Risks of Bromadiolone Use to

the Federally Threatened Alameda Whipsnake (*Masticophis lateralis euryxanthus*),

the Federally Endangered Salt Marsh Harvest Mouse (*Reithrodontomys raviventris*), and the Federally Endangered San Joaquin Kit Fox (*Vulpes macrotis mutica*)

Pesticide Effects Determinations
PC Code: 112001
CAS Number: 28772-56-7

Environmental Fate and Effects Division
Office of Pesticide Programs
Washington, D.C. 20460

9/28/11

Primary Authors:
Robin Sternberg, Ph.D., Wildlife Biologist
Robert Miller, Environmental Protection Specialist
Alicia Korol, Biologist

Secondary Review:
Stephen Wente, Ph.D., Biologist
Ed Odenkirchen, Ph.D., Biologist

Branch Chief, Environmental Risk Assessment Branch I:
Nancy Andrews, Ph.D.

Acknowledgement

We would like to acknowledge the contribution of the Litigation Steering Committee in compiling detailed information on the species and Geographic Information System analysis used to define the potential overlap between habitat and occurrence with the areas of potential effects. Additionally, the Steering Committee has provided invaluable guidance toward achieving greater consistency in format and content between chemicals being assessed.

Table of Contents

Appendices

Appendix A.	Verification Memo for Bromadiolone
Appendix B.	Risk Quotient (RQ) Method and Levels of Concern (LOCs)
Appendix C.	Summary of Ecotoxicity Data
Appendix D.	HED Toxicity Profile
Appendix E.	Bibliography of ECOTOX Open Literature
Appendix F.	Accepted ECOTOX Open Literature Data Table (sorted by effect)
Appendix G.	ECOTOX Open Literature Reviews
Appendix H.	Summary of Bromadiolone Incidents
Appendix I.	Summary of Labels

Attachments

Attachment I.	Supplemental Information on Standard Procedures for Threatened and Endangered Species Risk Assessments on the San Francisco Bay Species
Attachment II.	Status and Life History for the San Francisco Bay Species
Attachment III.	Baseline Status and Cumulative Effects for the San Francisco Bay Species

List of Tables

List of Figures

List of Commonly Used Abbreviations and Nomenclature

µg/kg	Symbol for "micrograms per kilogram"
µg/L	Symbol for "micrograms per liter"
ºC	Symbol for "degrees Celsius"
AAPCO	Association of American Pesticide Control Officials
a.i.	Active Ingredient
AIMS	Avian Monitoring Information System
Acc#	Accession Number
amu	Atomic Mass Unit
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
ESA	Endangered Species Act
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
FI	Food Intake
FIFRA	Federal Insecticide Fungicide and Rodenticide Act
FQPA	Food Quality Protection Act
ft	Feet
GENEEC	Generic Estimated Exposure Concentration model
HPLC	High Pressure Liquid Chromatography
IC_{05}	5% Inhibition Concentration
IC_{50}	50% (or median) Inhibition Concentration
i.e.	Latin for *id est* ("that is")
IECV1.1	Individual Effect Chance Model Version 1.1
KABAM	K_{OW} (based) Aquatic BioAccumulation Model
kg	Kilogram(s)
kJ/mole	Kilojoules per mole
km	Kilometer(s)
K_{AW}	Air-water Partition Coefficient
Kd	Solid-water Distribution Coefficient
KF	Freundlich Solid-Water Distribution Coefficient
K_{OC}	Organic-carbon Partition Coefficient
K_{OW}	Octanol–water Partition Coefficient
LAA	Likely to Adversely Affect
lb a.i./A	Pound(s) of active ingredient per acre
LC_{50}	50% (or Median) Lethal Concentration
LD_{50}	50% (or Median) Lethal Dose
LOAEC	Lowest Observable Adverse Effect Concentration
LOAEL	Lowest Observable Adverse Effect Level

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

	constant, dimensionless
ppb	Parts per Billion (equivalent to µg/L or µg/kg)
ppm	Parts per Million (equivalent to mg/L or mg/kg)
PCO	Pest Control Operator
PRD	Pesticide Re-Evaluation Division
PRZM	Pesticide Root Zone Model
PUR	Pesticide Use Reporting
RMD	Risk Mitigation Decision
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), the federally endangered salt marsh harvest mouse (SMHM), and the federally endangered San Joaquin kit fox (SJKF) arising from FIFRA regulatory actions regarding use of bromadiolone 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, 2004a), and consistent with a suit in which bromadiolone 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).

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

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

1.2. Scope of Assessment

1.2.1. Uses Assessed

Bromadiolone is an anticoagulant rodenticide that is used to control Norway rats (*Rattus norvegicus*), roof rats (*R. rattus*), and house mice (*Mus musculus*) in and around buildings and in transport vehicles (ships, trains, and aircraft), alleys, and sewers. Formulation types include meal bait, pellets, ready-to-use place packs, and paraffinized blocks. All formulations contain 0.005% bromadiolone. Bait products used outdoors must be placed inside tamper-resistant bait stations.

Bromadiolone has both indoor and outdoor use sites. Indoor use sites are classified as: **indoor food** (agricultural/farm structures/buildings and equipment); **indoor non-food** (commercial/institutional/industrial premises/equipment; commercial transportation facilities; public buildings/structures; food processing plant premises - nonfood contact; ships and boats); and **indoor residential** (household/domestic dwellings). Outdoor use sites are classified as: **outdoor residential** (household/domestic dwellings); **terrestrial non-food** (agricultural/farm structures/buildings and equipment; commercial/institutional/industrial premises/equipment; nonagricultural outdoor buildings/structures; food processing plant premises – nonfood contact); **terrestrial non-food + outdoor residential** (urban areas); and **aquatic non-food industrial** (sewage systems).

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. 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 Bromadiolone

Bromadiolone (3-[3-(4'-bromo[1,1'-biphenyl]-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-2H-1-benzopyran-2-one) may be considered moderately persistent in soil ($t_{1/2}$ = 14 days) and immobile (mean K_{oc} = 36824 L/Kg) in soil high in organic matter and clay content. The major route of dissipation on uneaten bait appears to be aerobic soil metabolism. The solubility in water at 25°C is 1.21 mg/L.

1.2.3. Evaluation of Degradates and Stressors of Concern

Two major degradates were identified in the aerobic soil metabolism study: degradate #1 (1,3-diphenyl-5(4'-bromo-biphenyl) pentane-1-ol) and degradate #3 (1,3-diphenyl-5(4'-bromo-biphenyl) pentane-1,5-diol) reached 19 and 25% of the applied at 120 and 270 days, respectively. Risk from exposure to degradation products was not considered because the majority of risk is expected to be from primary exposure due to direct consumption of intact bait products or from secondary exposure due to consumption of prey that ingested bait. Contamination of soil and water from use of bait products is expected to be minimal. Therefore, formation of degradation products in soil and water was not a major concern in this assessment.

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

The only aquatic taxon relevant to this assessment is aquatic plants for indirect effects to the SMHM; however, no aquatic plant toxicity data were available. Given that bromadiolone is used in very small quantities in bait-type products, the probability that it will come into contact with water is minimal. Even when used in sewers where the potential for contact with water is possible, little bromadiolone is expected to be released from the bait into water because: the water solubility of bromadiolone is very low; bromadiolone bait products used in sewers are formulated in highly hydrophobic, weather-resistant paraffinized blocks; and the maximum application rate of active ingredient in sewers is extremely small. Therefore, concentrations of bromadiolone in both freshwater and saltwater marshes are expected to be negligible and not impact aquatic vegetation, and models that estimate concentrations in surface water or calculate spray drift deposition of bromadiolone on aquatic habitats were not needed. No surface water monitoring data were available for bromadiolone.

1.3.1.b. Terrestrial Exposures

For this assessment, it was assumed that terrestrial animals could be exposed via two different pathways. Animals may directly consume bromadiolone bait (*i.e.*, primary exposure), or animals may consume prey that ingested bromadiolone bait (*i.e.*, secondary exposure). An assessment for primary exposure was conducted for the SMHM. An assessment for primary exposure of the AW was not conducted because snakes seldom consume anything except live prey, and thus the AW would not likely consume bromadiolone bait directly. An assessment for primary exposure of the SJKF was not conducted because all outdoor uses of bromadiolone require that bait be placed in bait stations. The use of bait stations should prevent access to bait by SJKFs and thus preclude their exposure to bromadiolone via primary consumption. An assessment for secondary exposure was conducted for the AW and SJKF.

Both acute and chronic exposures were considered. Primary exposure and secondary exposure were evaluated using a dose-based approach that is dependent on body weight and food consumption rates and a dietary approach that uses only the concentration of bromadiolone in the formulated bait itself (for primary exposure) or the maximum measured bromadiolone whole body residue in animals that ingested bromadiolone (for secondary exposure). Primary exposure was modeled using baits containing 0.005% (50 mg a.i./kg-bait) bromadiolone. On a dose basis, estimated environmental concentrations (EECs) from primary exposure ranged from 2.2 to 11.4 mg a.i./kg-bw/day. On a dietary basis, a bait concentration of 50 mg a.i./kg-bait was used as the EEC for primary exposure. Secondary exposure was modeled using a bromadiolone concentration of 1.83 mg a.i./kg-carcass, which was based on available data (MRID 48590803) for whole body residues of bromadiolone detected in carcasses of animals that ingested bromadiolone. On a dose basis, EECs ranged from 0.3 to 2.8 mg a.i./kg-bw/day for secondary exposure. On a dietary basis, a carcass concentration of 1.83 mg a.i./kg-carcass was used as the EEC for secondary exposure.

1.3.2. Toxicity Assessment

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

Section 4 summarizes available bromadiolone ecotoxicity data that are pertinent to the effects determination calls for the AW, SMHM, and SJKF. Bromadiolone is moderately toxic to birds on an acute oral basis, very highly toxic to birds on a sub-acute dietary exposure basis, and very highly toxic to mammals on an acute oral and sub-acute dietary exposure basis. Chronic toxicity data for birds and mammals were not available; however, in a mammalian developmental study (MRID 92196014), pregnant rats exhibited vaginal bleeding, hypotonicity, pale eyes, and death. No bromadiolone toxicity data were available for aquatic and terrestrial plants and terrestrial invertebrates. **Appendix C** tabulates all the ecotoxicity data available for bromadiolone.

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 bromadiolone use has the potential to adversely affect the assessed species or adversely modify their designated critical habitat. When RQs for a particular type of effect are below LOCs, the pesticide is considered to have "no effect" on the species and its designated critical habitat. Where RQs exceed LOCs, a potential to cause adverse effects or habitat modification is identified, leading to a conclusion of "may affect." If bromadiolone 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, Likely to Adversely Affect determination for the SMHM, SJKF and AW. Additionally, the Agency has determined that the potential exists for modification of the designated critical habitat for the AW from the use of the bromadiolone and makes a Habitat Modification determination for the AW. A summary of the risk conclusions and effects determinations for each 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 determinations and the potential modification of designated critical habitat for the AW, a description of the baseline status and cumulative effects for all three species is provided in **Attachment III**.

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

Species	Effects Determination	Basis for Determination
Alameda Whipsnake (AW) (*Masticophis lateralis euryxanthus*)	May Affect, Likely to Adversely Affect (LAA)	**Potential for Direct Effects**
		Calculated dose- and dietary-based acute RQs for secondary exposure did not exceed Acute Risk LOCs for AWs. However, the probability of an individual AW mortality event is high (1 in 7) for the dietary-based acute RQ for secondary exposure. In addition, sublethal effects were observed with acute oral or sub-acute dietary exposure of birds (surrogates for reptiles). Data were not available to assess AW chronic toxicity via secondary exposure.
		Potential for Indirect Effects
		Terrestrial prey items Risk assessment indicates that the registered uses of bromadiolone will adversely affect the number and quality of AW mammalian prey via primary and secondary exposure. • Calculated acute and chronic RQs for primary exposure exceeded Acute and Chronic Risk LOCs for all small (rodent) mammalian weight classes considered. • The probabilities of an individual small (rodent) mammal mortality event are high (1 in 1) for acute primary exposure RQs of all weight classes considered. • Incidents involving small mammals have been reported in association with the use of bromadiolone. ***Habitat modifications*** Risk assessment indicates that the registered uses of bromadiolone will adversely affect AW habitat by reducing the availability of small mammal burrows. • Calculated acute and chronic RQs from primary exposure exceeded Acute and Chronic Risk LOCs for all small (rodent) mammalian weight classes considered. • The probabilities of an individual small (rodent) mammal mortality event are high (1 in 1) for acute primary exposure RQs of all weight classes considered. • Incidents involving small mammals have been reported in association with the use of bromadiolone.

Species	Effects Determination	Basis for Determination
Salt Marsh Harvest Mouse (SMHM) (*Reithrodontomys raviventris*)	May Affect, Likely to Adversely Affect (LAA)	**Potential for Direct Effects** Risk assessment indicates that the registered uses of bromadiolone pose direct acute and chronic risks to the SMHM via primary exposure. • Calculated acute and chronic RQs for primary exposure exceeded Acute and Chronic Risk LOCs for the SMHM. • The probabilities of an individual SMHM mortality event are high (1 in 1) for acute primary exposure RQs. • Incidents involving non-predator/non-scavenger mammals have been reported in association with the use of bromadiolone. • Applications of bromadiolone as currently registered also place the SMHM at risk from sublethal effects. **Potential for Indirect Effects** ***Habitat modifications*** Risk assessment indicates that the registered uses of bromadiolone will reduce SMHM rearing sites by adversely affecting small mammals. • Calculated acute and chronic RQs for primary exceeded Acute and Chronic Risk LOCs for all small (rodent) mammalian weight classes considered. • The probabilities of an individual small (rodent) mammal mortality event are high (1 in 1) for acute primary exposure RQs of all weight classes considered. • Incidents involving small mammals have been reported in association with the use of bromadiolone.
San Joaquin Kit Fox (SJKF) (*Vulpes macrotis mutica)*	May Affect, Likely to Adversely Affect (LAA)	**Potential for Direct Effects** Risk assessment indicates that the registered uses of bromadiolone pose direct acute and chronic risks to the SJKF via secondary exposure. • Calculated acute and chronic RQs for secondary exposure exceeded Acute and Chronic Risk LOCs for the SJKF. • The probabilities of an individual SJKF mortality event are high (1 in 1) for acute secondary exposure RQs. • Incidents involving predator/scavenger mammals have been reported in association with use of bromadiolone. • Applications of bromadiolone as currently registered also place the SJKF at risk from sublethal effects. **Potential for Indirect Effects** ***Terrestrial prey items*** Risk assessment indicates that the registered uses of bromadiolone will adversely affect the number and quality of SJKF mammalian prey via primary and secondary exposure. • Calculated acute and chronic RQs for primary and secondary exposure exceeded Acute and Chronic Risk LOCs for all mammalian weight classes considered. • The probabilities of an individual small mammal mortality event are high for acute primary exposure RQs from primary exposure; the probabilities of an individual predator/scavenger mammal mortality range from high (1 in 1) to moderate (1 in 236) for acute secondary exposure RQs. • Incidents involving mammals have been reported in association with use of bromadiolone.

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 registered uses of bromadiolone 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 via toxicity to small mammals.

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

Uses	Potential for Effects to Identified Taxa:												
	SMHM and Small Mammals[1]		SJKF and Large Mammals[2]		Small Birds[3]		Herpetofauna except AW[4]		AW[5]		Invertebrates[6]	Terrestrial Plants[7]	Aquatic Plants[8]
	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic			
Bait for rat and mouse control in and around buildings and in alleys and transport vehicles	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No	No	No
Bait for rat control in sewers	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No	No	No

[1]A yes in this column indicates a potential for direct effects to the SMHM and indirect effects to the SMHM, SJKF, and AW.
[2]A yes in this column indicates a potential for direct and indirect effects to SJKF.
[3]A yes in this column indicates a potential for indirect effects to the SMHM, SJKF, and AW.
[4]A yes in this column indicates a potential for indirect effects to the SMHM, SJKF, and AW.
[5]A yes in this column indicates the potential for direct effects to the AW.
[6]A yes in this column indicates a potential for indirect effects to the SMHM, SJKF, and AW.
[7]A yes in this column indicates a potential for indirect effects to the SMHM, SJKF, and AW.
[8]A yes in this column indicates a potential for indirect effects to the SMHM.

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

When evaluating the significance of this risk assessment's direct/indirect and adverse habitat modification effects determinations, it is important to note that pesticide exposures and predicted risks to the listed species and its resources (*i.e.*, food and habitat) are not expected to be uniform across the action area. Exposure to bromadiolone and associated risks to the species and its resources are expected to decrease rapidly 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 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, sublethal effects, and 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) and the Services' *Endangered Species Consultation Handbook* (USFWS/NMFS, 1998), and is consistent with procedures and methodology outlined in the Overview Document (USEPA, 2004a) and reviewed by the U.S. Fish and Wildlife Service and National Marine Fisheries Service (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), the federally endangered salt marsh harvest mouse (SMHM), and the federally endangered San Joaquin kit fox (SJKF) arising from FIFRA regulatory actions regarding use of bromadiolone on all sites. In addition, this assessment evaluates whether these actions can be expected to result in modification of designated critical habitat for the AW. This ecological risk assessment has been prepared consistent with a stipulated injunction in the case *Center for Biological Diversity (CBD) vs. EPA et al*. 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, 2004a). A brief overview of each species is provided below:

- **Alameda Whipsnake (AW):** The AW was listed as threatened in 1997 by the USFWS. The species occurs in the Inner Coast Ranges in Contra Costa, Alameda, San Joaquin, and Santa Clara Counties in California. The PCEs for AWs are lands containing rock outcrops, talus, and small mammal burrows adjacent to woodland or annual grasslands contiguous with scrub/shrub communities with a mosaic of open and closed canopy.
- **Salt Marsh Harvest Mouse (SMHM):** The SMHM was listed by the USFWS as an endangered species in 1970. The species is found in tidal and non-tidal salt marshes along the San Francisco, San Pablo, and Suisun Bays in California.
- **San Joaquin Kit Fox (SJKF):** The SJKF was listed as endangered in 1967 by the USFWS. The species is found in a variety of habitats in the Central Valley area of California.

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 bromadiolone 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 bromadiolone 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 their designated critical habitat within the state of California. As part of the "effects determination", one of the following three conclusions will be reached separately for each of the assessed species in the lawsuits regarding the potential use of bromadiolone in accordance with current labels:

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

Additionally, for habitat and PCEs, a "No Effect" or a "Habitat Modification" determination is made.

A description of routine procedures for evaluating risk to the San Francisco Bay Species are 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 bromadiolone in accordance with the approved product labels for California is "the action" relevant to this ecological risk assessment.

Bromadiolone is a second generation anticoagulant rodenticide. It is registered for use in bait, pellets, ready-to-use place packs, and paraffinized blocks to control Norway rats (*Rattus norvegicus*), roof rats (*R. rattus*), and house mice (*Mus musculus*) in and around buildings and in transport vehicles (ships, trains, and aircraft), alleys, and sewers. Thus, bromadiolone has both indoor and outdoor uses with outdoor uses requiring that bait products be placed in bait stations. Indoor use sites are classified as: **indoor food** (agricultural/farm structures/buildings and equipment); **indoor non-food** (commercial/institutional/industrial premises/equipment; commercial transportation facilities; public buildings/structures; food processing plant premises - nonfood contact; ships and boats); and **indoor residential** (household/domestic dwellings). Outdoor use sites are classified as: **outdoor residential** (household/domestic dwellings); **terrestrial non-food** (agricultural/farm structures/buildings and equipment; commercial/institutional/industrial premises/equipment; nonagricultural outdoor buildings/structures; food processing plant premises – nonfood contact); **terrestrial non-food + outdoor residential** (urban areas); and **aquatic non-food industrial** (sewage systems).

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

2.2.1. Evaluation of Degradates

Two major degradates were identified in the aerobic soil metabolism study: degradate #1 (1,3-diphenyl-5(4'-bromo-biphenyl) pentane-1-ol) and degradate #3 (1,3-diphenyl-5(4'-bromo-biphenyl) pentane-1,5-diol) reached 19 and 25% of the applied at 120 and 270 days, respectively. This risk assessment evaluated the risk of exposure to the parent bromadiolone alone and did not attempt to evaluate the risk posed by environmental degradation products of bromadiolone. Risk from exposure to degradation products was not considered because the majority of risk is expected to be from primary exposure due to direct consumption of intact bait products or from secondary exposure due to consumption of prey that ingested bait. Contamination of soil and water from use of bait products is expected to be minimal. Therefore, formation of degradation products in soil and water was not a major concern in this assessment.

2.2.2. Evaluation of Mixtures

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

Bromadiolone does not have registered products that contain multiple active ingredients.

2.3. Previous Assessments

Two ecological risk assessments have been conducted by the Agency on bromadiolone since it was first registered in 1980. In addition, a Biological Opinion (BO) has been issued by the U.S. Fish and Wildlife Service (USFWS) on several listed species, and the Agency has issued a Risk Mitigation Decision for Ten Rodenticides. These assessments are described below.

The USFWS addressed the risk of bromadiolone use on endangered species in a BO issued in March of 1993 (USFWS, 1993). The USFWS produced the BO in response to a 1991 request by the EPA for formal consultation on 16 registered vertebrate control agents. Specific labels and application rates evaluated in the BO were not specified. The BO included an evaluation of the use of bromadiolone for control of Norway rats, roof rats, and house mice in urban areas in and around the periphery of homes, industrial, commercial and public buildings, alleys, and cargo areas of ships, trains, and aircraft.

The BO concluded that normal use of bromadiolone would not likely harm aquatic fauna because its formulations, application methods, and relative water insolubility should limit its susceptibility to runoff, leaching, or drift and thus preclude aquatic exposure. For terrestrial

animals, the BO discussed concerns for primary and secondary poisoning of listed scavengers and predators. The FWS issued a jeopardy call for species listed in **Table 2-1**. Furthermore, the BO contained the following Reasonable and Prudent Alternatives (RPAs):

- Prohibit use of bromadiolone within 100 yards of occupied habitat of the Choctawhatchee beach mouse, Alabama beach mouse, and Perdido Key beach mouse.
- Prohibit use of bromadiolone within 100 yards of occupied habitat of the Anastasia Island beach mouse and the Southeastern beach mouse.
- Prohibit the use of bromadiolone within 100 yards of the occupied habitat of the Morro Bay kangaroo rat.
- Prohibit outdoor bromadiolone use within 100 yards of all habitats known to be occupied by the SMHM.

The BO also contained the following Reasonable and Prudent Measure (RPM) for bromadiolone: to minimize anticipated incidental take, EPA must establish a monitoring enforcement program. The terms and conditions of such a program were outlined in the BO. Further detail on individual species is provided in the BO (http://www.fws.gov/sacramento/es/programmatic_consultations.htm).

