

**Risks of Diphacinone Use to the Federally Threatened  
Alameda Whipsnake (*Masticophis lateralis*  
*euryxanthus*), California Tiger Salamander  
(*Ambystoma californiense*),  
And  
the Federally Endangered  
Salt Marsh Harvest Mouse, California Tiger  
Salamander (*Ambystoma californiense*) Sonoma  
County Distinct Population Segment and Santa  
Barbara County Distinct Population Segment, and  
San Joaquin Kit Fox (*Vulpes macrotis mutica*)**

**Pesticide Effects Determinations  
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## Table of Contents

1.	EXECUTIVE SUMMARY .....	10
1.1.	PURPOSE OF ASSESSMENT .....	10
1.2.	SCOPE OF ASSESSMENT .....	11
1.3.	ASSESSMENT PROCEDURES .....	12
1.4.	SUMMARY OF CONCLUSIONS .....	13
2.	PROBLEM FORMULATION .....	18
2.1.	PURPOSE .....	18
2.2.	SCOPE .....	19
2.3.	PREVIOUS ASSESSMENTS .....	20
2.4.	ENVIRONMENTAL FATE PROPERTIES .....	21
2.5.	ASSESSED SPECIES .....	32
2.6.	DESIGNATED CRITICAL HABITAT .....	40
2.7.	ACTION AREA AND LAA EFFECTS DETERMINATION AREA .....	41
2.8.	ASSESSMENT ENDPOINTS AND MEASURES OF ECOLOGICAL EFFECT .....	43
2.9.	CONCEPTUAL MODEL .....	46
2.10.	ANALYSIS PLAN .....	49
3.	EXPOSURE ASSESSMENT .....	51
3.1.	LABEL APPLICATION RATES AND INTERVALS .....	51
3.2.	AQUATIC EXPOSURE ASSESSMENT .....	52
3.3.	TERRESTRIAL ANIMAL EXPOSURE ASSESSMENT .....	53
3.4.	TERRESTRIAL PLANT EXPOSURE ASSESSMENT .....	54
4.	EFFECTS ASSESSMENT .....	54
4.1.	ECOTOXICITY STUDY DATA SOURCES .....	54
4.2.	TOXICITY OF DIPHACINONE TO AQUATIC ORGANISMS .....	56
4.3.	TOXICITY OF DIPHACINONE TO TERRESTRIAL ORGANISMS .....	57
5.0	RISK CHARACTERIZATION .....	63
5.1	RISK ESTIMATION .....	64
5.2	RISK DESCRIPTION .....	71
6.0	UNCERTAINTIES .....	75
6.1	EXPOSURE ASSESSMENT UNCERTAINTIES .....	75
<a href="#">6.2</a>	EFFECTS ASSESSMENT UNCERTAINTIES .....	76
7.0	RISK CONCLUSIONS .....	77
8.0	REFERENCES .....	82
9.0	MRID LIST .....	84

## **Appendices**

- Appendix A. Verification Memo for Diphacinone
- Appendix B. Risk Quotient (RQ) Method and Levels of Concern (LOCs)
- Appendix C. Outputs from Tier 1 Water Models
- Appendix D. Bibliography of ECOTOX Open Literature
- Appendix E. Spreadsheet of Endpoints in Accepted ECOTOX Papers
- Appendix F. Summary of Diphacinone Incidents
- Appendix G. Multi active ingredient analysis

## **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

Table 1-1. Effects Determination Summary for Effects of Diphacinone on the SMHM, CTS, AW and SJKF. ....	14
Table 2-1. Physical-Chemical Properties of Diphacinone.....	21
Table 2-2. Summary of Diphacinone Environmental Fate Properties.....	22
Table 2-3. Diphacinone Uses Assessed for California .....	25
Table 2-4. Summary of California Department of Pesticide Registration (CDPR) Pesticide Use Reporting (PUR) Data from 1999 to 2009 for County Diphacinone Uses <sup>1</sup> .....	31
Table 2-5. Summary of Current Distribution, Habitat Requirements, and Life History Information for the Assessed Listed Species <sup>1</sup> .....	33
Table 2-6. Designated Critical Habitat PCEs for the CA Tiger Salamander and the Alameda Whipsnake <sup>1</sup> .....	40
Table 2-7. Taxa and Assessment Endpoints Used to Evaluate the Potential for Use of Diphacinone to Result in Direct and Indirect Effects to the Assessed Listed Species or Modification of Critical Habitat. ....	44
Table 3-1. Diphacinone Uses, Scenarios, and Application Information.....	51
Table 3-2. Summary of Environmental Fate Data Used for Aquatic Exposure Inputs for Diphacinone Endangered Species Assessment <sup>1</sup> .....	52
Table 4-1. Aquatic Toxicity Profile for Diphacinone.....	56
Table 4-2. Categories of Acute Toxicity for Fish and Aquatic Invertebrates.....	56
Table 4-3. Terrestrial Toxicity Profile for Diphacinone .....	58
Table 4-4. Categories of Acute Toxicity for Avian and Mammalian Studies .....	58
Table 5-1. Acute and Chronic RQs for Freshwater Fish .....	64
Table 5-2. Summary of Acute and Chronic RQs for Aquatic Invertebrates. (Example). ....	65
Table 5-3. RQs for Acute Effects to the AW from Consumption of a Small Mammal which Ingested Diphacinone Bait .....	67
Table 5-5. Risk Estimation Summary for Diphacinone - Direct and Indirect Effects .....	72
Table 5-6. Risk Estimation Summary for Diphacinone – Effects to Designated Critical Habitat. (PCEs) .....	72
Table 7-1. Effects Determination Summary for Effects of Diphacinone on the AW, CTS, SMHM and SJKF Effects Determination Summary for Effects of Diphacinone on the SMHM, CTS, AW and SJKF. ....	78
Table 7-2. Effects Determination Summary for the Critical Habitat Impact Analysis...	80

## List of Figures

Figure 2-1. Critical habitat and occurrence sections of the Alameda Whipsnake, as identified in Case No. 07-2794-JCS .....	36
Figure 2-2. Occurrences and occurrence sections of the salt marsh harvest mouse, as identified in Case No. 07-2794-JCS. ....	37
Figure 2-3. Occurrences and occurrence sections of the CA Tiger Salamander, as identified in Case No. 07-2794-JCS. ....	38

Figure 2-4. Occurrences and occurrence sections of the San Joaquin Kit Fox, as identified in Case No. 07-2794-JCS. ....	39
Figure 2-5. Conceptual model depicting stressors, exposure pathways, and potential effects to aquatic organisms from the use of diphacinone. ....	47
Figure 2-6. Conceptual model depicting stressors, exposure pathways, and potential effects to terrestrial organisms from the use of diphacinone. ....	48

## List of Commonly Used Abbreviations and Nomenclature

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

ECOTOX	EPA managed database of Ecotoxicology data
EEC	Estimated Environmental Concentration
EFED	Environmental Fate and Effects Division
<i>e.g.</i>	Latin <i>exempli gratia</i> (“for example”)
EIM	Environmental Information Management System
EPI	Estimation Programs Interface
ESU	Evolutionarily significant unit
<i>et al.</i>	Latin <i>et alii</i> (“and others”)
<i>etc.</i>	Latin <i>et cetera</i> (“and the rest” or “and so forth”)
EXAMS	Exposure Analysis Modeling System
FIFRA	Federal Insecticide Fungicide and Rodenticide Act
FQPA	Food Quality Protection Act
ft	Feet
GENEEC	Generic Estimated Exposure Concentration model
HPLC	High Pressure Liquid Chromatography
IC <sub>05</sub>	5% Inhibition Concentration
IC <sub>50</sub>	50% (or median) Inhibition Concentration
<i>i.e.</i>	Latin for <i>id est</i> (“that is”)
IECV1.1	Individual Effect Chance Model Version 1.1
KABAM	<u>K</u> <sub>ow</sub> (based) <u>A</u> quatic <u>B</u> io <u>A</u> ccumulation <u>M</u> odel
kg	Kilogram(s)
kJ/mole	Kilojoules per mole
km	Kilometer(s)
K <sub>AW</sub>	Air-water Partition Coefficient
K <sub>d</sub>	Solid-water Distribution Coefficient
K <sub>F</sub>	Freundlich Solid-Water Distribution Coefficient
K <sub>OC</sub>	Organic-carbon Partition Coefficient
K <sub>OW</sub>	Octanol–water Partition Coefficient
LAA	Likely to Adversely Affect
lb a.i./A	Pound(s) of active ingredient per acre
LC <sub>50</sub>	50% (or Median) Lethal Concentration
LD <sub>50</sub>	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 <sup>2</sup> /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
pptr	Parts per Trillion (equivalent to ng/L or ng/kg)



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

## 1. Executive Summary

### 1.1. Purpose of Assessment

The purpose of this assessment is to evaluate potential direct and indirect effects on the Alameda Whipsnake (AW), Salt Marsh Harvest Mouse (SMHM), CA Tiger Salamander (CTS) and the San Joaquin Kit Fox (SJKF) arising from FIFRA regulatory actions regarding use of diphacinone 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 Alameda Whipsnake, Salt Marsh Harvest Mouse, CA Tiger Salamander and the San Joaquin Kit Fox. This assessment was completed in accordance with the U.S. Fish and Wildlife Service (USFWS) and National Marine Fisheries Service (NMFS) *Endangered Species Consultation Handbook* (USFWS/NMFS, 1998), procedures outlined in the Agency's Overview Document (USEPA, 2004), and consistent with a suit in which diphacinone was alleged to be of concern to the AW, SMHM, CTS and the SJKF (*Center for Biological Diversity (CBD) vs. EPA et al.* (Case No. 07-2794-JCS).)

**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.

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

**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.

## **1.2. Scope of Assessment**

### **1.2.1. Uses Assessed**

Diphacinone labels may be categorized into two types: labels for manufacturing uses (including technical grade diphacinone) and end-use products. While technical products, which contain diphacinone of high purity, are not used directly in the environment, they are used to make formulated products (0.01%, 0.0025%, and 0.005% baits), which can be applied in specific areas to control rodents. The formulated product labels legally limit diphacinone's potential use to only those sites that are specified on the labels.

Diphacinone can be used for spot, broadcast, bait-station or in-burrow applications. Currently registered uses of diphacinone include non-agricultural areas and agricultural areas. Diphacinone may be used in and around buildings (placement must be within 50 feet of exterior walls), inside transport vehicles (ships, trains, and aircraft), alleys in urban areas, and sewers. Buildings where diphacinone bait may be used include residential, commercial, industrial, and agricultural premises, as well as port and terminal buildings. Labeled use sites for agricultural include: vegetables (unspecified), small grains (unspecified), alfalfa, apple, almond, walnut, pecan, pear, peach, nectarine, filbert, orchard (unspecified), and agricultural crops/soils (unspecified). Labeled uses sites for non-food and non-agricultural uses are sewage systems, drainage systems, forest trees, forest plantings (tree farms, tree plantations), household/domestic dwellings outdoor premises, rangeland, pastures, urban areas, nursery stock, ornamental woody shrubs and vines, ornamental lawns and turf, golf course turf, nonagricultural rights-of-way/hedgerows, and airports/landing fields. All of these uses are considered as part of the federal action evaluated in this assessment.

### **1.2.2. Environmental Fate Properties of Diphacinone**

Diphacinone has moderate leaching potential. However, the range of possible  $K_d$  values of 3 to 5 mL/g combined with a low solubility (0.3 mg/L) means that it is unlikely to contaminate ground water. Consistent with a relatively low vapor pressure of approximately  $1 \times 10^{-8}$  Torr and a Henry's Law constant of approximately  $2 \times 10^{-10}$  atm-m<sup>3</sup>/mole, volatilization is not expected to be a significant route of dissipation for diphacinone. Diphacinone is expected to be moderately mobile in soil. One clearly established route of transformation for diphacinone is aerobic soil metabolism. However, the primary dissipation route of diphacinone in many cases is likely to be consumption of bait.

### **1.2.3. Evaluation of Degradates and Stressors of Concern**

The major degradate detected in the aerobic soil metabolism study, diphenylglycolic acid, comprised up to 25% of the applied material about a month after application. This degradate appears to be persistent, but its mobility is unknown. Because diphacinone is formulated in pelleted bait or weather-resistant rodenticide products and primarily used in bait application or animal burrow treatment, it is probable that contact of this degradate with soil and water will be minimal. Furthermore, because risk to the assessed species is expected to result from direct consumption of bait products or from consumption of prey which directly consumed the bait, the

formation of degradation products in the soil and water is not a major concern in this assessment. Risk from the diphenylglycolic acid degradate was, therefore, not quantified.

### **1.3. Assessment Procedures**

#### **1.3.1. Exposure Assessment**

##### **1.3.1.a. Aquatic Exposures**

There are no aquatic monitoring data available for the use of diphacinone. For diphacinone usage as bait application or animal burrow treatment, it is possible to approximate a ‘high-end’ exposure scenario with two tier 1 models: GENEEC model for surface water exposure and SCIGROW model for ground water exposure. The peak surface water concentration (EEC) predicted by GENEEC is 483 ng/L. The peak ground water concentration (EEC) predicted by SCIGROW is 4.75 ng/L. The only effects on aquatic species that are relevant to this assessment are direct effects to the aquatic phase of the CTS (and aquatic plants and animals on which the CTS depends) and aquatic plants for indirect effects to the SMHM. Diphacinone is applied as bait, and models that estimate deposition on aquatic habitats from spray drift were therefore not needed (drift is assumed to be negligible for bait applications).

##### **1.3.1.b. Terrestrial Exposures**

To estimate diphacinone exposure to terrestrial species resulting from diphacinone applications, concentrations in bait and in animals that have consumed bait were compared with appropriate available toxicity values to calculate RQs. Exposures to terrestrial species resulting from application of baits were evaluated by assuming species directly consumed the baits (SMHM and SJKF). Since primary exposure takes place by direct consumption of baits, T-REX was not relevant to this assessment. The concentration of active ingredient in food was assumed simply to be the concentration of AI in the bait. The Agency does not have an approved standard method of predicting secondary exposure from terrestrial animals that eat other animals which have ingested bait. For this assessment, secondary exposure doses can then be estimated using allometric equations to estimate ingestion rates from the concentration of a.i. in the bait (ingestion rate in g food /kg bw x bait concentration in mg a.i./g food = mg a.i./kg bw). A conservative assumption was that the amount of active ingredient ingested by the AW and SJKF from secondary exposure was assumed to be equal to the amount of active ingredient that prey would ingest if it consumed bait at its daily ingestion rate. The prey was assumed to be a mammal or mammals that the AW and SJKF could ingest. The amount of bait these prey species could ingest was assumed based on their body weights. The weights of these prey species were the maximum percentage the consumer could ingest in one day or sitting (it was estimated that the AW can consume approximately 100% of its BW in one day and the SJKF could ingest 10% of its BW per day). All of the diphacinone ingested by the prey was assumed to be available to and assimilated by the AW and SJKF once prey is consumed. This is a reasonable assumption given that diphacinone can be relatively long-lived in target organisms.

### **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 and the CTS. Primary constituent elements (PCEs) were used to evaluate whether diphacinone has the potential to modify designated critical habitat. The Agency evaluated registrant-submitted studies and data from the open literature to characterize diphacinone toxicity. The assessment used 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.

Section 4 summarizes the ecotoxicity data available on Diphacinone. Diphacinone is moderately toxic to freshwater fish and invertebrates on an acute exposure basis. Diphacinone exhibits high to very high acute and dietary toxicity to small mammals ( $LD_{50}$ 's = 0.2 to 57 mg ai/kg bw;  $LC_{50}$ 's = 2.0 to 2.5 ppm) and moderate toxicity to birds ( $LD_{50}$ 's >400 to 3158 mg ai/kg bw;  $LC_{50}$ 's = 906 to >5000 ppm). Toxicity increases if diphacinone is ingested for several days rather than in a single dose.

### **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 Diphacinone 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 diphacinone 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 Likely to Adversely Affect determination for the AW, SJKF, CTS and SMHM from the use of diphacinone. Additionally, the Agency has determined that use of Diphacinone has the potential to cause modification of the designated critical habitat of the AW and CTS from the use of the chemical. (Critical habitat has not been designated for the SMHM or the SJKF). Given the LAA determination for the AW, SJKF, CTS and the SMHM and potential modification of designated critical habitat for the AW and CTS, a description of the baseline status and cumulative effects is provided in Attachment III.

