1 Uses assessed based on memorandum from Pesticide Re-evaluation Division (PRD) dated 08/08/2011 (**Appendix B**) and EFED Label Data report and associated Label Use Information Reports prepared on 06/07/2011.

3.2. Aquatic Exposure Assessment

3.2.1. Modeling Approach

Aquatic exposure of the AW, SMHM, and SJKF is considered negligible in this assessment. Therefore, no aquatic exposure assessment was carried out for difethialone.

3.2.2. Existing Monitoring Data

No monitoring data in surface water, groundwater, or in air were found from the USGS NAWQA program (http://water.usgs.gov/nawqa), or the California Department of Pesticide Regulation CDPR (http://www.cdpr.ca.gov/docs/emon/surfwtr/surfcont.htm). Water monitoring programs such as these generally monitor for conventional agricultural pesticides and typically do not include analysis for vertebrate control agents such as difethialone.

3.3. Terrestrial Animal Exposure Assessment

3.3.1. Exposure to Terrestrial Wildlife Prey from Primary Exposure

For assessing exposure of pesticides to terrestrial animals, the Agency typically uses T-REX to calculate EECs for dietary exposure of terrestrial wildlife, and T-HERPS to calculate refined EECs for dietary exposure to reptiles and amphibians. However, these models only calculate EECs (and risk quotient based on the EECs) for natural wildlife food items such as plants, seeds, and insects that are exposed from foliar application of pesticides. T-REX also models exposure from seed treatment and granular formulations. 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. Therefore, terrestrial animal exposure to difethialone was calculated without the use of these computer models.

For AW and SJKF, that consume prey animals, and for the SMHM that directly consumes difethialone bait (*i.e.*, primary exposure for prey), the EEC is simply the concentration of difethialone in the formulated bait. The assessment is based on the maximum concentration of difethialone in bait products used for rodent control. The maximum concentration is 0.0025% (25 mg a.i./kg product) for products used to control rats and mice. For dietary-based risk, the concentration of difethialone in the bait was compared directly to the toxicity endpoint from dietary toxicity studies (*i.e.*, 30-day LC_{50} studies due to delayed toxicity of difethialone). For dosed-based risk , the difethialone concentration in bait had to first be converted to a daily ingestion rate. This was done using the allometric equations of Nagy (1987), as provided in USEPA's Wildlife Exposure Factor Handbook (USEPA, 1993). Ingested doses of difethialone (mg a.i./kg-BW) were calculated for birds and mammals of various assumed body weights. The doses calculated for birds were also used for reptiles and terrestrial-phase amphibians.

The SMHM is likely to be exposed to difethialone residues from primary exposure that occurs from direct ingestion of difethialone bait. Bait with one concentration of difethialone was modeled, 0.0025%. The small mammals were assumed to consume their average daily food intake in the form of the bait. The average daily food intake rate was estimated using the following allometric equation:

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

Where FI is the food intake rate in g/d, and W is the body weight of the mammal in g.

This food ingestion rate was calculated for 10-g mammal which is representative of a typical SMHM. Food intake values for other mammals will be discussed later in the indirect effects section where reduction of mammalian prey to the AW and SJKF are discussed. These calculations yield a FI for the 10-g small mammal. The FI values were then converted into estimated daily doses using the following equation:

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

Where:

Dose is the dose of difethialone (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)

Table 3-2. Dietary EEC for Primary Exposure of Difethialone Bait.

	Formulation	% AI in Bait	Dietary EEC for Primary Exposure (mg ai/kg-diet)
Bait for rat and mouse control in and around buildings	Pellets or blocks, packs, pouches, *etc.*	0.0025	25

Direct acute effects from primary exposure to prey items for the AW and SJKF (mammals, birds, and reptiles) ingesting difethialone bait were evaluated by assuming an individual directly consumes a bait product containing difethialone at its daily ingestion rate. As all difethialone products are the same percent a.i, 0.0025%, only one concentration of difethialone was assessed. The average daily food intake rate was estimated using the following allometric equation for insectivorous reptiles (Nagy, 1987 as cited in USEPA, 1993):

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

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

Food ingestion rates were calculated for reptiles weighing 2, 20, and 800 g. These calculations yield FI values of 0.022, 0.13, and 2.3 g/d for small, medium and large reptiles, respectively. The FI values were then converted into estimated daily doses using the following equation:

$$Dose = FI \times \%A.I. \times (1/W) \times 10^4$$

Where:
Dose is the dose of difethialone (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)

Finally, acute risk quotients were calculated by dividing the expected dose of difethialone (mg-a.i./kg-bw) by the acute oral LD_{50} value for the northern bobwhite, 0.26 mg-a.i./kg-bw (surrogate for reptiles).

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 difethialone concentration of 0.0025%. The average daily food intake rate was estimated using the following allometric equation for birds (Nagy, 1987 as cited in USEPA, 1993):

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

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

Risk was assessed for birds of the standard default weights of 20, 100, and 1000 g, representing small, medium, and large birds. These calculated FI values for these weight classes were 4.56, 13.0, and 58.2 g/d, respectively. The FI values were then converted into estimated daily doses using the following equation:

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

Where:
Dose is the dose of difethialone (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)

Because the AW preys upon birds, they may be indirectly affected by adverse effects on bird populations. Dietary-based RQs for birds which consume difethialone bait were also calculated by dividing the concentration of difethialone in the bait by the subacute dietary LC_{50} value for the northern bobwhite (0.56 mg a.i./kg-diet). The difethialone concentrations in the bait, when expressed as parts-per-million (mg-a.i./kg), is 25 for rodenticide bait.

Risk quotients were calculated to assess risk to small mammals which directly consume difethialone 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 difethialone was modeled, 0.0025%. The small mammals were assumed to consume their average daily food intake in the form of the bait. The average daily food intake rate was estimated using the following allometric equation:

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

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

This food ingestion rate was calculated for 20-g mammal, which is representative of a typical small mammal on which the AW or SJKF might prey. These calculations yield a FI for the 20-g small mammal. The FI values were then converted into estimated daily doses using the following equation:

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

Where:
Dose is the dose of difethialone (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)

Finally, acute dose-based risk quotients were calculated by dividing the expected dose of difethialone (mg-a.i./kg-bw) by the acute oral LD_{50} value for the rat, 0.55 mg-a.i./kg-bw.

3.3.2. Exposure to Terrestrial Animals from 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 difethialone bait. Lizards in particular are believed to be the most important prey item of whipsnakes (USFWS, 2005), but lizards generally feed upon insects and other terrestrial arthropods and would not likely consume rodent bait. 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 after the prey had consumed difethialone bait. The prey animal was assumed to have consumed a quantity of bait equal to its daily ingestion rate. 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 difethialone bait.

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 of 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 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 difethialone 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 difethialone residues from secondary exposure that occurs from consumption of prey that has consumed difethialone bait. The AW and SJKF are capable of consuming all of the target small mammals species specified on the difethialone bait product labels, including 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 difethialone bait at their daily ingestion rate. The rat and the mouse were assumed to have consumed rodenticide bait with a difethialone concentration of 0.0025%. 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 difethialone 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[9]:

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

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

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 difethialone using the following equation:

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

Where:
Dose is the dose of difethialone (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 difethialone 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

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

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 difethialone (mg-a.i./kg-bw) by the acute oral LD_{50} value for the most sensitive bird species, 0.26 mg-a.i./kg-bw, which is a surrogate value to represent the snake.

For mammals consuming other mammals that have ingested difethialone 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. 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 residue studies available to estimate the concentration of difethialone 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 as 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 difethialone active ingredient (25 mg a.i./kg bait) to get an estimated dose for the small mammal of 1.72 mg a.i/day. This dose, divided by the weight of the 2.3 kg SJKF yields the dose-based EEC of 0.75 mg a.i/kg/day for a SJKF ingesting a poisoned 244 g mammal.

The AW and SJKF are likely to be exposed to difethialone residues from secondary exposure that occurs from consumption of prey that has consumed difethialone bait. The AW and SJKF are capable of consuming birds as prey that may have directly ingested difethialone 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 difethialone bait at their daily ingestion rate. Birds were assumed to have consumed rodenticide bait with a difethialone concentration of 0.0025%. 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[10]:

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

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

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

The calculated FI value for the small bird, medium bird, and large bird were 4.55, 13.00, and 58.1 g/d respectively. The FI values were then converted into estimated ingested doses of difethialone using the following equation:

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

Where:
Dose is the dose of difethialone (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 difethialone 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 difethialone (mg-a.i./kg-bw) by the acute oral LD_{50} value for the most sensitive bird species, 0.26 mg-a.i./kg-bw, which is used as a surrogate for snake.

For mammals consuming birds that have ingested difethialone 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 residue studies available to estimate the concentration of difethialone 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 difethialone active ingredient (25 mg a.i./kg bait) to get an estimated dose for the bird of 2.9 mg a.i/day. This dose, divided by the weight of the 2.3 kg SJKF yields the dose-based EEC of 1.26 mg a.i/kg/day for a SJKF ingesting a poisoned 244 g mammal.

The AW is likely to be exposed to difethialone residues from secondary exposure that occurs from consumption of prey that has consumed difethialone bait. The AW is capable of consuming other reptiles such as lizards (their chief preference of food) as prey that may have directly ingested difethialone bait. Therefore, risk based on secondary exposure was conducted

for a snake which feeds on lizards. These species were assumed to have consumed difethialone bait at their daily ingestion rate. Lizards and/or other reptiles were assumed to have consumed rodenticide bait with a difethialone concentration of 0.0025%. 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[11]:

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

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 difethialone using the following equation:

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

Where:
Dose is the dose of difethialone (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 difethialone 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 difethialone (mg-a.i./kg-bw) by the acute oral LD_{50} value for the most sensitive bird species, 0.26 mg-a.i./kg-bw, used as a surrogate to the whipsnake.

There has also been literature suggesting that difethialone residues are prevalent and widespread throughout wildlife. In an article by Lima and Salmon (2010), environmental impacts to raptor populations were evaluated based on anticoagulant rodenticide use in California. In this study, 53 birds from San Diego County were recovered and tested for the presence of four first generation anticoagulants (chlorophacinone, diphacinone, coumachlor, and warfarin) and three

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

second generation anticoagulants (difethialone, brodifacoum, and bromadiolone). The results of the analysis indicated that difethialone was present in 5 of the 53 carcasses (9.4%).

Albert *et al.* (2009) presented rodenticide residue information on three species of owls in Western Canada from the time period of 1988 – 2003. Barn owls (*Tyto alba*), barred owls, (*Strix varia*), and great horned owls (*Bulbo virginianus*) were collected during this time period in the provinces of British Columbia and the Yukon Territory. The livers of the birds were analyzed for brodifacoum, bromadiolone, chlorophacinone, diphacinone, difethialone, and warfarin. The results indicated that 10 out of 78 (13%) barred owls, 1 out of 25 barn owls (4%), and 3 out of 61 (5%) of great horned owls had detectable levels of difethialone.