Table 2-1. Jeopardy Calls for Species Evaluated in the 1993 FWS Biological Opinion on Bromadiolone Use

Species	N/NJ[1]	Species	N/NJ[1]
Alabama beach mouse (*Peromyscus polionotus ammobates*)	J	Point Arena mountain beaver (*Aplodontia rufa nigra*)	NJ
Anastasia Island beach mouse (*Peromyscus polionotus phasma*)	J	Salt marsh harvest mouse (*Reithrodontomys raviventris*)	J
Choctawhatchee beach mouse (*Peromyscus polionotus allophyrs*)	J	San Joaquin kit fox (*Vulpes macrotis mutica*)	NJ
Fresno kangaroo rat (*Dipodomys nitratoides exilis*)	NJ	Southeastern beach mouse (*Peromyscus polionotus niveiventris*)	J
Morro Bay kangaroo rat (*Dipodomys heermanni morroensis*)	J	Stephen's kangaroo rat (*Dipodomys stephensi*)	NJ
Perdido Key beach mouse (*Peromyscus polionotus trissyllepsis*)	J	Tipton kangaroo rat (*Dipodomys nitratoides nitratoides*)	NJ

[1]J= Jeopardy ; NJ = No Jeopardy

The Reregistration Eligibility Decision (RED) for bromadiolone was published in 1998 as part of the rodenticide cluster (USEPA, 1998b). The RED found that there was a high risk of primary and secondary poisoning in birds and mammals. Toxicity to aquatic organisms ranged from moderate to very high, but aquatic exposure was expected to be minimal. No ecological mitigations were immediately effectuated for bromadiolone, and no changes to bromadiolone labeled uses occurred as a result of the RED; however, the RED did contain mitigation measures for all rodenticides. These measures were later modified after further analysis within OPP and collaboration with a stakeholder group (see RMD for Ten Rodenticides below).

Another OPP risk assessment, titled "Potential Risks of Nine Rodenticides to Birds and Non-target Mammals: a Comparative Approach" (USEPA, 2004b), evaluated primary and secondary

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

In 2008, the Agency issued a Risk Mitigation Decision (RMD) for Ten Rodenticides (USEPA, 2008). The Decision issued the final reregistration eligibility status of rodenticide products containing ten different active ingredients (first- and second-generation anticoagulants and non-anticoagulants), including bromadiolone. The Decision also reflected the Agency's final action in response to the remand order in *West Harlem Environmental Action and Natural Resources Defense Council v. U.S. Environmental Protection Agency*, 380 F.Supp.2d 289 (S.D.N.Y. 2005). The 2008 RMD considered only commensal uses in and around buildings and underground manual application to pocket gopher and mole burrows. The two major mitigation components of the RMD were to minimize children's exposure to rodenticide products used in homes and to reduce wildlife exposure and ecological risks of these products. Mitigations measures relevant to this assessment include the following conditions of registration for second-generation anticoagulant rodenticides:

For Agricultural Use

(1) "consumer size" rodenticide bait products may not contain second-generation anticoagulants ("consumer size" bait products are defined as containing ≤ 1 lb of bait);
(2) products must contain ≥ 8 lbs of bait;
(3) products intended for professional applicators shall be sold in products containing ≥ 16 lbs bait;

(4) labels must state that products may only be used in and around, *i.e.* within 50 feet of, agricultural buildings and must bear the statement, "Do not use this product in homes or other human residences;"
(5) product labels must require use of bait stations for all outdoor, above-ground placements, however, bait need not be *sold* in bait stations;
(6) below ground uses are excluded from product requirements for product packaging within bait stations;
(7) any form of bait is acceptable, such as meal, pelleted, and block forms;
(8) registrants must agree to the terms of registration specifying that the registrants will control distribution of the products so that they are distributed or sold only to agricultural, farm, and tractor stores or directly to Pest Control Operators (PCO) and the products are not sold or distributed to hardware and home improvement stores, grocery stores, convenience stores, drug stores, club stores, big box stores, and other general retailers.

For Professional Applicator Use

(1) products must contain ≥ 16 lbs of bait;
(2) product labels must require use of bait stations for all outdoor, above-ground placements, however, bait need not be *sold* in bait stations;
(3) product labels must state, "Do not apply further than 50 feet from buildings."
(4) bait stations used in residential and institutional settings must meet the standards outlined in the RMD for the ability to isolate bait from children;
(5) any form of bait except liquid is acceptable (*e.g.*, paste formulations are acceptable);
(6) registrants must agree to the terms of registration specifying that the registrants will control distribution of the products so that they are distributed or sold only to agricultural, farm, and tractor stores or directly to Pest Control Operators (PCO) and the products are not sold or distributed to hardware and home improvement stores, grocery stores, convenience stores, drug stores, club stores, big box stores, and other general retailers.

The EPA requires all outdoor, above-ground placements of bait products containing second generation anticoagulants be contained in bait stations, in order to deny non-target animals ready access to rodenticide bait. Most baits 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 would be 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 (USEPA, 2008)

At the writing of this risk assessment, all active bromadiolone labels are compliant with the risk mitigations for second-generation anticoagulants as described in the 2008 RMD.

2.4. Environmental Fate Characterization

2.4.1. Environmental Fate Characterization

Bromadiolone (3-[3-(4'-bromo[1,1'-biphenyl]-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-2H-1-benzopyran-2-one) (**Figure 2-1**) may be considered moderately persistent in soil ($t_{1/2}$ = 14 days) and immobile in soil high in organic matter and clay. Two major degradates identified as #1 and #3 in the aerobic soil metabolism study are persistent, but there is no submitted information on mobility and toxicity. Aged and un-aged column leaching studies showed no movement of radioactive bromadiolone; 97% of radioactivity remained in the top one inch. The major route of dissipation appears to be consumption of bait by target organisms and aerobic soil metabolism for uneaten bait. **Table 2-2** lists the physical-chemical properties of bromadiolone.

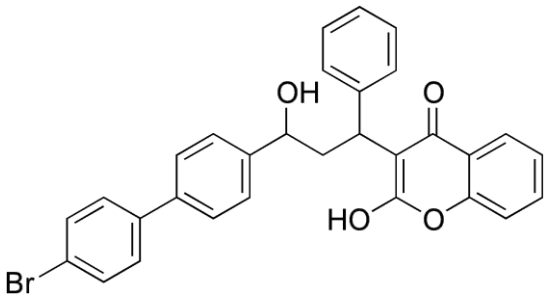

Figure 2-1. Bromadiolone Chemical Structure

Table 2-2. Physical-chemical Properties of Bromadiolone

Physical/Chemical Property	Value (unit)	Source
CAS Number	28772-56-7	
IUAPC	3-[3-(4'-bromo[1,1'-biphenyl]-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-2H-1-benzopyran-2-one	
Molecular Formula	$C_{30}H_{23}Br0_4$	
Molecular Weight	527.4 g/mole	
Physical State	Yellowish Powder	
Melting Point	196-210 ° C	MRID 46933604
Vapor Pressure (25°C)	10^{-7} torr @ 25°	MRID 46933604
Water Solubility (25°C)	1.21 mg/L	MRID 46933604

Table 2-3 lists other environmental fate properties of bromadiolone along with the major and minor degradates detected in the submitted environmental fate and transport studies.

Table 2-3. Environmental Fate Properties of Bromadiolone

Study	Value (units)	Major Degradates	MRID #
Hydrolysis	Stable at pH 5, pH 7, pH 9	None	42237501
Aerobic Soil Metabolism	$T_{1/2}$ = 14 days	Degradate #1 peaked at 19% on day 120 Degradate #3 peaked at 24% on day 270	43594301
Adsorption/ Desorption[1] K_{oc}	18824 to 59191 L/kg	None identified	43000702

Study	Value (units)	Major Degradates	MRID #
Leaching	Immobile in sandy loam and loam. Somewhat mobile in sand and clay. In all soil columns > 85% of the applied in the top 3 inches. Bromadiolone isomers >99% of recovered radio activity	None identified	43000702
Bioaccumulation	Bioaccumulation concentration factors (BCFs) of 460X, 160X, and 1658X were obtained for whole fish, edible, and non-edible tissues in bluegill sunfish	None identified	0161965

[1]Values are derived from leaching study. No desorption value. .

2.4.1.a. Degradation (Hydrolysis)

Bromadiolone parent is stable to hydrolysis at pH 5, 7, and 9 (MRID 42237501).

2.4.1.b. Aerobic Soil Metabolism

The half-life of the parent is 14 days. Two major degradates, #1 [1,3-diphenyl-5(4'-bromo-biphenyl) pentane-1-ol] and #3 [1,3-diphenyl-5(4'-bromo-biphenyl) pentane-1,5-diol], reached 19 and 25% of the applied at 120 and 270 days, respectively. The major route of dissipation for uneaten bait is aerobic soil metabolism. The data requirement for aerobic soil metabolism is satisfied (MRID 43594301).

2.4.1.c. Mobility (Leaching/Adsorption/Desorption)

Bromadiolone is immobile in sandy loam and loam soils, and somewhat mobile in sand and clay soils. In all soil columns, >85% of the applied radioactivity remained in the upper 3 inches of the soil and ≤0.8% leached below a depth of 4 inches; the column leachates contained a maximum of 0.3% radioactivity. Bromadiolone tends to adsorb strongly to soil (K_{OC} = 17,979-58,857 L/kg) and is expected to be hardly mobile according to FAO mobility classifications (FAO, 2000. Bromadiolone isomers comprised >99% of the radioactivity recovered from the soils following leaching (MRID 43000702). The mobility and toxicity are unknown for degradates #1 and # 3 identified in aerobic soil metabolism study.

2.4.1.d. Accumulation

Bioaccumulation concentration factors (BCFs) of 460X, 160X, and 1658X were obtained for the whole fish, edible, and non-edible tissues in bluegill sunfish, respectively. The BCF for the non-edible portion was 11.3X higher than the edible portion and 3.2X higher than the value obtained for the whole fish. Twenty-four percent, 35.8% and 16% of bromadiolone residues were retained in whole, edible tissues, and non-edible tissues, respectively, after 14 days of depuration (MRID 161965).

2.4.1.e. Degradates of Concern

Two major degradates were identified in the aerobic soil metabolism study: degradate # 1 (1,3-diphenyl-5(4' –bromo-biphenyl) pentane-1-ol) and degradate #3 (1,3-diphenyl-5(4' –bromo-biphenyl) pentane-1,5-diol) (MRID 43594301; **Figure 2-2**). The two major degradates are persistent, and their mobility and toxicity are unknown. Risk from exposure to degradation products was not considered because the majority of risk is expected to be from primary exposure due to direct consumption of intact bait products or from secondary exposure due to consumption of prey that ingested bait. Contamination of soil and water from use of bait products is expected to be minimal. Therefore, formation of degradation products in soil and water was not identified as a major concern in this assessment.

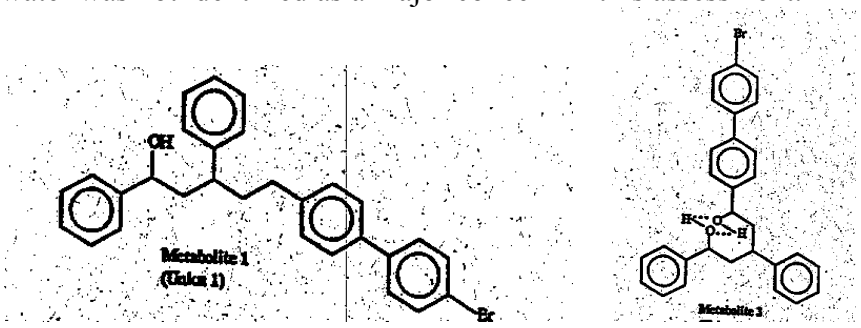

Figure 2-2. Chemical Structures for Degradates #1 and #3.

2.4.2. 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 bromadiolone is in bait for rodent control, no potential for runoff and spray drift exists. Exposure from volatilization (vapor pressure = 10^{-7}) is expected to be minimal. Bromadiolone bait used outdoors must be placed in bait stations. Therefore, the potential for contaminating surface water via leaching is assumed to be insignificant. Furthermore, since the active ingredient is often placed highly hydrophobic, weather-resistant paraffin blocks, volatilization from the bait is also expected to be insignificant.

Another possible route of transport is within the bodies of animals that ingest bromadiolone bait. Because poisoned animals do not die immediately, they may be able to travel some distance before death, thereby potentially exposing animals some distance away from the use site. This transport within animals is an important route of exposure for the AW and SJKF since their diets include small mammals, and thus they are vulnerable to secondary exposure from consuming rodents that ingested bromadiolone bait.

2.4.3. Mechanism of Action

Bromadiolone is an anticoagulant rodenticide that acts via antagonizing vitamin-K to 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). Bromadiolone is a second-generation anticoagulant and as such tends to be more acutely toxic than first-generation anticoagulants,

accumulates in livers of poisoned organisms, and is retained much longer in body tissues of primary consumers. Bromadiolone generally provides a lethal dose after a single feeding, although death is usually delayed 5 to 10 days during which the animal continues to feed.

2.4.4. Use Characterization

Analysis of labeled use information is the critical first step in evaluating the federal action. The current labels for bromadiolone 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, bromadiolone is registered for use in meal bait, pellets, ready-to-use place packs, and paraffinized blocks to control three commensal rodents: the Norway rat (*Rattus norvegicus*), the roof rat (*Rattus rattus*), and the house mouse (*Mus musculus*). Baits containing bromadiolone are registered for use in and around buildings and in alleys, transport vehicles, and sewers. All baits are to be placed not farther than 50 feet from buildings. Thus, bromadiolone has both indoor and outdoor uses with outdoor uses requiring that bait be placed in bait stations. Indoor use sites are classified as: **indoor food**, **indoor non-food**, and **indoor residential**. Outdoor use sites are classified as: **outdoor residential**, **terrestrial non-food**, **terrestrial non-food + outdoor residential** (urban areas), and **aquatic non-food industrial** (sewage systems).

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. 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. In California, all three of these commensal rodent species co-occur with the AW, SMHM, and SJKF. Therefore, all registered products of bromadiolone could be used in the area inhabited by the assessed species.

Labels of bromadiolone products 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 minimal time interval between applications. Labels generally state the number of bait stations, bait blocks, or bait packages that may be placed in one location as well as the linear interval between placements. The linear interval is generally 15 to 30 feet for rats and 8 to 12 feet for mice. The concentration of bromadiolone in bait is 0.005% for all rodent-control products. Label information for applications considered in this assessment is summarized in **Appendix I**.

The amount of active ingredient per placement or the amount of active ingredient per linear foot can be calculated for bromadiolone products. The maximum amount of active ingredient per placement for any product is 2.27 mg for rats and 0.28 mg for mice. The maximum amount of active ingredient per linear foot is 0.15 mg/ft for controlling rats and 0.04 mg/ft for controlling mice. The maximum amount of active ingredient per placement (*i.e.*, manhole) for any product

used in sewage systems is 13.61 mg. Use information for applications considered in this assessment is summarized in **Table 2-4.**

Table 2-4. Bromadiolone Uses Assessed for California

Use (Application Method)	Formulation	% a.i.	mg a.i./kg	Maximum Application Rate per Bait Placement (mg a.i./placement)	Bait Placement Interval
Bait for rat and mouse control in and around buildings and in alleys and transport vehicles (bait stations)	Meal bait, pellets, ready to use place packs, and paraffin blocks	0.005	50	2.27 (rats) 0.28 (mice)	15-30 ft (rats) 8-12 ft (mice)
Bait for rat control in sewers (hanging from manhole)	Paraffin blocks	0.005	50	13.61	Per manhole

2.4.1. Reported Usage Data

The Agency's Biological and Economic Analysis Division (BEAD) provides an analysis of both national- and county-level usage information using state-level usage data obtained from USDA-NASS[1], Doane (www.doane.com; the full dataset is not provided due to its proprietary nature) and the California's Department of Pesticide Regulation Pesticide Use Reporting (CDPR PUR) database[2]. CDPR PUR is considered a more comprehensive source of usage data than USDA-NASS or EPA proprietary databases, and thus the usage data reported for bromadiolone by county in this California-specific assessment were generated using CDPR PUR data. It should be noted that the CDPR PUR database does not include data on homeowner uses. Eleven years (1999-2009) of usage data were included in this analysis. Data from CDPR PUR were obtained for every agricultural pesticide application made on every use site at the section level (approximately one square mile) of the public land survey system[3]. BEAD summarized these data to the county level by site, pesticide, and unit 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 average and maximum application rate across all 11 years.

CDPR PUR data from 1999 to 2009 show that bromadiolone was used in all of the counties in California where the AW, SMHM, or SJKF may occur (**Table 2-5**). The counties of Solano, Santa Clara, and Alameda had the three highest average pounds applied per county with 1.13, 0.81, and 0.72 lbs. a.i., respectively. Average annual and maximum application rates and

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

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

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

average annual area treated were not summarized below because these values were reported in various units of area (*e.g.*, acres, square feet) or not reported at all (*e.g.*, "miscellaneous"). A summary of bromadiolone usage for California counties is provided below in **Table 2-5.**

Table 2-5. Summary of California Department of Pesticide Registration (CDPR) Pesticide Use Reporting (PUR) Data from 1999 to 2009[1]

County	Average Annual Pounds Applied	County	Average Annual Pounds Applied
Alameda	0.72	San Benito	0.07
Contra Costa	0.43	San Joaquin	0.37
Fresno	0.71	San Luis Obispo	0.11
Kern	0.28	San Mateo	0.47
Kings	0.50	Santa Barbara	0.53
Madera	0.04	Santa Clara	0.81
Marin	0.34	Solano	1.13
Merced	0.16	Sonoma	0.26
Monterey	0.47	Stanislaus	0.45
Napa	0.14	Tulare	0.11

[1]Based on data supplied by BEAD

[2]Highlighted cells indicate the highest average county usage in all counties in California where the AW, SMHM, or SJKF may occur.

2.5. Assessed Species

Table 2-6 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 2-3 to 2-5** for a map of the current range and designated critical habitat, if applicable, of the assessed listed species. A brief overview of each species is provided below:

- **Alameda Whipsnake (AW):** The AW was listed as threatened in 1997 by the USFWS. The species occurs in the Inner Coast Ranges in Contra Costa, Alameda, San Joaquin, and Santa Clara Counties in California.
- **Salt Marsh Harvest Mouse (SMHM):** The SMHM was listed by the USFWS as an endangered species in 1970. The species is found in tidal and non-tidal salt marshes along the San Francisco, San Pablo, and Suisun Bays in California.
- **San Joaquin Kit Fox (SJKF):** The SJKF was listed as endangered in 1967 by the USFWS. The species is found in a variety of habitats in the Central Valley area of California.

Table 2-6. Summary of Current Distribution, Habitat Requirements, and Life History Information for the Assessed Listed Species[1]

Assessed Species	Size	Current Range	Habitat Type	Designated Critical Habitat?	Reproductive Cycle	Diet
Salt Marsh Harvest Mouse (SMHM) (*Reithrodontomys raviventris*)	Adult 8 – 14 g	Northern subspecies can be found in Marin, Sonoma, Napa, Solano, and northern Contra Costa counties. The southern subspecies occurs in San Mateo, Alameda, and Santa Clara counties with some isolation populations in Marin and Contra Costa counties.	Dense, perennial cover with preference for habitat in the middle and upper parts of the marsh dominated by pickleweed and peripheral halophytes as well as similar vegetation in diked wetlands adjacent to the Bay	No	Breeding: March – November Gestation period: 21 – 24 days	Leaves, seeds, and plant stems; may eat insects; prefers "fresh green grasses" in the winter and pickleweed and saltgrass during the rest of the year; drinks both salt and fresh water
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	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	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	Small animals including blacktailed hares, desert cottontails, mice, kangaroo rats, squirrels, birds, lizards, insects and grass. It satisfies its moisture

Assessed Species	Size	Current Range	Habitat Type	Designated Critical Habitat?	Reproductive Cycle	Diet
		counties	annual grasslands. Kit foxes dig their own dens, modify and use those already constructed by other animals (ground squirrels, badgers, and coyotes), or use human-made structures (culverts, abandoned pipelines, or banks in sumps or roadbeds). They move to new dens within their home range often (likely to avoid predation by coyotes)		dens at about 1-month of age and may begin to disperse after 4 – 5 months usually in Aug. or Sept.	requirements from prey and does not depend on freshwater sources.
CA Alameda Whipsnake (AW) (*Masticophis lateralis euryxanthus*)	3 – 5 ft	Contra Costa and Alameda Counties in California (additional occurrences in San Joaquin and Santa Clara Counties)	Primarily, scrub and chaparral communities. Also found in grassland, oak savanna, oak-bay woodland, and riparian areas. Lands containing rock outcrops, talus, and small mammal burrows.	Yes	Emerge from hibernation and begin mating from late March through mid-June. Females lay eggs in May through July. Eggs hatch from August through November. Hibernate during the winter months.	Lizards, small mammals, nesting birds, other snakes including rattlesnakes, invertebrates

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

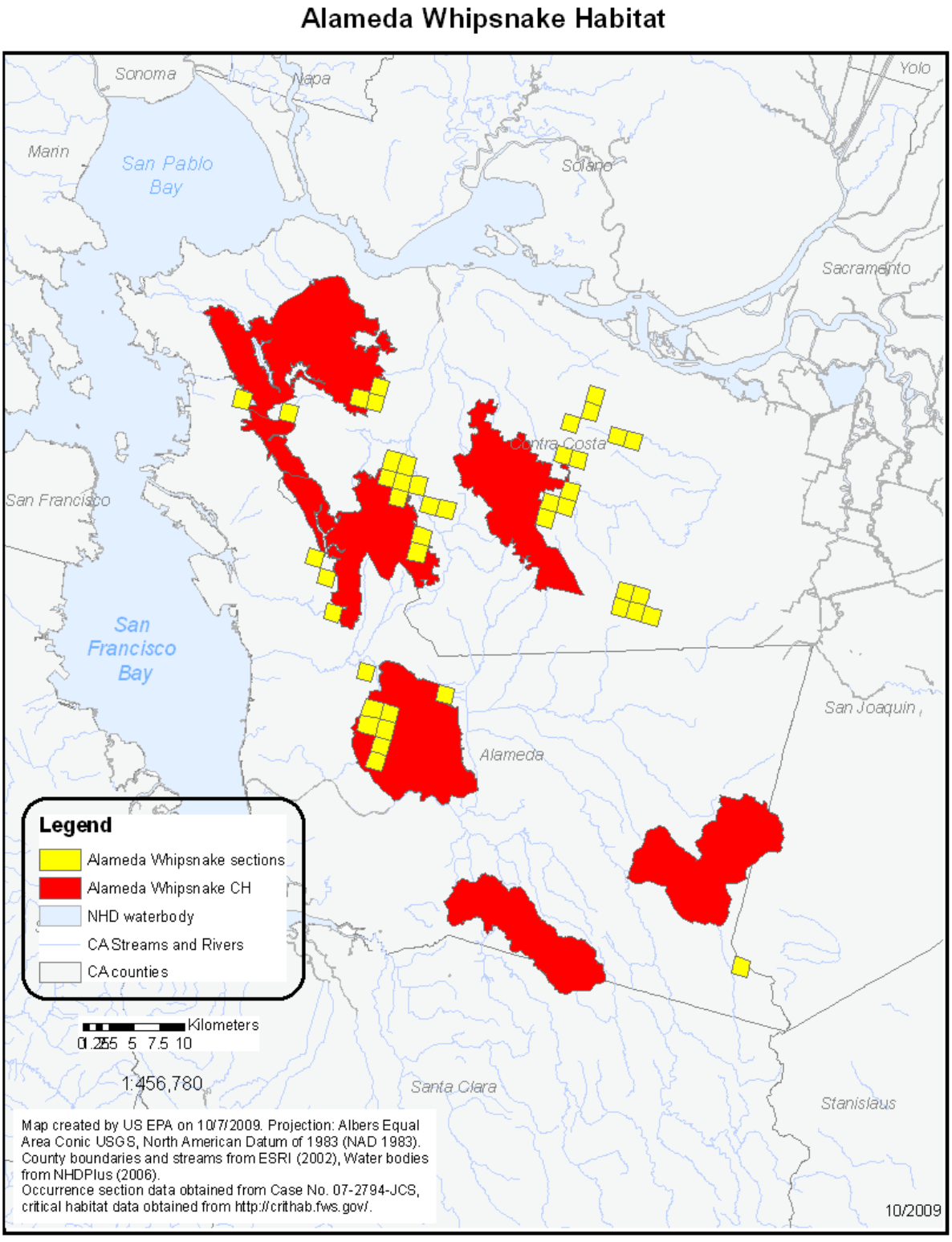

Figure 2-3. Critical habitat (CH) and occurrence sections of the Alameda Whipsnake, as identified in Case No. 07-2794-JCS