The labeled uses of Diphacinone may indirectly affect the AW, CTS and SJKF and/or affect designated critical habitat of the AW and CTS by reducing or changing the composition of the

food supply (mostly small mammals). It may also directly affect the SJKF and SMHM by direct mortality through ingestion of treated baits. In addition, Diphacinone may directly affect the AW and SJKF by direct mortality through secondary toxicity by ingestion of treated prey (small mammals). Lastly, Diphacinone may indirectly affect the CTS and/or modify its designated critical habitat by reducing or changing terrestrial habitat in its current range (via reduction in small burrowing mammals leading to reduction in underground refugia/cover). In addition, there have been many incidents of mortality to various mammals reported after ingestion of Diphacinone treated baits (including incidents of SJKF mortality) as well as from consuming prey that have eaten treated baits.

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

Species	Effects Determination	Basis for Determination
Alameda whipsnake ( <i>Masticophis lateralis euryxanthus</i> )	<i>May Affect and Likely to Adversely Affect (LAA)</i>	<b>Potential for Direct Effects</b>
		The risk assessment indicates use of diphacinone potentially will result in direct effects to the AW from acute toxicity. Dietary exposure estimates and acute toxicity to reptiles (based on acute toxicity data for birds) result in acute RQs that exceed the LOC for both primary and secondary exposure. While adverse acute effects are possible for both primary and secondary exposure, secondary exposure is considered the primary threat to this species. Data were not available to assess chronic toxicity, but since risk is predicted for acute effects, risk is also assumed for chronic effects.
		<b>Potential for Indirect Effects</b>
		<b><i>Terrestrial prey items</i></b> The risk assessment indicates use of diphacinone will likely reduce the abundance of terrestrial vertebrates which serve as prey for this species. This conclusion is based on acute RQs for birds and acute RQs for mammals, which exceed the LOC.
		<b><i>Habitat Modifications</i></b> The risk assessment indicates use of diphacinone may adversely modify the habitat of this species by reducing the availability of small mammal burrows. This conclusion is based on acute RQs for mammals that exceed the LOC. 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 and other prey eating reptiles.
Salt marsh harvest mouse ( <i>Reithrodontomys ravivertis</i> )	<i>May Affect and Likely to Adversely Affect (LAA)</i>	<b>Potential for Direct Effects</b>
		The risk assessment indicates use of diphacinone will likely result in direct effects to the SMHM from acute toxicity. Dietary exposure estimates and data on acute toxicity to small mammals results in acute RQs that exceed the LOC for primary exposure. This species is predicted to be susceptible to primary exposure through direct contact with treated bait products. This contact may result in ingestion of the bait, which would likely result in acute and possibly chronic toxic effects.
		<b>Potential for Indirect Effects</b>
		<b><i>Habitat Modifications</i></b> The risk assessment indicates use of diphacinone may adversely modify the habitat of this species by reducing the availability nest sites. This conclusion is based on acute RQs for mammals, that exceed the LOC. Adverse effects to mammals may result in a reduction of abandoned mammal nests, which are used as nest sites by this species.

Designated Critical Habitat for:	Effects Determination	Basis for Determination
San Joaquin Kit Fox ( <i>Vulpes macrotis mutica</i> )	<i>May Affect and Likely to Adversely Affect (LAA)</i>	<p><b>Potential for Direct Effects</b></p> <p>The risk assessment indicates use of diphacinone will likely result in direct effects to the SJKF from acute toxicity. Dietary exposure estimates and data on acute toxicity to small mammals results in acute RQs that exceed the LOC for primary exposure. This species is predicted to be susceptible to primary exposure through direct contact with treated bait products. This contact may result in ingestion of the bait, which would likely result in acute and possibly chronic toxic effects. Secondary exposure may also occur from consumption of contaminated prey animals. Incidents of SJKF mortality caused by diphacinone consumption have been reported.</p>
		<p><b>Potential for Indirect Effects</b></p> <p>The risk assessment indicates use of diphacinone may adversely modify the habitat of this species by reducing the availability of prey items. This conclusion is based on acute RQs for mammals that exceed the LOC.</p>
California Tiger Salamander ( <i>Ambystoma californiense</i> )	<i>May Affect and Likely to Adversely Affect (LAA)</i>	<p><b>Potential for Direct Effects</b></p> <p><b><i>Terrestrial-phase (Juveniles and Adults):</i></b></p> <p>The Risk Assessment indicates use of Diphacinone should not result in direct effects to the CTS from acute toxicity. Dietary exposure estimates and acute toxicity to terrestrial phase amphibians (based on acute toxicity data for snakes) result in acute RQs that exceed the LOC for primary exposure. However, it is unlikely that the CTS will ingest bait. Data were not available to assess chronic toxicity, but since risk is not predicted for acute effects, risk is also unlikely for chronic effects.</p> <p><b><i>Aquatic-phase (Eggs, Larvae, and Adults):</i></b></p> <p>The risk assessment indicates use of diphacinone should not result in direct effects to the CTS from acute toxicity. Dietary exposure estimates and acute toxicity to aquatic phase (based on acute toxicity data for fish) result in acute RQs that do not exceed the LOC for primary exposure. Data were not available to assess chronic toxicity, but since risk is not predicted for acute effects, risk is also unlikely for chronic effects.</p>
		<p><b>Potential for Indirect Effects</b></p> <p><b><i>Aquatic-phase (Eggs, Larvae, and Adults):</i></b></p> <p>The risk assessment indicates use of diphacinone should not result in indirect effects to the aquatic phase of the CTS.</p> <p><b><i>Terrestrial-phase (Juveniles and Adults):</i></b></p> <p>The risk assessment indicates use of diphacinone should not result in indirect effects to the CTS from acute toxicity. Dietary exposure estimates and acute toxicity to aquatic phase (based on acute toxicity data for fish) result in acute RQs that do not exceed the LOC for primary exposure. Data were not available to assess chronic toxicity, but since risk is not predicted for acute effects, risk is also unlikely for chronic effects. However, since the CTS can use small mammal burrows and Diphacinone kills small mammals, a loss of burrows may result and may indirectly affect the CTS through habitat modification.</p>

**Table 1-3. Use Specific Summary of The Potential for Adverse Effects to Terrestrial Taxa**

Uses	SMHM and Small Mammals <sup>1</sup>		SJKF and Large Mammals <sup>2</sup>		CTS-CC, CTS-SC, CTS-SB and Amphibians <sup>3</sup>		AW, and Reptiles <sup>4</sup>	
	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic
Rodent Control (all labeled use sites including residential, agricultural, and forestry)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

1 A yes in this column indicates a potential for direct effects to SMHM and indirect effects to SMHM, SJKF, AW, CTS-CC, CTS-SC, CTS, and CTS-SB.

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 CTS-CC, CTS-SC, CTS-SB and AW.

4 A yes in this column indicates the potential for direct and indirect effects to AW, and other reptiles.



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 species and its resources (*i.e.*, food and habitat) are not expected to be uniform across the action area. 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 the Alameda Whipsnake, Salt Marsh Harvest Mouse, CA Tiger Salamander and the San Joaquin Kit Fox life stages within the action area and/or applicable designated critical habitat. This information would allow for quantitative extrapolation of the present risk assessment's predictions of individual effects to the proportion of the population extant within geographical areas where those effects are predicted. Furthermore, such population information would allow for a more comprehensive evaluation of the significance of potential resource impairment to individuals of the assessed species.
- Quantitative information on prey base requirements for the assessed species. While existing information provides a preliminary picture of the types of food sources utilized by the assessed species, it does not establish minimal requirements to sustain healthy individuals at varying life stages. Such information could be used to establish biologically relevant thresholds of effects on the prey base, and ultimately establish geographical limits to those effects. This information could be used together with the density data discussed above to characterize the likelihood of adverse effects to individuals.
- Information on population responses of prey base organisms to the pesticide. Currently, methodologies are limited to predicting exposures and likely levels of direct mortality, growth or reproductive impairment immediately following exposure to the pesticide. The degree to which repeated exposure events and the inherent demographic characteristics of the prey population play into the extent to which prey resources may recover is not predictable. An enhanced understanding of long-term prey responses to pesticide exposure would allow for a more refined determination of the magnitude and duration of resource impairment, and together with the information described above, a more complete prediction of effects to individual species and potential modification to critical habitat.

## 2. Problem Formulation

Problem formulation provides a strategic framework for the risk assessment. By identifying the important components of the problem, it focuses the assessment on the most relevant life history stages, habitat components, chemical properties, exposure routes, and endpoints. The structure of this risk assessment is based on guidance contained in U.S. EPA's *Guidance for Ecological Risk Assessment* (USEPA, 1998), the Services' *Endangered Species Consultation Handbook* (USFWS/NMFS, 1998) and is consistent with procedures and methodology outlined in the Overview Document (USEPA, 2004) and reviewed by the U.S. Fish and Wildlife Service and National Marine Fisheries Service (USFWS/NMFS/NOAA, 2004).

### 2.1. Purpose

The purpose of this endangered species assessment is to evaluate potential direct and indirect effects on individuals of the AW, SMHM, CTS and the SJKF arising from FIFRA regulatory actions regarding use of Diphacinone in baits for pest control. This ecological risk assessment has been prepared consistent with a stipulated injunction settling the case *Center for Biological Diversity (CBD) vs. EPA et al.* (Case No. 07-2794-JCS).

In this assessment, direct and indirect effects to the Alameda Whipsnake, Salt Marsh Harvest Mouse, CA Tiger Salamander and the San Joaquin Kit Fox and potential modification to designated critical habitat for these species are evaluated in accordance with the methods described in the Agency's Overview Document (USEPA, 2004).

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 Diphacinone 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 diphacinone 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, CTS and the 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 Diphacinone 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.

## 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 Diphacinone in accordance with the approved product labels for California is “the action” relevant to this ecological risk assessment.

Although current registrations of diphacinone allow for use nationwide (at use sites permitted by registered labels), this ecological risk assessment and effects determination addresses currently registered uses of Diphacinone in portions of the action area that are reasonably assumed to be biologically relevant to the Alameda Whipsnake, Salt Marsh Harvest Mouse, CA Tiger Salamander and the San Joaquin Kit Fox and their designated critical habitat. Further discussion of the action area for these species and their critical habitat is provided in Section 2.7.

### **2.2.1. Evaluation of Degradates**

The major degradate detected in the aerobic soil metabolism study, diphenylglycolic acid, comprised up to 25% of the applied material in an aerobic soil metabolism study. This degradate appears to be persistent, but its mobility is unknown. Because diphacinone is formulated in pelleted bait or bait-block rodenticide products and primarily used in bait application or animal burrow treatment, it is probable that contact of this degradate with soil and water will be minimal. Furthermore, because the majority of risk to the assessed species is expected to result from direct consumption of bait products, or from consumption of prey which directly consumed the bait, the formation of degradation products in the soil and water is not a major concern in this assessment. Mobility and persistence data for the degradate diphenylglycolic acid are, therefore, not quantified for this assessment.

### **2.2.2. Evaluation of Mixtures**

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

Diphacinone does have registered products that contain multiple active ingredients. These products are formulations mixed with imidacloprid (Kaput-D Combo Bait Blocks and Kaput Field Rodent Bait B).

## **2.3. Previous Assessments**

- In March of 1993, a Biological Opinion (Effects of 16 Vertebrate Control Agents on Threatened and Endangered Species) was issued by the US Fish & Wildlife Service. The Agency determined that risk was minimal to avian species from direct consumption of treated bait. However, secondary toxicity to raptors was possible from consumption of treated rodents. Risk to mammals was also indicated. Specific jeopardy was indicated for the SMHM and the SJKF.
- The Agency assessed the risks of rodenticide uses of diphacinone, along with seven other rodenticides, in the Registration Eligibility Decision (RED): Rodenticide Cluster that was published in 1998. This document included an ecological effects risk assessment that was based on environmental fate and ecotoxicological studies that had been submitted by the registrants of diphacinone at that time.
- An assessment of potential risks of nine rodenticides, including diphacinone, to birds and mammals was completed in 2004 (Erickson and Urban 2004).

- In addition, a number of Section 18 (emergency exemption) assessments involving diphacinone were completed in 2009.

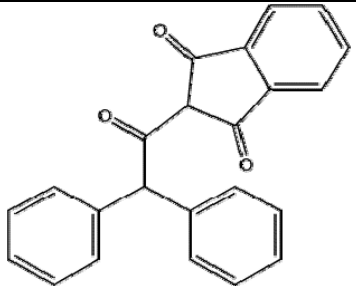
These assessments have consistently identified potential risks to birds and mammals from primary and secondary exposure as the predominant risk concern for diphacinone.

## 2.4. Environmental Fate Properties

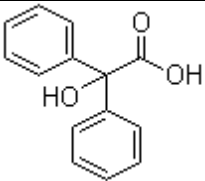
Diphacinone has moderate leaching potential. The range of possible K<sub>d</sub> values is 3 to 5 mL/g combined with a low solubility (0.3 mg/L) indicates that it is unlikely to contaminate ground water. Consistent with a relatively low vapor pressure of approximately  $1 \times 10^{-8}$  Torr and a Henry's Law constant of approximately  $2 \times 10^{-10}$  atm-m<sup>3</sup>/mole, volatilization is not expected to be a significant route of dissipation for diphacinone. Diphacinone is expected to be moderately mobile in soil. One clearly established route of transformation for diphacinone is aerobic soil metabolism. EFED has a half-life value of approximately 30 days for one soil tested in a single study. Diphacinone has a K<sub>ow</sub> of 20,000 indicating the potential for bioaccumulation in animal tissue. However, direct measures of bioaccumulation are not available. One primary dissipation pathway of diphacinone is expected to be consumption of bait by target and non-target animals.

Table 2-1 lists the physical-chemical properties of diphacinone. Table 2-2 lists the other environmental fate properties of diphacinone, along with the major and minor degradates detected in the submitted environmental fate and transport studies.

**Table 2-1. Physical-Chemical Properties of Diphacinone**

Property	Value	Source	Comment
Structure			Chemical Name: 2-(diphenylacetyl)indan-1,3-dione
Molecular Weight	340.48 g/mole		
Solubility	0.3 mg/L	EXTOXNET	<a href="http://extoxnet.orst.edu/pips/diphacin.htm">http://extoxnet.orst.edu/pips/diphacin.htm</a>
Vapor Pressure	$1 \times 10^{-8}$ torr	EUP Review 11/08/2000	
Henry's Law	$2 \times 10^{-10}$ atm-m <sup>3</sup> /mole	EUP Review 11/08/2000	
K <sub>ow</sub>	20,000	EUP Review 11/08/2000	

**Table 2-2. Summary of Diphacinone Environmental Fate Properties**

Study	Value and unit	Major Degradate <i>Minor Degradates</i>	MRID # or Citation	Study Classification, Comment
Hydrolysis	Stable	--	EUP Review 11/08/2000	at pH 5, 7, and 9
Photolysis in Water	Stable	--	EUP Review 11/08/2000	
Photodegradation on Soil	Stable	--		Assumed to be stable
Aerobic Soil Degradation	30 days (half-life)	 diphenylglycolic acid (25%)	EUP Review 11/08/2000	30 days in single soil study
Aerobic Aquatic Degradation	180 days (half-life)	--	2X aerobic soil value	No study submitted, assumed value
K <sub>d</sub>	3-5	--	EUP Review 11/08/2000	4 soils
K <sub>oc</sub>	300	--	EUP Review 11/08/2000	Supplemental column leaching study

Degradation/Metabolism.

Laboratory aerobic soil metabolism data are from a single sandy loam soil from North Dakota. The mean half-life of diphacinone in this soil was 30 days (average of 28 and 32 days) from studies done at two different radiolabeled positions. A major soil metabolite ( $\geq 10\%$  of dose) was diphenylglycolic acid which peaked at a maximum of approximately 20-25% of the initial dose around one month after treatment. This metabolite then remained at a relatively constant concentration until the end of the 3.5 month study. Up to eight minor organic metabolites were separated at various times, but not identified; these generally reached relatively constant concentrations ranging from a fraction of 1% to around 6% of the initial dose. (It is possible that some of these metabolites from separate labels may not be distinct compounds.) Volatile organics were negligible. Carbon dioxide was regularly and steadily produced from both radiolabels, and reached approximately 40% of the dose from both labels by the end of the study period. Recalcitrant soil residues also accounted for a large fraction of the dose from both labels, amounting to approximately 20-30% of the initial dose by study end. The evolved carbon dioxide and soil bound residuals are clearly indicative of ultimate biological deactivation of a large fraction of diphacinone and its transformation products. This study also indicated, however, that the major metabolite, diphenylglycolic acid, was somewhat resistant to further metabolism.