Riley *et al.* (2007) conducted a monitoring study in which bobcats and mountains lions in the Southern California were tested for anticoagulant exposure and the correlation of exposure to the parasitic disease mange. A total of 39 bobcats and 4 mountain lions were tested. Out of the 39 bobcats tested, 10 (26%) had trace amounts of difethialone present in their livers. Trace amounts were positive in the assay to detect but defined as being below the minimum detectable level (mdl) which for difethialone was 0.25 ppm. The primary attributed cause of death for these bobcats was either being struck by a car or mange and not difethialone poisoning. Difethialone was also detected at 0.66 ppm in two of the four (50%) mountain lions that were part of this study. Based on the above mentioned lines-of-evidence, bioaccumulation and the presence of sublethal levels of difethialone in wildlife appear likely.

3.3.3. Exposure to Terrestrial Invertebrates

As described in **Section 2.9,** indirect effects to the AW, SMHM, and SJKF mediated through exposure to terrestrial invertebrates are expected to be negligible. It is possible that some terrestrial invertebrates could directly consume or be exposed via direct contact to difethialone bait resulting in mortality of such invertebrates. However, given the outdoor use of difethialone bait is restricted to areas adjacent to walls of buildings, any mortality of invertebrates would be very localized. The impact to the invertebrate abundance throughout the range of the assessed species is expected to be negligible. Furthermore, although the AW, SMHM, and SJKF can consume terrestrial invertebrates that have potentially ingested or be exposed via direct contact to difethialone bait, their diet consists of many other non-invertebrate food items. These include lizards, small mammals, and birds for the AW and small mammals and birds for the SJKF. Also, although difethialone has the propensity to bioaccumulate in the livers of terrestrial vertebrates and persist, it is unknown whether the fat body, a similar structure to the liver, is found in insects and whether difethialone bioaccumulates in the same manner. There are also no submitted ecotoxicity studies for terrestrial invertebrates, and no method is current available to assess the consumption rate of difethialone bait by terrestrial invertebrates. 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.

3.3.4. Exposure to Aquatic Plants

Difethialone has a bait formulation nature as well as low application rates. Furthermore, its strong affinity to sorb to soil suggests that it is likely to strongly sorb to bait itself; consequently, off-field runoff and exposures in aquatic environments are expected to be negligible.

Concentrations of difethialone in both freshwater and saltwater marshes are expected to be negligible and not impact aquatic vegetation. Models that estimate concentrations in surface water or calculate spray drift deposition of difethialone on aquatic habitats were not applicable and not needed. No surface water monitoring data are readily available for difethialone. Based on this information, the aquatic habitat is not assessed in this document.

3.3.5. Exposure to Terrestrial Plants

The use of difethialone 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 discountable. 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 a snake. Thus any damage that might occur to plants would not be expected to cause significant vegetative damage which would significantly deteriorate the quality of the habitat of this species. Additionally, the amount of residues that would leach from difethialone bait applied at a maximum of 1 lb product/ placement or 0.000025 lb a.i./placement is expected to be small. Difethialone has very low mobility characteristic, based on a cursorily reviewed study (K_F range $1.6x10^5$ to $1.8x10^6$)]. Furthermore, bait products used for rodent control are oftentimes either placed within a plastic bait station that would minimize contact with rain water, or formulated into weather-resistant blocks which do not readily deteriorate.

Terrestrial plants serve several important habitat-related functions for the listed assessed species. In addition to providing habitat and cover for invertebrate and vertebrate prey items of the listed assessed species, terrestrial vegetation also provides shelter and cover from predators while foraging. Upland vegetation including grassland and woodlands provides cover during dispersal. Riparian vegetation helps to maintain the integrity of aquatic systems by providing bank and thermal stability, serving as a buffer to filter out sediment, nutrients, and contaminants before they reach the watershed, and serving as an energy source. As discussed in **Section 2.9.2.**, use of difethialone in bait products to rodent pests is not expected to result in significant exposure to plants. Bait are generally placed by hand in specific bait stations around buildings. The products containing difethialone cannot be broadcasted. Any exposure to plants would be minor and limited to the area immediately around the bait placement. Therefore, risk of indirect effects of difethialone to the AW mediates through damage to terrestrial plants is considered negligible.

4. Effects Assessment

This assessment evaluates the potential for difethialone to directly or indirectly affect AW, SMHM, and SJKF 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 (through secondary exposure for the AW and SJKF and through primary exposure to the SMHM) as well as indirect effects, such as reduction of the prey base, reduction of SMHM rearing sites or modification of habitat. In addition, potential modification

of the 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 the AW. Direct effects to the reptiles are based on avian toxicity data, given that birds are generally used as a surrogate for terrestrial-phase reptiles.

As described in the Agency's Overview Document (USEPA, 2004), the most sensitive endpoint for each taxon is used for risk estimation. For this assessment, evaluated taxa include birds (which are used as a surrogate for reptiles) and mammals. Acute (short-term) toxicity information is characterized based on registrant-submitted studies and a comprehensive review of the open literature on difethialone. There were no registrant submitted avian or mammalian chronic studies available for difethialone.

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, 2004). Open literature data presented in this assessment were obtained from registrant submitted studies as well as ECOTOX information obtained in April 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 difethialone to 'target' rodent species (the house mouse, the Norway rat, and the roof 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 D. Citations of open literature papers that provide toxicological data for target rodent species are listed in Appendix C with the code "TARGET" given after the citation. While toxicological findings were not included in the summary of acute and chronic 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 difethialone, such as information on the sublethal effects and the mode of action of difethialone.

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, 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 C. Appendix C also includes a rationale for rejection of those studies that did not pass the ECOTOX screen and those that were not evaluated as part of this endangered species risk assessment. A detailed spreadsheet of the available ECOTOX open literature data, including the full suite of lethal and sublethal endpoints is presented in **Appendix C.**

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 difethialone. A summary of the available aquatic and terrestrial ecotoxicity information and the incident information for difethialone is provided below.

4.2. Toxicity of Difethialone to Terrestrial Organisms

Toxicity of difethialone to terrestrial species is relevant for the SMHM given expected direct effects due to ingestion of the bait material (primary exposure) and to the AW and SJKF given ingestion of prey that ingests bait material such as mammalian and avian species (secondary exposure). Furthermore, toxicity of difethialone for mammals and birds is important due to the indirect effects of related prey reduction associated with difethialone use. **Table 4-1** summarizes the most sensitive terrestrial toxicity endpoints, based on an evaluation of the registrant submitted studies. A brief summary of submitted and open literature data considered relevant to this ecological risk assessment is presented below.

Table 4-1. Terrestrial Toxicity Profile for Difethialone

Endpoint	Acute/ Chronic	Species	Toxicity Value Used in Risk Assessment	Citation MRID/ ECOTOX reference No.	Comment
Birds (surrogate for terrestrial-phase amphibians and reptiles)	Acute	Japanese quail (*Coturnix coturnix*)	25.3 mg a.i/kg-bw	42687702	- Study classified as supplemental due to species not being one of recommended - No sublethal effects were noted - Exposure was followed by a 21-day observation period due to the

Endpoint	Acute/ Chronic	Species	Toxicity Value Used in Risk Assessment	Citation MRID/ ECOTOX reference No.	Comment
					delayed toxicity of difethialone
					- Study classified as acceptable - numerous sublethal effects reported including limping and bleeding from the mouth but the levels at which effects were observed were not reported - Significant decrease in body weight attributed to the 1.0 mg a.i/kg treatment group Exposure was followed by a 30-day observation period due to the delayed toxicity of difethialone
	Acute	Northern bobwhite (*Colinus virginius*)	0.26 mg a.i/kg-bw	40696901	
	Acute	Northern bobwhite	0.56 mg a.i./kg-bw	40696902	- Study classified as acceptable - Sublethal effects included anorexia in the 2.0 and 64.0 ppm dietary levels, and lethargy and piloerection in the 16 and 64 ppm dietary levels - Following a five day exposure, the birds were observed for 25 days due to the delayed toxicity of difethialone
	Acute	Mallard ducks (*Anas platyrhynchos)*	30-day LC_{50} 2.3 mg a.i/kg-bw	40268912	- Study was classified as acceptable - Clinical signs of toxicity included lethargy, weakness, green feces but the levels at which these effects occurred were not noted - Following a five day exposure, the birds were observed for 25 days due to the delayed toxicity of difethialone
	Chronic	--	--	--	No avian reproduction studies submitted
Mammals	Acute	Norway rat *(Rattus norvegicus)*	0.55 mg a.i/kg-bw	40268903	- Study classified as Acceptable - Sublethal effects were not noted during the study - An observation period of 21 days followed exposure due to the delayed toxicity of difethialone
	Chronic	--	--	--	No mammalian reproduction studies submitted

bw = body weight

Data are not available to characterize the toxicity of difethialone to nontarget invertebrates (*e.g.* honey bees) or to terrestrial or aquatic plants.

Acute toxicity to terrestrial animals is categorized using the classification system shown in **Table 4-2** (USEPA, 2004). Toxicity categories for terrestrial plants have not been defined. Toxicity data categorizes difethialone as *very highly toxic* to birds and mammals on an acute oral basis, and *highly toxic* to birds on a subacute dietary basis.

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

As specified in the Overview Document, the Agency uses birds as a surrogate for reptiles and terrestrial-phase amphibians when toxicity data for each specific taxon are not available (USEPA, 2004). A summary of acute and chronic bird data is provided below.

Table 4-3 summarizes findings of studies on acute toxicity to birds when difethialone is administered as a single oral dose. These data classify difethialone as *very highly toxic* to birds. **Table 4-4** summarizes findings of studies on subacute toxicity to birds when difethialone is administered in the diet. The results for the northern bobwhite categorize difethialone as *very highly toxic* to birds when administered through the diet.

Table 4-3. Acute Oral Toxicity of Difethialone to Birds

Species, Test substance	% AI	LD_{50} (mg/kg-bw) (95% confidence interval)	MRID or ECOTOX	Classification
Japanese quail difethialone in gelatin capsule	99.5	30-day LD_{50} = 23.5 (11.4 – 48.5) Slope: 3.19	MRID 40268913	Supplemental
Northern bobwhite quail, difethialone with corn oil vehicle	96	14-day LD_{50} = 0.26* (0.17 – 0.40) Slope: 5.9	MRID 40696901	Acceptable

*Endpoint used for quantitative assessment of risks.
N/R = Not reported

Table 4-4. Subacute Dietary Toxicity of Difethialone to Birds

Species	% AI	LC_{50} (mg/kg-diet) (95% confidence interval)	MRID or ECOTOX	Classification
Northern bobwhite	96	30-day LC_{50} = 0.56* (0.16 – 1.9) Slope: N/R	MRID 40696902	Acceptable
Mallard duck	99.5	30-day LC_{50} = 1.9 (0.74 – 5.1) Slope: N/R	MRID 40268912	Acceptable

*Endpoint used for quantitative assessment of risks.
[1] 30-day dietary studies were conducted instead of the standard 850.2200 8-day dietary studies due to the delayed toxicity of difethialone.
N/R = Not reported

In these studies, difethialone was observed to cause several sublethal effects in birds but the oral doses at which they occurred were only noted for loss of body weight (1.0 mg a.i./kg-diet) and severe foot depressions (0.68 and 1.0 mg a.i./kg-diet)[12]. Other sublethal effects observed in the acute oral toxicity studies at unspecified treatment levels were lethargy, piloerection, bloody diarrhea, limping, and bleeding from the tail feathers and the mouth. These sublethal effects were only noted in the bobwhite quail study as no sublethal effects were noted in the Japanese quail study. For the dietary toxicity studies, sublethal effects were noted in both studies (one with bobwhite quail and one with mallard duck) but the levels at which the sublethal effect occurred were not indicated. For the bobwhite quail dietary study, clinical signs of toxicity included, lethargy, weakness, green feces, and reduced food consumption. These effects occurred at all treatment levels except the two lowest levels tested (0.13, and 0.25 mg a.i/kg-diet). Food consumption depressions were noted in four of the treatment groups but it was not specified in the study report at which levels these occurred. Overall mean body weight changes were comparable to the controls in all but two treatment groups (2.0 mg a.i./kg-diet and the highest treatment group, 64 mg a.i./kg-diet)

For the dietary study with the mallard duck, clinical signs of toxicity included lethargy, weakness, green feces, and reduced food consumption yet the treatment levels that these effects occurred are not noted in the study report. Observed sublethal effects and the levels they occurred at where available are presented below in **Table 4-5** for the avian acute oral and avian acute dietary studies.