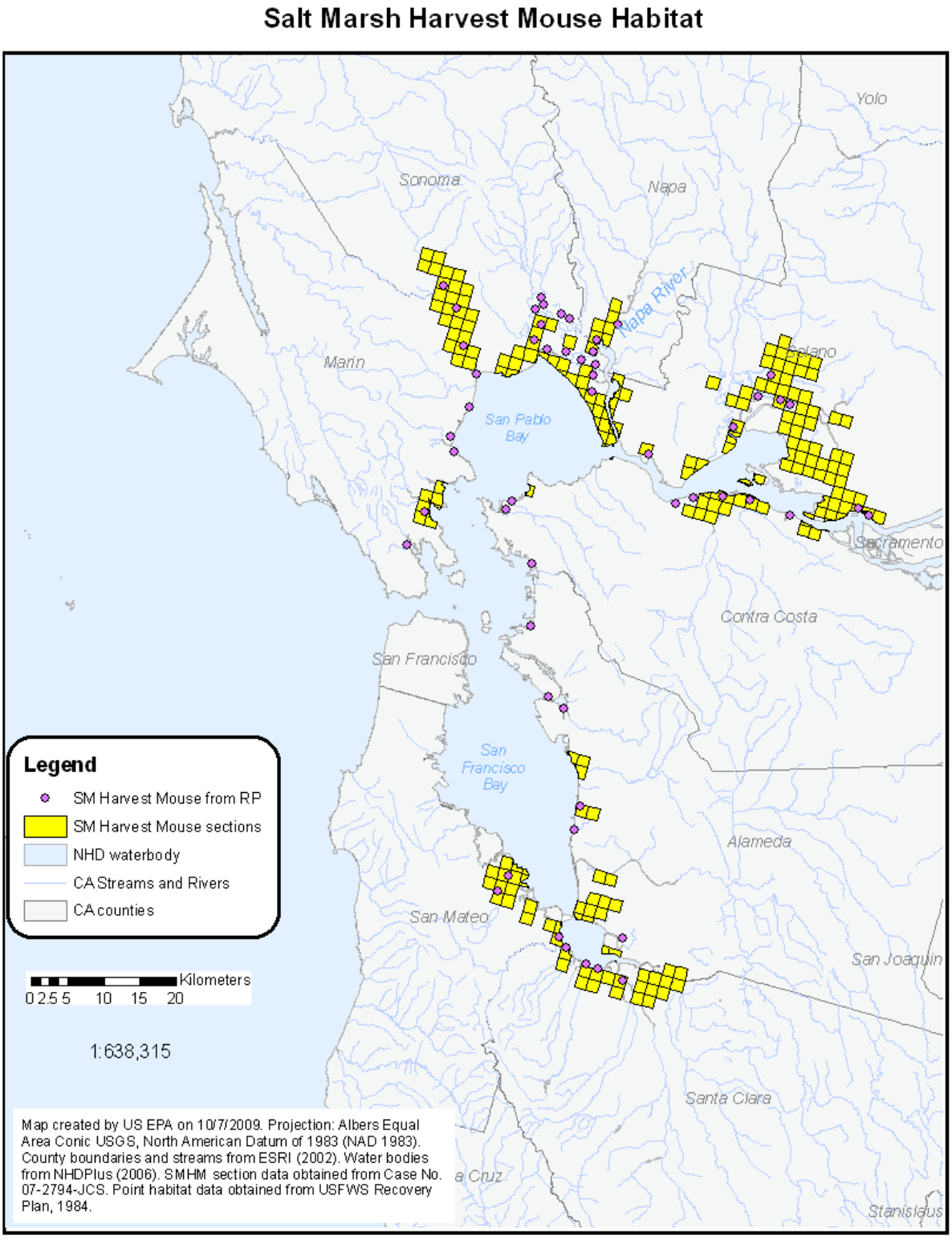

Figure 2-4. Occurrences and occurrence sections of the Salt Marsh Harvest Mouse, as identified in Case No. 07-2794-JCS

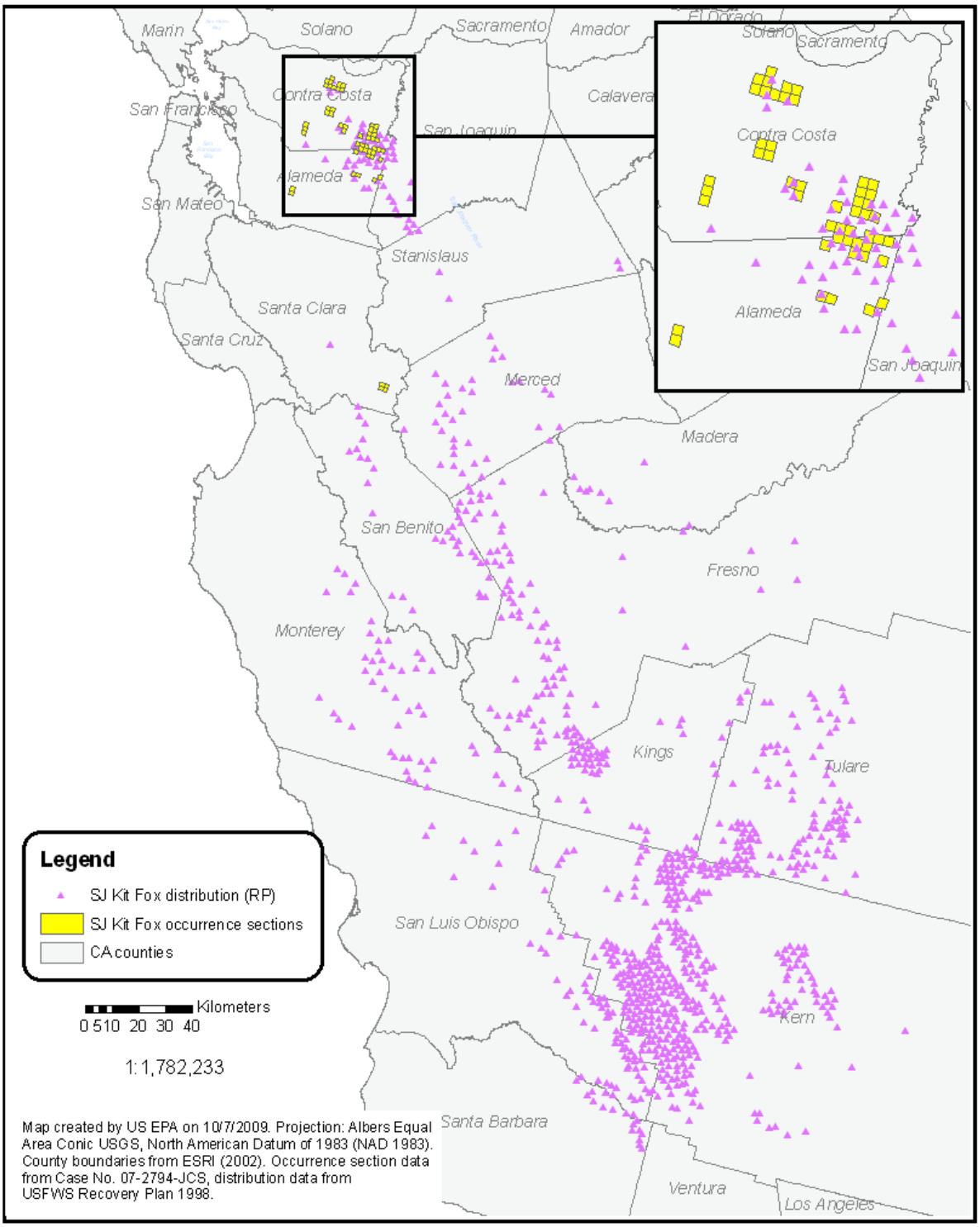

Figure 2-5. Occurrences and occurrence sections of the San Joaquin Kit Fox, as identified in Case No. 07-2794-JCS

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[1]

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

[1]These PCEs are in addition to more general requirements for habitat areas that provide essential life cycle needs of the species such as, space for individual and population growth and for normal behavior; food, water, air, light, minerals, or other nutritional or physiological requirements; cover or shelter; sites for breeding, reproduction, rearing (or development) of offspring; and habitats that are protected from disturbance or are representative of the historic geographical and ecological distributions of a species.
[2]PCEs that are abiotic, including, physical-chemical water quality parameters such as salinity, pH, and hardness are not evaluated.

More detail on the designated critical habitat applicable to this assessment can be found in **Attachment II**. Activities that may destroy or adversely modify critical habitat are those that alter the PCEs and jeopardize the continued existence of the species. Evaluation of actions related to use of bromadiolone 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 bromadiolone is expected to directly impact living organisms within the action area, critical habitat analysis for bromadiolone 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 bromadiolone 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 the 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 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 the AW and designated critical habitat of the AW 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. In the case of this assessment, however, the area of potential use of bromadiolone is not restricted spatially. Considering the use pattern of rodent control baits, bromadiolone potentially could be used in any terrestrial land use type. Thus, any area of the state of California is considered an area of potential use of bromadiolone bait, and thus the assessed species potentially could be exposed to bromadiolone wherever they occur. Based on CDPR Pesticide Usage Reporting data, bromadiolone has been used within the years 1999-2009 in all 20 of the

California counties in which occurrences or occurrence sections were identified for the SMHM, AW, or SJKF in Case No. 07-2794-JCS.

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 bromadiolone for each listed species assessed here. Birds that provide rearing sites to the SMHM (*e.g.*, passerine species) or that serve as prey to the AW and SJKF are not likely to be at risk from bromadiolone use because bromadiolone bait used outdoors must be placed in bait stations. Therefore, available avian toxicity data was only used as a surrogate for assessing direct effects to the AW. Although indirect effects to the AW, SMHM, and SJKF mediated through terrestrial invertebrates or herpetofauna are possible, they were assumed to have negligible contributions to overall risk to these species. Indirect effects to the AW, SMHM, and SJKF mediated through plants were also assumed to have negligible contributions to overall risk to these species. 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	Mammals	Birds
Salt Marsh Harvest Mouse	Direct Indirect (rearing sites)	Incomplete exposure pathway
San Joaquin Kit Fox	Direct Indirect (prey)	Incomplete exposure pathway
Alameda Whipsnake	Indirect (prey, habitat)	Direct

Table 2-9. Taxa and Assessment Endpoints Used to Evaluate the Potential for Use of Bromadiolone 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	a. Most sensitive bird* or terrestrial-phase reptile acute LC_{50} or LD_{50} (guideline or ECOTOX) b. Most sensitive bird* or terrestrial-phase reptile chronic NOAEC (guideline or ECOTOX)

Taxa Used to Assess Direct and Indirect Effects to Assessed Species and/or Modification to Critical Habitat or Habitat	Assessed Listed Species	Assessment Endpoints	Measures of Ecological Effects
Mammals	Direct Effect -Salt Marsh Harvest Mouse -San Joaquin Kit Fox	Survival, growth, and reproduction of individuals via direct effects	a. Most sensitive laboratory mammalian acute LC_{50} or LD_{50} (guideline or ECOTOX) b. Most sensitive laboratory mammalian chronic NOAEL (guideline or ECOTOX)
	Indirect Effect (prey/habitat from burrows/rearing sites) -Salt Marsh Harvest Mouse -San Joaquin Kit Fox - Alameda Whipsnake	Survival, growth, and reproduction of individuals or modification of critical habitat/habitat via indirect effects on terrestrial prey (mammals) and/or burrows/rearing sites	

*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 bromadiolone 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 bromadiolone 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 bromadiolone to the environment. The following risk hypotheses are presumed in this assessment.

The labeled use of bromadiolone within the action area may:

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

2.9.2. Diagram

The conceptual model is a graphic representation of the structure of the risk assessment. It specifies bromadiolone release mechanisms, biological receptor types, and effects endpoints of potential concern. The conceptual model for the possible effects of bromadiolone on the AW, SMHM, and SJKF is shown in **Figure 2-6**. Typically a separate diagram is created for terrestrial and aquatic exposure and effects. However, for this assessment, only a single diagram of terrestrial exposure and effects is depicted because use of bromadiolone bait is not expected to result in any significant exposure or effects to the assessed species through aquatic pathways. Although the conceptual models for direct/indirect effects and modification of designated critical habitat PCEs are shown on the same diagrams, the potential for direct/indirect effects and modification of PCEs will be evaluated separately in this assessment. Exposure routes shown in dashed lines are not quantitatively considered because the contribution of those potential exposure routes to potential risks to the AW, SMHM, and SJKF and modification to designated critical habitat for the AW are expected to be negligible.

As shown in the diagram, we consider exposure through direct consumption of intact bait (*i.e.*, primary exposure) and consumption of terrestrial mammals that have ingested intact bait (*i.e.*, secondary exposure) to be the main routes of exposure to the AW, SMHM, and SJKF. Therefore, the quantitative risk assessment focused on these routes of exposure. Indirect effects to these species mediated through birds, terrestrial invertebrates and herpetofauna are possible but were assumed to have negligible contributions to overall risk to these species. Exposure and indirect effects to these species mediated through plants were also assumed to have negligible contributions to overall risk to these species. These presumed negligible exposure routes and indirect effects include:

- Consumption of birds that have ingested bromadiolone bait.
- Consumption of herpetofauna that have ingested bromadiolone bait.
- Consumption of terrestrial invertebrates that have ingested bromadiolone bait.
- Indirect food chain effects resulting from bromadiolone reducing the abundance of plants, terrestrial invertebrates, birds, and herpetofauna.

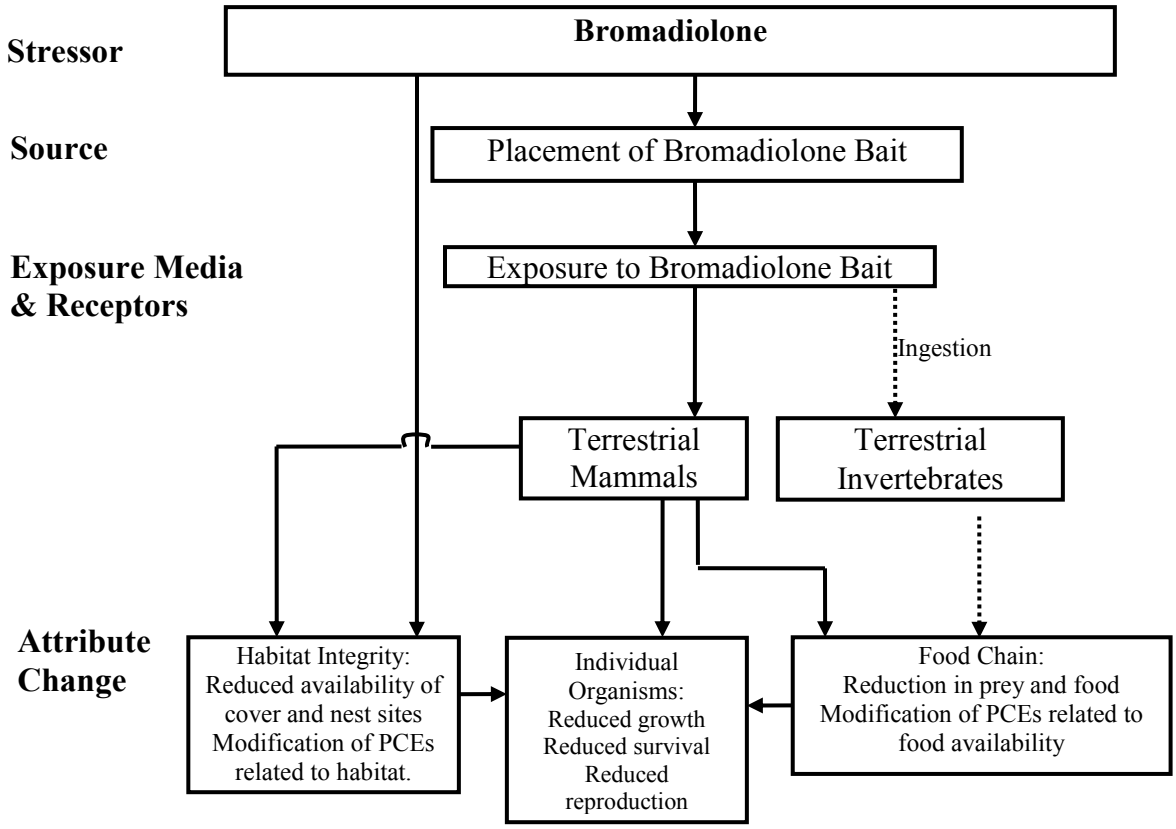

Figure 2-6. Conceptual Model Depicting Stressors, Exposure Pathways, and Potential Effects to Terrestrial Organisms from the Use of Bromadiolone
Dotted lines indicate exposure pathways that have a low likelihood of contributing to ecological risk.

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 bromadiolone are characterized and integrated to assess the risks. This is accomplished using a risk quotient (ratio of exposure concentration to effects concentration) approach. Although ecological risk is often defined as the likelihood and magnitude of adverse ecological effects, the risk quotient-based approach does not provide a quantitative estimate of likelihood and/or magnitude of an adverse effect. However, as outlined in the Overview Document (USEPA, 2004a), the likelihood of effects to individual organisms from particular uses of bromadiolone 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

Given that bromadiolone is used only in bait for vertebrate pest control; outdoor uses of bromadiolone are limited to in and around buildings and in alleys, transport vehicles, and sewers; and bromadiolone bait used outdoors must be placed in bait stations and not farther than 50 feet from buildings:

- Exposure of aquatic plants, terrestrial invertebrates, terrestrial plants, birds that provide rearing sites to the SMHM (*e.g.*, passerine species) or that serve as prey to the AW and SJKF, and herpetofauna to bromadiolone is expected to be minimal, and
- Exposure of terrestrial animals to bromadiolone is expected to occur via either direct consumption of bromadiolone bait (*i.e.*, primary exposure) or consumption of another animal that directly ingested bromadiolone bait (*i.e.*, secondary exposure).

2.10.1.a. Estimating Exposure in the Aquatic Environment

Since bromadiolone is used only in bait that is placed by hand, there is no potential for bromadiolone to be transported by drift. Furthermore, much of the bromadiolone bait used for rodent control is placed indoors or outdoors within plastic bait stations. These uses pose minimal potential for transport to surface or ground water and make leaching unlikely.

Even when used in sewers where the potential for contact with water is possible, little bromadiolone is expected to be released from the bait into water because: the water solubility of bromadiolone is very low (1.21 mg/L); bromadiolone bait products used in sewers are formulated in highly hydrophobic, weather-resistant paraffinized blocks; and the maximum application rate of active ingredient in sewers is extremely small (13.61 mg a.i. per manhole).

The only aquatic taxon relevant to this assessment is aquatic plants for indirect effects to the SMHM. Concentrations of bromadiolone in saltwater marshes are also expected to be negligible and not impact aquatic vegetation. Therefore, models that estimate concentrations of bromadiolone in surface water or calculate spray drift deposition of bromadiolone on aquatic habitats were not needed for this assessment. In addition, no surface water monitoring data were available for bromadiolone.

2.10.1.b. Estimating Exposure in the Terrestrial Environment

The Agency typically uses T-REX to calculate EECs for dietary exposure of terrestrial wildlife to pesticides, and T-HERPS to calculate refined EECs for dietary exposure to reptiles and amphibians. However, these models only calculate EECs (and risk quotients based on the EECs) for natural wildlife food items such as plants, seeds, and insects that are exposed from foliar application of pesticides. These models are not appropriate for calculating EECs for animals that directly consume bait products (*i.e.*, primary exposure) or consume prey that have ingested bait products (*i.e.*, secondary exposure). These models also calculate risk quotients (RQs) for application of granular pesticides and seed treatment uses but cannot be used to calculate RQs for bait products. Therefore, terrestrial animal exposure to bromadiolone was calculated without the use of T-REX and T-HERPS.

Instead, primary exposure and secondary exposure were evaluated using a dose-based approach that is dependent on body weight and food consumption rates and a dietary approach that uses only the concentration of bromadiolone in the formulated bait itself (for primary exposure) or the maximum measured bromadiolone whole body residue in animals that ingested bromadiolone (for secondary exposure). Food dry weight consumption estimates for the former approach were derived using allometric equations from USEPA's *Wildlife Exposure Factor Handbook* (1993).

2.10.2. Measures of Effect

Data identified in **Section 2.8** were 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 are used in assessments is available in **Attachment I**.

2.10.3. Integration of Exposure and Effects

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

2.10.4. 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, 2004a). 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 bromadiolone on par with the acute toxicity endpoint selected for RQ calculation. To accomplish this interpretation, the Agency uses the slope of the dose response relationship available from the toxicity study used to establish the acute toxicity measures of effect for each taxonomic group that is relevant to this assessment. The individual effects probability associated with the acute RQ is based on the mean estimate of the slope and an assumption of a probit dose response relationship. In addition to a single effects probability estimate based on the mean, upper and lower estimates of the effects probability are also provided to account for variance in the slope, if available.

Individual effect probabilities are calculated based on an Excel spreadsheet tool IECV1.1 (Individual Effect Chance Model Version 1.1) developed by the U.S. EPA, OPP, Environmental

Fate and Effects Division (June 22, 2004). The model allows for such calculations by entering the mean slope estimate (and the 95% confidence bounds of that estimate) as the slope parameter for the spreadsheet. In addition, the acute RQ is entered as the desired threshold.

2.10.5. Data Gaps

The environmental fate and ecological effects data requirements for bromadiolone have changed since the issuance of the RED in 1998. The data requirements are now consistent with the 40 CFR Part 158 guideline requirements for terrestrial, aquatic, and outdoor residential use patterns. Data gaps were assigned either a low or high potential to add value to the effects determination of bromadiolone risk to the AW, SMHM, and SJKF. While still considered data gaps according to 40 CFR Part 158, low potential studies are unlikely to change risk determinations because alternate methods and weight of evidence may possibly be used in the absence of data. High potential studies would enable the Agency to better characterize potential risks by eliminating uncertainties for both non-listed and listed species that cannot be accounted for using alternate methods or weights of evidence.

Data from the following guideline studies are considered to have **high potential** to add value to the ecological risk assessment:

- Avian Acute Oral Toxicity (850.2100). The acute oral toxicity of bromadiolone to passerine species is unknown. Risk quotients based on a weight-adjusted LD_{50} or LC_{50} for a galliform species may over- or underestimate risks to passerine species because passerines may metabolize the chemical differently than galliformes. Because bromadiolone is categorized as highly toxic to birds on an acute oral basis, data on the relative toxicity of bromadiolone to passerine species are necessary to further characterize risk to the AW, SMHM, and SJKF.
- Avian Reproduction (850.2300). Data on the reproductive toxicity of bromadiolone to birds is not available for risk assessment purposes. Under 40 CFR Part 158, two reproduction studies using waterfowl and upland game species are required. Because of the high toxicity documented for birds, including submitted data and reported incidents, information regarding chronic toxicity of bromadiolone to birds is essential to further characterize risk to the AW, SMHM, and SJKF.