Diphacinone was stable against simple hydrolysis at pH 7 and 9. At pH 5 hydrolysis proceeded with an extrapolated half-life of approximately 44 days. Products of hydrolysis were not satisfactorily identified or quantified. However, since the apparent hydrolysis was appreciable only at low pH and aerobic metabolism provides a viable alternative transformation

process for diphacinone, there is marginal value in additional information on hydrolytic degradates at low pH.

### Mobility

In Air. Volatilization of diphacinone or degradates was not detected in the aerobic soil metabolism study. A relatively low vapor pressure of approximately  $1 \times 10^{-8}$  torr and a Henry's law constant for diphacinone of approximately  $2 \times 10^{-10}$  atm-m<sup>3</sup>/mole also indicates that volatilization should not be a significant route of dissipation.

In Soil. Because of numerous deficiencies in the aged column leaching study (including not using radiolabeling, irregular soil columns, no reporting of elution rate, inability to detect less than 2% of applied diphacinone in the eluate or soil columns, no estimation of sorption coefficients, no attempt to identify and quantify degradates), the RED and previous reviews simply gave qualitative statements to the effect that diphacinone was "relatively immobile" because it was not detected in the leachates of the four soils tested and moved significantly only in a loamy sand soil. However, if we were to take the column results at face value and accept the limit of six centimeter soil increment study resolution, we can approximate sorption coefficients by estimating column retardation factors and using nominal assumptions about soil bulk density and porosity. For simplicity and convenience, we have assumed a nominal and uniform soil bulk density of 1.5 g/cm<sup>3</sup> and a porosity of 0.5 or 50%. Within error limits, choosing any reasonable specific values would not alter conclusions as explained in the following paragraph.

For three of the four soils (sandy loam, silt loam, sand), diphacinone was essentially confined to the top 6 cm of 30 cm columns (in one of these soils there was small migration into the 6 to 12 cm interval). There is the possibility that, within the stated experimental error of 2%, as much as 2% of the applied could be in each of four remaining increments. However, for the purposes of approximation we non-conservatively assume no chemical in these increments. We also assume the chemical was centered at the half-way point of each 6 cm increment—for example, at 3 cm for the top increment. This may or may not be a conservative assumption because of the 6 cm limit of resolution. For the loamy sand soil column in which leaching occurred down to the 18-24 cm increment, we calculated the center of mass of the incremental distribution of diphacinone and assumed that an undetected 2% reached the lowest 24-30 cm depth increment (which was essentially an inconsequential assumption). The resulting calculated sorption coefficient (Kd) for the three soils is roughly 5 mL/g with uncertain but "wide" error bars, if for no other reason than depth increment resolution. For the loamy sand soil, the corresponding calculated Kd is approximately 3 mL/g. Compounds with Kd values in this range are often classified as mobile. The loamy sand result should be a better approximation than that for the other soils because there were measurable concentrations at several depth increments. As stated previously, the registrant did not address the mobility of degradates in soil.

There is also an alternative approximation which can be made to estimate crudely the soil to water partitioning coefficient with the assumption that soil organic matter determines sorption. The estimation is based on an empirical correlation between the octanol to water partitioning ratio (Pow or Kow) and the soil organic matter to water sorption coefficient (Koc). The

relationships<sup>1</sup>, assuming soil organic matter is typically about 58% carbon [i.e., organic carbon (oc) = organic matter (om)/1.72)], are

$$\begin{aligned}\log (K_{om}) &= 0.904 \log (P_{ow}) - 0.779 \text{ (n = 12, r-squared = 0.989)} \\ \log (K_{oc}) &= 0.904 \log (P_{ow}) - 0.779 + \log 1.72\end{aligned}$$

Another empirical relationship given in a technical guidance document supported by the European Community (Directive 93/67/EEC) to calculate Koc from Pow is:

$$\log K_{oc} = 0.52 \log (P_{ow}) + 1.02$$

Log Pow for diphacinone is 4.27 (see comment on this value in Bioconcentration paragraph below). Thus, from the first relationship, log Koc = 3.32 and Koc is roughly 2100; from the second, log Koc = 3.24 and Koc is roughly 1700. These similar Koc values and the Kd values above place diphacinone in an intermediate range of mobility.

### Bioconcentration

The registrant has not submitted a bioconcentration study at this time. However, judging from the diphacinone octanol to water partitioning ratio (Pow or Kow) of approximately 20,000 (log Pow = 4.3), there is potential for bioconcentration.

#### **2.4.1. Environmental Transport Mechanisms**

Potential transport mechanisms typically include pesticide surface water runoff, spray drift, and secondary drift of volatilized or soil-bound residues leading to deposition onto nearby or more distant ecosystems. However, because the uses of diphacinone are in bait application or animal burrow treatment, spray drift is presumed to be negligible. Because diphacinone bait may be used outdoors, some potential exist for residues of diphacinone to leach from the bait that is exposed to rainwater or runoff. Due to the extremely low application rate (up to 0.0034 lb ai/ac), the low drift potential, and the formulation within a bait, the potential for contaminating surface water is believed to be insignificant, which is confirmed using Tier 1 (GENEEC) modeling.

Another possible route of transport is within the bodies of animals which feed on the diphacinone bait. Because poisoned animals would not be killed immediately, they would travel some distance before dying, thereby 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 diet includes small mammals, and thus it is vulnerable to secondary exposure from consuming poisoned rodents.

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<sup>1</sup>Sawhney, B. L. and Brown, K., Editors. Reactions and Movement of Organic Chemicals in Soils. SSSA Special Publication Number 22. Soil Science Society of America, Inc. Madison, WI. 1989.



### 2.4.2. Mechanism of Action

Diphacinone is first generation indanedione anticoagulant rodenticide and it is absorbed through the gut, inhibiting vitamin K. Indandiones are not only anticoagulants; they also uncouple oxidative phosphorylation (energy generation) in mammals. Diphacinone seems to have a different mode of action in reptiles. Mortality occurs but does not result from anticoagulation and its mode of action in snakes is unknown.

### 2.4.3. Use Characterization

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

Currently registered uses of diphacinone include non-agricultural areas and agricultural areas. Diphacinone may be used in and around buildings (placement must be within 50 feet of exterior walls), inside transport vehicles (ships, trains, and aircraft), alleys in urban areas, and sewers. Buildings where diphacinone bait may be used include residential, commercial, industrial, and agricultural premises, as well as port and terminal buildings. Labeled uses sites for agriculture include vegetables (unspecified), small grains (unspecified), alfalfa, apple, almond, walnut, pecan, pear, peach, nectarine, filbert, orchard (unspecified), and agricultural crops/soils (unspecified). Labeled uses sites for non-food and non-agricultural uses are sewage systems, drainage systems, forest trees, forest plantings (tree farms, tree plantations), household/domestic dwellings outdoor premises, rangeland, pastures, urban areas, nursery stock, ornamental woody shrubs and vines, ornamental lawns and turf, golf course turf, nonagricultural rights-of-way/hedgerows, and airports/landing fields.

Table 2-3 presents the uses and corresponding application rates considered in this assessment.

**Table 2-3. Diphacinone Uses Assessed for California**

Use	Original Maximum App. Rate	Converted Maximum App. Rate	+Maximum # App. /Unit of Time	+Minimum Retreatment Interval
Aquatic Non-Food Industrial				
Sewage Systems	16 oz/1 manhole or 1 placement	0.00005 lb ai/1 manhole or 1 placement	--	--
Drainage Systems	4 lb/1 manhole or 1 placement	0.0002 lb ai/1 manhole or 1 placement	--	14 days

Use	Original Maximum App. Rate	Converted Maximum App. Rate	+Maximum # App. /Unit of Time	+Minimum Retreatment Interval
Forestry				
Forest Trees (All or Unspecified)	2 lb / ac	0.0002 lb ai/ac	--	--
Forest Plantings (Reforestation Programs) (Tree Farms, Tree Plantations, etc.)	20 lb /ac	0.001 lb ai/ac	--	14 days
Indoor Food				
Agricultural/Farm Structures/Buildings and Equipment	16 oz / 1 station	0.00005 lb ai/1 station	--	--
Indoor Non-Food				
Ships and Boats	16 oz/ 1 placement	0.00005 lb ai/ 1 placement	--	--
Public Buildings/Structures (Vert. Pest Control)	16 oz/ 1 placement	0.00005 lb ai/ 1 placement	--	--
Commercial/Institutional/Industrial Premises/Equip. (Indoor)	0.5 oz / 72 sq in	5.42535 lb ai/ac	--	--
Commercial Transportation Facilities-Nonfeed/Nonfood	16 oz/ 1 placement	0.00005 lb ai/ 1 placement	--	--
Agricultural/Farm Premises	0.5 oz / 72 sq in	5.42535 lb ai/ac	--	--
Indoor Residential				
Household/Domestic Dwellings Indoor Premises	16 oz/ 1 placement	0.00005 lb ai/ 1 placement	--	--
Household/Domestic Dwellings Indoor Nonfood Handling Areas	1 oz / 1 placement	0.00000313 lb ai/ 1 placement	--	--
Household/domestic Dwellings Contents	1 oz / 1 placement	0.00000313 lb ai/ 1 placement	--	--
Household/Domestic Dwellings	1 oz / 1 placement	0.00000313 lb ai/ 1 placement	--	--
Outdoor Residential				
Household/Domestic Dwellings Outdoor Premises	16 oz/ 1 placement	0.00005 lb ai/ 1 placement	--	--

Use	Original Maximum App. Rate	Converted Maximum App. Rate	+Maximum # App. /Unit of Time	+Minimum Retreatment Interval
Terrestrial Feed Crop				
Rangeland	4 lb/ 1 placement	0.0002 lb ai/ 1 placement	--	14 days
Pastures	4 lb/ 1 placement	0.0002 lb ai/ 1 placement	--	--
Grass Forage/Fodder/Hay	8 lb /ac	0.0004 lb ai/ac	--	--
Alfalfa	8 lb /ac	0.0004 lb ai/ac	--	--
Terrestrial Food + Feed Crop				
Vegetables (Unspecified)	8 lb /ac	0.0004 lb ai/ac	--	14 days
Small Grains (Unspecified)	1 lb / 1 bait station	0.00005 lb ai/ 1 bait station	--	14 days
Small Grains	8 lb /ac	0.0004 lb ai/ac	--	--
Orchards (Unspecified)	20 lb /ac	0.001 lb ai/ac	--	14 days
Apple	20 lb /ac	0.001 lb ai/ac	--	30 days
Almond	20 lb /ac	0.001 lb ai/ac	--	30 days
Terrestrial Food Crop				
Walnut (English/Black)	20 lb /ac	0.001 lb ai/ac	--	30 days
Pecan	20 lb /ac	0.001 lb ai/ac	--	30 days
Pear	20 lb /ac	0.001 lb ai/ac	--	30 days
Peach	20 lb /ac	0.001 lb ai/ac	--	30 days
Orchards (Unspecified)	20 lb /ac	0.001 lb ai/ac	--	30 days
Nectarine	20 lb /ac	0.001 lb ai/ac	--	30 days
Filbert (Hazelnut)	20 lb /ac	0.001 lb ai/ac	--	30 days
Terrestrial Non-Food + Outdoor Residential				

Use	Original Maximum App. Rate	Converted Maximum App. Rate	+Maximum # App. /Unit of Time	+Minimum Retreatment Interval
Urban Areas	16 oz/ 1 placement	0.00005 lb ai/ 1 placement	--	--
Terrestrial Non-Food Crop				
Agricultural Crops/Soils (Unspecified)	4 lb/ 1 placement	0.0002 lb ai/ 1 placement	--	--
Refuse/Solid Waste Sites (Outdoor)	16 oz/ 1 placement	0.00005 lb ai/ 1 placement	--	--
Recreational Areas	2.5 oz / 50 sq ft	0.00340266 lb ai/ac	4	2 days
Recreational Lawns	16 oz / 1 bait station	0.00005 lb ai/ 1 bait station	--	14 days
Ornamental Woody Shrubs and Vines	7 oz /1 tree canopy	0.00002188 lb ai/1 tree canopy	--	--
Ornamental Lawns and Turf	8 lb /ac	0.0004 lb ai/ac	--	14 days
Ornamental and/or Shade Trees	7 oz /1 tree canopy	0.00002188 lb ai/1 tree canopy	--	--
Orchards (Unspecified)	20 lb /ac	0.001 lb ai/ac	4	2 days
Nursery Stock	20 lb /ac	0.001 lb ai/ac		14 days
Nonagricultural Uncultivated Areas/Soils	20 lb /ac	0.001 lb ai/ac	4	2 days
Nonagricultural Rights-Of- Way/Fencerows/Hedgerows	2.5 oz / 50 sq ft	0.00340266 lb ai/ac	4	2 days
Nonagricultural Outdoor Buildings/Structures	4 lb/ 1 placement	0.0002 lb ai/ 1 placement	--	--
Golf Course Turf	2.5 oz / 50 sq ft	0.00340266 lb ai/ac	4	2 days
Forest Nursery Plantings (For Transplant Purposes)	20 lb /ac	0.001 lb ai/ac	--	14 days
Fencerows/Hedgerows	4 lb/ 1 placement	0.0002 lb ai/ 1 placement	--	--

Use	Original Maximum App. Rate	Converted Maximum App. Rate	+Maximum # App. /Unit of Time	+Minimum Retreatment Interval
Commercial/Institutional/Industrial Premises/Equipment (Outdoor)	16 oz/ placement	0.00005 lb ai/ 1 placement	--	--
Christmas Tree Plantations	20 lb /ac	0.001 lb ai/ac	--	30 days
Airports/Landing Fields	16 oz/ 1 placement	0.00005 lb ai/ 1 placement	--	--
Agricultural/Farm Structures/Buildings and Equipment	4 lb/ 1 placement	0.0002 lb ai/ 1 placement	--	14 days
Agricultural/Farm Premises	8 lb/ 1 placement	0.0004 lb ai/ 1 placement	--	--
Agricultural Uncultivated Areas	16 oz / 1 bait station	0.00005 lb ai/ 1 bait station	--	--
Agricultural Crops/Soils (Unspecified)	4 lb/ 1 placement	0.0002 lb ai/ 1 placement	--	--

In the LUIS report, the following five columns (maximum number of applications per crop cycle; original seasonal maximum dose per year; converted seasonal maximum dose per year; original seasonal maximum dose per crop cycle; and converted seasonal maximum dose per crop cycle) are all entered with NS (Non-Specified). The converted maximum application rate is 5.425 lb ai/ac for agricultural/farm premises. This rate is converted from 0.5 oz/72 sq inch with 0.0025% AI. For each uses, only the highest labeled allowable rate is reported in this table. + All entries denoted with dashes in these two columns are denoted as NS (Non-Specifeid) in the LUIS report.

Neither the National Agricultural Statistics Service (NASS) nor the United States Geological Survey (USGS) is able to provide geographical data for diphacinone use.

The Agency's Biological and Economic Analysis Division (BEAD) provides an analysis of county level usage information (County-Level Usage for Chlorophacinone; Diphacinone; Diphacinone, Sodium Salt; and Mancozeb in California in Support of a San Francisco Bay Endangered Species Assessment, Draft, April 20, 2011) using state-level usage data obtained from California's Department of Pesticide Regulation Pesticide Use Reporting (CDPR PUR) database<sup>1</sup>. 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 diphacinone by county in this California-specific assessment were generated using CDPR PUR data. Eleven years (1999-2009) of usage data were included in this analysis. Data from CDPR PUR were obtained for every agricultural pesticide application made on every use site at the section level

<sup>1</sup> 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>.

(approximately one square mile) of the public land survey system.<sup>2</sup> 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, average annual area treated, and average and maximum application rate across all eleven years. The units of area treated are also provided where available.

According to the classification of “application rate by use site,” the analyses of the CDPR PUR data show all entries are either zero or blank for the columns of average application rate, 95 percentile application rate, and 99 percentile application rate. Only three entries in the maximum application rate column show a value of 0.1 lb per acre for pastureland, landscape maintenance, and rangeland. **Table 2-4** depicts the average annual use of diphacinone in various counties of California. San Diego and Orange Counties have the highest average annual uses, followed by Los Angeles County. According to all the reported average annual pounds applied, the diphacinone usage is very limited, with the highest reported amount of 6.1 pounds per year.