4.2.1.a. Birds: Secondary Hazard and Metabolism Studies

In, an assessment of the potential secondary hazard of difethialone (MRID 46656501), difethialone secondary hazard potential was estimated by comparing accumulated residues in rats to the dietary levels in magpies and ferrets. Based on the results of this study, the magpie was the more sensitive species. Both species were assessed in a 30-35 day (depending on species used and whether the study was a range finder or definitive test) exposure that consisted of a 3 day pre-treatment period, a 5 day treatment period, and a 25-27 day post-treatment period for observations. The accumulation of residues in rat carcasses was also assessed using two

[12] Foot depression, not further characterized in the study.

different feeding scenarios, one in which animals were offered bait feed for three days and then euthanized, and one in which animals were offered bait feed until poisoning and death occurred. Rats that were allowed to consume bait until death had a slightly higher body residue count (3.1 mg a.i./kg-diet compared to 2.8 mg a.i./kg-diet for euthanized rats). There was no statistical difference in these results.

In magpies, the LC_{50} was reported to be 4.48 mg a.i./kg diet (no reliable confidence intervals). Signs of toxicity were observed in birds consuming diets at the 8.5 mg a.i/kg-diet level and greater and included hemorrhaging (as indicated by blood around and under the cage, the food dish, and the perch), dark feces, lethargy, fluffed feathers, sterna recumbency, and wing droop. The NOAEC for mortality and clinical signs of toxicity was 0.95 mg a.i/kg-diet (the second lowest dose tested). Although necropsy was not included in the protocol, lack of observations of external hemorrhaging does not preclude the existence of internal hemorrhage that would also indicate toxicosis. According to US EPA classification, difethialone would be classified as very highly toxic to black billed magpies on an acute dietary basis.

Table 4-5. Sublethal Effects of Difethialone Observed in Acute Avian Toxicity Studies

Symptom	NOAEL	LOAEL	Reference (MRID)
Acute Oral Studies			
Lethargy	Not determined	Not determined	40696901
Piloerection	Not determined	Not determined	
Limping	Not determined	Not determined	
Loose feces/diarrhea	Not determined	Not determined	
Bleeding from tail feathers/mouth	Not determined	Not determined	
Severe foot depressions	0.46 (mg/kg-bw)	0.68 (mg/kg-bw)	
Reduced bodyweight gain	0.68 (mg/kg-bw)	1.0 (mg/kg-bw)	
Dietary Studies			
Lethargy	Not determined	Not determined	40696902
	Not determined	Not determined	
Weakness	Not determined	Not determined	
	Not determined	Not determined	
Green feces	Not determined	Not determined	
	Not determined	Not determined	
Reduced bodyweight gain	1.0 ppm	2.0 ppm	
Reduced food consumption	Not determined	Not determined	
	Not determined	Not determined	

No data are available on the effects of chronic exposure of birds to difethialone.

4.2.2. Toxicity to Mammals

A summary of acute and chronic mammalian data, including data published in the open literature, is provided below in **Sections 4.2.2.a** through **4.2.2.b**. To date, there has not been an HED chapter for difethialone, and it was not included in the Reregistration Eligibility Decision

(RED) process. The RED described in **Section 2.3** refers to a cluster of rodenticides that did not include difethialone; however, it indicated that the risk mitigation measures applied for the RED cluster would apply to new and other existing rodenticides, including difethialone specifically.

4.2.2.a. Mammals: Acute Exposure (Mortality) Studies

Table 4-6 summarizes findings of studies on the acute of difethialone to mammals. These data classify difethialone as *very highly toxic* to mammals. The lowest acute oral toxicity LD_{50} from a fully acceptable (MRID 40268903) study was 0.55 mg a.i./kg-bw. This value was used in the quantitative acute risk assessment for mammals. Other studies that were submitted yielded LD_{50} - that were non-definitive. It is unclear whether these studies were allowed to proceed for a longer observation period post treatment as difethialone has been shown to have delayed toxicity after treatment with time to death in studies ranging from 6-18 days.

Table 4-6. Summary of Findings of Acute Toxicity of Difethialone to Mammals

Species	Test Material	% AI	LD_{50} [1,2]	MRID, Citation	Classification
Norway Rat	TEP	0.0025	Female: >13.22 mg a.i/kg-bw Male: >13.22 mg a.i/kg-bw Combined[2]: >13.22 mg a.i/kg-bw Slope: N/R	45179601	Acceptable
Norway Rat	Co-formulated with Fipronil	0.0025	Female: >12.63 mg a.i/kg-bw Male: >12.63 mg a.i/kg-bw Combined: >12.63 mg a.i/kg-bw Slope: N/R	47303013	Acceptable
Norway Rat	TEP	0.0025	Female: >12.5 mg a.i/kg-bw Male: >12.5 mg a.i/kg-bw Combined: >12.5 mg a.i/kg-bw Slope: N/R	44961205	Acceptable
Norway Rat	TGAI	98.9	0.55 mg a.i/kg-bw* Slope: N/R	40268903/ 42687704	Acceptable

*Endpoint used for quantitative assessment of risks.
[1] 95% confidence intervals are given in parentheses when available.
[2] Results for combined sexes were calculated by the study author.
N/R = Not reported

Table 4-7. Sublethal Effects of Difethialone Observed in Mammalian Toxicity Studies

Symptom	Species	NOAEL (g/kg)	LOAEL (g/kg)	Reference
Acute Oral Studies				
Blood from ears/nostril discharge	Rat	<0.40	0.40	44649401
Lethargy	Rat	<0.40	0.40	

Symptom	Species	NOAEL (g/kg)	LOAEL (g/kg)	Reference
Dyspnea	Rat	<0.40	0.40	
Prostration	Rat	>0.50	0.50	
Lacrimation	Rat	0.50	0.45	
Piloerection	Rat	<0.40	0.40	
Tremors	Rat	<0.40	0.40	
Hyperactivity	Rat	>0.50	0.50	
Dry feces	Rat	0.50	0.45	
Pale eyes	Rat	>0.50	0.50	
Tachypnea	Rat	0.50	0.45	44649401
Catalepsy	Rat	>0.50	0.50	
Tremors	Rat	<0.40	0.40	
Toe walking	Rat	>0.50	0.50	
Watery stool	Rat	>0.50	0.50	
Pale lungs	Rat	>0.50	0.50	

In an ECOTOX run for difethialone conducted in April, 2011, one open literature paper was accepted by ECOTOX and OPP. This reference, ECOTOX citation 151509 (Chaudhary and Tripathi, 2006), evaluated the single dose efficacy of difethialone in the laboratory to the Indian gerbil (*Tatera indica*), the cutch rock rat (*Rattus cutchicus*), the Indian desert gerbil (*Meriones hurrianae*), and the Northern palm squirrel (*Funambulus pennanti*). All test rodents were exposed to pearl millet-based difethialone loose baits (0.0025%) for one day under no-choice and choice conditions. In the no-choice conditions, all test rodents were dead within 3-11 days of treatment. The minimum dose required to initiate mortality to *T. indica, R. cutchicus, M. hurrianae,* and *F. pennanti* was 0.50, 0.94, 0.60, and 1.42 mg/kg, respectively. Mortality was reduced to 80, 80, 70, and 50% for the four test species respectively when exposed under a choice test. There was no significant difference observed between the consumption of treated and untreated bait indicating that difethialone was fairly palatable to all rodent test species.

Although the 0.50 mg/kg endpoint to the Indian gerbil represents a lower endpoint than that of the registrant submitted Norway rat study (MRID 40268903), the endpoint is the minimum dose required to initiate mortality, whereas the registrant submitted study is LD_{50}. The intake of difethialone a.i to effect 100% mortality of test rodent species ranged from 1.04 (Indian gerbil) to 2.03 mg a.i/kg (cutch rock rat). As these data represent the amount of difethialone for total mortality and not an LD_{50} that is the traditional endpoint for ecotoxicity studies, these values will not be used quantitatively in this assessment.

4.2.2.b. Mammals: Chronic Exposure (Growth, Reproduction) Studies

There are no available chronic toxicity studies available to assess the effects of difethialone to mammals.

4.2.2.c. Mammals: Secondary Hazard and Metabolism Studies

In a metabolism study conducted by Lechevin *et al.*, (1988), radiolabeled difethialone was administered to rats in two doses (0.5 mg a.i/kg-bw, and 5 mg a.i/kg-bw). Half-life was assessed both in the plasma and the liver. It was concluded from the study that difethialone has a relatively short half life in the plasma (2.3 and 2.8 days for the two dose levels, respectively) and a longer half life in the liver (108 days at the 0.5 mg a.i/kg level, not assessed at the 5 mg a.i/kg-bw level). The elimination, which was essentially fecal was with an almost complete absence of metabolism. It was not possible to calculate the half life in blood at the 5 mg a.i./kg-bw level because of mortality in the test animals six days after administration.

As previously discussed, the potential secondary hazard of difethialone (MRID 46656501) was estimated by comparing accumulated residues in rats to the dietary levels in magpies and ferrets. Both species were assessed in a 30-35 day exposure that consisted of a 3 day pre-treatment period, a 5 day treatment period, and a 25-27 day post-treatment period for observations. The accumulation of residues in rat carcasses was assessed using two different feeding scenarios, one in which animals were offered bait feed for three days and then euthanized, and one in which animals were offered bait feed until poisoning and death occurred. Rats that were allowed to consume bait until death had a slightly higher (3.1 mg a.i/kg-diet compared to 2.8 mg a.i./kg-diet for euthanized rats) body residue count. There was no statistical difference in these results.