Data from the following guideline studies are considered to have **low potential** to add value to the ecological risk assessment because exposure of the associated taxa to bromadiolone is expected to be insignificant (Series 850 Ecological Effects Test Guidelines) or the environmental fate of bromadiolone is limited by placement of bait in bait stations for outdoor uses (Series 835 Fate, Transport and Transformation Test Guidelines):

- Algal Toxicity, Tier I (850.5400)
- Aquatic Plant Toxicity Test using *Lemna* spp., Tier I (850.4400)
- Seedling Emergence, Tier I (850.4100)
- Vegetative Vigor, Tier I (850.4150)
- Honey Bee Acute Contact Toxicity (850.3020)
- Freshwater Invertebrate Acute Toxicity (850.1010)

- Freshwater Fish Acute Toxicity, warm water species (850.1075)
- Freshwater Invertebrates Life Cycle (850.1300)
- Early Life Stage Toxicity of Freshwater fish (850.1400)
- Estuarine/Marine Acute Toxicity (850.1025, 850.1035, 850.1045, 850.1055, 850.1075)
- Photolysis in Water (835.2240)
- Photolysis in Soil (835.2410)
- Aerobic Aquatic Metabolism (835.4300)
- Anaerobic Aquatic Metabolism (835.4400)
- Terrestrial Field Dissipation (835.6100)

Although not an ecological effects data requirement, a chronic rodent study (*e.g.*, 870.4100) was not available to assess chronic risk to mammals. In lieu of this data, a developmental study with rats (MRID 92196014) was used for risk assessment purposes.

Data were limited regarding whole body residues of bromadiolone in target organisms for more fully characterizing secondary exposure. Only three studies providing residue data for rodents that ingested bromadiolone were available for estimating secondary exposure (MRIDs 46750931/40077202, 48590801, 48590803). These studies examined non-target rodents (*i.e.*, water voles, ground squirrels). Only 2 of these studies provided whole body residues (MRIDs 48590801, 48590803); the third study only provided residues of bromadiolone in individual tissues (MRIDs 46750931, 40077202).

Data regarding the toxicity of bromadiolone to reptiles was also lacking. In lieu of this data, toxicity data for birds was used as a surrogate for the AW.

3. Exposure Assessment

Bromadiolone is formulated as meal bait, pellets, ready-to-use place packs, and paraffinized blocks. Baits are placed by hand; they may not be broadcasted. Since there is no potential for spray drift and/or runoff, these routes of exposure were not considered in this assessment.

3.1. Label Application Rates and Intervals

Bromadiolone labels may be categorized into two types: labels for manufacturing uses (including technical grade bromadiolone) and end-use products. While technical products, which contain bromadiolone 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 rodents. The formulated product labels legally limit bromadiolone's potential use to only those sites that are specified on the labels.

Labels of bromadiolone products 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 minimal time interval between applications. However, labels do state that bromadiolone bait must not be applied farther than 50 feet from buildings and that, for outdoor uses, bait must be placed in bait stations. Labels generally state the number of bait stations, bait blocks, or bait

packages that may be placed in one location as well as the linear interval between placements. The linear interval is generally 15 to 30 feet for rats and 8 to 12 feet for mice. The concentration of bromadiolone in bait is 0.005% (50 mg/kg) for all products. The amount of active ingredient per placement or the amount of active ingredient per linear foot can be calculated for bromadiolone products. The maximum amount of active ingredient per placement for any product is 2.27 mg for rats and 0.28 mg for mice. The maximum amount of active ingredient per linear foot is 0.15 mg/ft for controlling rats and 0.04 mg/ft for controlling mice. The maximum amount of active ingredient per placement (*i.e.*, manhole) for use in sewage systems is 13.61 mg. Use information for applications considered in this assessment is summarized in **Table 2-4.**

3.2. Aquatic Exposure Assessment

3.2.1. Modeling Approach

Aquatic exposure from use of bromadiolone was assumed to be negligible (see **Section 2.10.1.a**). Therefore, aquatic exposures were not assessed.

3.2.2. Existing Monitoring Data

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

3.3. Terrestrial Animal Exposure Assessment

For this assessment, it was assumed that terrestrial animals could be exposed via two different pathways. Animals may directly consume bait (*i.e.*, primary exposure), or animals may consume prey that have ingested bait (*i.e.*, secondary exposure). Primary exposure and secondary exposure were evaluated using two methodologies, when possible:

- a dose-based approach that is dependent on body weight and food consumption rates resulting in an estimate of bromadiolone intake as mg a.i./kg-bw/day, where kg-bw is the kilograms of the consuming individual; and
- a dietary approach that uses only the concentration of bromadiolone in the formulated bait itself (for primary exposure) or the maximum measured bromadiolone whole body residue in animals that ingested bromadiolone (for secondary exposure).

Both approaches and the expected exposure levels are detailed below.

3.3.1. Primary Exposure of Terrestrial Wildlife

Primary exposure was evaluated for assessing direct risk to the SMHM and other mammals that may indirectly affect the AW, SMHM, or SJKF. An assessment for primary exposure of the AW was not conducted because snakes seldom consume anything except live prey, and thus the AW would not likely consume bromadiolone bait directly. Assessments for primary exposure of the

SJKF, birds, and herpetofauna that may indirectly affect the AW, SMHM, and SJKF were not conducted because all outdoor uses of bromadiolone require that bait be placed in bait stations. The use of bait stations should prevent access to bait by the SJKF, birds, and herpetofauna and thus preclude their exposure to bromadiolone via primary consumption.

Primary exposure through bromadiolone bait consumption was calculated using two methodologies. For the first method, bromadiolone exposure was calculated as mg a.i./kg-bw/day, where kg-bw is the kilograms of the consuming individual for three standard weight classes of mammals and for a typical weight of the SMHM. Exposure (food dry weight consumption) estimates were derived using allometric equations from USEPA's *Wildlife Exposure Factor Handbook* (1993). The allometric equation for rodents was used as this would best approximate those individuals with a high potential for consuming grain and thus give the most conservative exposure estimates. Bromadiolone bait products were assumed to be 100% dry material. Thus, food dry weight was assumed to be equivalent to food wet weight. Formulas for calculation of intake estimates are provided in **Table 3-1**, and bromadiolone exposure estimates (on a dose basis) are provided in **Table 3-2**.

The EEC for direct effects to the SMHM was calculated based on an estimated average body weight of 10 g. EECs for indirect effects of reduction in prey (*i.e.*, rodents) were also calculated. EFED default weight classes for mammals (*e.g.*, rodents) are usually small (15 g), medium (35 g), and large (1000 g). However, the large weight class for mammals (*i.e.*, 1000 g) may exceed the size of a rodent capable of entering a bait station to consume bait as reported body weights of house mice, roof rats, and Norway rats, the three target rodent species for bromadiolone bait, are 18-23 g (Whitaker, 1996), 142-283 g (ICWDM, online)[4], and 195-485 g (Whitaker, 1996), respectively. Therefore, the upper weight bound (*i.e.*, 485 g) for the largest target rodent species (*i.e.*, the Norway rat) was used as the large weight class for this analysis.

Acute and chronic RQs, presented in the Risk Estimation section, are generated by dividing these estimates of bromadiolone exposure (mg a.i./kg-bw/day) for a given weight class by the most conservative toxicity endpoint for the relevant taxa adjusted for the default body weights.

Table 3-1. Formulas for Calculating Bromadiolone Intake Based on Consumption of Bait

Rodent food intake (kg, dry weight)[1]

$$FI\ (kg\ dry\text{-}wt/day) = 0.621 * Wt(kg)^{0.564}$$

Food intake (kg, wet weight)

$$FI\ (kg\ wet\text{-}wt/day) = FI\ (kg\ dry\text{-}wt/day)$$

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

Where:

Wt(g) = weight (in kilograms) of the rodent consumer

C (mg a.i./kg-bait) = concentration of bromadiolone in bait

FI = Food intake

[1]Equation for SMHM and generic bait-consuming rodents

[4] http://icwdm.org/handbook/rodents/RoofRats.asp

Table 3-2. Expected Bromadiolone Intake for SMHM and Generic Rodents Weights Based on Primary Consumption of Bait

Species or Taxa	% a.i. in bait	Bromadiolone Concentration in Bait (mg a.i./kg)	Weight (g)	Food Intake (g dry-wt/day)	Food Intake (g wet-wt/day)	Bromadiolone Intake (mg a.i./kg-bw/day)*
SMHM	0.005	50	10	2.3	2.3	11.4
Rodents	0.005	50	15	2.9	2.9	9.5
			35	4.6	4.6	6.6
			435	19.1	19.1	2.2

*See Table 3-2 for derivation

A second method for assessing primary exposure uses the concentration of bromadiolone in the formulated bait itself, resulting in a dietary concentration of 50 mg a.i./kg-bait. Since these EECs are not dependent on body weight or food consumption rates, they apply to both direct effects to the SMHM and indirect effects to all three evaluated species (*i.e.*, effects to rodents). Acute and chronic RQs for dietary-based calculations of primary exposure were generated by dividing this exposure estimate of bromadiolone (mg a.i./kg-bait) by the most conservative dietary toxicity endpoint for the relevant taxa.

3.3.2. Secondary Exposure of Terrestrial Wildlife

Secondary exposure was evaluated for assessing direct risk to the AW and SJKF from consumption of prey that ingested bromadiolone bait. An assessment for secondary exposure of the SMHM was not conducted because the SMHM's diet consists primarily of plants and insects, taxa for which uptake or ingestion of bromadiolone is unlikely.

Indirect effects to the AW, SMHM, and SJKF mediated through secondary exposure of mammals were evaluated. However, indirect effects to the AW, SMHM, and SJKF mediated through secondary exposure of birds were not evaluated because adverse effects to birds that are likely to be at risk from secondary exposure (*i.e.*, birds consuming prey that ingested bromadiolone bait) would not impact the AW, SMHM, or SJKF.

Secondary exposure to the AW and SJKF from consuming bromadiolone residues in reptilian, amphibian, or invertebrate prey items was not evaluated because the majority of secondary exposure is expected to be from residues in target organisms (*i.e.*, rats and mice). Although lizards in particular are believed to be the most important prey item of whipsnakes (Stebbins, 2003; Swaim, 1994), lizards generally feed upon insects and other terrestrial arthropods and would be less likely to consume rodent bait. Therefore, secondary exposure was based on consumption of small mammals only.

The determination of bromadiolone intake for individuals consuming prey that have ingested bromadiolone bait was calculated in a manner similar to the approach for individuals consuming bait (**Section 3.3.1**). Empirical residue data were used instead of bait concentration of bromadiolone (**Table 3-3**), assuming that concentrations were reported using wet weights.

Three studies providing residue data for rodents that ingested bromadiolone bait were available for ascertaining secondary exposure from consumption of mammalian prey (**Table 3-3**). In one study, California ground squirrels (*Otospermophilus beecheyi*), not of uniform sex or weight, were offered 15-g portions of 0.005% (50 mg a.i./kg) bromadiolone bait for three consecutive days and monitored until death (MRIDs 162324 and 162325). Seven squirrel carcasses were analyzed for bromadiolone residues in loin muscle, heart muscle, spleen, kidney, and liver. The residue values for the squirrel carcass containing the highest residues in each of these organs except for the spleen were 0.20, 2.62, 1.50, 1.90, and 3.75 mg a.i./kg-tissue, respectively (**Table 3-3**); spleen tissue concentrations in two other squirrel carcasses measured 2.00 and 1.75 mg a.i./kg-tissue.

In a second study, bromadiolone residues were measured in water voles (*Arvicola terrestris*) that were allowed to feed on bromadiolone bait after a single field treatment (MRID 48590801). Wheat bait (50 mg a.i./kg, Lipatech) was placed in artificial burrows at a rate of 1 kg bait per 100 m over 21 acres. Rodents were trapped or collected above and below ground every day from days 1 through 3, days 6 through 8, and days 10 through 20 post-treatment. The average concentrations of bromadiolone in 40 voles were: 5.95 mg/kg (95% CI 4.30–7.79; maximum 30.23) in the liver; 2.28 mg/kg (95% CI 1.70–2.95; maximum 12.37) in the digestive tract; and 0.75 mg/kg (95% CI 0.58–0.92; maximum 3.19) in the rest of the body. In this study, the average whole body weight of *A. terrestris* was 77.02 g (SE 1.89), and the average quantity of bromadiolone per vole was 93.97 µg (95% CI: 73.8–113.5) yielding an average whole body bromadiolone residue of 1.22 mg a.i./kg-carcass (**Table 3-3**).

In third study, residues of bromadiolone were measured in both water voles (*Arvicola terrestris*) and common voles (*Microtus arvalis*) that were allowed to feed on bromadiolone bait after a single field treatment (MRID 48590803). Bait was prepared by mixing dried wheat grains with a commercial formulation of bromadiolone (Super Caid® A659, ref. R227002, France) to achieve a concentration of 50 mg a.i./kg-bait and placed in artificial burrows at a rate of 8.1 kg bait/A over 24 acres. Rodents were trapped or collected above and below ground every day for 10 days after treatment, and then every three to four or more days until day 135 (sampling occurred on days 1 to 10, day 13, day 16, day 19, day 24, day 27, day 49, and day 135). The study focused on the kinetics of bromadiolone residues within rodent populations and thus did not present measured residue data with the exception of one water vole trapped underground on day 135. The concentrations in the whole body, liver, and digestive tract for this one vole were 1.43, 16.63, and 0.86 mg a.i./kg-tissue, respectively. The study also reported that the average whole body residue in water voles collected on day 1 was 1.83 ± 0.96 mg a.i./kg-carcass (**Table 3-3**).

To give the most conservative estimate of secondary exposure, the maximum reported whole body bromadiolone residue of 1.83 mg a.i./kg-carcass was used for risk assessment (**Table 3-3**).

Table 3-3. Bromadiolone Residue Levels in Primary Consumers

Exposure Information	Species	Sample Size	Dietary Concentration (mg a.i./ kg-bait)	Bromadiolone Residue (tissue) (mg a.i./kg)	Reference/ Classification
Voles allowed to feed on bait placed in artificial burrows	Water vole (*Arvicola terrestris*)	1	50	16.63 (liver day 135) 0.86 (digestive tract day 135) 1.43 (whole body day 135)	MRID 48590803 (Sage *et.al.*, 2008) Quantitative
		6		1.83 (whole body average day1)	
Voles allowed to feed on bait placed in artificial burrows	Water vole (*Arvicola terrestris*)	40	50	5.95 (liver average) 30.23 (liver max) 2.28 (GI average) 12.37 (GI max) 0.75 (rest of body average) 3.19 (rest of body max) 1.22 (whole body average)	MRID 48590801 (Giraudoux *et al.*, 2006) Quantitative
Bait offered in 15-g portions, with no alternative, for 3 days	California ground squirrel (*Otosperm-ophilus beecheyi*)	1 (of 7)	50	0.201* (loin muscle) 2.616* (heart muscle) 1.504* (spleen) 1.897* (kidney) 3.748* (liver)	MRIDs 162324 and 162325 (Marsh and Howard, 1986) Core

Abbreviations : GI = digestive tract
*Values for squirrel carcass containing the highest residues in each of these organs except for the spleen.

Secondary exposure of mammals and the SJKF via consumption of mammalian prey that ingested bromadiolone bait was calculated as mg a.i./kg-bw/day, where kg-bw is the kilograms of the consuming individual for three weight classes of mammals and for the estimated average body weight of the SJKF (*i.e.*, 2300 g) and AW (see estimates below). For this analysis, mammal weight classes of 50, 1000, and 3000 g individuals were used to better represent the larger size of carnivores and scavengers relative to the full range of mammal weights. Exposure (food dry weight consumption) estimates were derived using the generic mammal allometric equations (USEPA, 1993). Food dry weight was converted to wet weight assuming the consumed prey/carcass contained 68% water (USEPA, 1993). Formulas for calculation of dose estimates are provided in **Table 3-4**, and bromadiolone exposure estimates (on a dose basis) are provided in **Table 3-5**. To make this assessment protective for the AW, the exponent used in the snake food intake equation is the upper limit of the 95% confidence interval that King (2002) reported for this parameter (**Table 3-4**).

Table 3-4. Formulas for Calculating Bromadiolone Intake Based on Consumption of Animals that Ingested Bromadiolone Bait

Mammal food intake (kg, dry weight)[1]
FI (kg dry-wt/day) = 0.235 * $Wt(kg)^{0.822}$

Snake food intake (kg, wet weight)[2]
FI (kg dry-wt/day) = $Wt(kg)^{1.071}$

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

Bromadiolone intake (mg a.i./kg-bw/day) = (FI (kg wet-wt/day) * 1.83 mg a.i./kg-carcass) / Wt(kg)

Where:
Wt(kg) = weight (in kilograms) of the mammal or snake consumer

FI = Food intake
[1]Equation for SJKF and generic mammals
[2]Equation for AW

Table 3-5. Expected Bromadiolone Intake for the AW, SJKF, and Generic Mammal Weights Based on Consumption of Prey That Ingested Bromadiolone Bait

Species or Taxa	Weight (g)	Bromadiolone Residue in Carcass of Prey (mg a.i./kg-carcass)	Food Intake (g dry-wt/day)	Food Intake (g wet-wt/day)	Bromadiolone Intake (mg a.i./kg-bw/day)**
AW	18.6*	1.83	-	23	2.3
	195*		-	283	2.7
	322*		-	485	2.8
SJKF	2300	1.83	136	425	0.3
Mammals	50	1.83	5.9	18.3	0.7
	1000		68.7	214.7	0.4
	3000		169.5	529.8	0.3

* Minimum snake sizes needed to consume the three target species: house mouse, roof rat, and Norway rat
** See Table 3-5 for derivation

Secondary exposure of the AW via consumption of prey that ingested bromadiolone bait was calculated as mg a.i./kg-bw/day; however the weight of the AW was not available. The Agency estimated the body weight of this species from its length using the method presented in the *Wildlife Exposure Factor Handbook* (USEPA, 1993). The body weight of this species was estimated to range from 2.5 to 176 g for juveniles and 46 to 897 g for adults (USEPA, 2010a). Using the upper bounds of these ranges and the allometric food intake (*i.e.*, prey intake) equation in **Table 3-4**, the maximum prey size for the AW was estimated to be 254 g for juvenile snakes and 1450 g for adult snakes. As stated previously, reported body weights of house mice, roof rats, and Norway rats, the three target rodent species for bromadiolone bait, are 18-23 g (Whitaker, 1996), 142-283 g (ICWDM, online)[5], and 195-485 g (Whitaker, 1996), respectively. Therefore, the AW is predicted to be able to consume all three of these prey species.

[5] http://icwdm.org/handbook/rodents/RoofRats.asp

Using the upper limit of the reported body weight ranges of each prey species, *i.e.*, 23 g for the house mouse, 283 g for the roof rat, and 485 g for the Norway rat, AW size was set at the minimum size snake that could consume these size prey items. Deriving the minimum AW size was accomplished by setting the prey size in the allometric equation for maximum prey size, given above, and solving for snake body weight. The minimum snake sizes to consume the mouse, roof rat, and Norway rat were calculated to be 18.6, 195, and 322 g, respectively. The 18.6-g snake is plausible for a juvenile AW, and the 195 and 322-g snakes are plausible for an adult AW.

RQs for dosed-based calculations of secondary exposure were generated by dividing these exposure estimates of bromadiolone (mg a.i./kg-bw/day) for a given weight class by the most conservative oral toxicity endpoint for the relevant taxa adjusted for the default body weights. RQs using these exposure estimates were generated for acute and chronic mammal toxicity.

The second exposure method for estimating secondary exposure is dietary-based, considering only the maximum bromadiolone whole body residue measured in an animal that has ingested bromadiolone bait, *i.e.*, 1.83 mg a.i./kg-carcass. RQs for dietary-based calculations of secondary exposure were generated by dividing this exposure estimate of bromadiolone (mg a.i./kg-bw/kg-carcass) by the most conservative dietary toxicity endpoint for the relevant taxa. RQs using these exposure estimates were generated for acute and chronic mammal toxicity.

3.3.3. Exposure of Terrestrial Invertebrates

Exposure of terrestrial invertebrates can occur through ingestion and contact with bait. Exposed individuals could be adversely affected or be available for consumption by the AW, SMHM, and SJKF as well as other animals. However, indirect effects to the AW, SMHM, and SJKF mediated through exposure of terrestrial invertebrates are expected to be negligible given that invertebrates represent only one type of prey item among many for the assessed species. Therefore no terrestrial invertebrate exposure assessment was conducted. It should be noted that EFED does not currently have a methodology for estimation of terrestrial invertebrate exposure through contact with bait. In addition, EFED does not currently have any bromadiolone data for estimation of invertebrate body burden of bromadiolone.

3.4. Terrestrial Plant Exposure Assessment

As described in **Section 2.9**, indirect effects to the AW, SMHM, and SJKF mediated through exposure of terrestrial plants are expected to be negligible because of the use pattern of bromadiolone. Therefore no terrestrial plant exposure assessment was conducted.

4. Effects Assessment

This assessment evaluates the potential for bromadiolone to directly or indirectly affect the AW, SMHM, and SJKT or modify their designated critical habitat. Assessment endpoints for the effects determination for each assessed species include direct toxic effects on the survival, reproduction, and growth, as well as indirect effects, such as reduction of the prey base or modification of its habitat. In addition, potential modification of critical habitat is assessed by

evaluating effects to the PCEs, which are components of the critical habitat areas that provide essential life cycle needs of each assessed species. Direct effects to the AW are based on avian toxicity data, given that birds are generally used as a surrogate for terrestrial-phase amphibians and reptiles.

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

4.1. Ecotoxicity Study Data Sources

Toxicity endpoints are established based on data generated from guideline studies submitted by the registrant, and from open literature studies that meet the criteria for inclusion into the ECOTOX database maintained by EPA/Office of Research and Development (ORD) (USEPA, 2004a). Open literature studies presented in this assessment were obtained from citations in previous risk assessments as well as ECOTOX information obtained on 15 March 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 acute oral or dietary effects of bromadiolone 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 identified during the ECOTOX open literature search that the Agency conducted are not included in the summary table provided in **Appendix F**. Rather citations of open literature papers that provide toxicological data for target rodent species are listed in **Appendix E** with the code "TARGET" given after the citation. While toxicological findings were not included in the summary of acute and chronic toxicity endpoints in this document, some of these papers which were deemed useful and were obtained to provide supplemental information for characterizing the toxicity of bromadiolone, such as information on the sublethal effects and the mode of action of bromadiolone.

Data that pass the ECOTOX screen are evaluated along with the registrant-submitted data, and may be incorporated qualitatively or quantitatively into this endangered species assessment. In

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

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

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 bromadiolone. A summary of the available aquatic and terrestrial ecotoxicity information and the incident information for bromadiolone are provided in **Sections 4.2** through **4.4**.

A detailed summary of all available ecotoxicity information for bromadiolone technical grade and end use product formulations can be found **Appendix C**. No toxicity data are available for bromadiolone's degradates.