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<sup>2</sup> Most pesticide applications to parks, golf courses, cemeteries, rangeland, pastures, and along roadside and railroad rights of way, and postharvest treatments of agricultural commodities are reported in the database. The primary exceptions to the reporting requirement are home-and-garden use and most industrial and institutional uses (<http://www.cdpr.ca.gov/docs/pur/purmain.htm>).

**Table 2-4. Summary of California Department of Pesticide Registration (CDPR) Pesticide Use Reporting (PUR) Data from 1999 to 2009 for County Diphacinone Uses<sup>1</sup>**

County Name	Average Annual Pounds Applied
SAN DIEGO	6.1
ORANGE	6.1
LOS ANGELES	4.4
CONTRA COSTA	2.5
RIVERSIDE	2.1
FRESNO	1.8
KERN	1.5
SAN LUIS OBISPO	1.4
SANTA BARBARA	1.3
TULARE	1.3
SAN BERNARDINO	0.9
SAN JOAQUIN	0.9
MERCED	0.9
STANISLAUS	0.8
ALAMEDA	0.7
VENTURA	0.6
SOLANO	0.5
MADERA	0.4
SACRAMENTO	0.3
SAN MATEO	0.3
MONTEREY	0.3
YOLO	0.3
SANTA CLARA	0.3
SAN BERNARDINO	0.2
KINGS	0.2
SUTTER	0.2
MARIN	0.2
PLACER	0.1
SONOMA	0.1
STANISLAUS	0.1
MARIPOSA	0.1
SAN FRANCISCO	0.1
SANTA CRUZ	0.1
COLUSA	0.1
BUTTE	0.1

1- Based on data supplied by BEAD (County-Level Usage for Chlorophacinone; Diphacinone; Diphacinone, Sodium Salt; and Mancozeb in California in Support of a San Francisco Bay Endangered Species Assessment, Draft, April 20, 2011).

## 2.5. Assessed Species

The following section provides a summary of the current distribution, habitat requirements, and life history parameters for the listed species being assessed. See Figure 2-.1 – 2.4 below for maps of the current range and designated critical habitat, if applicable, of the assessed listed species.

**Alameda Whipsnake (AW):** The AW, a subspecies of the California Whipsnake (*Masticophis lateralis*), was listed as threatened in 1997 by the USFWS. 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. A recovery plan for the SMHM was approved by the USFWS in 1973. No critical habitat has been designated for this species. The species is found in tidal and non-tidal salt marshes along the San Francisco, San Pablo, and Suisun Bays in California.

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

**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 2-5. Summary of Current Distribution, Habitat Requirements, and Life History Information for the Assessed Listed Species<sup>1</sup>**

Assessed Species	Size	Current Range	Habitat Type	Designated Critical Habitat?	Reproductive Cycle	Diet
Salt Marsh Harvest Mouse (SMHM) ( <a href="#"><i>Reithrodontomys raviventris</i></a> )	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	<u>Breeding:</u> March – November <u>Gestation period:</u> 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) ( <a href="#"><i>Vulpes macrotis mutica</i></a> )	Adult ~2 kg	Alameda, Contra Costa, Fresno, Kern, Kings, Madera, Merced, Monterey, San Benito, San Joaquin, San Luis Obispo, Santa Barbara, Santa Clara, Stanislaus, Tulare and Ventura counties	A variety of habitats, including grasslands, scrublands ( <i>e.g.</i> , chenopod scrub and sub-shrub scrub), vernal pool areas, oak woodland, alkali meadows and playas, and an agricultural matrix of row crops, irrigated pastures, orchards, vineyards, and grazed annual grasslands. Kit foxes dig their own dens, modify and use those already constructed by other animals (ground squirrels, badgers, and coyotes), or use human-made structures (culverts,	No, but has designated core areas	<u>Mating and conception:</u> late December - March. <u>Gestation period:</u> 48 to 52 days. <u>Litters born:</u> February - late March  Pups emerge from their dens at about 1-month of age and may begin to disperse after 4 – 5 months usually in Aug. or Sept.	Small animals including blacktailed hares, desert cottontails, mice, kangaroo rats, squirrels, birds, lizards, insects and grass. It satisfies its moisture requirements from prey and does not depend on freshwater sources.

Assessed Species	Size	Current Range	Habitat Type	Designated Critical Habitat?	Reproductive Cycle	Diet
			abandoned pipelines, or banks in sumps or roadbeds). They move to new dens within their home range often (likely to avoid predation by coyotes)			
Alameda Whipsnake (AW) ( <a href="#">Masticophis lateralis euryxanthus</a> )	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
California Tiger Salamander (CTS) ( <a href="#">Ambystoma californiense</a> )	Adult 14.2-80.5 g <sup>3</sup>	CTS-SC are primarily found on the Santa Rosa Plain in Sonoma County.  CTS-CC occupies the Bay Area (central and southern Alameda, Santa Clara, western Stanislaus, western Merced, and the majority of San Benito Counties), Central Valley (Yolo, Sacramento, Solano, eastern Contra Costa, northeast Alameda, San Joaquin, Stanislaus, Merced, and northwestern Madera Counties), southern San Joaquin Valley (portions of Madera, central Fresno, and northern Tulare and Kings Counties), and the Central Coast Range (southern Santa Cruz,	Freshwater pools or ponds (natural or man-made, vernal pools, ranch stock ponds, other fishless ponds); Grassland or oak savannah communities, in low foothill regions; Small mammal burrows	Yes	<u>Emerge from burrows and breed:</u> fall and winter rains <u>Eggs:</u> laid in pond Dec. – Feb., hatch: after 10 to 14 days <u>Larval stage:</u> 3-6 months, until the ponds dry out, metamorphose late spring or early summer, migrate to small mammal burrows	<u>Aquatic Phase:</u> algae, snails, zooplankton, small crustaceans, and aquatic larvae and invertebrates, smaller tadpoles of Pacific tree frogs, CRLF, toads; <u>Terrestrial Phase:</u> terrestrial invertebrates, insects, frogs, and worms

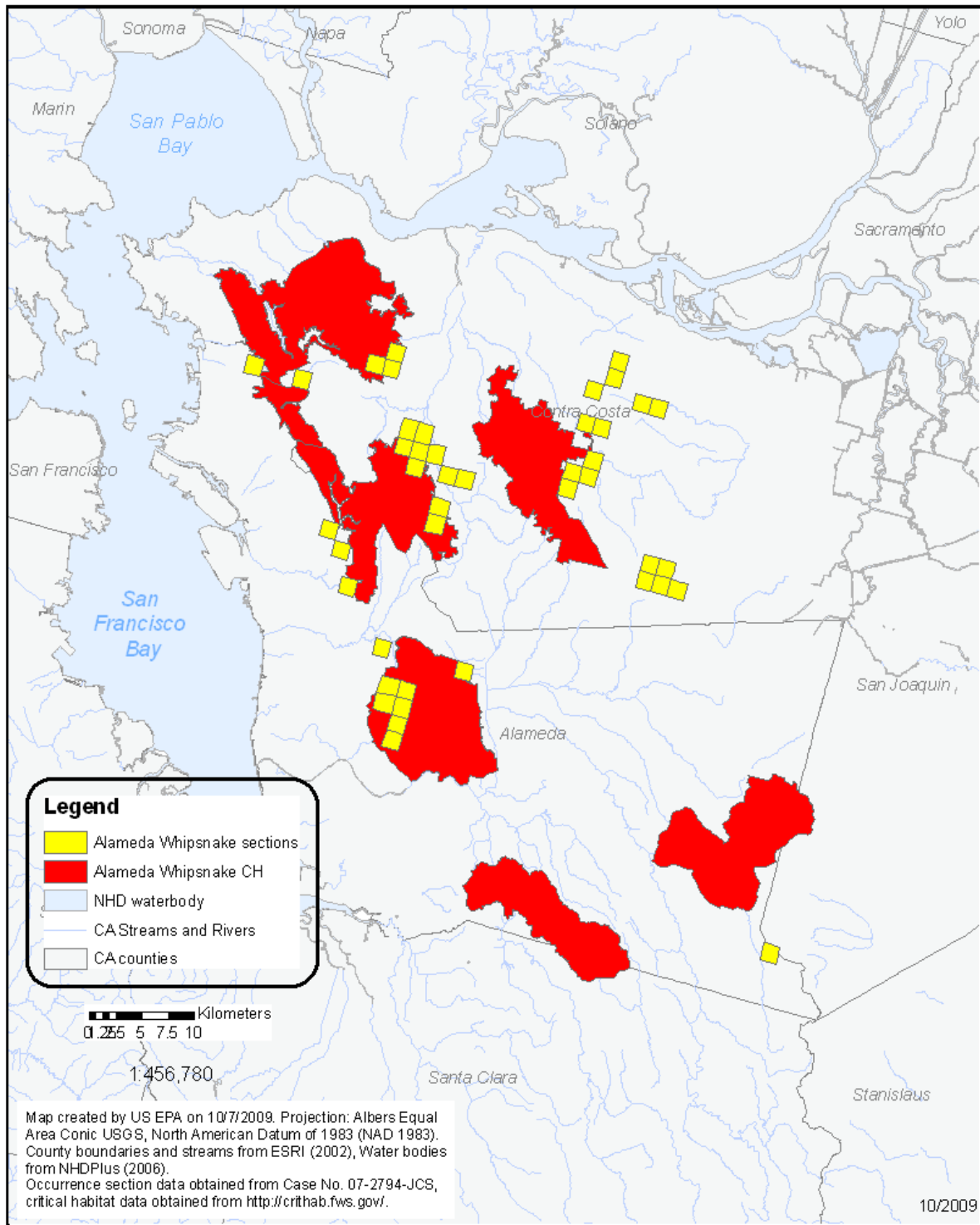
Assessed Species	Size	Current Range	Habitat Type	Designated Critical Habitat?	Reproductive Cycle	Diet
		Monterey, northern San Luis Obispo, and portions of western San Benito, Fresno, and Kern Counties).  CTS-SB are found in Santa Barbara County.				

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

<sup>2</sup> Oviparous = eggs hatch within the female's body and young are born live.

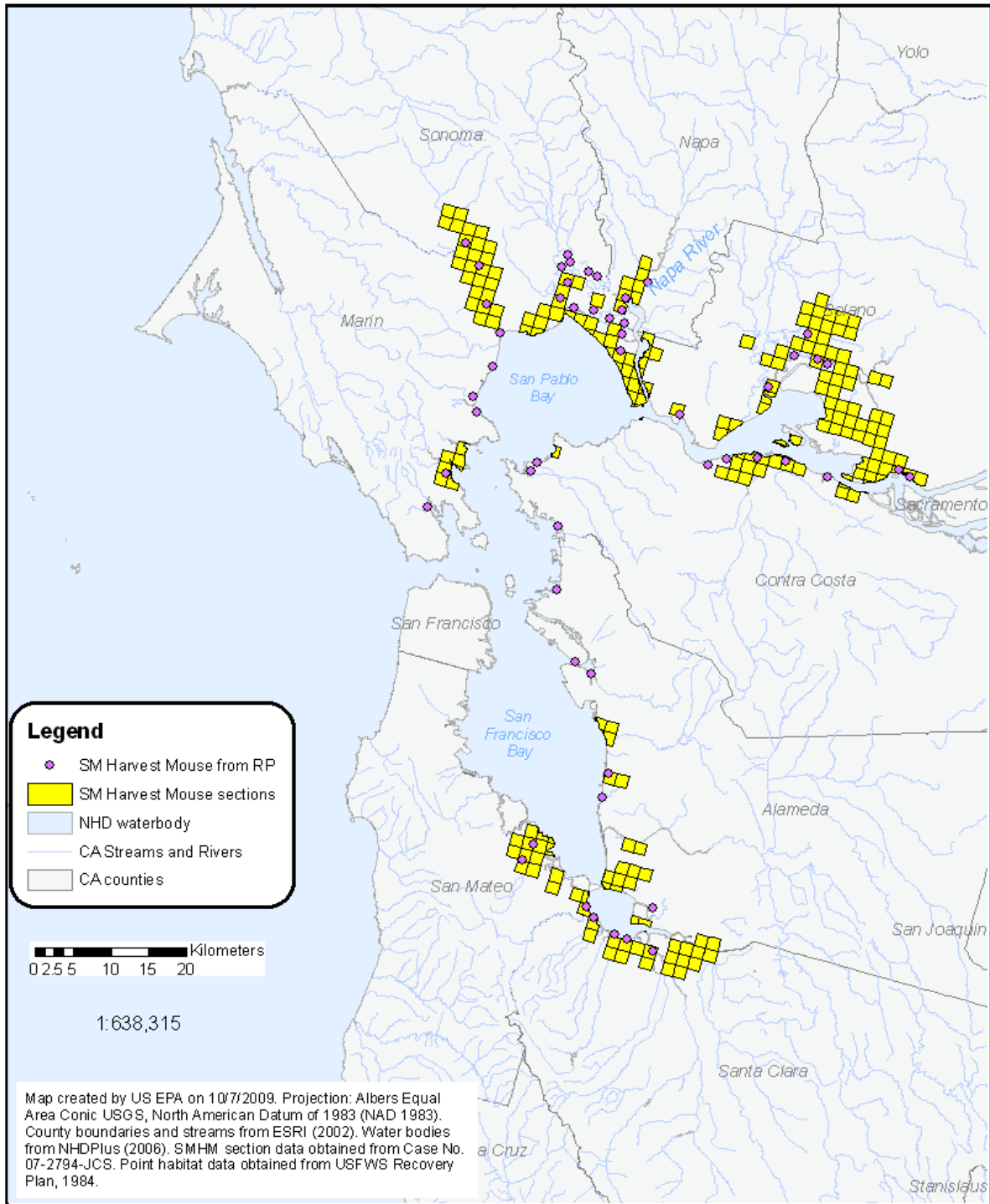
<sup>3</sup> See Page 369 of Trenham *et al.* (Trenham *et al.*, 2000).