In ferrets, an LC_{50} of 97.7 mg a.i/kg was estimated with no reliable confidence intervals. There was no mortality during the range finding study where the LC_{50} was greater than 112 mg a.i./kg. In a second study, mortality data were variable; however, the sample size was low and factors precluded the ability to derive a reliable toxicity estimate. Red urine (presumably from blood), blood under cage, lethargy, ataxia, and labored breathing were observed in animals exposed at greater than 1.02 mg a.i/kg diet levels (the second lowest dose tested). The NOAEC for mortality and clinical signs of toxicity was 0.089 mg a.i./kg diet (the lowest dose tested). Due to the small samples size and lack of confidence intervals, caution should be taken in utilizing the result as a definitive LC_{50} point estimate for ferrets.

4.2.3. Toxicity to Terrestrial Invertebrates

No data are available on the toxicity of difethialone to terrestrial invertebrates.

4.3. Toxicity of Chemical Mixtures

The Agency does not routinely include, in its risk assessments, an evaluation of mixtures of active ingredients, either those mixtures of multiple active ingredients in product formulations or those in the applicator's tank. In the case of the product formulations of active ingredients (that is, a registered product containing more than one active ingredient), each active ingredient is subject to an individual risk assessment for regulatory decision regarding the active ingredient on a particular use site. If effects data are available for a formulated product containing more than one active ingredient, they may be used qualitatively or quantitatively in accordance with the Agency's Overview Document and the Services' Evaluation Memorandum (USEPA, 2004; USFWS/NMFS/NOAA, 2004).

Difethialone has one registered product that contains two active ingredients (the other active ingredient is fipronil). There is one oral mammalian study for this product (MRID No. 47303013) in which the acute oral LD_{50} was > 5,050 mg a.i./kg-bw (male, female and combined). Fipronil is an insecticide that causes nerve excitation by targeting the γ-aminobutyric acid type A receptor system (GABA). It blocks the voltage-gated chloride channels in the neurons, resulting in removal of inhibitory mechanism and high neuronal activity. It has more binding affinity to the GABA regulated channels in the insects than in the mammals, resulting in selectivity (http://www.pw.ucr.edu/textfiles/fipronil.pdf, accessed 08/16/2011). In contrast, difethialone is an anticoagulant vitamin-K antagonist that disrupts normal blood-clotting mechanism and induces capillary damage. It would appear that no important interactions would occur between these active ingredients (*e.g.*, synergism) given that the active and inert ingredients would not be expected to have similar mechanisms of action, metabolites or toxicokinetic behavior. It appears reasonable to conclude that an assumption of dose-addition would be inappropriate; however, there is not enough data to make conclusive evaluations of mixture toxicity and it is unknown whether this formulation is reflecting an independent additive toxicity response and not an interactive effect. In the absence of detailed information, an assessment of difethialone's potential effect on the AW, SMHM, and SJKF when it is co-formulated with fipronil will be based on the toxicity of difethialone.

4.4. Incident Database Review

A review of the Ecological Incident Information System (EIIS, version 2.1), the 'Aggregate Incident Reports' (v. 1.0) database, and the Avian Monitoring Information System (AIMS) for ecological incidents involving difethialone, was completed on June 21, 2011. The results of this review for terrestrial incidents are tabulated below in **Table 4-8**.

Table 4-8. Reported terrestrial incidents for difethialone

Incident ID	Year	Legality	Certainty	State	Residential / Urban or field area?	Generic Species and (Number Affected)	Comments
I018414-002	2007	N/R	Probable	CA	Urban	Hawk (1)	A red-shouldered hawk was found dead in San Francisco's Golden Gate Park after ingesting rats poisoned by difethialone that was used to control rodents in the park.
I018414-003	2007	N/R	Probable	CA	Urban	Hawk (1)	A red-tailed hawk was found dead in San Francisco's Golden Gate Park after ingesting rats poisoned by difethialone that was used to control rodents in the park.
I018414-004	2007	N/R	Probable	CA	Urban	Hawk (1)	A red-tailed hawk was found dead in San Francisco's Golden Gate Park after ingesting rats poisoned by difethialone that

Incident ID	Year	Legality	Certainty	State	Residential / Urban or field area?	Generic Species and (Number Affected)	Comments
							was used to control rodents in the park.
I018414-005	2007	N/R	Probable	CA	Urban	Fox (1)	A red fox was found dead in San Francisco's Golden Gate Park after ingesting rats poisoned by difethialone that was used to control rodents in the park.
I020713-002	2009	N/R	Possible	FL	Residential	Deer (1)	A key deer was found dead near a private residence and submitted for necropsy. There was a moderate amount of dark red to black fluid in the thoracic cavity. The lungs are dark red and edematous and the heart was flaccid and dark red with similar fluid to that seen in the thoracic cavity. An analysis of the liver detected brodifacoum residues at 1.3 ppm and trace amounts of difethialone. The final diagnosis was brodifacoum and difethialone poisoning.

The first four incidents (I018414-002 – 005) occurred in Golden Gate Park, San Francisco, California. The San Francisco Recreation and Park Department had pressure put on it to only use pesticides on municipal properties as last resorts. In the fifteen months that difethialone bait stations were used, 3 hawks and one red fox were found dead in the park, later prompting the city to put a temporary ban on the rodenticide use in the park. While there was no chemical residue analysis to confirm difethialone residues in the liver, the birds and fox died in a park that had been extensively baited with difethialone (36 of the city's rodenticide bait stations were used in the park). These incidents occurred in 2007 but wildlife deaths were also reported in 2005 when baits were previously used in the park.

The most recent incident in Florida (I020713-002) involved the death of a key deer *(Odocoileus virginianus clavium)*, a listed species. While a chemical residue analysis showed only trace amounts of difethialone and 1.3 ppm of brodifacoum concentrations, the ultimate diagnosis was poisoning by both chemicals. As such, this incident is classified as "possible," for difethialone.

While a small sample size, it is worth noting that the majority (4/5 or 80%) of the incidents involve secondary poisoning in which a secondary consumer preys upon another animal that has ingested difethialone bait. The delayed nature of toxicity of difethialone combined with prolonged liver retention times make animals who feed upon rodents, such as the AW and the

SJKF especially susceptible from difethialone poisoning in areas where bait is used for rodent control.

The incidents used for this analysis were drawn from primarily one source. The incidents came from the Environmental Fate and Effects Divisions (EFED) maintained incident database EIIS (Environmental Incident Information System, Version 2.1). Incidents reports for non-target organisms typically provide information only on mortality events. A search in August, 2011 of the American Bird Conservancy maintained Avian Incident Monitoring System (AIMS) did not yield any results. Except for phytotoxic effects in terrestrial plants, sublethal effects for organisms such as reduced growth or impaired reproduction are rarely reported. The EPA's changes in the registrant reporting requirements for incidents in 1998 may account for a reduced number of reported incidents. Registrants are now only required to submit detailed information on "major," wildlife incidents. Minor wildlife incidents as well as other non-target incidents are generally reported aggregately and not included in EIIS. In addition, there have been changes in state monitoring efforts due to a lack of resources. Essentially a lack of incidents does not necessarily indicate a lack of exposure or toxicity events but rather could be the result of a lack of reporting.

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

The Agency uses the probit dose response relationship as a tool for providing additional information on the potential for acute direct effects to individual listed species and aquatic animals that may indirectly affect the listed species of concern (USEPA, 2004). 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 difethialone 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. The upper and lower bounds of the effects probability are based on available information on the 95% confidence interval of the slope. A statement regarding the confidence in the estimated event probabilities is also included. Studies with good probit fit characteristics (*i.e.*, statistically appropriate for the data set) are associated with a high degree of confidence. Conversely, a low degree of confidence is associated with data from studies that do not statistically support a probit dose response relationship. In addition, confidence in the data set may be reduced by high variance in the slope (i.e., large 95% confidence intervals), despite good probit fit characteristics. In the event that dose response information is not available to estimate a slope, a default slope assumption of 4.5 (lower and upper bounds of 2 to 9) (Urban and Cook, 1986) is used.

Individual effect probabilities are calculated based on an Excel spreadsheet tool IECV1.1 (Individual Effect Chance Model Version 1.1) developed by the U.S. EPA, OPP, Environmental Fate and Effects Division (June 22, 2004). The model allows for such calculations by entering

the mean slope estimate (and the 95% confidence bounds of that estimate) as the slope parameter for the spreadsheet. In addition, the acute RQ is entered as the desired threshold.

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 AW, SMHM, and SJKF or for modification to the AW designated critical habitat from the use of difethialone 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 modification of 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 standard EFED procedures and models. 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 aquatic animals, as well as terrestrial invertebrates, the LOC is 0.05. For acute exposures to the birds (and, thus, reptiles and terrestrial-phase amphibians) and mammals, the LOC is 0.1. The LOC for chronic exposures to animals, as well as acute exposures to plants is 1.0.

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 difethialone for use in and around homes and residential buildings, industrial, commercial and public buildings, food processing facilities, transport vehicles (ships, trains, aircraft) and their related ports, in and around agricultural buildings, alleys and sewers.

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 difethialone 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 difethialone bait) and to the AW and SJKF through secondary exposure, that is, consuming a bird, mammal, or reptile that has directly ingested difethialone bait. Although not confirmed by research, the probability of the AW and SJKF, which typically consumes live prey, consuming rodenticide bait pellets 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 in this assessment have

shown, difethialone 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 3.3.1**, the SMHM is likely to be exposed to difethialone residues from primary exposure that occurs from direct ingestion of difethialone bait. Bait with one concentration of difethialone was modeled, 0.0025%.

Acute dose-based risk quotients were calculated by dividing the expected dose of difethialone (mg-a.i./kg-bw) by the acute oral LD_{50} value for the rat, 0.55 mg-a.i./kg-bw (**Table 4-7**). Results of these calculations are presented in **Table 5-1**. No acute dietary-based or chronic RQs were calculated for mammals because no acute dietary toxicity or chronic toxicity data were available.

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. RQs for Acute Effects to the SMHM from Consumption of Difethialone Bait

Mammal (bodyweight)	Bait Type	%AI	FI[1] (g/d)	Dose (mg/kg-bw)	Acute RQ[2]
SMHM (10 g)	Rodent Control	0.0025	2.28	5.7	**10.36**

LOC exceedances (acute RQ > 0.1) are bolded.
[1] Daily food ingestion rate.
[2] Based on the FI and the rat acute oral LD_{50} of 0.55 mg/kg-bw

Since the acute RQs for the SMHM exceeded the LOC (RQ of **10.36**) for listed and non-listed species, use of difethialone has the potential to cause direct effects to the SMHM.

Based on the RQs and the LD_{50} in the most sensitive acute oral toxicity study to mammals (a default slope of 4.5 was used as the study did not provide one), calculations were made to determine the probability that a particular individual would be killed by the dose predicted for a SMHM consuming difethilone bait. The individual probability of death is 100% (1 in 1) for SMHM that ingested difethialone bait.