4.2. Toxicity of Bromadiolone to Terrestrial Organisms

Table 4-1 summarizes the most sensitive toxicity endpoints for primary bromadiolone exposure, based on an evaluation of both submitted studies and the open literature. A brief summary of submitted and open literature data considered relevant to this ecological risk assessment is presented below. Additional information is provided in **Appendix C**.

Table 4-1. Terrestrial Toxicity Profile for Bromadiolone

Taxa	Study Type	Species Tested	Toxicity Value Used in Risk Assessment	Citation MRID/ ECOTOX Reference No.	Classification and Comment
Birds	Acute oral (single dose by gavage)	Northern bobwhite (*Colinus virginianus*)	30-day LD_{50} = 170 mg a.i./kg-bw Slope = n/a	143279	Acceptable

Taxa	Study Type	Species Tested	Toxicity Value Used in Risk Assessment	Citation MRID/ ECOTOX Reference No.	Classification and Comment
	Dietary (5-day exposure)	Northern bobwhite (*Colinus virginianus*)	30-day LC_{50} = 37.6 mg a.i./kg-diet Slope = 0.83 (95% CI = 0.43 – 1.24)	143280	Acceptable
Mammals	Acute oral (single dose by gavage)	Rat, Wistar albino	14-day LD_{50} = 0.6 mg a.i./kg-bw Slope = n/a	241703	Supplemental (Minimally satisfies data requirements)
	Dietary (5-day exposure)	Rat, albino	14-day LC_{50} = 0.94 mg a.i./kg-diet Slope = 5.20 (95% CI = 1.91 – 8.50)	Teeters, 1981 TNM 105*	Supplemental
	Developmental (10-day oral exposure by gavage)	Rat, Sprague-Dawley	NOEL = 0.035 mg a.i./kg-bw	92196014	Acceptable LOEL = 0.070 mg a.i./kg-bw (dams) Based on vaginal bleeding, hypotonicity, pale eyes, and deaths.

n/a: not applicable; bw = body weight

* Teeters, W.R. 1981. Bromadiolone technical: Toxicity to Laboratory Rat: Test No. 105. (U.S. Environmental Protection Agency, Pesticides Regulation Div., Agricultural Research Center, Animal Biology Laboratory, unpublished report.)

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

Table 4-2. Categories of Acute Toxicity for Avian and Mammalian Studies

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

4.2.1. Toxicity to Birds

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, 2004a). A summary bird toxicity data, including data published in the open literature, is provided below in **Sections 4.3.1.a** through **4.3.1.c**.

4.2.1.a. Primary Toxicity to Birds: Acute Exposure (Mortality) Studies

Bromadiolone is classified moderately toxic to birds for acute oral exposures based on data from a study with bobwhite quail that yielded a 30-day LD_{50} of 170 mg a.i./kg-bw (MRID 143279). Birds were given a single oral dose ranging from 25 to 2400 mg a.i./kg-bw and observed for 30 days. Mortality was due to hemorrhaging, and all birds except in one instance died within 9 days of test initiation. Sublethal effects were not reported for the surviving birds, although there may have been an effect on body weights. Results from two other studies available on bromadiolone's acute avian oral toxicity included undefined LD_{50} values (MRIDs 30961 and 145323); reported sublethal effects from these two studies include lethargy, reduced reaction to stimuli, wing droop, ruffled appearance, loss of coordination, and lower limb weakness. A 90-day acute oral toxicity study on the Mallard was available but has not been reviewed by the Agency (MRID 44000201). A cursory review of the data indicate that the study LD_{50} is not lower than the most sensitive endpoint (LD_{50} = 170 mg a.i./kg-bw).

Bromadiolone is classified as highly toxic to birds based on acute dietary exposures; the most sensitive sub-acute dietary LC_{50} is 37.6 mg a.i./kg-diet (MRID 143280). Bobwhite quail were exposed to bromadiolone for 5 days and monitored for signs of toxicity for thirty. Mortality occurred at all test concentrations (ranging 10 to 2560 mg a.i./kg-diet), and coincided with internal and external hemorrhaging for the majority of birds. Except for one incidence (10 mg a.i./kg-diet, Day 21), all mortalities occurred within the first 15 days of the test. Clinical signs of toxicity were observed at all doses consisting of external (foot, beak) and internal hemorrhaging, emaciation, and swollen or bruised appendages. Acute dietary toxicity to birds was evaluated in additional Agency guideline studies with resulting LC_{50} values of >10, >20, 158, and 440 mg a.i./kg-diet (MRIDs 145321, 145322, 143278, and 249995, respectively). Sublethal effects noted in these studies included lethargy, depression, reduced reaction to external stimulus, wing droop, loss of coordination, lower limb weakness, prostrate posture and loss of righting reflex, hematomas (dermal, subcutaneous, and thoracic), hemorrhaging, loss of water consumption, anorexia, blooding droppings, and blood filled cysts. A 90-day acute dietary toxicity study on the Mallard was available but has not been reviewed by the Agency (MRID 44000202). A cursory review of the data indicates that the pattern of mortality observed in the study is not suitable for calculation of a LC_{50} value.

4.2.1.b. Primary Toxicity to Birds: Chronic Exposure (Growth, Reproduction) Studies

No acceptable or supplemental data were available to characterize chronic toxicity to birds.

4.2.1.c. Secondary Toxicity to Birds

A supplemental study by Mendenhall and Pank (MRID 46750931) examined secondary poisoning of barn owls (*Tyto alba*) by bromadiolone-fed rats (see **Table 4-3**). Rats were given a free choice of poisoned baits or lab chow for 5 days. Owls were then fed these bromadiolone-laced, CO_2-euthenized rats for a period of 1, 3, 6, or 10 days. Bromadiolone levels in rats fed to owls were not quantified, but some of the consumed bromadiolone was presumably metabolized and excreted before death. One of six owls fed bromadiolone-laced rats died. The dead bird showed hemorrhaging and heart lesions. The surviving owls fed bromadiolone-laced rats did not show hemorrhaging when necropsied after 10 or more days of being fed bromadiolone laced rats. In a second study, six barn owls were fed bromadiolone-fed rats for either 1, 3, or 6 days (MRID 48590804). None of the barn owls died; the incidence of sublethal effects included a temporary increase in blood coagulation time that returned to normal after 10 days. **Table 4-3** summarizes the birds in which mortality or sublethal effects occurred.

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

Predator/ Scavenger Species	Prey Offered	No. Prey Offered Daily per Bird	Predator Exposure Duration	No. Dead Predators	Signs of Toxicity in Survivors	Reference/ Classification
Barn owl (*Tyto alba*)	Rats fed choice of 0.005% bait or untreated bait for 5 days	1-2	1 for 1 day 2 for 3 days 1 for 6 days 2 for 10 days All followed for 20 days.	0 0 0 1	None	MRIDs 46750931 and 40072202 (Mendenhall and Pank, 1980) Qualitative
Barn owl (*T. alba*)	Mice fed commercial bait (% a.i. not reported) and allowed to die	2-3	6 for 1 day 6 for 3 days 6 for 6 days	0 0 0	Increased blood coagulation time, returning to normal after 10 days	MRID 48590804 (Wyllie, 1995) Qualitative

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 4.3.2.a** through **4.3.2.c**. A more complete analysis of toxicity data to mammals is available in **Appendix D**, which provides the Health Effects Division (HED) chapter prepared in support of the 1998 Reregistration Eligibility Decision.

4.2.2.a. Primary Toxicity to Mammals: Acute Exposure (Mortality) Studies

Bromadiolone is classified very highly toxic to mammals on an acute oral exposure basis, based on data from a study with Wistar albino rats that yielded a 14-day LD_{50} of 0.6 mg a.i./kg-bw (MRID 241703). Wistar albino rats were dosed with a 1% bromadiolone, 99% corn starch formulation and observed for 14 days. Sublethal effects were not reported. This study was classified supplemental. In a second acute oral toxicity study, male and female rats were given one dose of bromadiolone in concentrate form and observed for 21 days (MRID 41900001); the

study LD_{50} fell between 0.56 and 0.84 mg a.i./kg-bw. This study minimally satisfied data requirements because a concentrate was used instead of technical grade bromadiolone (USEPA, 1998b). Clinical observations of toxicity included internal hemorrhage, extreme physical weakness, pale mucous membranes, difficulty breathing, impaired equilibrium, reduced responsiveness to noise, reduced weight gain, swelling, hematoma, and piloerection. The former study endpoint was used for risk quotient derivation (LD_{50} = 0.6 mg a.i./kg-bw).

Acute oral toxicity of bromadiolone was assessed in other studies with mice (LD_{50} = 1.75 mg a.i./kg-bw; MRID 226423 or 007425), rats (LD_{50} = 1.13 mg a.i./kg-bw; MRID 226423), domestic cats (LD_{50} > 25 mg a.i./kg-bw; MRID 142699), and domestic beagle dogs (LD_{50} > 10 mg a.i./kg-bw; MRID 142699). Reported sublethal effects to these test mammals included polydipsia, bleeding, hemorrhaging, anorexia, and exhaustion. Two studies evaluated the repeated dosing of bromadiolone to beagle dogs and pigs. Beagle dogs were dosed by gavage each day for 90 days with 0.008, 0.02, or 50 mg a.i./kg-bw (MRID 160954). Although there was insufficient dose levels and dose progression, an LD_{50} was calculated to be 16.7 mg a.i./kg-bw/day based on available data. Tested dogs exhibited changes in body weight, food consumption, and hemorrhaging. Pigs were dosed in two tests at either 0.02 mg a.i./kg-bw/day for 45 days or at 1.0 mg a.i./kg-bw/day for 5 days, and 5 days again after 15 days without dosing (MRID 143276). The LD_{50} in either case was greater than the dose given. The only sublethal effect reported was anorexia.

Bromadiolone is classified as very highly toxic to mammals for acute dietary exposures; the most sensitive sub-acute dietary 14-day LC_{50} is 0.94 mg a.i./kg-diet (Teeters, 1981). Specifically, four acute dietary studies were conducted in the same laboratory using very similar methodology: Albino rats were followed for 14 days after starting a diet treated with technical bromadiolone (5 days treated diet, followed by 9 days clean diet). All mortalities occurred between days 5 and 9 after the initiation of feeding with treated diet. Although the study reports included data on body weight and food consumption, statistical analysis was not conducted for these parameters because of the high rate of mortality. Sublethal effects were not reported.

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

A chronic study with rats is unavailable to evaluate the chronic toxicity of bromadiolone. However, a developmental toxicity study with Sprague-Dawley rats is available and is quantitatively linked to the ecological risk assessment endpoints: survival, growth, and reproduction. Groups of pregnant rats received technical grade bromadiolone from gestation days 6 to 16 via oral gavage. The LOAEL and NOAEL were 0.070 and 0.035 mg a.i./kg-bw, respectively, based on vaginal bleeding, hypotonicity, pale eyes, and death (MRID 92196014).

4.2.2.c. Secondary Toxicity to Mammals

Two open literature studies are available that evaluate the secondary toxicity of bromadiolone to mammalian predators (**Table 4-4**). Of the 19 animals tested among studies with stone martens (*Martes foina*), mongooses (*Herpestes auropunctatus*), and coyotes (*Canis latrans*), seven (37%) animals died. Four stone martens were fed 4 to 8 bromadiolone-fed, yellow-necked field mice

for 1 or 4 days; none died during the 3-week observation period (MRID 48590802). However, increased fragility of the small blood vessels of the muscles on top of the skull was noted for the 2 individuals exposed for 4 days. The stone martens ingested 3.4 to 13.9 mg a.i./kg-bw bromadiolone. Two mongooses were each fed one bromadiolone-laced rat for 1, 3, 5, or 6 days; five died at the end of 10 days (MRID 157732). The five mongooses that died ingested a 13.2 to 24.4 mg a.i./kg-bw bromadiolone whereas the three mongooses that survived ingested 4.07 to 11.6 mg a.i./kg-bw bromadiolone. Four adult coyotes ingested a mean of 2.0 mg a.i./kg-bw (1.3-3.0 mg a.i./kg-bw) bromadiolone over the 5-day testing period and survived; the two sub-adult coyotes that died ingested 4.2 and 4.8 mg a.i./kg-bw bromadiolone, and the one sub-adult survived ingested 3.7 mg a.i./kg-bw bromadiolone (MRID 162324).

Table 4-4. Secondary Toxicity of Bromadiolone to Mammals in Laboratory Studies

Predator/ Scavenger Species	Prey Offered	No. Prey Offered Daily Per Predator	Predator Exposure Duration	No. Dead Predators	Signs of Toxicity in Survivors	Reference / Acceptability Classification
Stone marten (*Martes foina*)	Yellow-necked field mice fed 0.005% bait in excess to feed for 4 days	4 - 8	2 for 1 day 2 for 4 days All followed for 3 weeks.	0 0	Increased fragility of small blood vessels of the muscles on top of the skull for individuals exposed for 4 days	MRID 48590802 (Lund and Rasmussen, 1986) Qualitative
Mongoose (*Herpestes auropunctatus*)	Black or Norway rats fed 0.005% bait for 5 days	1	2 for 1 day 2 for 3 days 2 for 5 days 2 for 6 days All followed until death or 10 days.	0 1 2 2	Not reported	MRID 157732 (Pank and Hirata, 1976) Qualitative
Coyote (*Canis latrans*)	Ground squirrels fed 15 g of 0.01% bait for 3 days	1	4 adults for 5 days 3 sub-adults for 5 days All followed until death or 30 days post-treatment.	0 2	2 coyotes stopped feeding for 8 and 16 days, but resumed feeding and survived until the end of the study	MRID 162324 (Marsh and Howard, 1986) Core

4.3. Incident Database Review

A review of the Ecological Incident Information System (EIIS, version 2.1.1), which is maintained by the Agency's Office of Pesticide Programs (OPP), the Avian Monitoring Information System (AIMS), which is maintained by the American Bird Conservancy, and the Aggregate Summary Module (ASM) (v.1.0) of OPP's incident database, which is maintained by the Information Technology and Resource Management Division, for ecological incidents involving bromadiolone was completed on 15 July 2011. The results of this review are discussed

below **Sections 4.4.1** and **4.4.2**. A complete list of the incidents involving bromadiolone including associated uncertainties is included as **Appendix H**.

4.3.1. Incidents from the EIIS and AIMS Databases

A total of 66 ecological incidents were reported in association with the use of bromadiolone (**Appendix H**). This total excludes incidents classified as „unlikely' or „unrelated' and only includes those incidents with certainty categories of „possible', „probable', and „highly probable' (for EIIS) and „possible', „probable', „likely', 'highly likely' and „certain' (for AIMS). All of the bromadiolone incidents, excluding those classified as „unlikely' or „unrelated', occurred between 1983 and 2009 (4 had no date associated with them) and involved mammals or birds. The EIIS certainty categories regarding the likelihood that the use of bromadiolone caused the 66 incidents ranged from „highly probable' and „possible' (27 incidents each) to „probable' (12 incidents). Twenty-one of the incidents were considered registered uses at the time of the incident, 2 involved misuses, and the legality of use was undetermined in 43 incidents.

4.3.2. Incidents from the ASM Database

In addition to the incidents recorded in EIIS and AIMS, additional incidents have been reported to the Agency in aggregated incident reports. Pesticide registrants report certain types of incidents to the Agency as aggregate counts of incidents occurring per product per quarter. Ecological incidents reported in aggregate reports include those categorized as „minor fish and wildlife' (W-B), „minor plant' (P-B), and „other non-target' (ONT) incidents. „Other non-target' incidents include reports of adverse effects to insects and other terrestrial invertebrates. For bromadiolone, registrants have reported 3 minor fish and wildlife incidents.

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 designated critical habitat for the AW from the use of bromadiolone in CA. The risk characterization provides an estimation (**Section 5.1**) and a description (**Section 5.2**) of the likelihood of adverse effects; articulates risk assessment assumptions, limitations, and uncertainties; and synthesizes an overall conclusion regarding the likelihood of adverse effects to the assessed species or their designated critical habitat (*i.e.*, "no effect," "likely to adversely affect," or "may affect, but not likely to adversely affect"). In the risk estimation section, risk quotients are calculated using 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 birds (surrogates for reptiles and

terrestrial-phase amphibians) and mammals, the Endangered Species LOC is 0.1. The LOC for chronic exposures to animals is 1.0.

Acute and chronic risks to terrestrial animals are estimated based on primary and secondary exposures resulting from application of bromadiolone (**Section 3.3**) and the appropriate toxicity endpoints from **Table 4-1**.

5.1.1. Exposures in the Aquatic Habitat

The only aquatic taxon relevant to this assessment is aquatic plants for indirect effects to the SMHM, and no aquatic plant toxicity data were available. Given that bromadiolone is used in very small quantities in bait-type products, the probability that it will come into contact with water is minimal. Even when used in sewers where the potential for contact with water is possible, little bromadiolone is expected to be released from the bait into water. Therefore, no significant aquatic exposure is expected; no aquatic exposure assessment was performed; and indirect effects to the SMHM from exposure of aquatic plants to bromadiolone are not expected to occur.

5.1.2. Exposures in the Terrestrial Habitat: Birds (surrogate for Reptiles, including the AW, and Terrestrial-phase Amphibians)

Potential direct effects to birds were not evaluated because all outdoor uses for bromadiolone require that bait be placed in bait stations which should preclude primary exposure of birds. Additionally, only birds that consume prey that ingested bromadiolone bait, which would not indirectly impact the SMHM, SJKF, or AW, are expected to be at risk from secondary exposure.

Potential direct effects to the AW were evaluated by using avian toxicity data (adjusted for AW weight as necessary) and considering dose- and dietary-based EECs for secondary exposure.

5.1.2.a. AW: Dose-based Acute Risks from Secondary Exposure

To determine EECs for AW secondary exposure, available literature concerning whole body bromadiolone residues in animals that ingested bromadiolone were considered. For this assessment, a bromadiolone whole body residue of 1.83 mg a.i./kg-carcass was used to estimate bromadiolone intake (*i.e.*, exposure) on a dose basis as mg a.i./kg-bw/day (**Table 3-5**).

Toxicity of bromadiolone to the AW was estimated by the LD_{50} obtained from single-dose gavage studies with birds which serve as surrogates for reptiles. For a toxic response elicited from the gavage exposure route, toxicity is measured as mg a.i./kg-bw. The avian LD_{50} of 170 mg a.i./kg-bw (**Table 4-1**) was adjusted for various weights of the AW (18.6, 195, and 322 g – see **Section 3.3.2**) according to the formula in **Table 5-1**.

Dosed-based acute RQs for secondary exposure of the AW were calculated as the ratio of bromadiolone intake (exposure) to adjusted LD_{50} (toxicity) and are provided in **Table 5-2**; Based

on RQs that ranged between 0.015 – 0.019, Acute Endangered Species, Acute Restricted Use, and Acute Risk LOCs were not exceeded for AWs ranging in size from 18.6 g (*i.e.*, juvenile) to 322 g (*i.e.*, adult).

Table 5-1. Formula for Calculation of Weight-Adjusted Bromadiolone LD_{50}'s for the AW

$$\text{Adj. AW } LD_{50} = \text{Avian } LD_{50} \left(\frac{SW}{TW}\right)^{(x-1)}$$

Where:

Adj. AW LD_{50} = adjusted AW LD_{50} (mg/kg-bw)
Avian LD_{50} = endpoint reported from bird study (mg/kg-bw)
TW = body weight of tested animal (203 g bobwhite)
SW = body weight of the assessed animal (18.6, 101, and 322 g AW)
x = Mineau scaling factor for birds; EFED default 1.15

Table 5-2. Dose-based Acute RQs for Secondary Exposure of the AW

Species	Weight (g)	Bromadiolone Intake (mg a.i./kg-bw/day)[1]	Adjusted LD_{50} (mg a.i./kg-bw)[2]	Dose-based Acute RQs
AW	18.6	2.3	119	0.019
	195	2.7	169	0.016
	322	2.8	182	0.015

[1]See Tables 3-5 and 3-6 for derivation.
[2]See Table 5-1 for derivation with assessed body weights used in this table.

5.1.2.b. AW: Dietary-based Acute Risks from Secondary Exposure

As stated above, available literature concerning whole body bromadiolone residues in animals that ingested bromadiolone were considered for estimating AW secondary exposure. A bromadiolone whole body residue of 1.83 mg a.i./kg-carcass was used to estimate dietary exposure of the AW (**Section 3.3.2**).

Dietary toxicity of bromadiolone to the AW was estimated by the LC_{50} obtained from dietary studies (5 days on treated diet) with birds which are surrogates for reptiles. For a toxic response elicited from the dietary exposure route extended over several days, toxicity is measured as mg a.i./kg-diet.

Dietary-based acute RQs for secondary exposure of the AW were calculated as the ratio of bromadiolone whole body residue in carcass of prey (exposure) to LC_{50} (toxicity) and are provided in **Table 5-3**; Acute Endangered Species, Acute Restricted Use, and Acute Risk LOCs were not exceeded for the AW.

Table 5-3. Dietary-Based Acute RQ for Secondary Exposure of the AW

Species	Bromadiolone Whole body Residue in Carcass of Prey (mg a.i./kg-carcass)	LC_{50} (mg a.i./kg-diet)	Dietary-based Acute RQ
AW	1.83	37.6	0.049

5.1.2.c. AW: Chronic Risk from Secondary Exposure

Since no acceptable or supplemental data were available to characterize chronic toxicity to birds, which are surrogates for reptiles, a chronic RQ for secondary exposure of the AW could not be calculated.

5.1.3. Exposures in the Terrestrial Habitat: Mammals

Potential direct effects to the SMHM were evaluated by considering dose- and dietary-based EECs for primary exposure (**Sections 5.1.3.a**). Potential direct effects to the SJKF were evaluated by considering dose- and dietary-based EECs for secondary exposure (**Sections 5.1.3.b**).

Direct effects to mammals, particularly rodents, may result in indirect effects to the AW (due to a reduction in prey and habitat), SJKF (due to a reduction in prey), and SMHM (due to a reduction in rearing sites). Therefore, potential direct effects to mammals were evaluated by considering dose- and dietary-based EECs for both primary and secondary exposure (Sections **5.1.3.a** and **5.1.3.b**).

5.1.3.a. Mammals: Dose-based Acute and Chronic Risks from Primary Exposure

For primary exposure, it was assumed that bromadiolone bait is ingested by non-target animals, *i.e.*, rodent mammals, and evokes a toxic response. Estimates of bromadiolone intake (exposure) for the SMHM and rodent mammals were calculated as mg a.i./kg-bw/day in **Table 3-2**.

Acute toxicity of bromadiolone to rodent mammals was estimated by the LD_{50} obtained from single-dose gavage studies with mammals. For a toxic response elicited from the gavage exposure route, toxicity is measured as mg a.i./kg-bw. Chronic toxicity of bromadiolone to rodent mammals was estimated by the NOEL obtained from a developmental study with rats and measured as mg a.i./kg-bw. The LD_{50} and NOEL were adjusted for the weight of the assessed rodent mammals (10, 15, 35, 435 g) according to the formulas in **Table 5-4**.

Dose-based acute and chronic RQs for primary exposure of the SMHM and rodent mammals were calculated as the ratio of bromadiolone intake (exposure) to adjusted LD_{50} or NOEL (toxicity) and are provided in **Table 5-5**; the Acute Endangered Species, Acute Restricted Use, Acute Risk, and Chronic Risk LOCs were exceeded for primary exposure of the SMHM and all small rodent mammals.