## Alameda Whipsnake Habitat



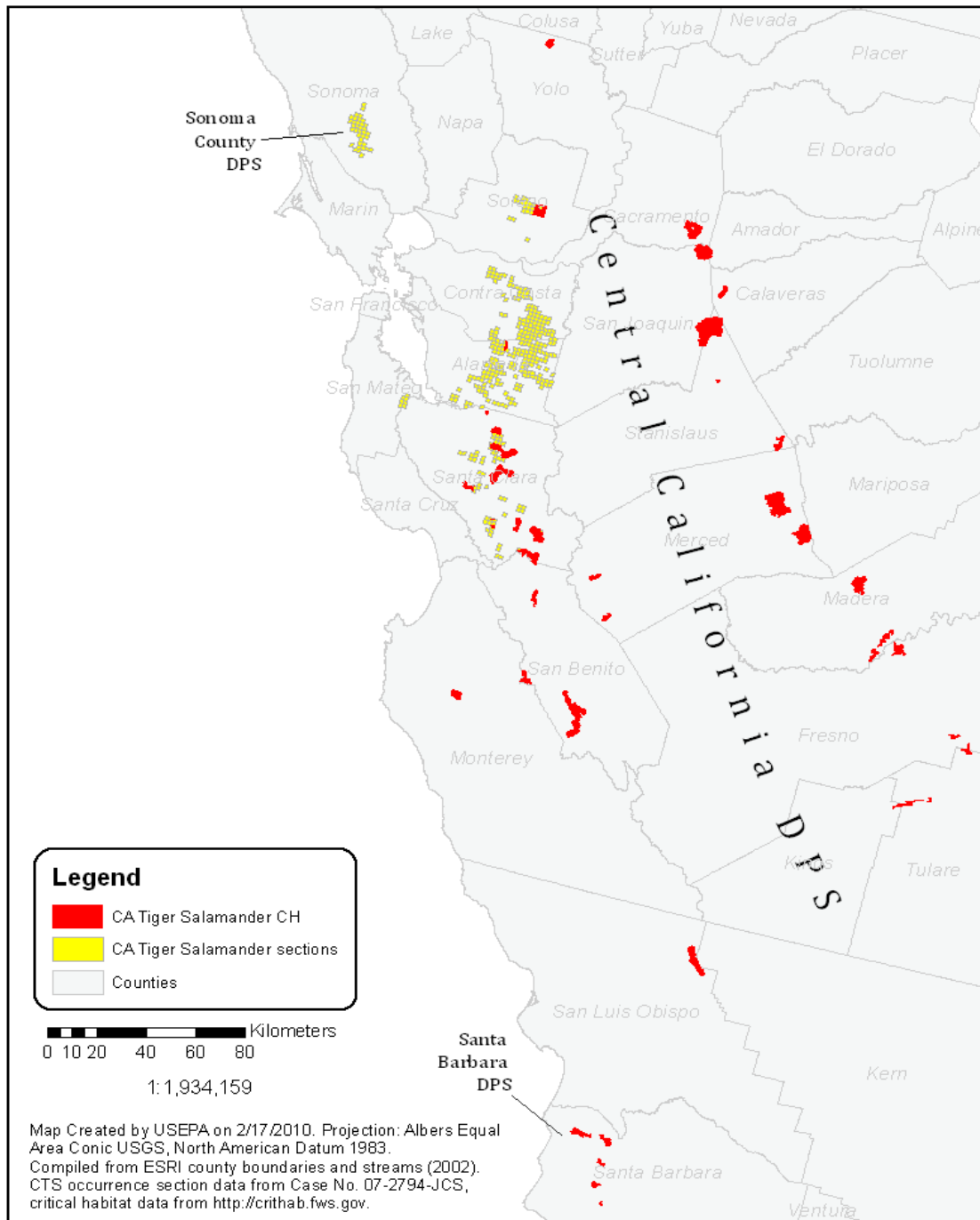
**Figure 2-1. Critical habitat and occurrence sections of the Alameda Whipsnake, as identified in Case No. 07-2794-JCS**

## Salt Marsh Harvest Mouse Habitat



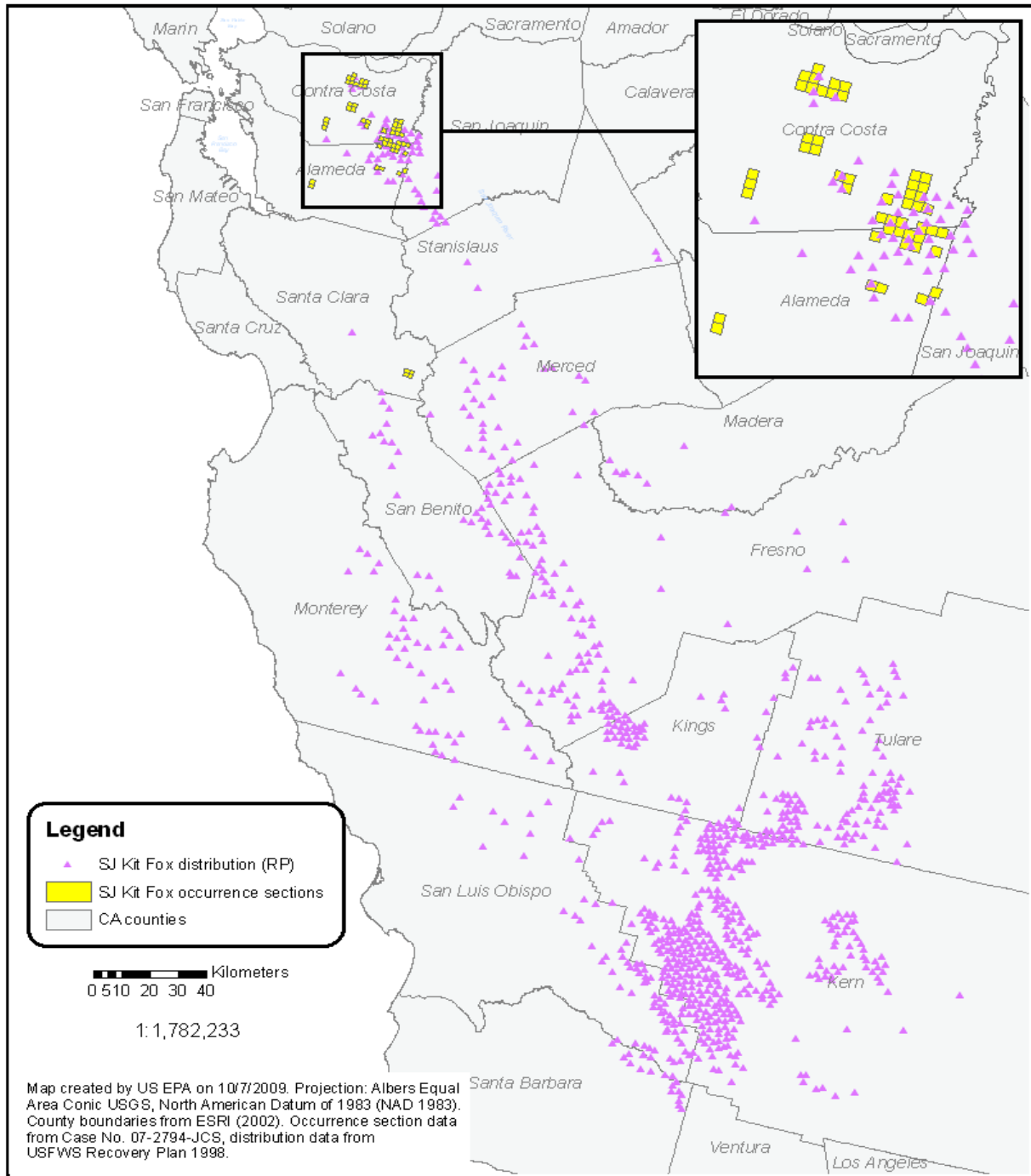
**Figure 2-2. Occurrences and occurrence sections of the salt marsh harvest mouse, as identified in Case No. 07-2794-JCS.**

## CA Tiger Salamander Habitat



**Figure 2-3. Occurrences and occurrence sections of the CA Tiger Salamander, as identified in Case No. 07-2794-JCS.**

## San Joaquin Kit Fox Habitat



**Figure 2-4. 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 and the CTS. Risk to critical habitat is evaluated separately from risk to effects on the species. Critical habitat is defined in the ESA as the geographic area occupied by the species at the time of the listing where the physical and biological features necessary for the conservation of the species exist, and there is a need for special management to protect the listed species. It may also include areas outside the occupied area at the time of listing if such areas are essential to the conservation of the species. Critical habitat designations identify, to the extent known using the best scientific and commercial data available, habitat areas that provide essential life cycle needs of the species or areas that contain certain primary constituent elements (PCEs) (as defined in 50 CFR 414.12(b)). Table 2-6 describes the PCEs for the critical habitats designated for the AW and the CTS.

**Table 2-6. Designated Critical Habitat PCEs for the CA Tiger Salamander and the Alameda Whipsnake<sup>1</sup>.**

Species	PCEs	Reference
California tiger salamander	Standing bodies of fresh water, including natural and man-made (e.g., stock) ponds, vernal pools, and dune ponds, and other ephemeral or permanent water bodies that typically become inundated during winter rains and hold water for a sufficient length of time (i.e., 12 weeks) necessary for the species to complete the aquatic (egg and larval) portion of its life cycle <sup>2</sup>	FR Vol. 69 No. 226 CTS, 68584, 2004
	Barrier-free uplands adjacent to breeding ponds that contain small mammal burrows. Small mammals are essential in creating the underground habitat that juvenile and adult California tiger salamanders depend upon for food, shelter, and protection from the elements and predation	
	Upland areas between breeding locations (PCE 1) and areas with small mammal burrows (PCE 2) that allow for dispersal among such sites	
Alameda whipsnake	Scrub/shrub communities with a mosaic of open and closed canopy	71 FR 58175 58231, 2006
	Woodland or annual grassland plant communities contiguous to lands containing PCE 1	
	Lands containing rock outcrops, talus, and small mammal burrows within or adjacent to PCE 1 and or PCE 2	

<sup>1</sup> 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.

<sup>2</sup> PCEs that are abiotic, including, physical-chemical water quality parameters such as salinity, pH, and hardness are not evaluated.

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 Diphacinone that may alter the PCEs of the designated critical habitat for the AW and the CTS 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 Diphacinone is expected to directly impact living organisms within the action area, critical habitat analysis for Diphacinone 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 diphacinone is likely to encompass considerable portions of the United States based on the large array of agricultural and non-agricultural uses. 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, SJKF, SMHM and CTS and their designated critical habitat within the state of California. For this assessment, the entire state of California is considered the action area given the large extent of potential use sites (residential, agricultural, and forestry uses). 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. 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, SJKF, SMHM and CTS and designated critical habitat may be affected or modified via endpoints associated with reduced survival, growth, or reproduction.

### **2.7.2 LAA Effects Determination Area**

A stepwise approach is used to define the Likely to Adversely Affect (LAA) Effects Determination Area. An LAA effects determination applies to those areas where it is expected that the pesticide’s use will directly or indirectly affect the species and/or modify its designated

critical habitat using EFED's standard assessment procedures (see Attachment 1) and effects endpoints related to survival, growth, and reproduction. This is the area where the "Potential Area of LAA Effects" (initial area of concern + drift distance or downstream dilution distance) overlaps with the range and/or designated critical habitat for the species being assessed. If there is no overlap between the potential area of LAA effects and the habitat or occurrence areas, a No Effect determination is made. The first step in defining the LAA Effects Determination Area is to understand the federal action. The federal action is defined by the currently labeled uses for diphacinone. An analysis of labeled uses and review of available product labels was completed. Some of the currently labeled uses are special local needs (SLN) uses not specified for use in California or are restricted to specific states and are excluded from this assessment. In addition, a distinction has been made between food use crops and those that are non-food/non-agricultural uses. For those uses relevant to the assessed species, the analysis indicates that, for diphacinone, the following agricultural uses are considered as part of the federal action evaluated in this assessment:

- Vegetables (unspecified)
- Small Grains (unspecified)
- Alfalfa
- Apple
- Almond
- Walnut
- Pecan
- Pear
- Peach
- Nectarine
- Filbert
- Orchard (unspecified)
- Agricultural Crops/Soils (unspecified)

In addition, the following non-food and non-agricultural uses are considered:

- Sewage Systems
- Drainage Systems
- Forest Trees
- Forest Plantings (Tree Farms, Tree Plantations)
- Household/Domestic Dwellings Outdoor Premises
- Rangeland
- Pastures
- Urban Areas
- Nursery Stock
- Ornamental Woody Shrubs and Vines
- Ornamental Lawns and turf (including Golf Course turf)
- Nonagricultural Rights-of-Way/Hedgerows
- Airports/Landing Fields

Following a determination of the assessed uses, an evaluation of the potential “footprint” of diphacinone use patterns (*i.e.*, the area where pesticide application may occur) is determined. This “footprint” represents the initial area of concern, based on an analysis of available land cover data for the state of California. The initial area of concern is defined as all land cover types and the stream reaches within the land cover areas that represent the labeled uses described above. For diphacinone, the land cover types include cultivated, orchard, developed open/developed low/developed medium/developed high, forestry, pasture, urban and turf. The land cover types that make up the initial area of concern for diphacinone is broad, almost representing the whole state, so no specific map was generated.

## 2.8. Assessment Endpoints and Measures of Ecological Effect

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

**Table 2-7. Taxa Used in the Analyses of Direct and Indirect Effects for the Assessed Listed Species.**

Listed Species	Birds	Mammals	Terr. Plants	Terr. Inverts.	FW Fish	FW Inverts.	Estuarine /Marine Fish	Estuarine /Marine Inverts.	Aquatic Plants
San Joaquin kit fox	Indirect (prey)	Direct  Indirect (prey)	Indirect (food/habitat)	Indirect (prey)	n/a	n/a	n/a	n/a	n/a
California Alameda whipsnake	Direct  Indirect (prey)	Indirect (prey/habitat)	Indirect (habitat)	Indirect (prey)	n/a	n/a	n/a	n/a	n/a
California tiger salamander	Direct	Indirect (prey/habitat)	Indirect (habitat)	Indirect (prey)	Direct  Indirect (prey)	Indirect (prey)	n/a	n/a	Indirect (food/habitat)
Salt marsh harvest mouse	Indirect (rearing sites)	Direct  Indirect (rearing sites)	Indirect (food, habitat)	Indirect (prey)	n/a	n/a	n/a	n/a	Indirect (habitat)

Abbreviations: n/a = Not applicable; Terr. = Terrestrial; Invert. = Invertebrate; FW = Freshwater

**Table 2-7. Taxa and Assessment Endpoints Used to Evaluate the Potential for Use of Diphacinone to Result in Direct and Indirect Effects to the Assessed Listed Species or Modification of Critical Habitat.**

Taxa Used to Assess Direct and Indirect Effects to Assessed Species and/or Modification to Critical Habitat or Habitat	Assessed Listed Species	Assessment Endpoints	Measures of Ecological Effects
1. Freshwater Fish and Aquatic-Phase Amphibians	<u>Direct Effect</u> – -California Tiger Salamander	Survival, growth, and reproduction of individuals via direct effects	Most sensitive fish acute LC <sub>50</sub> (guideline or ECOTOX)
	<u>Indirect Effect (prey)</u> None	Survival, growth, and reproduction of individuals or modification of critical habitat/habitat via indirect effects on aquatic prey food supply ( <i>i.e.</i> , fish and aquatic-phase amphibians)	
2. Freshwater Invertebrates	<u>Direct Effect</u> – <u>None</u>	Survival, growth, and reproduction of individuals via direct effects	Most sensitive freshwater invertebrate EC <sub>50</sub> (guideline or ECOTOX)
	<u>Indirect Effect (prey)</u> - CA Tiger Salamander	Survival, growth, and reproduction of individuals or modification of critical habitat/habitat via indirect effects on aquatic prey food supply ( <i>i.e.</i> , freshwater invertebrates)	
3. Aquatic Plants (freshwater/marine)	<u>Indirect Effect (food/habitat)</u> -Salt Marsh Harvest Mouse -CA Tiger Salamander	Survival, growth, and reproduction of individuals or modification of critical habitat/habitat via indirect effects on habitat, cover, food supply, and/or primary productivity ( <i>i.e.</i> , aquatic plant community)	No data
4. Birds	<u>Direct Effect</u> -Alameda Whipsnake -CA Tiger Salamander	Survival, growth, and reproduction of individuals via direct effects	Most sensitive laboratory avian acute LC <sub>50</sub> or LD <sub>50</sub> (guideline or ECOTOX)
	<u>Indirect Effect (prey/rearing sites)</u> -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 (birds)	
5. Mammals	<u>Direct Effect</u> -Salt Marsh Harvest Mouse -San Joaquin Kit Fox	Survival, growth, and reproduction of individuals via direct effects	Most sensitive laboratory mammalian acute LC <sub>50</sub> or LD <sub>50</sub> (guideline or ECOTOX)
	<u>Indirect Effect (prey/habitat from</u>	Survival, growth, and reproduction of individuals	

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
	<u>burrows/rearing sites</u> -Salt Marsh Harvest Mouse -San Joaquin Kit Fox - Alameda Whipsnake -CA Tiger Salamander	or modification of critical habitat/habitat via indirect effects on terrestrial prey (mammals) and/or burrows/rearing sites	
6. Terrestrial Invertebrates	<u>Direct Effect</u> <u>None</u>	Survival, growth, and reproduction of individuals via direct effects	No data
	<u>Indirect Effect (prey)</u> -Salt Marsh Harvest Mouse -San Joaquin Kit Fox -Alameda Whipsnake -CA Tiger Salamander	Survival, growth, and reproduction of individuals or modification of critical habitat/habitat via indirect effects on terrestrial prey (terrestrial invertebrates)	
7. Terrestrial Plants	<u>Indirect Effect (food/habitat) (non-obligate relationship)</u> -Salt Marsh Harvest Mouse -San Joaquin Kit Fox -Alameda Whipsnake -CA Tiger Salamander	Survival, growth, and reproduction of individuals or modification of critical habitat/habitat via indirect effects on food and habitat ( <i>i.e.</i> , riparian and upland vegetation)	No data
	<u>Indirect Effect (food/habitat) (obligate relationship)</u> None		

Abbreviations: SF=San Francisco

\*The most sensitive fish species across freshwater and estuarine/marine environments is used to assess effects for these species because they may be found in freshwater or estuarine/marine environments.