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

As discussed in **Section 3.3.2**, the AW and SJKF are likely to be exposed to difethialone residues from secondary exposure that occurs from consumption of prey that has consumed difethialone bait. The AW and SJKF are capable of consuming all of the target small mammals species specified on the difethialone bait product labels, including 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 difethialone (mg-a.i./kg-bw) by the acute oral LD_{50} value for the most sensitive bird species, 0.26 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. RQs for Acute Effects to the AW from Consumption of Mammals which Ingested Difethialone 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	0.0025	20.3	1.57	**6.04**
	House mouse (23 g)	18.6 g	0.0025	3.64	4.89	**18.81**

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 exceed 0.1 (RQs ranged from **6.04 – 18.81**), the LOC for acute effects to listed species, use of difethialone has the potential to directly affect the AW that feeds upon small mammals which ingested difethialone bait, by way of secondary exposure.

For mammals consuming other mammals that have ingested difethialone 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 3.3.2.

When the dose based EEC (as derived in **Section 3.3.2**) is divided by the adjusted LD_{50} of 0.34 mg a.i/kg for the 244-g mammal, the resulting RQ is **2.2** (Table 5-3).

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

Bait Type	Prey Species	Assumed weight of AWSJKF	%AI in Bait	Dose received by prey FI[1] (mg a.i./d)	Dose-based EEC for SJKF (mg a.i./kg BW)	Adjusted LD_{50} (mg a.i./kg BW)	Acute RQ[2]
Rodent control	Small mammal (244 g)	2300 g	0.0025	1.72	0.75	0.34	**2.2**

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 difethialone in the bait (25 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 0.34 mg/kg-bw

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

Based on the RQs and the LD_{50} in the most sensitive acute oral toxicity study to mammals (a default slope of 4.5 was used as the original study report did not provide one), 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 100% (1 in 1) for a SJKF consuming a small mammal that ingested difethialone bait.

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

The AW and SJKF are likely to be exposed to difethialone residues from secondary exposure that occurs from consumption of prey that has consumed difethialone bait. The AW and SJKF are capable of consuming birds as prey that may have directly ingested difethialone bait. Therefore, risk based on secondary exposure was conducted for a snake and mammal which feeds on birds as discussed in **Section 3.3.2**.

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 difethialone (mg-a.i./kg-bw) by the acute oral LD_{50} value for the most sensitive bird species, 0.26 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 Difethialone 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	0.0025	4.55	7.02	**27.00**
	Medium Bird (100 g)	74.1	0.0025	13.00	4.39	**16.88**
	Large Bird (1000 g)	631	0.0025	58.1	2.30	**8.85**

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 exceed 0.1 (RQs ranged from **8.85 – 27.00**), the LOC for acute effects to listed species, use of difethialone has the potential to directly affect the AW that feed upon birds which ingested difethialone bait by way of secondary exposure.

Based on the RQs and the LD_{50} in the most sensitive acute oral toxicity study to birds (a slope of 5.9 was used per the study report), calculations were made to determine the probability that a particular individual would be killed by the dose predicted for a AW consuming a contaminated bird. The individual probability of death is 100% (1 in 1) for an AW consuming either a small, medium, or large bird that ingested difethialone bait.

For mammals consuming birds that have ingested difethialone 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 3.3.2.

When the dose-based EEC (as derived in **Section 3.3.2**) is divided by the adjusted LD_{50} of 0.20 mg a.i/kg for the 244-g bird, the resulting RQ is **6.30** (**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 mammals which have ingested difethialone bait.

Table 5-5. RQs for Acute Effects to the SJKF from Consumption of Mammals which Ingested Difethialone 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	0.0025	2.9	1.26	0.20	**6.30**

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 difethialone in the bait (25 mg a.i.)

[2] Based on the dose-based EEC in the ingested prey and the adjusted LD_{50} of a 244-g bird of 0.20 mg/kg-bw

Based on the RQs and the LD_{50} in the most sensitive acute oral toxicity study to birds (a slope of 5.9 was used per the study report), calculations were made to determine the probability that a particular individual would be killed by the dose predicted for a AW consuming a contaminated bird. The individual probability of death is 100% (1 in 1) for an AW consuming either a small, medium, or large bird that ingested difethialone bait.

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

The AW is likely to be exposed to difethialone residues from secondary exposure that occurs from consumption of prey that has consumed difethialone bait. The AW is capable of consuming other reptiles such as lizards (their chief preference of food) as prey that may have directly ingested difethialone 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 3.3.2.**

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 difethialone (mg-a.i./kg-bw) by the acute oral LD_{50} value for the most sensitive bird species, 0.26 mg-a.i./kg-bw, used as a surrogate to 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 Difethialone 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	0.0025	0.02	0.26	**1.00**
	Medium reptile (20 g)	16.2	0.0025	0.13	0.20	**0.77**
	Large reptile (800 g)	513	0.0025	2.28	0.11	**0.42**

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 exceed 0.1 (RQs ranged from **0.42 – 1.00**), the LOC for acute effects to listed species, use of difethialone has the potential to directly affect the AW that feed upon reptiles which ingested difethialone bait by way of secondary exposure.

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 difethialone bait were evaluated by assuming an individual directly consumes a bait product containing difethialone at its daily ingestion rate. As all difethialone products are the same percent a.i, 0.0025%, only one concentration of difethialone 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 3.3.1**.

Acute risk quotients were calculated by dividing the expected dose of difethialone (mg-a.i./kg-bw) by the acute oral LD_{50} value for the northern bobwhite, 0.26 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 difethialone 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 (0.01) from direct consumption of difethialone bait is exceeded with RQs ranging from **0.27** for the largest size reptile to **1.08** for the smallest size reptile.

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

Size (bodyweight)	%AI	FI[1] (g/d)	Dose (mg ai/kg-BW)	Acute Dose-Based RQ[2]
Small (2 g)	0.0025	0.022	0.28	**1.08**
Medium (20 g)	0.0025	0.13	0.16	**0.62**
Large (800 g)	0.0025	2.3	0.07	**0.27**

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 0.26 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 difethialone concentration of 0.0025%. 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 3.3.1.**

Acute RQs were calculated by dividing the expected dose of difethialone (mg-a.i./kg-bw) by the acute oral LD_{50} value for the northern bobwhite, 0.26 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 difethialone bait were also calculated by simply dividing the concentration of difethialone in the bait by the subacute dietary LC_{50} value for the northern bobwhite (0.56 mg a.i./kg-diet). The difethialone concentrations in the bait, when expressed as parts-per-million (mg-a.i./kg), is 25 for rodenticide bait. Therefore, the dietary RQs for indirect effects resulting from toxicity to birds is **44.64** for rodenticide bait.

Table 5-8. Acute RQs for Birds that Consume Difethialone 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)	0.0025	4.56	5.70	**21.92**	**44.64**
Medium (100 g)	0.0025	13.0	3.25	**12.50**	**44.64**
Large (1000 g)	0.0025	58.2	1.46	**5.61**	**44.64**

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 0.26 mg/kg-bw
[3] Based on the %AI of the bait and the northern bobwhite dietary LC_{50} of 0.56mg/kg-diet.

No chronic avian risk quotients could be calculated because toxicity data on the chronic effects of difethialone to birds are not available.

Because the acute risk quotient for reptiles exceed the Agency's listed and non listed species LOCs (dose-based acute RQs ranged from **5.61 – 21.92**, and diet-based acute RQs were **44.64**), use of difethialone has the potential to cause indirect effects to the AW. Additionally, since the acute RQs for birds exceeded the non-endangered acute risk LOC of 0.5, the use of difethialone also has the potential to cause indirect effects to the AW through a reduction of prey available to the AW

Mammals

Risk quotients were calculated to assess risk to small mammals which directly consume difethialone 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 difethialone was modeled, 0.0025%. The small mammals were assumed to consume their average daily food intake in the form of the bait as discussed in **Section 3.3.1**.

Acute dose-based risk quotients were calculated by dividing the expected dose of difethialone (mg-a.i./kg-bw) by the acute oral LD_{50} value for the rat, 0.55 mg-a.i./kg-bw (**Table 4-7**). Results of these calculations are presented in **Table 5-9**. No acute dietary-based or chronic RQs were calculated for mammals because no acute dietary toxicity or chronic toxicity data were available. RQs 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. RQs for Acute Effects to Mammals from Consumption of Difethialone Bait

Mammal (bodyweight)	Bait Type	%AI	FI[1] (g/d)	Dose (mg/kg-bw)	Acute RQ[2]
House mouse (23g)	Rodent control	0.0025	3.64	3.95	**7.19**
Norway rat (485 g)	Rodent Control	0.0025	20.3	1.05	**1.90**

LOC exceedances (acute RQ > 0.1) are bolded.
[1] Daily food ingestion rate.
[2] Based on the FI and the rat acute oral LD_{50} of 0.55 mg/kg-bw

Since the acute RQs for a 23-g mammal and a 485 g mammal exceeded the LOCs (RQs ranged from **1.90 – 7.19**) for listed and non-listed species, use of difethialone 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.

Based on the RQs and the LD_{50} in the most sensitive acute oral toxicity study to mammals (a default slope of 4.5 was used as the study did not provide one), calculations were made to determine the probability that a particular individual would be killed by the dose predicted for a AW consuming a contaminated small mammal. The individual probability of death is 100% (1 in 1) for an AW consuming a small mammal that ingested difethialone bait.

5.1.2. Primary Constituent Elements of Designated Critical Habitat

For difethialone use, the assessment endpoints for 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 final No Effect/May Affect determination is made after the spatial analysis is completed at the end of the risk description, **Section 5.2.2**. In **Section 5.2.2**, a discussion of 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) 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.

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 difethialone's use within the action area. However, if LOCs for direct or indirect effect are exceeded or effects may modify the PCEs of the critical habitat, the Agency concludes a preliminary "may affect" determination for the FIFRA regulatory action regarding difethialone. For this assessment of the use of vertebrate control bait products containing difethialone, a preliminary May Affect determination was made for the AW, SMHM, and SJKF. A preliminary May Affect determination was also made for adverse effects on the PCE's of the critical habitat of the AW. A summary of the risk estimation results are provided in **Table 5-7** for direct and indirect effects to the AW and in **Table 5-8** for the PCEs of their designated critical habitat.

Table 5-10. Risk Estimation Summary for Difethialone - 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 birds that feed on any difethialone bait, and to reptiles and terrestrial-phase amphibians which feed on bait used for rodent control.	Indirect Effects: SMHM and AW
	Listed Species: Yes	Risk of acute secondary poisoning to snakes feeding on prey which ingested difethialone bait.	Direct Effects: AW

Taxa	LOC Exceedances (Yes/No)	Description of Results of Risk Estimation	Assessed Species Potentially Affected
Mammals	Non-listed Species: Yes	Risk of acute effects small mammals that feed on difethialone bait. Risk of acute toxic effects to mammals that serve as prey that feed on difethialone bait.	Direct Effects: SMHM Indirect Effects: AW, SJKF
	Listed Species: Yes	Risk of acute effects small mammals that feed on difethialone bait.	Direct Effects: SMHM

Table 5-11. Risk Estimation Summary for Difethialone – 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 difethialone bait that may be consumed by the AW.	AW
Mammals	Non-listed Species: Yes	Risk of acute effects small mammals that feed on difethialone bait that serve as prey for the AW. Risk of acute effects to small mammals that feed on difethialone 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.