Table 5-4. Formulas for Calculation of Weight-Adjusted Mammalian Bromadiolone LD_{50}'s and NOAELs

Adjusted mammalian LD_{50}

$$\text{Adj. } LD_{50} = LD_{50} \left(\frac{AW}{TW}\right)^{(0.25)}$$

Where:

Adj. LD_{50} = adjusted LD_{50} (mg/kg-bw)
LD_{50} = endpoint reported from mammal study (mg/kg-bw)
TW = body weight of tested animal (232 g rat, Wistar albino)
AW = body weight of assessed animal (10 g SMHM; 2300 g SJKF; 15, 135, and 435 g rodent mammal; 50, 1000, and 3000 g mammal)

Adjusted mammalian NOAEL

$$\text{Adj. NOAEL} = \text{NOAEL} \left(\frac{AW}{TW}\right)^{(0.25)}$$

Where:

Adj. LD_{50} = adjusted NOAEL (mg/kg-bw)
NOAEL = endpoint reported from mammal study (mg/kg-bw)
TW = body weight of tested animal (215g rat, Sprague-Dawley)
AW = body weight of assessed animal (10g SMHM; 2300 SJKF; 15g, 135g, and 435g rodent mammal; 50g, 1000g, 3000g mammal)

Table 5-5. Dose-based Acute and Chronic RQs for Primary Exposure of the SMHM and Small (Rodent) Mammals

Species or Taxa	Bromadiolone Concentration in Bait (mg a.i./ kg-bait)	Weight (g)	Bromadiolone Intake (mg a.i./ kg-bw/day)[1]	Adjusted LD_{50} (mg a.i./ kg-bw)[2]	Dose-based Acute RQ[3]	Adjusted NOAEL (mg a.i./kg-bw)[2]	Dose-based Chronic RQ[4]
SMHM	50	10	11.4	0.27	**42***	0.016	**711****
Rodent mammals	50	15	9.5	0.30	**32***	0.018	**530****
		35	6.6	0.37	**18***	0.022	**300****
		435	2.2	0.70	**3***	0.042	**53****

[1]See Table 3-2 and 3-3 for derivation.
[2]See Table 5-4 for derivation.
[3]Bolded * RQs exceed Acute Endangered Species, Acute Restricted Use, and Acute Risk LOCs.
[4]Bolded ** RQs exceed Chronic Risk LOC.

5.1.3.b. Mammals: Dietary-based Acute Risks from Primary Exposure

Dietary exposure of the SMHM and rodent mammals was estimated as the concentration of bromodiolone in the bait itself – 50 mg a.i./kg-bait.

Dietary toxicity of bromadiolone to the SMHM and rodent mammals was estimated by the LC_{50} obtained from dietary studies (5 days on treated diet) with rats. For a toxic response elicited from the dietary exposure route extended over several days, toxicity is measured as mg a.i./kg-diet

Dietary-based acute RQs for primary exposure of the SMHM and rodent mammals were calculated as the ratio of concentration of bromadiolone in bait (exposure) to LC_{50} (toxicity) and are provided in **Table 5-6**; Acute Endangered Species, Acute Restricted Use, and Acute Risk LOCs were exceeded for the SMHM and rodent mammals.

Table 5-6. Dietary-Based Acute RQs for Primary Exposure of the SMHM and Small (Rodent) Mammals

Species	Bromadiolone Concentration in bait (mg a.i./kg-bait)	LC_{50} (mg a.i./kg-diet)	Dietary-based Acute RQs[1]
SMHM	50	0.94	**53***
Rodent mammals	50	0.94	**53***

[1]Bolded * RQs exceed Acute Endangered Species, Acute Restricted Use, and Acute Risk LOCs.

5.1.3.c. Mammals: Dose-based Acute and Chronic Risks from Secondary Exposure

To determine secondary exposure EECs for mammals, available literature concerning bromadiolone residue concentrations in animals that ingested bromadiolone were considered. For this assessment, a residue concentration of 1.83 mg a.i./kg-carcass was used to estimate bromadiolone intake (*i.e.*, exposure) on a dose basis as mg a.i./kg-bw/day (**Table 3-5**).

As in primary exposure risk estimation, the LD_{50} and NOEL were adjusted for the weight of the assessed mammals (2300, 50, 1000, 3000 g). Heavier body weights for mammals were used to better represent predators and scavengers which tend to be larger individuals.

Dose-based acute and chronic RQs for secondary exposure of the SJKF and predator/scavenger mammals were calculated as the ratio of bromadiolone intake (exposure) to adjusted LD_{50} or NOEL (toxicity) and are provided in **Table 5-7;** the Acute Endangered Species Risk and the Chronic Risk LOCs were exceeded for secondary exposure of the SJKF. Acute Endangered Species Risk, Acute Restricted Use, Acute Risk, and Chronic Risk LOCs were exceeded for secondary exposure of 50-g mammals; Acute Restricted Use, Acute Risk, and Chronic Risk LOCs were exceeded for secondary exposure of 1000-g mammals; and Acute Risk and Chronic Risk LOCs were exceeded for secondary exposure of 3000-g mammals.

Table 5-7. Dosed-based SJKF and Predator/Scavenger Mammal Acute and Chronic RQs for Secondary Exposure

Species or Taxa	Weight (g)	Acute Bromadiolone Intake (mg a.i./kg-bw/day)[1]	Adjusted LD_{50} (mg a.i./kg-bw)[2]	Dose-based Acute RQ[3]	Adjusted NOAEL (mg a.i./kg-bw)[2]	Dose-based Chronic RQ[4]
SJKF	2300	0.3	1.06	**0.28***	0.063	**4***
Mammals	50	0.7	0.41	**1.71*****	0.024	**29***
	1000	0.4	0.86	**0.46****	0.051	**8***
	3000	0.3	1.14	**0.26***	0.068	**4***

[1]See Table 3-5 and 3-6 for derivation.
[2]See Table 5-4 for derivation.
[3]Bolded * RQs exceed Acute Endangered Species Risk LOC only; bolded ** RQs exceed Acute Endangered Species and Acute Restricted Use Risk LOCs; bolded *** RQs exceed Acute Endangered Species, Acute Restricted Use, and Acute Risk LOCs.
[4]Bolded * RQs exceed Chronic Risk LOC.

5.1.3.d. Mammals: Dietary-based Risks from Secondary Exposure

Similar to estimating dietary exposure of the AW, a bromadiolone whole body residue of 1.83 mg a.i./kg-carcass was used to estimate dietary exposure of mammals via consumption of prey that ingested bromadiolone bait (**Section 3.3.2**).

Dietary toxicity of bromadiolone to mammals was estimated by the LC_{50} obtained from dietary studies (5 days on treated diet) with rats. For a toxic response elicited from the dietary exposure route extended over several days, toxicity is measured as mg a.i./kg-diet.

Dietary-based acute RQs for secondary exposure of the SJKF and predator/scavenger mammals were calculated as the ratio of bromadiolone whole body residue in carcass of prey (exposure) to LC_{50} (toxicity) and are provided in **Table 5-8**; Acute Endangered Species, Acute Restricted Use, and Acute Risk LOCs were exceed for the SJKF and predator/scavenger mammals.

Table 5-8. Dietary-Based Acute RQs for Secondary Exposure of the SJKF and Predator/Scavenger Mammals

Species	Bromadiolone Residue in Carcass of Prey (mg a.i./kg-carcass)	LC_{50} (mg a.i./kg-diet)	Dietary-based Acute RQs[1]
SJKF	1.83	0.94	**2***
Mammals	1.83	0.94	**2***

[1]Bolded * RQs exceed Acute Endangered Species, Acute Restricted Use, and Acute Risk LOCs.

5.1.4. Exposures in the Terrestrial Habitat: Terrestrial Invertebrates

Although invertebrates may be exposed to bait, they represent only one type of prey item among many for the AW, SMHM, and SJKF. Therefore, indirect effects to the AW, SMHM, and SJKF via reduction in terrestrial invertebrate food items are expected to be negligible.

5.1.4.a. Exposures in the Terrestrial Habitat: Terrestrial Plants

Since bromadiolone is not applied directly onto plants, and because so little is expected to leach from the bait and be available for plant uptake, exposure is expected to be minimal. Therefore, the Agency presumes that direct risks to terrestrial plants are low. Based on this analysis, indirect effects to the AW, SMHM, and SJKF from exposure of terrestrial plants to bromadiolone are expected to be negligible.

5.1.5. Primary Constituent Elements of Designated Critical Habitat

For bromadiolone 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 spatial extent of potential effects is discussed for each of the listed species including any potential overlap between areas where potential usage may result in LAA effects and areas where species are expected to occur (including any designated critical habitat). The final No Effect/May Affect determination is made after the spatial analysis is completed at the end of the risk description for each species. If there is no overlap of the species habitat and occurrence sections with the Potential Area of LAA Effects a No Effect determination is made.

If the RQs presented in the Risk Estimation (**Section 5.1**) show no direct or indirect effects for the assessed species, and no modification to PCEs of the designated critical habitat, a preliminary "no effect" determination is made, based on bromadiolone'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 bromadiolone. A preliminary effects determination of "may effect" was made for each of the assessed species (AW, SMHM, SJKF) and for the critical habitats of the AW. A summary of the risk estimation results are provided in **Table 5-9** for the assessed taxa that may directly or indirectly affect the listed species, and in **Table 5-10** for the PCEs of their designated critical habitat.

Table 5-9. Risk Estimation Summary for Bromadiolone - Direct and Indirect Effects

Taxa	LOC Exceedances (Yes/No)	Description of Results of Risk Estimation	Assessed Species Potentially Affected
Reptiles (AW)	Listed Species (No)	No acute LOCs for the AW were exceeded. Chronic RQs could not be calculated.	Direct Effects: none
Mammals	Non-listed Species (Yes)	Risks of acute and chronic effects via consumption of bait (*i.e.*, primary exposure) and consumption of prey that ingested bait (*i.e.*, secondary exposure).	Indirect Effects: AW, SMHM, SJKF
	Listed Species (Yes)	Risks of acute and chronic effects via consumption of bait (*i.e.*, primary exposure) or consumption of prey that ingested bait (*i.e.*, secondary exposure).	Direct Effects: SMHM, SJKF
Birds	RQs were not calculated for non-listed species	There is no complete exposure pathway for birds for estimation of risk.	Indirect Effects: None
Terrestrial Invertebrates	RQs were not calculated for non-listed species	Exposure is expected to be minimal.	Indirect Effects: none
Terrestrial Plants	RQs were not calculated for non-listed species	Exposure is expected to be minimal..	Indirect Effects: none
Aquatic Plants	RQs were not calculated for non-listed species	Exposure is expected to be minimal..	Indirect Effects: none

Table 5-10. Risk Estimation Summary for Bromadiolone – Effects to Designated Critical Habitat (PCEs) for the AW.

Taxa	LOC Exceedances (Yes/No)	Description of Results of Risk Estimation
Reptiles (AW)	Listed Species (No)	No acute LOCs for the AW were exceeded. Chronic RQs could not be calculated.
Mammals	Non-listed Species (Yes)	Risks of acute and chronic effects via consumption of bait (*i.e.*, primary exposure) and consumption of prey that ingested bait (*i.e.*, secondary exposure).
	Listed Species (Yes)	Risks of acute and chronic effects via consumption of bait (*i.e.*, primary exposure) or consumption of prey that ingested bait (*i.e.*, secondary exposure).
Birds	RQs were not calculated for non-listed species	There is no complete exposure pathway for birds for estimation of risk.
Terrestrial Invertebrates	RQs were not calculated for non-listed species	Exposure is expected to be minimal
Terrestrial Plants	RQs were not calculated for non-listed species	Exposure is expected to be minimal

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

The criteria used to make determinations that the effects of an action are "not likely to adversely affect" the assessed species or modify its designated critical habitat include the following:

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

A description of the risk and effects determination for each of the established assessment endpoints for the assessed species and their designated critical habitat is provided in **Sections 5.2.1** through **5.2.3**. The effects determination section for each listed species assessed will follow a similar pattern. Each will start with a discussion of the potential for direct effects, followed by a discussion of the potential for indirect effects. These discussions do not consider the spatial analysis. For those listed species that have designated critical habitat, the section will end with a discussion on the potential for modification to the critical habitat from the use of bromadiolone. Finally, a discussion of any potential overlap between areas of concern and the species (including any designated critical habitat) is presented. If there is no overlap of the species habitat and occurrence sections with the Potential Area of LAA Effects a No Effect determination is made.

5.2.1. Alameda Whipsnake

5.2.1.a. Direct Effects

The AW is directly exposed to bromadiolone through consumption of prey that ingested bromadiolone bait. However, Acute Endangered Species, Acute Restricted Use, and Acute Risk LOCs for secondary exposure were not exceeded for juvenile- or adult-sized AWs (**Tables 5-2** and **5-3**). The LD_{50} used to calculate the RQs was based on granivorous species. These species prefer to eat a relatively consistent quantity of food on a daily basis. Carnivores and scavengers

may consume a large meal (an entire carcass) once every several days. Given this feeding strategy, the AW may get a larger single-dose exposure of bromadiolone than is currently estimated in the risk quotient methodology. Therefore, the acute RQs for bromadiolone secondary exposure may underestimate risk to the AW.

Bromadiolone is a second-generation anticoagulant. This class of rodenticide tends to be retained in primary consumers after a single feeding and death occurs 5 to 10 days later. The toxicity pattern observed in the avian acute oral toxicity studies and mammalian toxicity studies corroborates this finding. In the acute single oral dose study (MRID 14379), all birds except in one instance died within 9 days of test initiation. In the acute dietary feeding study (MRID 14380), bobwhite quail were exposed to bromadiolone for 5 days and monitored for signs of toxicity for 30 days. Mortality coincided with internal and external hemorrhaging for the majority of birds and except for one incidence (10 mg a.i./kg diet, Day 21), all mortalities occurred within the first 15 days of the test. Mammals exhibited similar mortality patterns. Rats were given a single oral dose in one acute study (MRID 41900001) and observed for 21 days. All rats died by day 10, with the exception of one rat that died on day 15. In a second acute oral test, rats were observed for 14 days post-gavage. For those rats that died, death occurred between days 5 and 12 (MRID 241703). Risk for secondary exposure of the AW is high when bromadiolone-intoxicated prey items remain alive. The whole body residue value for primary consumers was based on water voles consuming bromadiolone for 1 day (MRID 48590803). Modeling in the water vole study indicated that bromadiolone concentrations in water voles are highest between 3.3 and 6.5 days after treatment. However, the maximum whole body value was not used in this assessment because it was only presented in a graph rather than specified in the text. Thus, whole body residues could have been higher than currently estimated if data from days 3 through 7 were available. In this respect, risk from secondary exposure of the AW to bromadiolone may be greater than what is estimated by the RQ methodology.

Bromadiolone intoxicated birds (surrogates for reptiles) exhibited sublethal signs of toxicity in acute oral and subacute dietary studies. Mortality and sublethal effects were sometimes seen at all test concentrations in the avian acute oral and dietary studies (*e.g.*, MRID 143280) and thus the extent of sublethal effects in unknown. Avian sublethal effects reported in the acute toxicity studies included lethargy, depression, reduced reaction to external stimulus, wing droop, loss of coordination, lower limb weakness, prostrate posture and loss of righting reflex, hematomas (dermal, subcutaneous, and thoracic), hemorrhaging, loss of water consumption, anorexia, blooding droppings, and blood filled cysts. In a secondary hazard study, owls exhibited increased blood coagulation time for 10 days after secondary exposure to bromadiolone, after which none of them died (n=18; MRID 48590804).
Collectively, these documented sublethal effects in birds, and by extension herpetofauna, indicate that the AW could be at risk from sublethal effects of bromadiolone via consumption of prey that ingested bromadiolone bait.

The Agency uses the probit dose-response relationship as a tool for providing additional information on the potential for acute direct effects to individual listed species and aquatic animals that may indirectly affect the listed species of concern (USEPA, 2004a). Effects in this instance refer to acute lethality. For these calculations, acute RQs calculated for secondary exposure of the AW based on a range of weights of the consuming individual (*i.e.*, dose-based

RQs) and on the dietary concentration of bromadiolone (*i.e.*, dietary-based RQ) were used. The probabilities of an individual effect to the AW using dose-based RQs were 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 (**Table 4-1**; MRID 143279). The probability of an individual effect to the AW using the dietary-based RQ was calculated using a probit slope of 0.83 with 95% confidence bounds (0.43, 1.24) (**Table 4-1**; MRID 143208). The probabilities of an individual effect are presented in **Table 5-11** below. The calculations indicate that the probability of an individual AW mortality event is low for dose-based RQs (1 in 2.10×10^{14} to 1 in 8.86×10^{15}). In contrast, the probability of an individual AW mortality event is high (*i.e.*, 1 in 7) for dietary-based acute RQs indicating that although acute LOCs for secondary exposure of the AW were not exceeded, there is still potential for direct acute effects to the AW from registered uses of bromadiolone.

Table 5-11. Individual Chance of Effect to the AW for Acute Secondary Exposure RQs

Species	Weight (g)	Acute Dose-based RQ[1]	Acute Dietary-based RQ[2]	Individual Chance of Effect (Upper and Lower Bounds)
AW	18.6	0.019	n/a	1 in 2.10×10^{14} (1 in 3.47×10^{3} to 1 in 5.03×10^{53})
	195	0.016	n/a	1 in 3.13×10^{15} (1 in 6.09×10^{3} to 1 in 2.17×10^{58})
	322	0.015	n/a	1 in 8.86×10^{15} (1 in 7.56×10^{3} to 1 in 1.34×10^{60})
	n/a	n/a	0.049	1 in 7.22 (1 in 3.49 to 1 in 19.2)

n/a = not applicable
[1]based on weight of the consuming individual
[2]based on a dietary concentration of bromadiolone (1.83 mg a.i./kg-carcass)

5.2.1.b. Indirect Effects

Indirect effects to the AW may occur through the potential for bromadiolone use to adversely affect small mammals that serve as prey or provide burrows for the AW. For small mammalian prey ingesting bait directly, acute dose- and dietary based RQs for primary exceeded the Acute Endangered Species, Acute Restricted Use, and Acute Risk LOCs, and chronic dose-based RQs exceeded the Chronic Risk LOC (**Tables 5-5** and **5-6**).

The probabilities of an individual effect to small mammals using dose-based RQs were 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 (**Table 4-1**; MRID 241703). The probability of an individual effect to small mammals using the dietary-based RQ was calculated using a probit slope of 5.20 with 95% confidence bounds (1.91, 8.50) (**Table 4-1**; Teeters, 1981). The calculations for the probability of mortality of an individual small mammal consuming bromadiolone bait directly is essentially 1 for all RQs (**Table 5-12**).

Table 5-12. Individual Chance of Effect to Small (Rodent) Mammals for Acute Primary Exposure RQs

Taxon	Weight (g)	Acute Dose-based RQ[1,3]	Acute Dietary-based RQ[2,3]	Individual Chance of Effect (upper and lower bounds)
Rodent Mammals	15	**32***	n/a	1 in 1 (1 in 1 to 1 in 1)
	35	**18***	n/a	1 in 1 (1 in 1 to 1 in 1)
	1000	**3***	n/a	1 in 1 (1 in 1 to 1 in 1.2)
	n/a	n/a	**53***	1 in 1 (1 in 1 to 1 in 1)

n/a = not applicable
[1]based on weight of the consuming individual
[2]based on a dietary concentration of bromadiolone (50 mg a.i./kg-diet)
[3]Bolded * RQs exceed Acute Endangered Species, Acute Restricted Use, and Acute Risk LOCs

Incidents of mortality from bromadiolone use with likelihood categories of „highly probable' to „possible' have been reported for the following small mammalian species: squirrel, hare, badger, opossum, rabbit, stone marten, and skunk (**Appendix H**). These incidents support the conclusion that adverse effects to small mammals via primary exposure are expected at levels of concern.

Risks of bromadiolone use to birds, reptiles, and terrestrial invertebrates that serve as AW prey and terrestrial plants that serve as AW habitat were not evaluated. Terrestrial plants are unlikely to be exposed to bromadiolone, and thus indirect effects to the AW via the potential of bromadiolone to adversely affect plants are expected to be insignificant. Although exposure of birds, reptiles, and terrestrial invertebrates is possible, indirect effects to the AW via the potential of bromadiolone to adversely affect these taxa are also expected to be insignificant.

5.2.1.c. Modification of Designated Critical Habitat

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. Because direct and indirect effects may potentially occur to the AW, as discussed above, there is a potential for the modification of designated critical habitat.

5.2.1.d. Spatial Extent of Potential Effects

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

An alternative type of spatial analysis was conducted to characterize the potential use of bromadiolone bait products within the region where the AW may occur. Since outdoor use of bromadiolone bait is restricted to within 50 feet of buildings, the extent of bromadiolone use is expected to be highly correlated with human development. Therefore, a spatial analysis was 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 AW and its critical habitat. The results of this spatial analysis are shown in **Figure 5-1** for the AW.

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

5.2.1.a. Effects Determinations

The results of this risk assessment indicate that the applications of bromadiolone as currently registered place the AW at risk from sublethal effects. Additionally, indirect effects to AW prey base and habitat (*i.e.*, burrows) may occur from bromadiolone effects to small mammals. Finally, the AW occurs in areas where bromadiolone bait may be intensively used. Therefore, the Agency makes a "**May Affect**" and "**Likely to Adversely Affect**" determination for the AW, and a "**Habitat Modification**" determination for AW designated critical habitat based on direct and indirect effects to the AW and effects to AW critical habitat PCEs.