\*\* 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 diphacinone 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 diphacinone 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 Diphacinone to the environment. The following risk hypotheses are presumed in this assessment:

The labeled use of diphacinone within the action area may:

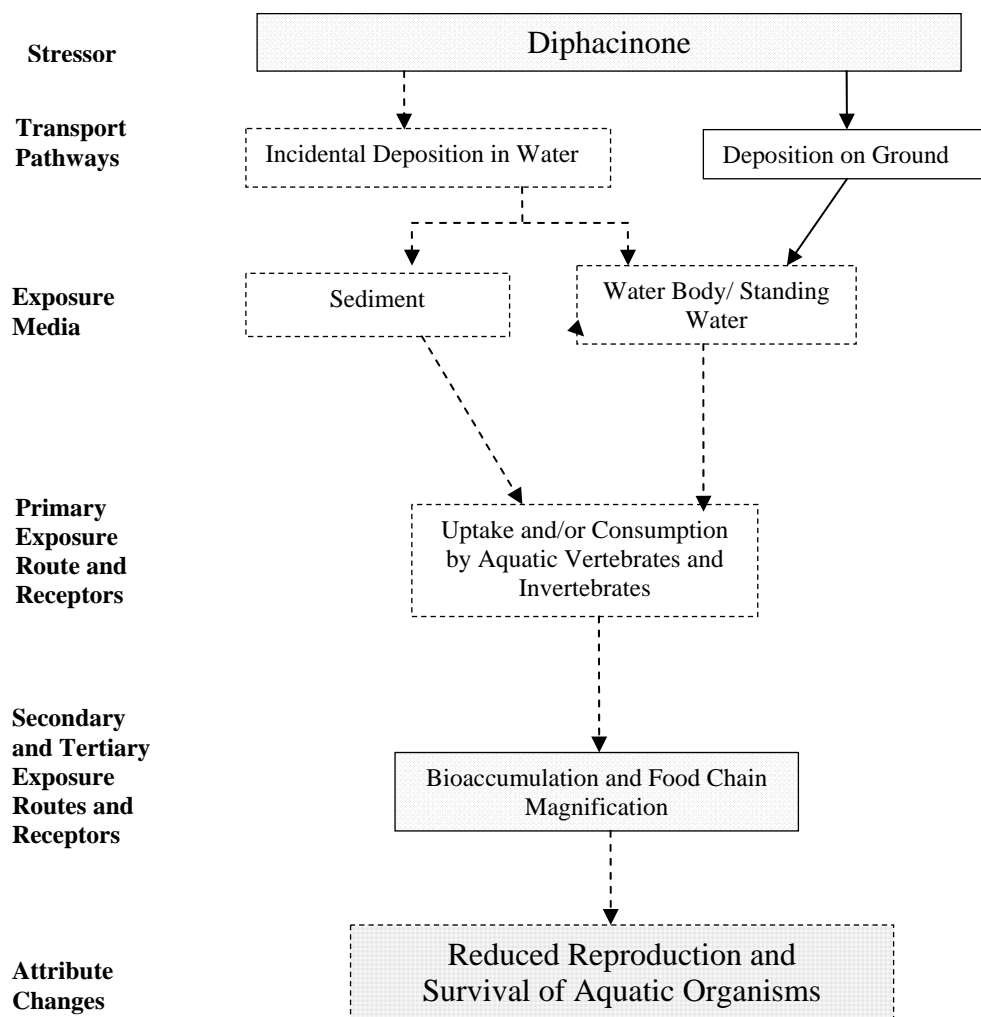
- directly affect the SJKF, AW, CTS, and the SMHM by causing mortality or by adversely affecting growth or fecundity;
- indirectly affect the SJKF, CTS and the AW and/or modify their designated critical habitat by reducing or changing the composition of food supply;
- indirectly affect CTS and/or modify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

### **2.9.2. Diagram**

The conceptual model is a graphic representation of the structure of the risk assessment. It specifies the diphacinone release mechanisms, biological receptor types, and effects endpoints of potential concern. The conceptual models for the Alameda Whipsnake, Salt Marsh Harvest Mouse, CA Tiger Salamander and the San Joaquin Kit Fox and the conceptual models for the aquatic and terrestrial PCE components of critical habitat are shown in Figures 2-5 and 2-6. 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 is expected to be negligible.

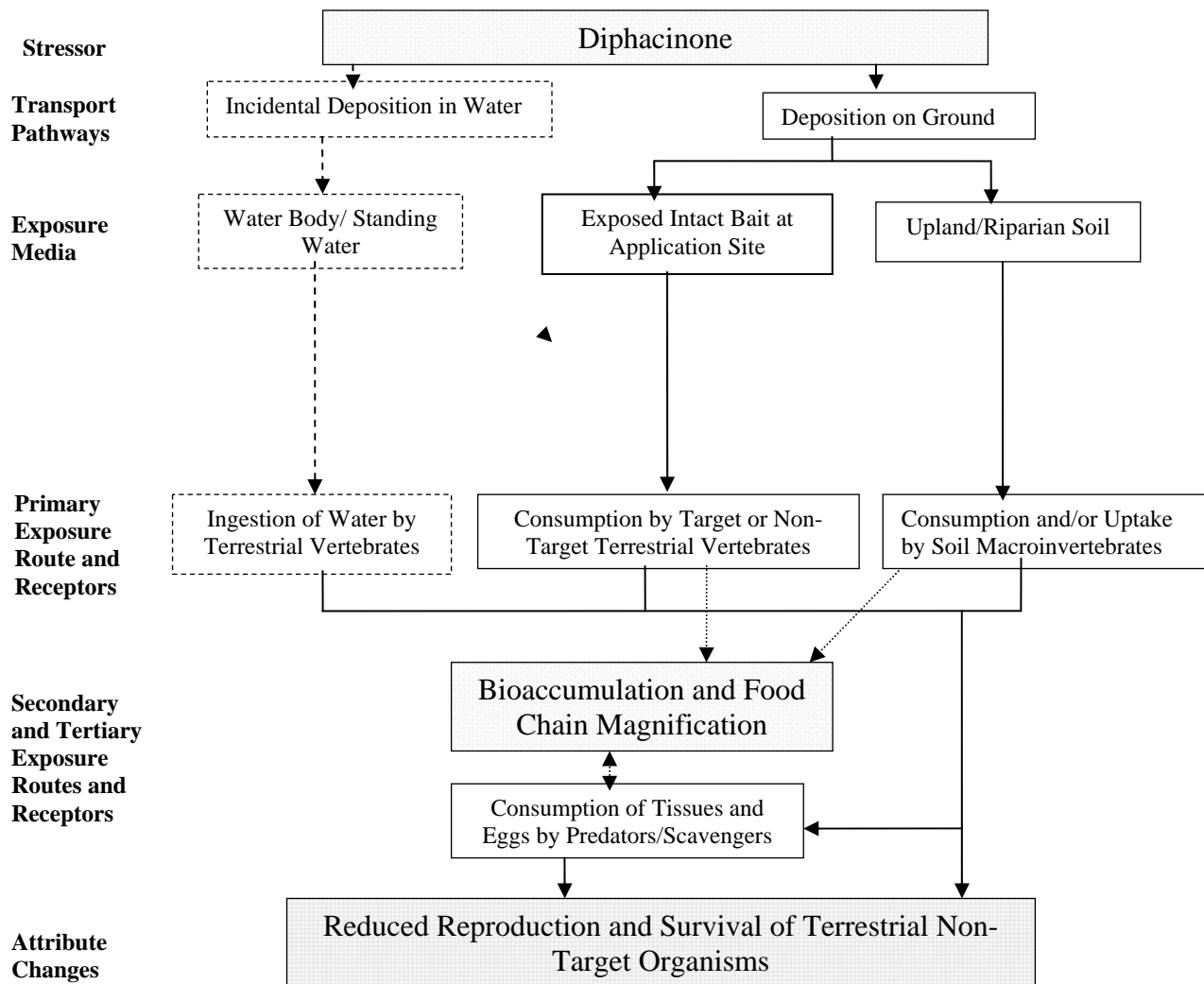
Terrestrial species may ingest diphacinone by drinking contaminated water. Diphacinone is applied as a bait, but little is expected to partition into drinking water sources (*e.g.* puddles) compared to that which is available for direct consumption on the bait itself; therefore, this route of exposure was not assessed. Since diphacinone is not sprayed directly onto plants, and because so little is expected to leach from the bait and then be available for plant uptake, consumption of diphacinone on plants is also not being considered as a route of exposure. Dermal and inhalation routes of exposure occur for some pesticides (*e.g.*, foliar sprays). However, these are not

expected to be important routes of exposure for bait because diphacinone is not volatile and is not expected to absorb appreciably through the skin.



**Figure 2-5. Conceptual model depicting stressors, exposure pathways, and potential effects to aquatic organisms from the use of diphacinone.**

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



**Figure 2-6. Conceptual model depicting stressors, exposure pathways, and potential effects to terrestrial organisms from the use of diphacinone.**

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 diphacinone are characterized and integrated to assess the risks. This is accomplished using a risk quotient (ratio of exposure concentration to effects concentration) approach. Although risk is often defined as the likelihood and magnitude of adverse ecological effects, the risk quotient-based approach does not provide a quantitative estimate of likelihood and/or magnitude of an adverse effect. However, as outlined in the Overview Document (USEPA, 2004), the likelihood of effects to individual organisms from particular uses of diphacinone is estimated using the probit dose-response slope and either the level of concern (discussed below) or actual calculated risk quotient value.

### **2.10.1. Measures of Exposure**

According to the physical chemical properties of diphacinone, the potential aquatic exposure will be limited due to the low solubility and low application rates. In order to provide high-end exposure estimates, the tier 1 aquatic models will be used.

For terrestrial exposure, the primary pathways of terrestrial animals to diphacinone are through direct ingestion of diphacinone bait, or consumption of another animal that directly ingested the bait. Because diphacinone is used as bait application or animal burrow treatment, the exposure to plants is expected to be minimal. Therefore, exposure to diphacinone from consumption of plants is expected to be minimal.

Measures of exposure are based on aquatic and terrestrial models that predict estimated environmental concentrations (EECs) of diphacinone using maximum labeled application rates and methods of application. The model used to predict aquatic EECs was the Generic Estimated Environmental Concentration model (GENEEC, version 2.3). GENEEC output are point estimates of peak and mean values that are expected to recur once every ten years at a site which is more vulnerable than most sites used to grow crops in the United States. This model is parameterized using relevant reviewed registrant-submitted environmental fate data.

#### **2.10.1.a. Estimating Exposure in the Aquatic Environment**

For diphacinone usage as bait application or animal burrow treatment, it is possible to approximate a 'high-end' exposure scenario with two tier 1 models: GENEEC model for surface water exposure and SCIGROW model for ground water exposure.

#### **2.10.1.b. Estimating Exposure in the Terrestrial Environment**

For this assessment, applications of diphacinone treated baits are considered. Terrestrial EECs were derived for birds and mammals consuming treated bait as well as species consuming treated mammals (primary and secondary toxicity). Exposure estimates generated are for the parent

alone. For primary consumption of bait, measures of exposure include concentrations of diphacinone in bait ( $EEC = \text{concentration of a.i. in bait}$ ). Resulting doses in animals that consume bait are estimated using food ingestion allometric equations ( $\text{concentration in bait} \times \text{food ingestion rate} = \text{dose-based EEC}$ ). For secondary toxicity risk, the resulting concentrations of diphacinone in primary consumers (i.e., the dose) are used to estimate potential exposures to predators that may consume contaminated prey items.

### **2.10.2. Measures of Effect**

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

#### **2.10.2.a. Integration of Exposure and Effects**

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

### **2.10.3. Data Gaps**

#### **Environmental Fate**

As cited in the RED (USEPA, 1998b), the only available environmental fate data from studies for diphacinone are 1) physicochemical properties, 2) supplemental hydrolysis, 3) satisfactory aerobic soil metabolism, and 4) supplemental aged column leaching. However, because of the specific results of studies and because the specific use and application rates for diphacinone, relative to established end-point toxicities, is indeed low, EFED can satisfactorily evaluate/screen limits of potential environmental concentrations and ecological effects without additional environmental fate data.

#### **Ecological Effects**

Avian reproduction studies were not required for rodenticides in the past. Diphacinone does have field uses where wildlife may be exposed, and exposure could occur repeatedly and during breeding seasons. It is not known what effects low non-lethal doses may have on reproduction in wildlife.

### 3. Exposure Assessment

Diphacinone is formulated as solid bait products. The usages are for bait application or animal burrow treatment, so potential for spray drift is minimal, and spray drift analysis was not conducted for this assessment.

#### 3.1. Label Application Rates and Intervals

Diphacinone labels may be categorized into two types: labels for manufacturing uses (including technical grade diphacinone) and end-use products. While technical products, which contain diphacinone 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 diphacinone's potential use to only those sites that are specified on the labels.

Currently registered uses of diphacinone include non-agricultural areas and agricultural areas. Diphacinone may be used in and around buildings (placement must be within 50 feet of exterior walls), inside transport vehicles (ships, trains, and aircraft), alleys in urban areas, and sewers. Buildings where diphacinone bait may be used include residential, commercial, industrial, and agricultural premises, as well as port and terminal buildings. Labeled uses sites for agricultural include vegetables (unspecified), small grains (unspecified), alfalfa, apple, almond, walnut, pecan, pear, peach, nectarine, filbert, orchard (unspecified), and agricultural crops/soils (unspecified). Labeled uses sites for non-food and non-agricultural uses are sewage systems, drainage systems, forest trees, forest plantings (tree farms, tree plantations), household/domestic dwellings outdoor premises, rangeland, pastures, urban areas, nursery stock, ornamental woody shrubs and vines, ornamental lawns and turf, golf course turf, nonagricultural rights-of-way/hedgerows, and airports/landing fields. All of these uses are considered as part of the federal action evaluated in this assessment. The only usage being assessed to represent the highest aquatic exposure is summarized in Table 3-1. The modeled scenario was chosen to represent a high-end use pattern. If the high-end scenario resulted in potential risks to aquatic organisms, then additional scenarios would be explored. For terrestrial exposures, estimates were based on maximum label rates and concentration of diphacinone in the bait in the same manner as the aquatic assessment. In addition, estimates of daily doses for terrestrial animals were calculated for both primary exposure (direct consumption of bait) and secondary exposures (consumption of contaminated animals) using food intake equations as described in Section 5.1.2.

**Table 3-1. Diphacinone Uses, Scenarios, and Application Information to Estimate Aquatic Exposures**

Uses Represented by Scenario	Application Method/ Formulation	Application Rate	Maximum Number of Applications	Application Interval
Recreational Areas, Nonagricultural Rights-Of-Way/Fencerows/Hedgerows, Golf Course Turf	Field Rodent Bait	0.0034 lb ai/ac (based on 2.5 oz / 50 sq ft)	4	2 days

<sup>1</sup> Uses assessed based on the verification memorandum from Pesticide Re-evaluation Division (PRD) dated (April 25, 2011).

## 3.2. Aquatic Exposure Assessment

### 3.2.1 Modeling Approach

Based on the low water solubility of diphacinone (0.3 mg/L), the highest potential aquatic exposure will be at this solubility limit. Due to the relatively small application rates of diphacinone, the aquatic exposure will be relatively small as well. However, for diphacinone usage as bait application or animal burrow treatment, it is possible to approximate a ‘high-end’ exposure scenario with two tier 1 models: GENEEC model for surface water exposure and SCIGROW model for ground water exposure.

### 3.2.2 Model Inputs

Several conservative estimates and assumptions regarding input parameters and usage patterns allow the tier 1 models to be used for exposure estimates. Label instructions state for recreational areas and golf course turf, the maximum application rate for diphacinone is 0.0034 lb ai/ac. Four applications with 2-day intervals is the maximum total allowed. The most conservative available physical/chemical input values were used for modeling (see Table below).