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 difethialone. Finally, in **Section 5.2.2**, 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. Alameda Whipsnake

5.2.1.a. Direct Effects

The primary risk of direct effect of difethialone 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 difethialone bait products are meant to control (rats and mice). Whether a whipsnake would scavenge upon a dead mammal that was killed by difethialone bait is unknown, but since rats and mice poisoned by difethialone 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 difethialone exposure would likely be attractive prey to the snakes. Sublethal effects of difethialone include lethargy and tremors. These sublethal symptoms likely would make poisoned rodents easier to catch.

Extensive use of difethialone 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 (**see Section 2.4.3**). Placement of difethialone 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 difethialone 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 difethialone. 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 difethialone bait may not die for several days. Poisoned small mammals may 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 rat and mouse ingests would pose a risk of acute toxicity to a whipsnake that feeds on it (acute RQs: **6.04 – 18.81**). An assessment was also conducted to predict the length of time that toxicity in a prey animal would remain at levels that would yield a RQ above the LOC of 0.1, and thus may pose a risk of secondary poisoning to the AW. Since laboratory studies show that mammals that die from difethialone poisoning usually die within 7-10 days, prey which ingest a lethal dose would not be expected to remain alive for this long. Still, risk of secondary hazard would likely be high while the prey is intoxicated and immobilized by difethialone poisoning, but remains alive. Small mammals exposed to difethialone may be immobilized during this time, showing symptoms of difethialone toxicity such as lethargy and tremors. This could make them attractive prey which a snake could easily catch. Once the prey animal dies, it is not expected to pose a significant risk of secondary poisoning to the AW. Whipsnakes hunt by sight and are attracted to prey by movement, and thus would be unlikely to consume a dead carcass.

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 difethialone 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.

The results of the risk assessment indicate that non-target reptiles like the AW would be susceptible to secondary poisoning from difethialone, and this risk has been confirmed by documented incidents. As discussed in **Section 4.4**, five wildlife mortality incidents have been documented in which primary or secondary exposure to difethialone was identified as the cause.

Risk quotients indicate that direct consumption of difethialone bait by the AW also would pose an acute risk to the AW. Acute RQs for a reptile that directly ingested the bait ranged from **0.27 -1.1.** This risk is much less certain than the secondary exposure risk, however, because it is 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 difethialone 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 difethialone, in particular from secondary exposure that may occur from consumption of poisoned prey.

5.2.1.b. Indirect Effects

The risk assessment also identified the potential for difethialone 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 (USFWS, 2006), 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 is expected to be great enough to cause significant declines in the populations of small mammal species including the three target species occurring in this region (the Norway rat, roof rat, the house mouse). This is based on another second generation anticoagulant rodenticide, brodifacoum, with very similar chemistry, use patterns, and toxicity profile. Furthermore, brodifacoum has over 260 reported ecological incidents, many of which involve other small mammals like chipmunks and squirrels ingesting brodifacoum bait that results in mortality. Even for the target species, 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 (USFWS, 2005). 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 mammals (a default slope of 4.5 was used as the study did not provide one), calculations were made to determine the probability that a particular individual would be killed by the dose predicted for a AW consuming a contaminated small mammal. The individual probability of death is 100% (1 in 1) for an AW consuming a small mammal that ingested difethialone bait.

5.2.1.c. Modification of Designated Critical Habitat

Critical habitat has been defined for the AW. As discussed above, the potential for difethialone use to 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 certainly has the potential to adversely affect the abundance of small mammals within the critical habitat. Since the AW preys on small mammals (along with other types of terrestrial vertebrates and invertebrates), adverse effects on small mammal 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 is uncertain. In addition to prey effect, 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.

5.2.1.d. Spatial Extent of Potential Effects

Since LOCs are exceeded, analysis of the spatial extent of potential LAA effects is needed to determine where effects may occur in relation to the treated site. If the potential area of usage and subsequent Potential Area of LAA Effects overlaps with AW habitat or areas of occurrence and/or critical habitat, a likely to adversely affect determination is made. If the Potential Area of LAA Effects and the AW habitat and areas of occurrence and/or critical habitat, do not overlap, a no effect determination is made.

To determine this area, the footprint of difethialone's use pattern is identified using corresponding land cover data (**see Section 2.7**). Difethialone is used in and around any type of building, including residential, commercial, industrial, and commercial structures, as well as transportation ports and terminals. For these uses, potential land cover classes include, developed high intensity, developed low intensity, developed medium intensity and possibly developed open space. Because difethialone may be used in and around agricultural buildings, other potential land cover categories include cultivated crops, orchards/ vineyards, pasture/ hay. Actual usage is expected to occur in a smaller area as the chemical is only expected to be used on a portion of the identified area.

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 difethialone is a vertebrate pest control that may be used in a wide variety of urban and non-urban areas, the spatial extent of difethialone cannot be limited to defined areas. The Agency assumes that difethialone potentially may be used in any area of the state, as any area could potentially be adjacent to some kind of building where difethialone rodenticide bait may be placed. Difethialone use may occur in any of the land use categories that are identified in the NLCD. Therefore, a spatial analysis was not conducted to identify this overlap. All areas where these species occur, and all areas of the critical habitat of the AW, are assumed to lie within the potential use area of difethialone.

An alternative type of spatial analysis was conducted to characterize the potential use of difethialone bait products within the region where the assessed species may occur. Since outdoor use of difethialone 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 5-1.**

5.2.1.e. Effects Determinations for Alameda Whipsnake (AW)

The results of this risk assessment indicate that use of difethialone in baits for vertebrate pest control poses a high risk of acute toxicity to the AW resulting from secondary exposure. Secondary poisoning may occur when the AW preys upon small mammals, birds, reptiles or other vertebrate prey species which have ingested the difethialone bait. Further risk to this species would be posed by sublethal behavioral and neurological effects which may result from acute exposure to difethialone. Although less certain, some additional risk also may exist for direct effects from primary exposure to difethialone bait. Finally, indirect effects are also possible from use of this product reducing the abundance of vertebrate prey, and reducing the availability of small mammal burrows where the AW could inhabit.

Therefore, the Agency makes **"may affect"** and **"likely to adversely affect"** determination 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 the AW's critical habitat.

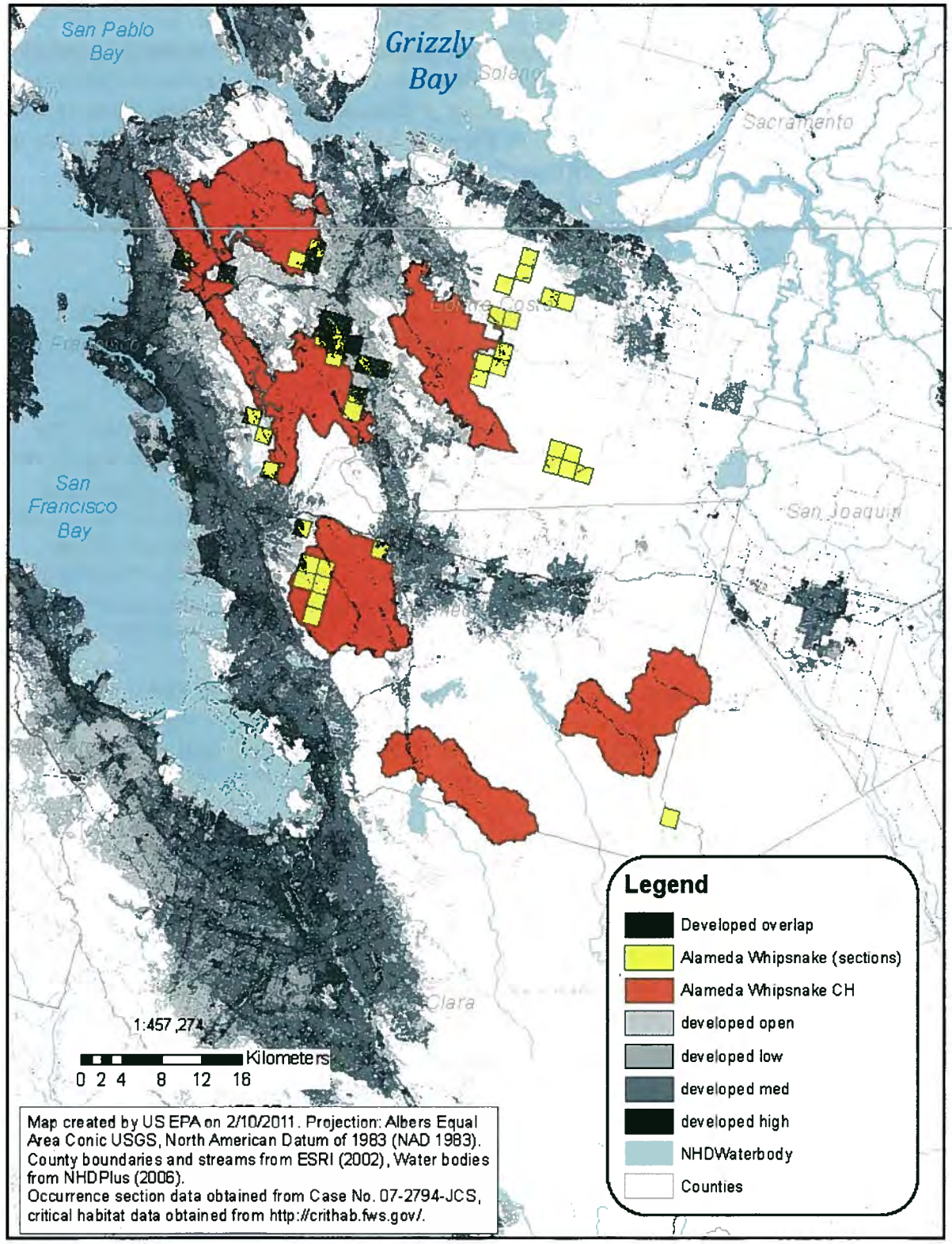

Figure 5-1. Map showing the occurrence of Alameda whipsnake, and its critical habitat, in relation to the intensity of human development.

Areas with higher human development are expected to represent areas where difethialone bait would be more intensively used and where species would be most vulnerable to exposure to difethialone. On the scale displayed, the maps have limited usefulness for identifying specific areas of vulnerability. However, the map shows that the AW occurs in a region of California where development is widespread, and a significant portion of range of both species occurs in areas with moderate to high development. As the species occurs in areas where difethialone bait may be intensively used, AW would be susceptible to exposure to difethialone.

5.2.2. Salt Marsh Harvest Mouse

5.2.2.a. Direct Effects

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

Extensive use of difethialone bait products is believed to be possible in the region where the SMHM occurs. The counties where the SMHM occurs (Marin, Sonoma, Napa, Solano, Contra Costa, San Mateo, Alameda, and Santa Clara counties) include many highly developed and densely populated areas (**see Section 2.4.3**). Placement of difethialone rodenticide bait around residential homes, farm buildings, commercial buildings, and recreation buildings in these counties would provide widespread opportunities for the small mammals like the SMHM to encounter difethialone 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 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 difethialone bait but to what extent is uncertain. Having an average weight of 8 – 14g, the SMHM could easily still get into tamper resistant bait station and consume difethialone bait.