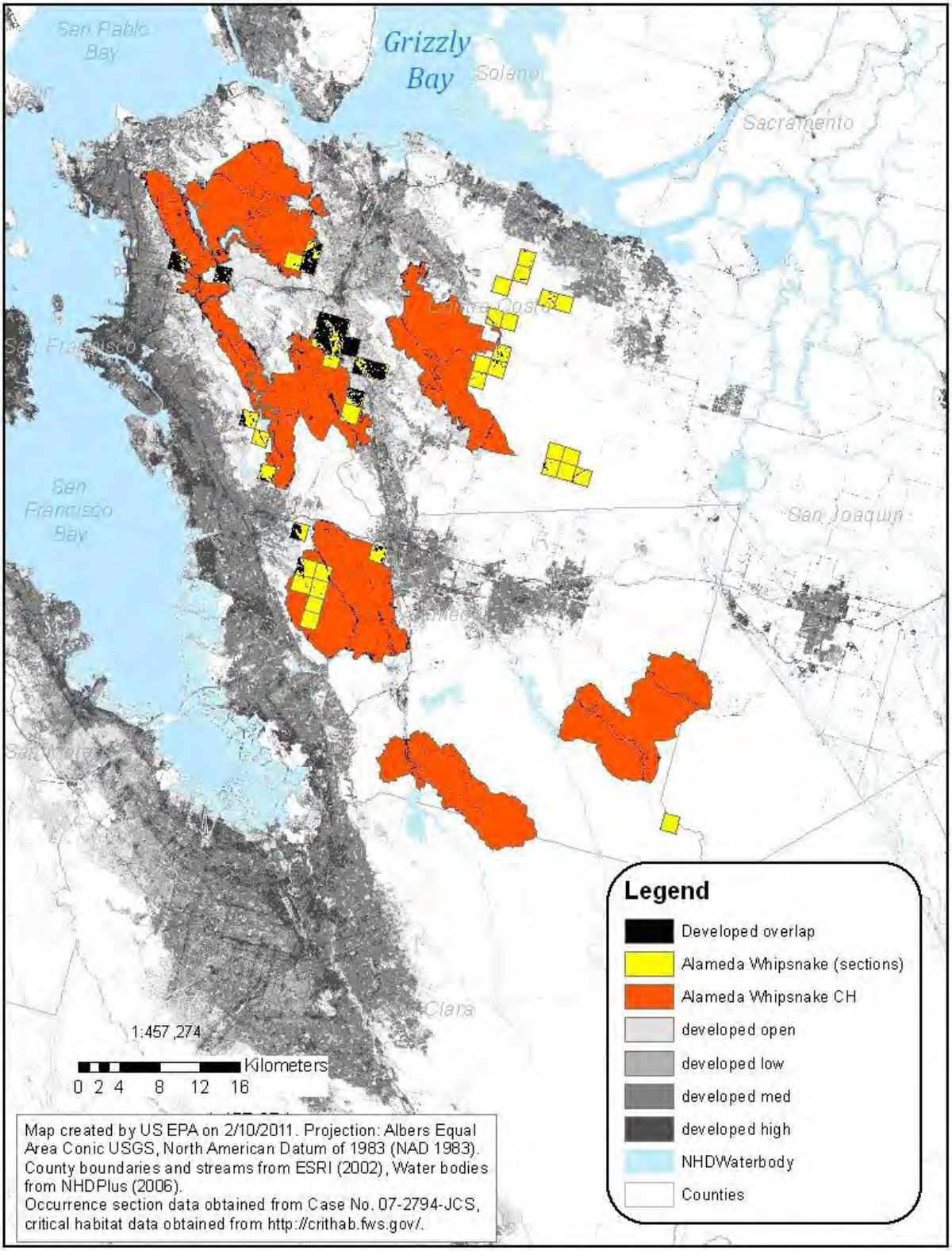

Figure 5-1. Map showing sections of the Alameda Whipsnake, and its critical habitat (CH), in relation to the intensity of human development

5.2.2. Salt Marsh Harvest Mouse

5.2.2.a. Direct Effects

The SMHM is at risk from use of bromadiolone. The SMHM may directly consume bromadiolone bait as the bait is formulated specifically as grain for attracting rodents, and SMHM's diet has a grain-based component (*e.g.*, seeds). Dose- and dietary based acute RQs for primary exposure of the SMHM exceeded the Acute Endangered Species, Acute Restricted Use, and Acute Risk LOCs (**Tables 5-5** and **5-6**). Therefore, there is potential for mortality to the SMHM through consumption of bait for a single day.

In the rat developmental study (MRID 92196014), significant mortality of dams occurred at exposure levels greater than 0.035 mg a.i./kg-bw. Based on a bait concentration of 50 mg a.i./kg-bait and an adjusted NOAEL of 0.016 mg a.i./kg-bw, a chronic RQ of 711 was calculated for the SMHM, which exceeds the Chronic Risk LOC of 1. Other observed effects in this study included vaginal bleeding, hypotonicity, and pale eyes at the dose level of 0.070 mg a.i./kg-bw, and vaginal bleeding was often a precursor to death. Therefore, there is potential for mortality to the SMHM through consumption of bait for periods of time longer than a day.

Incidents of mortality from bromadiolone use with likelihood categories of „highly probable' to „possible' have been reported for the following non-predator/non-scavenger mammals: squirrels, rabbits, hares, and stone martens (**Appendix H**). These incidents support the conclusion that adverse effects to the SMHM via primary exposure are expected at levels of concern,

For individual chance of effect calculations, dose- and dietary-based acute RQs for primary exposure of the SMHM were used. The probability of an individual effect to the SMHM 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 (**Table 4-1**; MRID 241703). The probability of an individual effect to the SMHM using the dietary-based RQ was calculated using a probit slope of 5.20 with 95% confidence bounds (1.91, 8.50) (**Table 4-1**; Teeters, 1981). The estimated chance of an individual acute mortality of the SMHM at a dose-based acute RQ of 42 or a dietary-based acute RQ of 53 is 1 in 1 with respective upper and lower bounds of 1 in 1 to 1 in 1. These results indicate that the probability of an individual mortality occurrence is high and that bromadiolone has the potential to directly affect the SMHM via primary exposure.

5.2.2.b. Indirect Effects

Direct effects of bromadiolone use to mammals/birds, terrestrial invertebrates, and terrestrial/ aquatic plants may result in indirect effects to the SMHM due to a reduction in rearing sites, reduction in prey, and reduction in food and/or alteration in habitat, respectively.

Potential direct effects of bromadiolone use to birds were not evaluated because all outdoor uses for bromadiolone require that bait be placed in bait stations. This requirement should preclude birds that provide rearing sites for the SMHM (*e.g.*, passerine species) from directly

ingesting bromadiolone bait. Additionally, only birds consuming prey that ingested bromadiolone bait, which do not generally provide rearing sites to the SMHM, are expected to be at risk from secondary exposure. Thus indirect effects to the SMHM from avian exposure to bromadiolone are expected to be insignificant.

Risks of bromadiolone use to terrestrial invertebrates that serve as SMHM prey and plants (terrestrial and aquatic) that serve as SMHM habitat were not evaluated. Terrestrial plants are unlikely to be exposed to bromadiolone, and thus indirect effects to the SMHM via the potential of bromadiolone to adversely affect plants are expected to be insignificant. Although exposure of terrestrial invertebrates is possible, indirect effects to the SMHM via the potential of bromadiolone to adversely affect this taxon are also expected to be insignificant.

Potential direct effects of bromadiolone to small mammals that provide rearing sites for the SMHM were evaluated. For small mammals that consume bait directly, dose- and dietary based acute and chronic RQs exceeded Acute Endangered Species, Acute Restricted Use, Acute, and Chronic Risk LOCs (**Tables 5-5** and **5-6**). In addition, the probability of an individual mortality occurrence is very high for small mammals ingesting bromadiolone bait directly (see **Table 5-12**). Finally, incidents of mortality from bromadiolone use with likelihood categories of „highly probable' to „possible' have been reported for the following mammalian species: squirrel, hare, badger, opossum, rabbit, stone marten, and skunk (**Appendix H**). These incidents support the conclusion that adverse effects to small mammals via primary exposure are expected at levels of concern. Overall, 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 Potential 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 bromadiolone is a vertebrate pest control agent that may be used in a wide variety of urban and non-urban areas, the spatial extent of bromadiolone cannot be limited to defined areas. The Agency assumes that bromadiolone 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 bromadiolone.

An alternative type of spatial analysis was conducted to characterize the potential use of bromadiolone bait products within the region where the SMHM may occur. Since outdoor use of bromadiolone bait is restricted to within 50 feet of buildings, the extent of bromadiolone use is expected to be highly correlated with human development. Therefore, a spatial analysis was conducted in which the occurrence locations of the SMHM were overlaid with a representation of human development. The "developed" land cover classes of the NLCD were used to represent the intensity of human development. These land cover classes were displayed with gray shading, with darker grays representing areas of more intense development. This layer was overlaid on

segments and points that represent the location of the SMHM. The results of this spatial analysis are shown in **Figure 5-2** for the SMHM.

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

5.2.2.a. Effects Determination

The results of this risk assessment indicate that the registered uses of bromadiolone pose direct acute and chronic risks to the SMHM based on primary exposure. Applications of bromadiolone as currently registered also place the SMHM at risk from sublethal effects such as polydipsia, hemorrhaging, anorexia, exhaustion, and hypotonicity. Furthermore, indirect effects to the SMHM, in the form of a reduction in rearing sites, may occur from effects of bromadiolone use to small mammals. Finally, the SMHM occurs in areas where bromadiolone bait may be intensively used. Therefore, the Agency makes a "**May Affect**" and "**Likely to Adversely Affect**" determination for the SMHM based on direct and indirect effects to the SMHM.

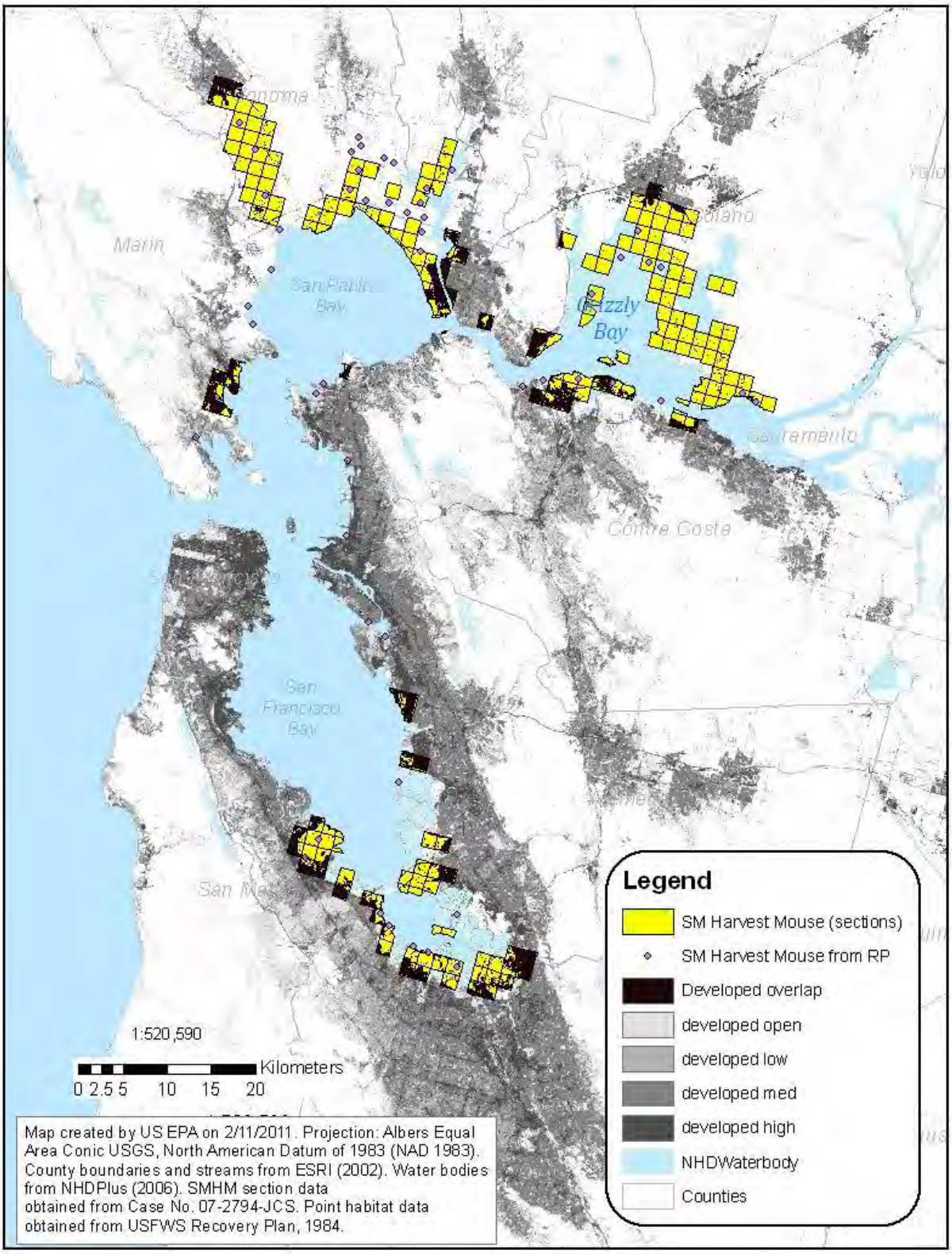

Figure 5-2. Map showing occurrences of the Salt Marsh Harvest Mouse in relation to the intensity of human development

5.2.3. San Joaquin Kit Fox

5.2.3.a. Direct Effects

The SJKF is at risk from use of bromadiolone. The dose- and dietary-based acute RQs calculated in the risk estimation for secondary exposure of the SJKF to bromadiolone exceeded the Acute Endangered Species LOC (**Tables 5-7** and **5-8**). Therefore, there is potential for mortality to the SJKF through consumption of prey that ingested bromadiolone bait. Because a chronic study was not available, a no-effect level based on mortality and other signs of toxicity from a developmental study with rats was used instead for calculations of a dose-based chronic RQ. The dosed-based chronic RQ exceeded the Chronic LOC (**Table 5-7**) indicating the potential for chronic risk to the SJKF due to bromadiolone use.

Acute toxicity studies on numerous mammals have been conducted with bromadiolone. Sublethal effects observed in these toxicity studies include internal hemorrhage, extreme physical weakness, pale mucous membranes, difficulty breathing, impaired equilibrium, reduced responsiveness to noise, reduced weight gain, swelling, hematoma, piloerection, polydipsia, bleeding, hemorrhaging, anorexia, and exhaustion. Secondary toxicity feeding studies demonstrate that both the mongoose and coyote are vulnerable to bromadiolone poisoning from consuming prey that ingested bromadiolone (rats and squirrels, respectively). These adverse effects increase the vulnerability of the SJKF and may deteriorate survival and reproductive capabilities. These documented sublethal effects support the conclusion that bromadiolone has the potential to adversely affect the SJKF via secondary exposure.

Numerous incidents of mammalian toxicity have been reported to the Agency (see **Section 4.4.2** and **Appendix H**). These incidents provide one piece of evidence that both primary and secondary exposures of bromadiolone are occurring to non-target organisms, as well as evidence that exposures of bromadiolone are occurring to the SJKF. Three of the incidents involved the kit fox (*Vulpes mascotis*) and two others involved either the red fox or an unspecified species of fox. Two of these incidents entailed kit fox consumption of bromadiolone although the outcome of ingestion, such as mortality or other signs of intoxication, is unknown and/or not reported. A third incident concerned the reported mortality of 36 kit foxes due to bromadiolone secondary exposure. The incident occurred in San Joaquin, CA, and was rated ‚probable.' The date of the incident is unknown, along with the legality of the implicated bromadiolone usage. In 1991, 22 individual red foxes (*Vulpes fulva*) were reported dead from ingestion of bromadiolone in an incident rated ‚probable.'

For individual chance of effect calculations, dose- and dietary-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 (**Table 4-1**; MRID 241703). The probability of an individual effect to the SJKF using the dietary-based RQ was calculated using a probit slope of 5.20 with 95% confidence bounds (1.91, 8.50) (**Table 4-1**; Teeters, 1981). The estimated chance of an individual acute mortality of the SJKF at a dose-based acute RQ of 4 or a dietary-based acute RQ of 2 is essentially 1 in 1 with respective upper and lower bounds of 1

in 1 to 1 in 1. These results indicate that the probability of an individual mortality occurrence is high and that bromadiolone 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 bromadiolone to adversely affect the number and quality of mammalian prey items. For non-listed mammalian prey consuming bait directly, acute dose-and dietary based RQs and chronic dose-based RQs exceeded Acute and Chronic LOCs (**Tables 5-6** and **5-7**). The probability of mortality for an individual small mammal consuming bromadiolone bait directly is essentially 1 (**Table 5-12**). Incidents of mortality from bromadiolone use with likelihood categories of „highly probable' to „possible' have been reported for the following species: squirrel, hare, badger, opossum, rabbit, and skunk. These incidents support the conclusion that adverse effects to mammalian prey item are expected at levels of concern for the SJKF.

For non-listed mammalian prey consuming other animals that have ingested bromadiolone bait, acute dose-and dietary based RQs and chronic dose-based RQs exceeded Acute and Chronic LOCs (**Tables 5-7** and **5-8**). The probability of an individual effect to predator/scavenger mammals 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 (**Table 4-1**; MRID 241703). The probability of an individual effect to predator/scavenger mammals using the dietary-based RQ was calculated using a probit slope of 5.20 with 95% confidence bounds (1.91, 8.50) (**Table 4-1**; Teeters, 1981). For acute dose-based RQs, the probability of mortality for an individual 50, 1000, and 3000 g scavenger/predator mammal from consuming prey that ingested bromadiolone bait is 1 in 1, 1 in 15, and 1 in 236, respectively (**Table 5-13**). For an acute dietary-based RQ of 2, the probability of mortality for an scavenger/predator mammal from consuming prey that ingested bromadiolone bait is 1 in 1 (**Table 5-13**). These high to moderate probabilities of individual mortality provide further support for adverse effects of bromadiolone to SJKF prey via secondary exposure.

Table 5-13. Individual Chance of Effect to Predator/Scavenger Mammalian Prey of the SJKF for Acute Secondary Exposure RQs

Taxon	Weight (g)	Acute Dose-based RQ[1,3]	Acute Dietary-based RQ[2,3]	Individual Chance of Effect (upper and lower bounds)
Mammals	50	**1.71***	n/a	1 in 1 (1 in 1 to 1 in 1.5)
	1000	**0.46***	n/a	1 in 15 (1 in 4 to 1 in 832)
	3000	**0.26***	n/a	1 in 236 (1 in 8 to 1.43 x 10^7 in)
	n/a	n/a	**2***	1 in 1 (1 in 1 to 1 in 1.4)

n/a = not applicable
[1]based on weight of the consuming individual
[2]based on a dietary concentration of bromadiolone (1.83 mg a.i./kg-carcass)
[3]Bolded * RQs exceed Acute Endangered Species Risk LOC.

Incidents of bromadiolone exposure to predators and scavengers have been reported for "ingestion" and "secondary exposure". In addition to reports for foxes as described above, incidents of mortality have been reported for the opossum (n=1) and coyote (n=2) with a confidence rating of "highly probable"; for the stone marten (n=1), lynx (n=1), badger (n=1), and opossum (n=1) with a confidence rating of "probable"; and for bobcat (n=1) and skunk (n=2)

with a confidence rating of "possible". Because adverse effects from secondary exposure are expected to mammals based on RQ and individual chance of effect calculations, and because there are incidents that demonstrate mortality can occur from secondary exposure, indirect effects to the SJKF at levels of concern are likely to occur from bromadiolone use.

The SJKF consumes lizards, birds, and terrestrial invertebrates and uses terrestrial plants as habitat. Risks of bromadiolone use to herpetofauna, birds, terrestrial invertebrates and plants were not evaluated. Terrestrial plants are unlikely to be exposed to bromadiolone, and thus indirect effects to the SJKF via the potential of bromadiolone to adversely affect plants are expected to be insignificant. Although exposure of terrestrial invertebrates, birds, and herpetofauna is possible, indirect effects to the SJKF via the potential of bromadiolone to adversely affect these prey items are expected to be insignificant compared to indirect effects to the SJKF from reductions in mammalian prey.

5.2.3.c. Spatial Extent of Potential 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 bromadiolone is a vertebrate pest control agent that may be used in a wide variety of urban and non-urban areas, the spatial extent of bromadiolone cannot be limited to defined areas. The Agency assumes that bromadiolone 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 AW occurs are assumed to lie within the potential use area of bromadiolone.

An alternative type of spatial analysis was conducted to characterize the potential use of bromadiolone bait products within the region where the SJKF may occur. Since outdoor use of bromadiolone bait is restricted to within 50 feet of buildings, the extent of bromadiolone use is expected to be highly correlated with human development. Therefore, a spatial analysis was conducted in which the occurrence locations of the SJKF were overlaid with a representation of human development. The "developed" land cover classes of the NLCD were used to represent human development. This layer was overlaid on segments and points that represent the location of the SJKF. The results of this spatial analysis are shown in **Figure 5-3** for the SJKF.

Areas with higher human development are expected to represent areas where bromadiolone bait would be more intensively used and where the SJKF would be most vulnerable to exposure to bromadiolone. On the scale displayed, the map has limited usefulness for identifying specific areas of vulnerability. However, the map does show that the SJKF occurs in a region of California where development is widespread, and a significant portion of the range of the species occurs in areas with development. Since the SJKF occurs in areas where bromadiolone bait may be intensively used, the SJKF would be susceptible to exposure to bromadiolone.

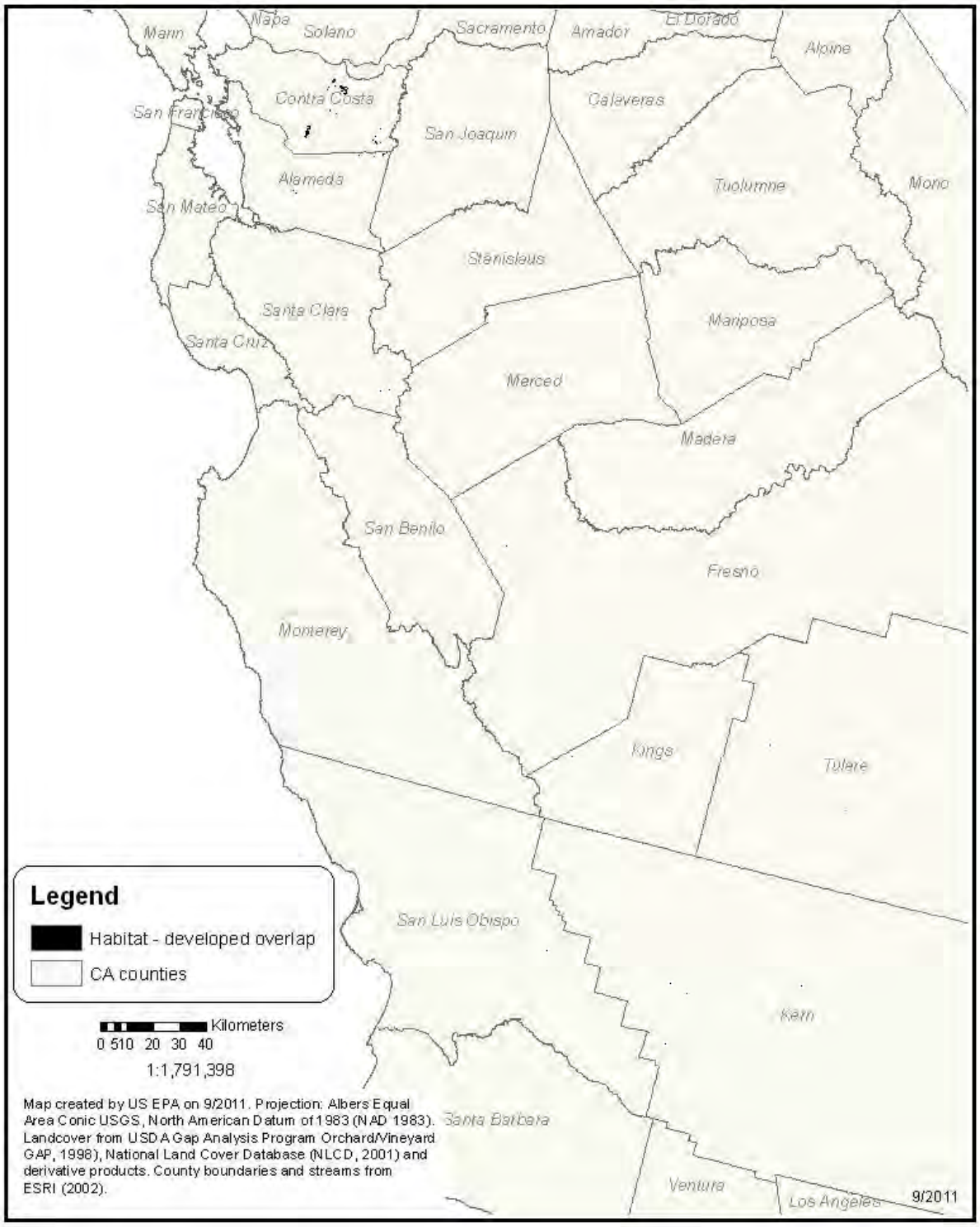

Figure 5-3. Map showing occurrences of the San Joaquin Kit Fox in relation to human development

5.2.3.d. Effects Determination

The results of this risk assessment indicate that the registered uses of bromadiolone pose direct acute and chronic risk to the SJKF based on secondary exposures. Applications of bromadiolone as currently registered also place the SKJF at risk from sublethal effects. Additionally, indirect effects to SJKF prey base may occur from bromadiolone effects to small mammals. Finally, the SJKF occurs in areas where bromadiolone bait may be intensively used. Therefore, the Agency makes a "**May Affect**" and "**Likely to Adversely Affect**" determination for the SJKF.