**Table 3-2. Summary of Environmental Fate Data Used for Aquatic Exposure Inputs for Diphacinone Endangered Species Assessment<sup>1</sup>**

GENEEC INPUTS		
Property	Value	Comment
Solubility	0.3 mg/L	<a href="http://extoxnet.orst.edu/pips/diphacin.htm">http://extoxnet.orst.edu/pips/diphacin.htm</a>
Hydrolysis	Stable	at pH 7 and 9 (EUP Review 11/08/2000)
Photolysis in Water	Stable	EUP Review 11/08/2000
Photodegradation on Soil	Stable	Conservation assumption
Aerobic Soil Degradation	90 days (half-life)	30 days in single soil study – adjusted to 90 days (3X) for model input (EUP Review 11/08/2000)
Aerobic Aquatic Degradation	180 days (half-life)	2X aerobic soil value
K <sub>oc</sub>	300 L/kg	Supplemental column leaching study (EUP Review 11/08/2000)
Incorporation Depth	0.0 inches	Label says “scatter by hand near burrows”.
Application Rate	0.0034 lb ai/ac	Label
Number of Apps.	4	Label states: “3-4 applications”
Application Interval	2 days	Label
SCIGROW INPUTS		
Property	Value	Comment
Aerobic Soil Degradation	90 days (half-life)	30 days in single soil study – adjusted to 90 days (3X) for model input (EUP Review 11/08/2000)
K <sub>oc</sub>	300	Supplemental column leaching study (EUP Review 11/08/2000)
Application Rate	0.0034 lb ai/ac	Label
Number of Apps.	4	Label states: “3-4 applications”

<sup>1</sup> – Inputs determined in accordance with EFED “Guidance for Selecting Input Parameters in Modeling the Environmental Fate and Transport of Pesticides. Version 2.1” dated October 22, 2009.

### **3.2.3 Results**

The peak surface water concentration (EEC) predicted by GENEEC is 483 pptr (ng/L). The peak ground water concentration (EEC) predicted by SCIGROW is 4.75 pptr (ng/L). The full complement of predicted exposure values are shown in Appendix B. These values represent a very 'high-end' exposure scenario, in that they reflect only maximum potential exposure associated with use for rodent control on recreation areas and golf course turfs; this is but one of many uses, most of which should result in much lower exposure estimates.

### **3.2.4 Existing Monitoring Data**

No monitoring data in surface water, in groundwater or in air were found from the USGS NAWQA program (<http://water.usgs.gov/nawqa>), the California Department of Pesticide Regulation CDPR (<http://www.cdpr.ca.gov/docs/emon/surfwttr/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 rodenticide such as diphacinone.

## **3.3. Terrestrial Animal Exposure**

### **3.3.1. Exposure to Residues in Terrestrial Food Items**

#### **Primary Exposure From Consumption of Treated Bait**

For this assessment, applications of diphacinone treated baits are considered. Terrestrial EECs and RQs were derived for birds (surrogates for the AW and terrestrial phase CTS) and mammals (SJKF and SMHM) consuming treated bait. Exposure estimates generated are for the parent alone. The AW and CTS are not capable consumers of treated baits. However, the SJKF and SMHM can consume treated bait. On a dietary basis, estimates of exposure are simply the concentration of diphacinone in the bait. On a dose basis, allometric equations that estimate average daily food intake was used to estimate a daily dose. See Section 5.1.2 for further details.

#### **Secondary Exposure From Consumption of Treated Prey**

For this assessment, diphacinone treated bait is assumed to be consumed by the prey species, which is then consumed by the SJKF, CTS and AW. RQs are derived from comparison of the concentration in prey (eaten by the SJKF and AW) and then to the most sensitive mammalian (for the SJKF) and snake (for the AW) LD50. The amount of diphacinone in prey was estimated using allometric equations as was done for primary exposure. The resulting estimated concentration of diphacinone in prey species served as the basis for secondary exposure to predators along with allometric equations for the predator species to estimate food intake. See Section 5.1.2 for further details

#### **Terrestrial Organism Exposure to Residues in Aquatic Food Items (KABAM)**

Aquatic EECs are in the parts per trillion (pptr) with some very conservative assumptions regarding how much chemical gets off the bait and enters the water. There is no drift concern

given the formulation types (baits). Therefore, water contamination and resulting bioaccumulation is not a concern.

### **3.4. Terrestrial Plant Exposure Assessment**

Potential exposures to terrestrial plants were not estimated for the following reasons: (1) the mode of action of diphacinone is not consistent with a mode of action that would affect terrestrial plants (anticoagulant); (2) application rates are low such that diphacinone would need to be relatively toxic to terrestrial plants to result in an LOC exceedance, and (3) no incidents in terrestrial plants have been reported. Therefore, risk to terrestrial plants and organisms that depend on terrestrial plants (indirect effects) is presumably lower than concern levels.

## **4. Effects Assessment**

This assessment evaluates the potential for diphacinone to directly or indirectly affect the Alameda Whipsnake, Salt Marsh Harvest Mouse, CA Tiger Salamander and the San Joaquin Kit Fox 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 aquatic-phase tiger salamander are based on toxicity information for freshwater fish, while terrestrial-phase amphibian effects (tiger salamander) and reptiles (Alameda Whipsnake) 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, 2004), the most sensitive endpoint for each taxon is used for risk estimation. For this assessment, evaluated taxa include freshwater fish (used as a surrogate for aquatic-phase amphibians), freshwater invertebrates, birds (used as a surrogate for terrestrial-phase amphibians and reptiles) and mammals. 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 diphacinone.

### **4.1. Ecotoxicity Study Data Sources**

Toxicity endpoints are established based on data generated from guideline studies submitted by the registrant, and from open literature studies that meet the criteria for inclusion into the ECOTOX database maintained by EPA/Office of Research and Development (ORD) (USEPA, 2004). Open literature data presented in this assessment were obtained from ECOTOX information obtained on 11/30/10. 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 for other ‘target’ insect species (not including bees, butterflies, beetles, and non-insect invertebrates including soil arthropods and worms), which include efficacy studies, are not currently considered in deriving the most sensitive endpoint for terrestrial insects. Efficacy studies do not typically provide endpoint values that are useful for risk assessment (*e.g.*, NOAEC, EC50, *etc.*), but rather are intended to identify a dose that maximizes a particular effect (*e.g.*, EC100). Therefore, efficacy data and non-efficacy toxicological target insect data are not included in the ECOTOX open literature. For the purposes of this assessment, ‘target’ insect species are defined as all terrestrial insects with the exception of bees, butterflies, beetles, and non-insect invertebrates (*i.e.*, soil arthropods, worms, *etc.*).

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 D 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 E.

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

Available toxicity of degradates and other stressors of concern are summarized for each taxa in the appropriate Sections for the taxa.

## 4.2. Toxicity of Diphacinone to Aquatic Organisms

Table 4-1 summarizes the most sensitive aquatic toxicity endpoints, based on an evaluation of both the submitted studies and the open literature, as previously discussed. A brief summary of submitted and open literature data considered relevant to this ecological risk assessment for the Alameda Whipsnake, Salt Marsh Harvest Mouse, CA Tiger Salamander and the San Joaquin Kit Fox is presented below. All endpoints are expressed in terms of the active ingredient (a.i.) unless otherwise specified. Diphacinone is moderately toxic to freshwater fish (Rainbow Trout LC<sub>50</sub> = 2.6 ppm) and aquatic invertebrates (water flea EC<sub>50</sub> = 1.8 ppm).

**Table 4-1. Aquatic Toxicity Profile for Diphacinone**

Assessment Endpoint	Acute/ Chronic	Species TGAI/TEP % a.i.	Toxicity Value Used in Risk Assessment	Citation or MRID # (Author, Date) <sup>1</sup>	Classification
Freshwater fish (surrogate for aquatic-phase amphibians)	Acute	Bluegill sunfish <i>Lepomis macrochirus</i> TGAI 95.8%	96-hr LC <sub>50</sub> = 7.5 mg/L	MRID 432495-01 (Machado 1994)	Acceptable (moderately toxic)
		Rainbow Trout <i>Oncorhynchus mykiss</i> TGAI 95.8%	96-hr LC <sub>50</sub> = 2.6 mg/L	MRID 432495-02 (Machado 1994)	Acceptable (moderately toxic)
Freshwater invertebrates	Acute	Daphnia <i>Daphnia magna</i> TGAI 98.7%	EC <sub>50</sub> = 1.8 mg/L	MRID 422822-01 (Putt 1992)	Acceptable (moderately toxic)

1-ECOTOX references are designated with an E followed by the ECOTOX reference number.

Note: There were many other studies submitted but they were not listed here because they were classified as INVALID.

Toxicity to fish and aquatic invertebrates is categorized using the system shown in Table 4.2 (USEPA, 2004). Toxicity categories for aquatic plants have not been defined.

**Table 4-2. Categories of Acute Toxicity for Fish and Aquatic Invertebrates**

LC <sub>50</sub> (mg/L)	Toxicity Category
< 0.1	Very highly toxic
> 0.1 - 1	Highly toxic
> 1 - 10	Moderately toxic
> 10 - 100	Slightly toxic
> 100	Practically nontoxic

### 4.2.1. Toxicity to Aquatic Animals

A summary of acute and chronic freshwater fish data is provided below. No data from the open literature was found.



Diphacinone is moderately toxic to freshwater fish (Rainbow Trout LC50 = 2.6 ppm and Bluegill Sunfish LC50 = 7.5 ppm). Diphacinone is moderately toxic to freshwater aquatic invertebrates (Water flea EC50 = 1.8 ppm). No valid data have been submitted for estuarine/marine organisms.

#### **4.2.2. Toxicity to Aquatic Plants**

No toxicity data have been submitted for aquatic plants and risk is assumed to be low due to the mode of action of diphacinone (an anticoagulant), the low application rate, the low potential for spray drift, and formulation type (bait).

#### **4.3. Toxicity of Diphacinone to Terrestrial Organisms**

Table 4-3 summarizes the most sensitive terrestrial toxicity endpoints, based on an evaluation of both the 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.

**Table 4-3. Terrestrial Toxicity Profile for Diphacinone**

Species	Acute/ Chronic	Species	Toxicity Value Used in Risk Assessment	Citation MRID/ECOTOX reference No.	Classification
Birds (surrogate for terrestrial- phase amphibians and reptiles)	Acute oral LD50	Bobwhite Quail	>400<2000 mg/kg (reported as $\geq 1630$ mg/kg)	422452-01	Supplemental
	Acute Dietary LC50 <sup>1</sup>	Mallard Duck	906 ppm <sup>3</sup> (LOAEC=1.6 ppm)	424088-02	Core
		Bobwhite Quail	>5000 ppm <sup>2</sup>	424088-01	Core
Mammals	Acute	Rat	2.5 mg/kg M 2.1 mg/kg F	00060605	Core
	Acute Dietary LC50	Rat Rat	2.31 ppm 2.5 ppm	TMN 075 TMN 051	Supplemental Supplemental
	Acute oral LD50	Coyote Mongoose	0.6 0.2	Savarie <i>et al.</i> 1979 <sup>4</sup> DWRC <sup>5</sup>	Quantitative Supplemental
Reptile	Acute oral LD50 <sup>6</sup>	Brown tree snake	Ethanol carrier 20.75 mg/kg  Prop glycol carrier 32.2 mg/kg	Brooks <i>et al.</i> 1998	Quantitative

n/a: not applicable; ND = not determined; bw = body weight

1 test organisms (10/level; 6 test concentrations, 3 control groups) were observed an additional 20 days while on untreated feed.

2 all mortality (10% at 5000 ppm, 30% at 1667 ppm, and 10% at 185 ppm) occurred within 18 days.

3 all mortality (20 of 60 birds dosed) occurred within 16 days.

4 reported by the Denver Wildlife Research Center (Savarie *et al.* 1979, ASTM STP 693, pp. 69-79)

5 reported by the Denver Wildlife Research Center in EUP application for mongoose control in Hawaii

6 no hemorrhaging was reported. All snakes died within 24hrs. Diphacinone had no dermal toxicity to brown tree snakes.

Note: There were other studies submitted but they were not listed here because they were classified as INVALID.

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

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

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

#### **4.3.1. Toxicity to Birds**

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

##### **4.3.1.a. Birds: Acute Exposure (Mortality) Studies**

Diphacinone is acutely moderately toxic to avian species. However, a reliable LD<sub>50</sub> was not determined in the available study (MRID 422452-01). The 95% confidence interval ranged from 0 to ∞, in part because test concentrations were separated by a factor of 5X rather than the 1.6X separation recommended in the study guideline. Visual inspection of the data indicates that the LD<sub>50</sub> is less than 2000 mg/kg but greater than 400 mg/kg. Therefore, until an adequate study is submitted, 400 mg/kg will be used as a conservative estimate of the LD<sub>50</sub>.

Subacutely, diphacinone is considered moderately toxic (Mallard Duck LC<sub>50</sub> = 906 ppm) to practically non-toxic (Bobwhite Quail LC<sub>50</sub> = 5000 ppm).

##### **4.3.1.b. Birds: Chronic Exposure (Growth, Reproduction) Studies**

No data have been submitted or were located in the open literature.

#### **4.3.2. 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 0.

##### **4.3.2.a. Mammals: Acute Exposure (Mortality) Studies**

Acute oral and dietary toxicity to mammals is classified as very highly toxic. Symptoms occurred at all doses, and were not necessarily associated with subsequent mortality. These included clear or colored nasal discharge, soft stool and/or diarrhea (possibly associated with the corn oil vehicle used), decreased motor activity and occasional drying of the corneal surface. Symptoms at higher dose levels included lacrimation, ataxia, cyanosis and bloody exudate from nose and eyes. Hemorrhage into the body cavities and of various organs was observed in animals which died. The acute oral LD<sub>50</sub> for males was calculated as 2.50 mg/kg, with 95% confidence limits of 1.82-3.44 mg/kg; for females it was 2.10 mg/kg, with 95% confidence limits of 1.55-2.86 mg/kg. The combined oral LD<sub>50</sub> for both sexes was calculated as 2.31 mg/kg (95% confidence limits of 1.86 to 2.88 mg/kg).

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

No data have been submitted and no data were located in the open literature to allow for an evaluation of potential reproductive effects.

#### 4.3.3 Birds and Mammals, Secondary Toxicity Tests

Secondary poisoning studies with a mammalian predator and a predacious bird are required only if a biologically significant toxicant load exists. Studies were previously submitted or published. Collectively (Erickson and Urban 2004), these studies indicate that some mammals are susceptible to secondary poisoning from consuming diphacinone residue in animal tissue. The toxicant loads of the rats, mice, and nutria fed to raptors, mustelids, and dogs are not known but were sufficiently high to cause mortality in most species. The relationship between target loads in poisoned target species and the levels presented in coyote and sheep muscle was not established. Moreover, the amount of toxicant present in the coyote muscle was found to be considerably lower than that contained in the small intestine, liver, kidney, and heart tissues. The data are adequate to demonstrate that avian and mammalian predators may be killed from consuming target species poisoned with 0.01% a.i. bait.