Since the acute RQs for the SMHM exceeded the LOC (**RQ of 10.36**) for listed and non-listed species, use of difethialone has the potential to cause direct effects to the SMHM.

Based on the RQs and the LD_{50} in the most sensitive acute oral toxicity study to mammals (a default slope of 4.5 was used as the study did not provide one), 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 difethialone bait.

5.2.2.b. Indirect Effects

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

Potential effects of difethialone 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 difethialone 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.

5.2.2.c. Spatial Extent of Risks

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 difethialone is a vertebrate pest control agent that may be used in a wide variety of urban and non-urban areas, the spatial extent of difethialone cannot be limited to defined areas. The Agency assumes that difethialone potentially may be used in any area of the state and that use may occur in any of the land use categories that are identified in the NLCD. Therefore, a spatial analysis was not conducted to identify this overlap. All areas where the SMHM occurs are assumed to lie within the potential use area of difethialone. Based on CDPR Pesticide Usage Reporting data, difethialone has been used within the years 1999-2009 in all 8 of the California counties in which occurrences or occurrence sections were identified for the SMHM in Case No. 07-2794-JCS. See Section 5.2.1.d for an explanation of the spatial analysis that is represented in the land use cover maps. The map pertaining to the SMHM is presented below in **Figure 5-2**.

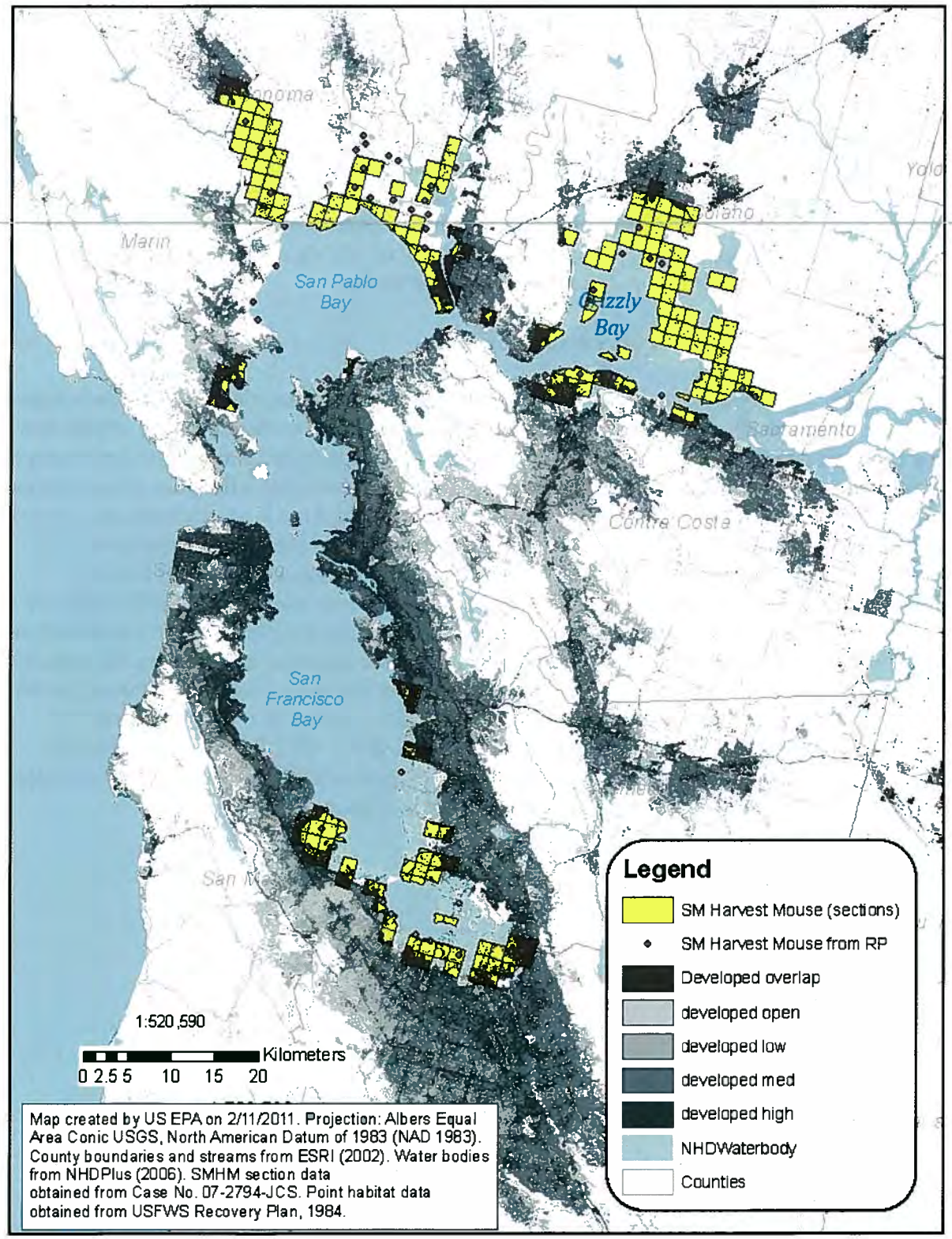

Figure 5-2. Map showing the occurrence of the salt marsh harvest mouse in relation to the intensity of human development.

5.2.2.d. Effects Determinations for Salt Marsh Harvest Mouse (SMHM)

The results of this risk assessment indicates that use of difethialone in baits for vertebrate pest control poses a high risk of acute toxicity to the SMHM resulting from primary exposure through direct consumption of difethialone bait. Further risk to this species would be posed by sublethal behavioral and neurological effects which may result from acute exposure to difethialone. Finally, indirect effects are also possible from use of this product reducing the abundance of rearing sites for the SMHM.

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

5.2.3. San Joaquin Kit Fox

5.2.3.a. Direct Effects

The SJKF is at risk from use of difethialone. The dose-based acute RQs calculated in the risk estimation for secondary exposure of the SJKF to difethialone 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 difethialone bait. Chronic studies for the toxicity of difethialone to mammals were not available but due to the high acute toxicity and effects of difethialone to mammals, chronic risk is also assumed.

In an acute oral toxicity study with the Norway rat, the highest treatment group (0.5 mg a.i./kg-bw ppm) showed signs of toxicity that included bleeding from the ears, dyspnea (shortness of breath), lethargy, prostration, lacrimation, piloerection, dry feces, pale eyes, nostril discharge, tachypnea (rapid breathing), catalepsy, tremors, hyperactivity, somnolence, tow walking, and watery stool. Clinical signs of toxicity for the middle treatment group (0.45 mg a.i./kg-bw) included blood from ears, dyspnea, lethargy, dry feces, nostril discharge, piloerection, lacrimation, tremors, and tachypnea. Clinical signs of toxicity from the lowest treatment group (0.40 mg a.i./kg-bw) included blood from ears, dyspnea, lethargy, loose stool, piloerection, and tremors.

Two wildlife incidents of mammalian toxicity have been reported to the Agency. These incidents provide one piece of evidence that both primary and secondary exposures of difethialone are occurring to non-target organisms, as well as evidence that exposures of difethialone are occurring to the SJKF. One of the incidents involved a red fox (*Vulpes vulpes*). This incident entailed the fox consuming difethialone poisoned rats in a city park where difethialone had been used for rodent control. The incident occurred in San Francisco, CA, and was rated 'probable.'

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 default slope estimate of 4.5 because a probit slope for the dose-response could not be calculated for the associated acute oral study. The RQ for a SJKF ingesting a 244 g small mammal is 6.30 (Table 5-5). The estimated chance of an individual acute mortality of the SJKF ingesting a difethialone poisoned mammal is 1 in 1. These results indicate that the

probability of an individual mortality occurrence is high and that difethialone 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 difethialone 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 **1.9 – 7.19**) (**Table 5-9**). The probability of mortality for an individual small mammal or bird consuming difethialone bait directly is 100% (1 in 1).

5.2.3.c. Spatial Extent of Risks

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 difethialone is a vertebrate pest control agent that may be used in a wide variety of urban and non-urban areas, the spatial extent of difethialone cannot be limited to defined areas. The Agency assumes that difethialone potentially may be used in any area of the state and that use may occur in any of the land use categories that are identified in the NLCD. Therefore, a spatial analysis was not conducted to identify this overlap. All areas where the SJKF occurs are assumed to lie within the potential use area of difethialone. Based on CDPR Pesticide Usage Reporting data, difethialone has been used within the years 1999-2009 in all 16 of the California counties in which occurrences or occurrence sections were identified for the SJKF in Case No. 07-2794-JCS. The map pertaining to the SJKF is presented below in **Figure 5-3**.

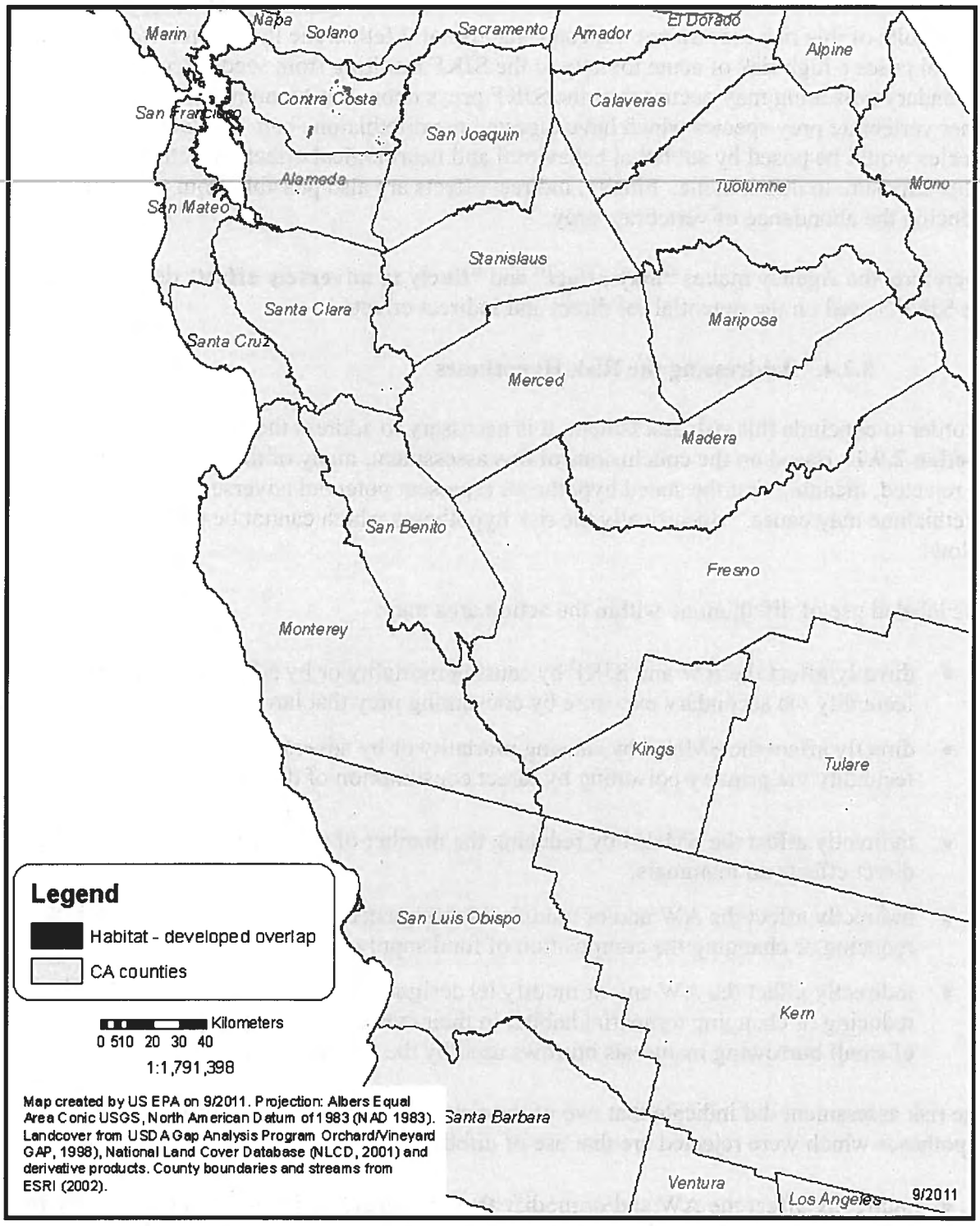

Figure 5-3. Map showing the occurrence of the San Joaquin Kit Fox in relation to the intensity of human development.