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, the hypotheses cannot be rejected, meaning that the stated hypotheses represent concerns in terms of direct and indirect effects of bromadiolone to the AW, SMHM, and SJKF and designated critical habitat for the AW.

The labeled use of bromadiolone with the action area may:

- directly affect the AW, SMHM, and SJKF by causing acute mortality or by adversely affecting chronic growth or fecundity;
- indirectly affect the AW, SMHM, and SJKF and/or affect their designated critical habitat by reducing or changing the composition of the food supply.

6. Uncertainties

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.

6.1. Exposure Assessment Uncertainties

Information regarding whole body residues of bromadiolone in prey was limited to data from three studies. For two of these studies, complete raw data was not available, and only numerical values presented in the text could be used. Therefore, the whole body residue value chosen for calculating secondary exposure (*i.e.*, 1.83 mg a.i./kg-carcass, MRID 48590803; Sage *et al.*, 2008) was an average whole body residue rather than a maximum whole body value. Modeling by Sage *et al.* (MRID 48590803) indicated that concentrations in water voles are maximal between 3.3 and 6.5 days after treatment. However, a maximal value couldn't be used in this assessment because it was only presented in a graph rather than as a numerical value in the text. Thus, the bromadiolone whole body residue value used in this assessment may underestimate secondary exposure from consumption of prey that ingested bromadiolone bait.

6.2. Use of Surrogate Species Effects Data

Guideline toxicity tests and open literature data on bromadiolone were not available for reptiles, or snakes in particular; therefore, birds were used as surrogate species for the terrestrial AW.

Endpoints based on avian toxicity data were assumed to be protective of potential direct effects to the AW. Efforts were made to select the organisms most likely to be affected by the type of compound and usage pattern; however, there is an inherent uncertainty in extrapolating across phyla. In addition, the Agency's LOCs are intentionally set very low, and conservative estimates are made to account for these uncertainties.

6.3. Data Gaps and Uncertainties

Data gaps were assigned either a low or high potential to add value to the effects determination of bromadiolone risk to SMHM, SJKF, and AW. While still considered data gaps according to 40 CFR part 158, low potential studies are unlikely to change risk determinations because alternate methods and weight of evidence may possibly be used in the absence of data. High potential studies would enable the Agency to better characterize potential risks by eliminating uncertainties for both non-listed and listed species that cannot be accounted for using alternate methods or weights of evidence.

Data from the following guideline studies were considered to have **high potential** to add value to the ecological risk assessment:

- Avian Acute Oral Toxicity (850.2100).
- Avian Reproduction (850.2300).

Data from the following guideline studies were considered to have **low potential** to add value to the ecological risk assessment because exposure of the associated taxa to bromadiolone is expected to be insignificant (Series 850 Ecological Effects Test Guidelines) or the environmental fate of bromadiolone is limited by placement of bait in bait stations for outdoor uses (Series 835 Fate, Transport and Transformation Test Guidelines):
:

- Algal Toxicity, Tier I (850.5400)
- Aquatic Plant Toxicity Test using *Lemna* spp., Tier I (850.4400)
- Seedling Emergence, Tier I (850.4100)
- Vegetative Vigor, Tier I (850.4150)
- Honey Bee Acute Contact Toxicity (850.3020)
- Freshwater Invertebrate Acute Toxicity (850.1010)
- Freshwater Fish Acute Toxicity, warm water species (850.1075)
- Freshwater Invertebrates Life Cycle (850.1300)
- Early Life Stage Toxicity of Freshwater fish (850.1400)
- Estuarine/Marine Acute Toxicity (850.1025, 850.1035, 850.1045, 850.1055, 850.1075)
- Photolysis in Water (835.2240)
- Photolysis in Soil (835.2410)
- Aerobic Aquatic Metabolism (835.4300)
- Anaerobic Aquatic Metabolism (835.4400)
- Terrestrial Field Dissipation (835.6100)

Although not an ecological effects data gap, additional data is also lacking to fully characterize whole body residues of bromadiolone in target organisms (see **Section 6.1**) and dietary toxicity within mammals for determination of an LC_{50} value.

6.4. Sublethal Effects

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

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 bromadiolone to the AW, SMHM, and SJKF, and AW designated critical habitat.

Based on the best available information, the Agency makes a May Affect, Likely to Adversely Affect determination for the AW, SMHM, and SJKF. Additionally, the Agency has determined that there the potential exists for modification of the designated critical habitat for the AW from the use of the chemical and makes a Habitat Modification determination for the AW. Given the LAA determinations and the potential modification of designated critical habitat for the AW, a description of the baseline status and cumulative effects for all three species is provided in **Attachment III**.

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

Table 7-1. Effects Determination Summary for Effects of Bromadiolone on the AW, SMHM, and SJKF.

Species	Effects Determination	Basis for Determination
Alameda Whipsnake (AW) (*Masticophis lateralis euryxanthus*)	May Affect, Likely to Adversely Affect (LAA)	**Potential for Direct Effects**
		Calculated dose- and dietary-based acute RQs for secondary exposure did not exceed Acute Risk LOCs for AWs. However, the probability of an individual AW mortality event is high (1 in 7) for the dietary-based acute RQ for secondary exposure. In addition, sublethal effects were observed with acute oral or sub-acute dietary exposure of birds (surrogates for reptiles). Data were not available to assess AW chronic toxicity via secondary exposure.
		Potential for Indirect Effects
		Terrestrial prey items Risk assessment indicates that the registered uses of bromadiolone will adversely affect the number and quality of AW mammalian prey via primary and secondary exposure. • Calculated acute and chronic RQs for primary exposure exceeded Acute and Chronic Risk LOCs for all small (rodent) mammalian weight classes considered. • The probabilities of an individual small (rodent) mammal mortality event are high (1 in 1) for acute primary exposure RQs of all weight classes considered. • Incidents involving small mammals have been reported in association with the use of bromadiolone. ***Habitat modifications*** Risk assessment indicates that the registered uses of bromadiolone will adversely affect AW habitat by reducing the availability of small mammal burrows. • Calculated acute and chronic RQs from primary exposure exceeded Acute and Chronic Risk LOCs for all small (rodent) mammalian weight classes considered. • The probabilities of an individual small (rodent) mammal mortality event are high (1 in 1) for acute primary exposure RQs of all weight classes considered. • Incidents involving small mammals have been reported in association with the use of bromadiolone.

Species	Effects Determination	Basis for Determination
Salt Marsh Harvest Mouse (SMHM) (*Reithrodontomys raviventris*)	May Affect, Likely to Adversely Affect (LAA)	**Potential for Direct Effects** Risk assessment indicates that the registered uses of bromadiolone pose direct acute and chronic risks to the SMHM via primary exposure. • Calculated acute and chronic RQs for primary exposure exceeded Acute and Chronic Risk LOCs for the SMHM. • The probabilities of an individual SMHM mortality event are high (1 in 1) for acute primary exposure RQs. • Incidents involving non-predator/non-scavenger mammals have been reported in association with the use of bromadiolone. • Applications of bromadiolone as currently registered also place the SMHM at risk from sublethal effects. **Potential for Indirect Effects** ***Habitat modifications*** Risk assessment indicates that the registered uses of bromadiolone will reduce SMHM rearing sites by adversely affecting small mammals. • Calculated acute and chronic RQs for primary exceeded Acute and Chronic Risk LOCs for all small (rodent) mammalian weight classes considered. • The probabilities of an individual small (rodent) mammal mortality event are high (1 in 1) for acute primary exposure RQs of all weight classes considered. • Incidents involving small mammals have been reported in association with the use of bromadiolone.
San Joaquin Kit Fox (SJKF) (*Vulpes macrotis mutica)*	May Affect, Likely to Adversely Affect (LAA)	**Potential for Direct Effects** Risk assessment indicates that the registered uses of bromadiolone pose direct acute and chronic risks to the SJKF via secondary exposure. • Calculated acute and chronic RQs for secondary exposure exceeded Acute and Chronic Risk LOCs for the SJKF. • The probabilities of an individual SJKF mortality event are high (1 in 1) for acute secondary exposure RQs. • Incidents involving predator/scavenger mammals have been reported in association with use of bromadiolone. Applications of bromadiolone as currently registered also place the SJKF at risk from sublethal effects. **Potential for Indirect Effects** ***Terrestrial prey items*** Risk assessment indicates that the registered uses of bromadiolone will adversely affect the number and quality of SJKF mammalian prey via primary and secondary exposure. • Calculated acute and chronic RQs for primary and secondary exposure exceeded Acute and Chronic Risk LOCs for all mammalian weight classes considered. • The probabilities of an individual small mammal mortality event are high for acute primary exposure RQs from primary exposure; the probabilities of an individual predator/scavenger mammal mortality range from high (1 in 1) to moderate (1 in 236) for acute secondary exposure RQs. • Incidents involving mammals have been reported in association with use of bromadiolone.

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 registered uses of bromadiolone may adversely 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 mammals.

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

Uses	Potential for Effects to Identified Taxa:												
	SMHM and Small Mammals[1]		SJKF and Large Mammals[2]		Small Birds[3]		Herpetofauna except AW[4]		AW[5]		Invertebrates[6]	Terrestrial Plants[7]	Aquatic Plants[8]
	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic			
Bait for rat and mouse control in and around buildings and in alleys and transport vehicles	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No	No	No
Bait for rat control in sewers	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No	No	No

[1]A yes in this column indicates a potential for direct effects to the SMHM and indirect effects to the SMHM, SJKF, and AW.
[2]A yes in this column indicates a potential for direct and indirect effects to SJKF.
[3]A yes in this column indicates a potential for indirect effects to the SMHM, SJKF, and AW.
[4]A yes in this column indicates a potential for indirect effects to the SMHM, SJKF, and AW.
[5]A yes in this column indicates the potential for direct effects to the AW.
[6]A yes in this column indicates a potential for indirect effects to the SMHM, SJKF, and AW.
[7]A yes in this column indicates a potential for indirect effects to the SMHM, SJKF, and AW.
[8]A yes in this column indicates a potential for indirect effects to the SMHM.

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

When evaluating the significance of this risk assessment's direct/indirect and adverse habitat modification effects determinations, it is important to note that pesticide exposures and predicted risks to the listed species and its resources (*i.e.*, food and habitat) are not expected to be uniform across the action area. Exposure to bromadiolone and associated risks to the species and its resources are expected to decrease rapidly 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 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, sublethal effects, and 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.

8. References

A bibliography of ECOTOX references, identified by the letter E followed by a number, is located in **Appendix E**.

Cox, P. and R.H. Smith. 1992. Rodenticide ecotoxicology: pre-lethal effects of anticoagulants on rat behavior. Proceedings of the Vertebrate Pest Conference 15:165-170.

Food and Agriculture Organization of the United Nations. FAO Pesticide Disposal Series. Assessing Soil Contamination: A Reference Manual. Appendix 2. Parameters of Pesticides that Influence Processes in the Soil. Editorial Group, FAO Information Division: Rome 2000. http://www.fao.org/DOCREP/003/X2570E/X2570E00.htm

ICWDM. © 2005. Internet Center for Wildlife Damage Management. Online at http://icwdm.org/handbook/rodents/RoofRats.asp

King, R.B. 2002. Predicted and observed maximum prey size - snake size allometry. Functional Ecology 16:766-772.

Lindstedt, S.L. and P.J. Schaeffer. 2002. Use of allometry in predicting anatomical and physiological parameters of mammals. Laboratory Animals 36:1-19.

Pelfrene, A.F. 1991. Synthetic organic rodenticides. Handbook of Pesticide Toxicology, vol. 3: Classes of Pesticides, pages 1271-1316. Academic Press, Inc.

Stebbins, R.C. 2003. A field guide to western amphibians and reptiles. Houghton Mifflin Company, Boston, Massachusetts, USA.

Swaim, K.E. 1994. Aspects of the Ecology of the Alameda Whipsnake (*Masticophis lateralis euryxanthus*). Unpublished Master's Thesis, California State University, Hayward, California. 140 pp.

Teeters, W.R. 1981. Bromadiolone technical: Toxicity to Laboratory Rat: Test No. 105. (U.S. Environmental Protection Agency, Pesticides Regulation Div., Agricultural Research Center, Animal Biology Laboratory, unpublished report.)

USEPA. 1993. Wildlife Exposure Handbook. Office of Research and Development, United States Environmental Protection Agency. Available at http://www.epa.gov/ncea/pdfs/toc2-37.pdf (Accessed June 19, 2009).

USEPA. 1998. Guidelines for Ecological Risk Assessment. United States Environmental Protection Agency (USEPA). Risk Assessment Forum. Office of Research and Development. Available at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=12460 (Accessed June 19, 2009).

USEPA. 1998b. Reregistration Eligibility Decision (RED): Rodenticide Cluster. July 1998. EPA 738-R-98-007.

USEPA. 2004a. Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs. United States Environmental Protection Agency (USEPA). Environmental Fate and Effects Division. Office of Pesticide Programs. Available at http://www.epa.gov/espp/consultation/ecorisk-overview.pdf (Accessed June 19, 2009).

USEPA. 2004b. Potential Risks of Nine Rodenticides to Birds and Nontarget Mammals: A Comparative Approach. July 2004. US EPA Office of Pesticide Programs, Environmental Fate and Effects Division.

USEPA. 2008. Risk Mitigation Decision for Ten Rodenticides. May 28, 2008. Office of Pesticide Programs, Special Review and Reregistration Division.

USEPA. 2010a. Guidance on calculating risk quotients (RQs) for San Francisco Bay species litigation assessments, uncertainty language for the terrestrial exposure assessment, and release of an updated version of T-HERPS. Memorandum from Donald Brady to the Environmental Fate and Effects Division. February 5, 2010.

USFWS. 1993. U.S. Fish and Wildlife Service Biological Opinion. Effects of 16 Vertebrate Control Agents on Threatened and Endangered Species. U.S. Fish and Wildlife Service.

USFWS/NMFS. 1998. Endangered Species Consultation Handbook: Procedures for Conducting Consultation and Conference Activities Under Section 7 of the Endangered Species Act. Final Draft. United States Fish and Wildlife Service (USFWS) and National Marine Fisheries Service (NMFS). Available at http://www.fws.gov/endangered/consultations/s7hndbk/s7hndbk.htm (Accessed June 19, 2009).

USFWS/NMFS/NOAA. 2004. 50 CFR Part 402. Joint Counterpart Endangered Species Act Section 7 Consultation Regulations; Final Rule. Federal Register Volume 69. Number 20.Pages 47731-47762. August 5, 2004.

Whitaker, J. O., Jr. 1996. National Audubon Society® Field Guide to North American Mammals. Alfred A. Knopf, New York. 937 pp.

9. MRID List

00030961 Beavers, J.B.; Fink, R.; Brown, R. (1977) Final Report: Acute Oral LD50--Bobwhite Quail: Project No. 154-105. (Unpublished study received May 23, 1978 under 40868-1; prepared by Wildlife Inter- national, Ltd. in cooperation with Washington College, submitted by Interstate Sales, Edgewater, N.J.; CDL:234001-E)

00142699 Chabert, J. (1975) Study of Acute Toxicity in Domestic Cats and Dogs.

EPA Accession No. 254000 (Species Domestic Cats/Beagle Dog)

00143276 Chempar (1984) Mammalian Oral Ingestion Test with Pigs. (Comparative Toxicity in the Pig After Repeated Oral Ingestion of Brodifacoum, Bromadiolone and Warfarin. Laboratory of Toxicology. Lyon Nat'l Veterinary School. Accession No. 257769

00143278 Fletcher, D. (1985) 35-Day Dietary LC50 Study with Bromadiolone in Mallard Ducklings: BLAL No. 84 DC 50. Unpublished study pre- pared by Bio-Life Associates, Ltd. Lipha report no. 85-009. 37 p.

00143279 Shapiro, R. and R. Roth(1985) Avian Single Dose Oral LD50 (Bob White Quail): Lab Report No. T-5100. Unpublished study prepared by Product Safety Labs. Lipha report no. 85-010. 27 p.

00143280 Shapiro, R. (1985) Avian Dietary LC50 (Bob White Quail): Lab Report No. T-5099. Unpublished study prepared by Product Safety Labs. Lipha report no. 85-011. 16 p.

00145321 Beavers, J. (1984) A Dietary LC50 Study in the Bobwhite With Rat-A-Rest: Final Report: Wildlife International Ltd. Project No.199- 101. Unpublished study prepared by Wildlife International Ltd. 16 p.

00145322 Beavers, J. (1984) A Dietary LC50 Study in the Mallard with Rat-A- Rest: Final Report: Wildlife International Ltd. Project No. 199-102. Unpublished study prepared by Wildlife International Ltd. 15 p.

00145323 Beavers, J. (1984) An Acute Oral Toxicity Study in the Bobwhite with Rat-A-Rest: Final Report: Wildlife International Project No. 199-103. Unpublished study prepared by Wildlife International Ltd. 14 p.

00157732 Pank, L.F.; Hirata, D.H. (1976) Primary and Secondary Toxicity of Anticoagulant Rodenticides. Job Completion Report. Work Unit DF-103.7. U.S. Fish and Wildlife Service. Denver Wildlife Research Center. Wildlife Damage Research Station. Hilo, HA. 25 p.

00160954 Chempar Chemical (1981) Bromadiolone II-2.5 90-Day Toxicity Study in the Beagle Dog. Fixed Dosage. EPA Accession No. 254001. Also: Lorgue, G. (1979) Report on Test Performed on Toxicity of LM637 on Beagle Dogs-Increased Dosages. Ministere De 1'Agriculture, Ecole Nationale Veterinaire, Service Pharmacie et Toxicologie. EPA Accession No. 253121.

00161965 Rhoderick, J. (1981) [Carbon 14]-Bromadiolone Bluegill Sunfish Lepomis macrochirus Rafinesque: Flowthrough Bioconcentration Study: Project No. 80PL-85-BG. Unpublished study prepared by Biospherics, Inc. 85 p.

00161966 Rhoderick, J. (1981) [Carbon 14]-Bromadiolone Channel Catfish Ictalurus punctatus: Static Bioconcentration Study: Project No. 80PL-86-CAT. Unpublished study prepared by Biospherics, Inc. 93 p.

00162324 Marsh, R.; Howard, W. (1986) Ground Squirrel-coyote Secondary Toxicity Studies with Chlorophacinone and Bromadiolone (An Administrative Report of Laboratory Findings). Unpublished study prepared by Univ. of CA, Davis, Wildlife and Fisheries Biology in cooperation with Lipha Chemicals, Inc. 64 p.

00162325 Spare, W. (1986) Bromadiolone Residue Levels in Coyote and Ground Squirrel Tissues from Davis, CA: Project No. 1410. Unpublished study prepared by Agrisearch, Inc. 105 p.

00226423 (also 0007425) Author unknown. (1976) Determination of Acute Oral Toxicity on Mice, Rats, Rabbits, Dogs. Unpublished study by Chempar Chemical Co., Inc. 18 p.

00241703 Verlangieri, A.J.; Shapiro, R.; Stern, A. (1979) Evaluation of the Acute Toxicity of a 1% Formulation of Bromadiolone: Oral Toxicity (Rats). Report No. T-716. Unpublished study by Product Safety Labs. 46 p.

00249995 Beavers, J.B.; Fink, R.; Brown, R. (1979) Final Report: Twenty Five-Day Dietary LC50 – Mallard Duck; Bromadiolone. Submitted by Chempar Chemical Co. 14 p.

41900001 Mally, Ch.; Porret-Blanc, G. (1987) LM637 (Bromadiolone) Determination of LD50 of LM 637 Orally in Rats: Lab Project Number: 87.04 .LM 637 RPL. Unpublished study prepared by Lipha Centre de Recherches. 83 p.

44000201 Palmer, S.; Beavers, J. (1995) Technical Bromadiolone: An Acute Oral Toxicity Study with the Mallard with a 90-Day Observation Period: Amended Report: Lab Project Number: 284-105. Unpublished study prepared by Wildlife International Ltd. 72 p.

44000202 Palmer, S.; Beavers, J. (1995) Technical Bromadiolone: A Dietary LC50 Study with the Mallard with a 90-Day Observation Period: Amended Report: Lab Project Number: 284-104. Unpublished study prepared by Wildlife International Ltd. 72 p.

42237501 Spare, W. (1992) Hydrolysis of Bromadiolone: Lab Project Number: 1414. Unpublished study prepared by Agrisearch Inc. 62 p.

43000702 Spare, W. (1993) Leaching Characteristics of Bromadiolone: Lab Project Number: 1422. Unpublished study prepared by Agrisearch Inc. 123 p.

43594301 Misra, B. (1995) Aerobic Soil Metabolism of Bromadiolone: Amended Final Report: Lab Project Number: ME 9200154. Unpublished study prepared by Pittsburgh Environmental Research Lab, Inc. 106 p.

46750931, (also 40077202) Mendenhall, V.M.; Pank, L.F. (1980) Secondary poisoning of owls by anticoagulant rodenticides. Wildlife Society Bulletin 8(4):311- 315. Also USFWS, Denver Wildlife Research Center. No Lab ID No. Submitted by Chempar-Lipha Chemicals, 3101 West Custer Ave., Milwaukee, WI 53209. Document No. 5. Case No. 047415. S331496.

48590801 Giraudoux, P., Tremollieres, C., Barbier, B., Defaut, R., Rieffel, D., Bernard, N., Lucot, E., Berny, P. (2006) Persistence of bromadiolone anticoagulant rodenticide in *Arvicola terrestris* populations after field control. Environmental Research 102: 291-298.

48590802 Lund, M., and Rasmussen, A.M. (1986) Secondary Poisoning Hazards to Stone Martens (*Martes foina*) Fed Bromadiolone-Poisoned Mice. Nordisk Veterinaermedicin 38, 241-243.

48590803 Sage, M., Coerdassier, M., Defaut, R., Gimbert, F., Berny, Giraudoux, P. (2008) Kinetics of bromadiolone in rodent populations and implications for predators after field control of the water vole, *Arvicola terrestris*. Science of the Total Environment 407: 211-222.

48590804 Wyllie, I. (1995) Potential secondary poisoning of barn owls by rodenticides. Pesticide Outlook 6:19-25.

92190614 Monnot, R.; Fave, A.; T. Illat, T.; Briet, Ph. (1981) Teratology Study in the Rat with LM 637. Original Study No. 103204. Unpublished study prepared by Institut Francais de Researches et Essais Biologiques. Domaine des Oncins. BP109-962101 'Arborasle, France. 125 p.