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

The results of this risk assessment indicates that use of difethialone 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 the difethialone bait. Further risk to this species would be posed by sublethal behavioral and neurological effects which may result from acute exposure to difethialone. 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 2.9.1**. Based on the conclusions of this assessment, many of the risk hypotheses cannot be rejected, meaning that the stated hypotheses represent potential adverse effects that use of difethialone may cause. Specifically the risk hypotheses which cannot be rejected are listed below.

The labeled use of difethialone within the action area may:

- 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 difethialone bait;
- directly affect the SMHM by causing mortality or by adversely affecting growth or fecundity via primary poisoning by direct consumption of difethialone 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.

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

- indirectly affect the AW and/or modify their designated critical habitat of the AW by reducing or changing the composition of the terrestrial plant community in the species' current range;

- indirectly affect the SMHM by reducing or changing the composition of the aquatic plant community in the species' current range, thus affecting primary productivity and/or cover;

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

6. Uncertainties

6.1. Exposure Assessment Uncertainties

Uncertainty in the exposure assessment stems mainly from assumption made in the assessment related to the consumption of difethialone 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. The consumption of difethialone 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 predicts 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, studies discussed earlier in this assessment have shown difethialone to have a long half life in the liver tissue (108 days).

The dose of difethialone 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 3.3.2**, 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 difethialone in terrestrial animals brings uncertainty in the conclusions of the secondary poisoning assessment. A secondary poisoning study, 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 difethialone.

Finally, avian reproduction data have not been submitted for difethialone. 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 difethialone 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. However, given the acute toxicity of difethialone to birds and mammals, and the persistence it is expected to exhibit once consumed, the use of equations that would account for carnivorous mammals and reptiles would not be expected to change the LOC exceedances.

6.2.2. Use of Surrogate Species Effects Data

While the available toxicity data provides fairly certain information on the acute toxicity of difethialone 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.

Difethialone 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 difethialone 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 difethialone to the AW, SMHM, and SJKF, and to the designated critical habitat of the 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 difethialone relative to the AW, SMHM and SJKF. Additionally, the Agency has determined use of difethialone 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 Difethialone 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**
		Risk assessment indicates use of difethialone potentially will 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 both primary and secondary exposure. Secondary exposure is considered the primary threat to 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.
		Potential for Indirect Effects
		Terrestrial prey items Risk assessment indicates use of difethialone will likely reduce the abundance of terrestrial vertebrates which serve as prey for this species. This conclusion is based on acute RQs for birds, mammals, and reptiles which exceed the LOCs. Data were not available to assess chronic

Species	Effects Determination	Basis for Determination
		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 involving small mammals have been reported in association with the use of difethialone. ***Habitat Modification*** Risk assessment indicates use of difethialone 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 raviventris*)	May Affect, Likely to Adversely Affect (LAA)	**Potential for Direct Effects** Risk assessment indicates that use of difethialone will 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 to the SMHM. Primary exposure is considered the primary threat to 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 involving small mammals have been reported in association with the use of difethialone. **Potential for Indirect Effects** ***Terrestrial Habitat*** Risk assessment indicates that the registered uses of difethialone will reduce SMHM rearing sites by adversely affecting small mammals. 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** Risk assessment indicates use of difethialone potentially will result in direct effects to the SJKF from acute toxicity via secondary exposure. Secondary exposure is considered the primary threat to 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 involving mammals have been reported in association with the use of difethialone. **Potential for Indirect Effects** ***Terrestrial prey items*** Risk assessment indicates use of difethialone will likely reduce the abundance of terrestrial vertebrates which serve as prey for this species. 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. Data were not available to assess chronic toxicity; however, since risk is predicted for acute exposure based on mortality, and

Species	Effects Determination	Basis for Determination
		sublethal effects were observed with sublethal acute exposure, risk of chronic effects is also assumed. Furthermore, incidents involving birds and mammals have been reported in association with the use of difethialone.

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 difethialone may modify the critical habitat of this species by reducing the availability of small mammal burrows. This may result in modification of PCE 3: "Lands containing rock outcrops, talus, and small mammal burrows within or adjacent to PCE 1 and or PCE 2." In addition, the availability of prey may be reduced in the critical habitat by toxicity to small birds, mammals, reptiles, and terrestrial-phase amphibians.

Table 7-3. Use Specific Summary of the Potential for Adverse Effects 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, SMHM 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 habitat modification effects determinations, it is important to note that pesticide exposures and predicted risks to the species and its resources (*i.e.*, food and habitat) are not expected to be uniform across the action area. Difethialone exposure and associated risks to the species and its resources are expected to rapidly decrease with increasing distance away from the sites of bait placement. 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 AW, SMHM, and SJKF 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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8. MRID List

71-1 Avian Single Dose Oral Toxicity

MRID	Citation Reference
40696901	Fletcher, D. (1988) 30-Day Acute Oral Toxicity Study with LM-2219 Technical in Bobwhite Quail: Project ID: 87 QD 93. Unpublished study prepared by Bio-Life Associates, Ltd. 39 p.
42065004	Fletcher, D. (1988) 30-Day Acute Oral Toxicity Study with LM-2219 Technical in Bobwhite Quail: Lab Project Number: 87 QD 93. Unpublished study prepared by Bio-Life Associates, Ltd. 42 p.
42687702	Lorgue, G. (1987) Determination of the LD50 of LM-2219 Given Orally to the Japanese Quail (Coturnix coturnix): A Supplement: Lab Project Number: 86/3. Unpublished study prepared by Lyon National Veterinary School, France. 4 p.
40268913	Lorgue, G. (1987) Determination of LD-50 of LM-2219 Given Orally to the Japanese Quail (Coturnix coturnix): Lab Project ID: 86/3. Unpublished study prepared by Ecotoxicology Lab. 37 p.

71-2 Avian Dietary Toxicity

MRID	Citation Reference
40268912	Fletcher, D. (1986) 30-day Dietary LC50 Study with LM-2219 Technical in Mallard Ducklings: [Final Report]: Lab Project ID: BLAL No. 85 DC 64. Unpublished study prepared by Bio-Life Associates, Ltd. 111 p.
40696902	Fletcher, D. (1988) 30-Day Dietary LC50 Study with LM-2219 Technical in Bobwhite Quail: Project ID: 87 QC 89. Unpublished study prepared by Bio-Life Associates, Ltd. 38 p.
42065005	Fletcher, D. (1988) 30-Day Dietary LC50 Study with LM-2219 Technical in Bobwhite Quail: Lab Project Number: 87-QC 89. Unpublished study prepared by Bio-Life Associates, Ltd. 41 p.
42687703	Fletcher, D. (1986) 30-Day Dietary LC50 Study with LM-2219 Technical in Mallard Ducklings: A Supplement: Lab Project Number: 85 DC 64. Unpublished study prepared by Bio-Life Assocs. 5 p.

72-1 Acute Toxicity to Freshwater Fish

MRID	Citation Reference
40268914	Nicholson, R.; Surprenant, D. (1986) Acute Toxicity of LM-2219 to Rainbow Trout, Bluegill, and Daphnids: Lab Project IDs: BW-86-7-2081, BW-86-7-2079, BQ-86-7-2080. Unpublished study prepared by Springborn Bionomics,

Inc. 59 p.

72-2 Acute Toxicity to Freshwater Invertebrates

MRID	Citation Reference
40268914	Nicholson, R.; Surprenant, D. (1986) Acute Toxicity of LM-2219 to Rainbow Trout, Bluegill, and Daphnids: Lab Project IDs: BW-86-7-2081, BW-86-7-2079, BQ-86-7-2080. Unpublished study prepared by Springborn Bionomics, Inc. 59 p.

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40268916	Lorgue, G. (1985) Activity and Attractiveness Studies of LM-2219 Rodenticide Compound Against Field Rodents: (Translation from Original French Report): Lab Project ID: 22-6-85. Unpublished study prepared by National Veterinarian School of Lyon. 33 p.
46656501	Savarie, P. (2005) Secondary Toxicity Hazard Assessment of Difethialone in Black-Billed Magpies (Pica pica) and European Ferrets (*Mustela putoris furo*). Project Number: QA/385. Unpublished study prepared by U.S. Dept. of Agriculture, APHIS, ADC. 194 p.
48396203	Byers, R.; Carbaugh, D. (1991) Rodenticides for the Control of Pine and Meadow Voles in Orchards (Diphacinone, Chlorophacinone, Bromadiolone, Difethialone, Zinc Phosphide, Cholecalciferol, and Oxytetracycline). Journal of Environmental Horticulture 9 (3): 167-172.

161-1 Hydrolysis

MRID	Citation Reference
40268902	Spare, W. (1986) Determination of the Hydrolysis Rate Constants of LM-2219: [Final Report]: Lab Project ID: 1403. Unpublished study prepared by Agrisearch Inc. 37 p.

161-2 Photodegradation-water

MRID	Citation Reference
42785801	Spare, W. (1987) Determination of the Solution Photolysis Rate of LM-2219 (Difethialone): Lab Project Number: 1404. Unpublished study prepared by Agrisearch Inc. 53 p.

162-1 Aerobic soil metabolism

MRID	Citation Reference

42785802	Spare, W. (1987) Aerobic Soil Metabolism of LM-2219 (Difethialone): Lab Project Number: 1401. Unpublished study prepared by Agrisearch Inc. 72 p.

163-1 Leach/adsorp/desorption

MRID	Citation Reference
42628109	Spare, W. (1992) Adsorption/Desorption of Difethialone: Amended Final Report: Lab Project Number: 1421. Unpublished study prepared by Agrisearch Inc. 98 p.