#### Summary of Secondary Toxicity of Diphacinone to Mammals and Birds (from Erickson and Urban 2004)

Predator/ scavenger (p/s)	Prey offered to p/s	No. prey offered daily per p/s	No. days p/s exposed	No. p/s exposed	No. p/s dead	No. survivors with signs of diphacinone toxicity <sup>a</sup>
Mink	nutria fed 0.01% carrot bait for up to 10 days	ad lib.	5-18	3	3	no survivors
Mongoose	rats fed 0.005% bait for 5 days	1	1 3 5 6 7 8 10	1 1 2 1 1 1 1	0 1 2 1 1 1 1	nr no survivors no survivors no survivors no survivors no survivors no survivors
Ermine	deer mice fed 0.01% bait for 10 days	2	5	2	1	nr
Striped skunk	deer mice fed 0.01% bait for 10 days	2	5	5	0	nr
Deer mouse	liver from diphacinone- poisoned owls	1 g daily	7	4	1	3 (ct)
Rat	meat containing 0.5 ppm ai	ad lib.	6	8	4	nr
Dog (domestic)	nutria fed 0.01% carrot bait for up to 10 days	ad lib.	6-10	3	3	no survivors

<sup>a</sup> eb = external bleeding; ih = internal hematoma; bl = bleeding (unspecified); ct = increased blood coagulation time; nr = not reported

Predator/ scavenger (p/s)	Prey offered to p/s	No. prey offered daily per p/s	No. days p/s exposed	No. p/s exposed	No. p/s dead	No. survivors with signs of diphacinone toxicity <sup>a</sup>
Great horned owl	mice fed choice of 0.01% bait or untreated food for 10 days	2	5	3	2	1 (ct)
Saw-whet owl	mice fed choice of 0.01% bait or untreated food for 10 days	2	5	1	1	no survivors
Barn owl	rats fed choice of 0.005% bait or untreated food for 5 days	ad lib.	10	2	0	0
American crow	rats fed 0.005% bait until death	1 1-2 <sup>b</sup>	1 6	10 11	0 0	0 5 (eb/ct)
Golden eagle	meat laced at 2.7 ppm ai	454 g	5 10	4 3	0 0	4 (eb/ct) 3 (eb/ct) <sup>c</sup>

<sup>a</sup> eb = external bleeding; ih = internal hematoma; bl = bleeding (unspecified); ct = increased blood coagulation time; nr = not reported

<sup>b</sup> offered 1 rat per crow for 5 days and 2 rats per crow on day 6

<sup>c</sup> general weakness of all eagles was observed after 5 days

Predator/ scavenger (p/s)	Prey offered to p/s	No. prey offered daily per p/s	No. days p/s exposed	No. p/s exposed	No. p/s dead	No. survivors with signs of diphacinone toxicity <sup>a</sup>
Great horned owl	mice fed choice of 0.01% bait or untreated food for 10 days	2	5	3	2	1 (ct)
Saw-whet owl	mice fed choice of 0.01% bait or untreated food for 10 days	2	5	1	1	no survivors
Barn owl	rats fed choice of 0.005% bait or untreated food for 5 days	ad lib.	10	2	0	0
American crow	rats fed 0.005% bait until death	1 1-2 <sup>b</sup>	1 6	10 11	0 0	0 5 (eb/ct)
Golden eagle	meat laced at 2.7 ppm ai	454 g	5 10	4 3	0 0	4 (eb/ct) 3 (eb/ct) <sup>c</sup>

<sup>a</sup> eb = external bleeding; ih = internal hematoma; bl = bleeding (unspecified); ct = increased blood coagulation time; nr = not reported

<sup>b</sup> offered 1 rat per crow for 5 days and 2 rats per crow on day 6

<sup>c</sup> general weakness of all eagles was observed after 5 days

#### 4.3.4 Toxicity to Reptiles

Brooks *et al.* (1998) conducted a study to determine the acute oral and dermal toxicity of 18 chemicals to Brown Tree Snakes. Diphacinone was one of the chemicals used. Acute exposure to diphacinone resulted in LD50s of 20.75 mg/kg (95% CI=10.145 to 44.876) with a slope of 4.227 (ethanol used as a carrier) and 32.21 mg/kg (95% CI=18.756 to 55.915) with a slope of 5.0 (propylene glycol used as a carrier) as calculated by the EFED probit slope analysis program. Five snakes of either sex were used in each treatment group. All snakes were observed for 7 days after administration of Diphacinone. Diphacinone produced mortalities within 24 hrs of administration of doses of 10-40 mg/kg. At necropsy, there was no evidence of hemorrhaging. Diphacinone produced no mortalities from dermal application.

#### **4.3.5 Toxicity to Terrestrial Invertebrates**

No toxicity data have been submitted for terrestrial invertebrates.

#### **4.3.6 Toxicity to Terrestrial Plants**

No toxicity data have been submitted for terrestrial plants.

#### **4.3.7 Toxicity of Chemical Mixtures**

No data are available regarding toxicity data for mixtures. Diphacinone currently has products that contain multiple active ingredients (diphacinone + imidicloprid). However, sufficient data are not available to allow for an evaluation of the potential interaction between these two active ingredients (Appendix G).

### **4.4 Incident Database Review**

A review of the Ecological Incident Information System (EIIS, version 2.1), the ‘Aggregate Incident Reports’ (v. 1.0) database, and the Avian Monitoring Information System (AIMS) for ecological incidents involving diphacinone was completed on 2/11/2011. The results of this review for terrestrial, plant, and aquatic incidents are discussed below in Sections 4.4.1 through 4.4.3. Incidents are further summarized in Appendix F.

There were a total of 29 incidents listed in the database. Eleven (38%) were deemed “highly probable”, six (21%) were deemed as “probable”, nine (31%) were deemed “possible”, two (7%) were deemed “unlikely” and one (3%) was deemed “unrelated”. Descriptions of the incidents that are most likely associated with diphacinone are described below. These incidents confirm that exposure to and effects from diphacinone exposure can occur from both primary consumers and secondary poisoning.

#### **4.4.1 Terrestrial Incidents**

There were a number of terrestrial incidents reported for diphacinone and involved both primary and secondary toxicity. Only a small fraction of wildlife incidents are believed to be reported to the Agency.

Eleven incidents were deemed “highly probable”. These included mortality to a mountain lion, a raccoon and a snowy owl through secondary poisoning, as well as nineteen gray squirrels, two white-tailed deer, a ground squirrel, a kit fox and a rat through bait consumption. Listed below are descriptions of “highly probable” incidents as well as those that resulted in measurable residues.

Incidents located in EIIS are summarized below. Additional information is included in Appenbix F.

- Testing found diphacinone residue concentrations of 45 ppm and 55 ppm from blood and liver samples in a mountain lion and raccoon found dead in Northern CA respectively (B000641-001).
- Diphacinone was also found in a dead adult kit fox (found in Bakersfield CA and weighing 5.75 lbs) by immunoassay in liver and blood at a concentration of 0.18 ppm (B000640-001).
- A male deer fawn (found approximately 2 weeks post mortem) and an adult buck (weighing 202 lbs) was found dead on Fire Island NY and contained 0.2 ppm and 0.93 ppm diphacinone in liver samples respectively (I004768-001 and I004769-001).
- An adult male gray squirrel was found dead in Brookhaven NY and contained 2.0 ppm diphacinone (I005353-001).
- A male snowy owl was found dead in Rhinebeck NY of likely secondary poisoning (I006306-01). Diphacinone was found in the liver but the concentration was not reported.
- A number of gray squirrels were found dead in Richmond VA and pooled liver samples from two individuals (one weighed 490g and the other weighed 567g) contained 4.94 and 3.4 ppm diphacinone respectively (I007488-004).
- Although listed as a “probable” incident, a red-tailed hawk was found moribund (dying the next day) in Roslyn NY and contained 0.34 ppm diphacinone in a liver sample (I009270-001).
- A gray squirrel was found dead in Albany NY of apparent blunt impact trauma (listed as a “possible” incident). However, the liver contained 1.02 ppm diphacinone (I011518-001).
- Eight squirrels were found dead in Conyers GA and 3.6 ppm diphacinone was found in the liver of one (410g) of the squirrels that was sampled (I019839-001).
- A rat and a ground squirrel were found dead in Ventura CA and contained 4.12 and 1.99 ppm diphacinone in liver samples respectively (I014886-001).
- Two deer found dead in Suffolk NY contained 0.2 and 0.93 ppm diphacinone in liver samples respectively (I013810-007 and I013810-008, both incidents deemed as “probable”).
- A radio-collared yearling female coyote was found dead in Ventura CA and contained diphacinone residues of 1.3 ppm in liver, 0.1 ppm in blood and 0.16 ppm in stomach contents but also contained 0.083 ppm Brodifacoum in the liver (I007107-014, I007165-003).

#### **4.4.2 Plant Incidents**

There were no incidents reported for diphacinone involving plants.

#### **4.4.3 Aquatic Incidents**

There were no aquatic incidents reported for diphacinone.

### **5.0 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

Alameda Whipsnake, Salt Marsh Harvest Mouse, CA Tiger Salamander and the San Joaquin Kit Fox or for modification to their designated critical habitat from the use of diphacinone 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. For acute exposures to the aquatic animals the LOC is 0.05. For acute exposures to birds (and, thus, reptiles and terrestrial-phase amphibians) and mammals, the LOC is 0.1. The LOC for chronic exposures to animals is 1.0.

### 5.1.1 Exposures in the Aquatic Habitat

#### 5.1.1.1 Freshwater Fish and Aquatic-phase Amphibians

Acute risk to fish and aquatic-phase amphibians and reptiles is based on peak EECs in the standard pond and the lowest acute toxicity value for freshwater fish. Risk quotients for freshwater fish are shown in Table 5-1.

**Table 5-1. Acute and Chronic RQs for Freshwater Fish**

Uses/Application Rate	Species	Peak EEC (mg/L)	60-day EEC (mg/L)	Acute RQ*	Chronic RQ
0.0034 lb ai/ac bait	Rainbow trout	0.000483	N/A	<0.01	N/A
* Acute RQ = peak EEC/2.6 ppm.					

Based on data from the rainbow trout, diphacinone does not have the potential to directly affect the aquatic phase of the CA Tiger Salamander. Additionally, since the acute RQs are not exceeded, there is not a potential for indirect effects to those listed species that rely on fish (and/or aquatic-phase amphibians) during at least some portion of their life-cycle (*i.e.*, CTS, AW, or SJKF).

#### 5.1.1.2 Freshwater Invertebrates

Acute risk to freshwater invertebrates is based on 1 in 10 year peak EECs in the standard pond and the lowest acute toxicity value for freshwater invertebrates. Risk quotients for freshwater invertebrates are shown in Table 5-2.



**Table 5-2. Summary of Acute and Chronic RQs for Aquatic Invertebrates.**

Uses/Application Rate	Species	Peak EEC (mg/L)	21-day EEC (µg/L)	Acute RQ*	Chronic RQ*
0.0034 lb ai/ac bait	Daphnid	0.000483	N/A	<0.01	N/A
* Acute RQ = peak EEC/1.8 ppm.					

Based on data for the daphnia, diphacinone does not have the potential to directly affect the aquatic phase of the CA Tiger Salamander. Additionally, since the acute RQs are not exceeded, there is not a potential for indirect effects to those listed species that rely on freshwater invertebrates during at least some portion of their life-cycle (*i.e.*, CTS, AW, or SJKF).

### 5.1.1.3 Aquatic Plants

No aquatic toxicity data are available. However, risk to aquatic plants is assumed to be lower than concern levels given the mode of action of diphacinone and the very low aquatic EECs. Diphacinone would need to be more toxic than a potent herbicide (EC50s of <483 ng/L) to result in potential effects to aquatic plants and resulting indirect effects to the assessed species. Therefore, since the acute RQs are presumably not exceeded, there is not a potential for indirect effects to those listed species that rely on aquatic plants during at least some portion of their life-cycle.

## 5.1.2 Exposures in the Terrestrial Habitat

### 5.1.2.1. Primary Exposure to AW

Diphacinone is not likely to have the potential to directly affect the AW or the terrestrial phase of the CTS. Both of these species feed on live moving prey and would not ingest treated bait. Additionally, since the acute RQs (0.5) are not exceeded, indirect effects to listed species that rely on birds (and, thus, reptiles and/or terrestrial-phase amphibians) during at least some portion of their life-cycle (*i.e.*, CTS, AW, SMHM and SJKF) are unlikely to occur.

Based on the acute dietary toxicity of diphacinone and potential exposure to 0.005% ai bait, primary risk to birds is not expected. The dietary risk quotient (RQ), a comparison of potential exposure (bait, ppm ai) to dietary toxicity (LC50), does not exceed 0.06 (50 ppm ai/906 ppm). That RQ does not exceed the Agency's level of concern (LOC) for acute risk to non-endangered (LOC = 0.5) or endangered (LOC = 0.1) birds. For a single feeding, based on the most sensitive LD50 (using 400 mg/kg as a conservative estimate), a 50-g bird would need to consume more than 800 g of bait to receive an LD50 dose. Since there are no direct effects to birds, and birds being surrogates to reptiles and amphibians, there should not be effects to either the AW or terrestrial phase of the CTS from direct consumption of baits. Both these species are consumers of live prey, and are not expected to eat baits, which further supports this risk conclusion.

### 5.1.2.2. Secondary Exposure to AW

Since the AW eats live prey, the assumption is that it could consume a small mammal that has eaten treated bait. The AW may be exposed to residues from secondary exposure from consumption of prey that has consumed treated bait. The AW is capable of consuming all of the target species for diphacinone bait products as well as other non-target species that may also feed on treated bait.

As a conservative assumption, the amount of active ingredient ingested by the AW from secondary exposure was assumed to be equal to the amount of active ingredient that prey would ingest if it consumed bait at its daily ingestion rate. The prey was assumed to be a mammal that the AW could ingest. The amount of bait the prey species could ingest was assumed based on its body weight. The weight of the prey species was the maximum percentage the consumer could ingest in one day or sitting (the AW can consume approximately 100% of its BW per day) as reported by King (2002), who reported the following allometric equation for the maximum size prey animal that can be consumed:  $BW \text{ of snake}^{1.071}$  (King 2002). According to this equation, a 400-gram snake can consume a prey animal that is approximately 600 grams (150% of the snake's body weight). An assumption of 100% was used for convenience, and even though it is less conservative than an assumption of a prey animal that is 150% of the snake's mass, the conclusions would be the same regardless of the assumption of the prey's body weight.

All of the diphacinone ingested by the prey was assumed to be available to and assimilated by the AW once the prey is consumed.

Calculations for secondary exposure were conducted for a 400 g AW that has fed on prey that is equivalent to its mass (i.e., 400 grams). The prey species was assumed to have consumed treated bait at its daily ingestion rate. The prey species was assumed to have consumed rodenticide bait with a diphacinone concentration of 0.01%.

The average daily food intake rates of prey species were estimated using the following allometric equation for mammals:

$$\text{Food intake (g dry weight): } FI = 0.621 W^{0.564}$$

$$\text{Food intake (g, wet weight): } FI \text{ (g wet-wt/day)} = FI \text{ (g dry-wt/day)} / 0.32$$

Where FI is the food intake rate in g/d, and W is the bodyweight of the rodent in g. 0.32 is the water content for wet weight calculation.

Using this equation, the calculated FI for a 400 g mammal was 57 g/day wet weight.

Assuming that a 400 g AW can eat 100% of its bw at one sitting, and a 400 g mammal eats 57 g/day wet wt. or 57,000 mg/day, then the resulting consumption of a.i. is 5.7 mg a.i./day (57,000 x 0.01% diphacinone bait = 5.7 mg ai/day).

The resulting dose based EEC can be calculated using the following equation: 5.7 mg ai per day / 0.400 kg AW b.w. = 14.25 mg ai/kg bw.

The RQ can then be calculated using the following equation: EEC of 14.25 mg ai/kg bw / AW LD50 of 20.75 mg/kg bw = 0.68 RQ.

To assess the maximum exposure that an AW could receive through secondary exposure, 100% of the active ingredient which was ingested by the mammalian prey source was assumed to be present in the animal when it was consumed by the snake. This is not implausible because a snake could prey upon the small mammal very soon after it has ingested the bait, with all of the diphacinone contamination present in the ingesta within the gastrointestinal tract of animal, before it has had a chance to metabolize or excrete any of it. Also, diphacinone is expected to persist in the prey animal and multiple feedings may occur. Thus the amount of active ingredient the prey was assumed to ingest was also the dose, in mg, which the AW was assumed to ingest. This dose was then divided by the assumed body weight of the snake to convert the dose into units of mg ai/kg BW. Finally, the acute secondary exposure risk quotients were calculated by dividing the predicted dose of diphacinone (mg-ai/kg-bw) by the acute oral LD<sub>50</sub> value for the Brown Tree snake (surrogate for the AW).

An AW, which after receiving a single dose from preying on a small mammal, would not likely feed again for several weeks. Thus, even if the snake would feed exclusively on small mammals that ingested treated bait, it would receive only one dose every several weeks.

The RQ would be exceeded even if the snake only consumed one 50 g mouse (RQ = 0.22). The calculated FI for a 50 g mammal was 18.3 g/day wet weight. The resulting dose based EEC can be calculated using the following equation: 1.83 mg ai per day / 0.400 kg AW bw = 4.575 mg ai/kg bw. The RQ can then be calculated using the following equation: EEC 4.575 mg ai/kg bw / AW 20.75 mg/kg bw LD50 = 0.22 RQ.

**Table 5-3. RQs for Acute Effects to the AW from Consumption of a Small Mammal which Ingested Diphacinone Bait**

Bait Type	Prey Species	Assumed weight of consumer	%AI in Bait	Prey FI <sup>1</sup> (mg/d)	Dose (mg ai/kg BW)	Acute RQ <sup>2</sup>
Rodent bait	400 g	400 g AW	0.01	57,000	14.25	0.68
	400 g	400 g AW	0.005	57,000	7.125	0.34
	50 g	400 g AW	0.01	18,300	4.575	0.22

<sup>1</sup> Daily food ingestion rate.

<sup>2</sup> Based on the dose in the ingested prey and the acute oral LD<sub>50</sub> in mg/kg-bw for the Brown Tree snake (Brooks et al 1998)

Because the acute RQs for secondary exposure exceed 0.1, the LOC for acute effects to listed species, use of diphacinone has the potential to directly affect the AW by way of secondary exposure.

#### **5.1.2.3. Secondary Exposure to CTS**

Since the terrestrial phase CTS is listed as being capable of eating live mammalian prey, the assumption is that it could consume a small mammal that has eaten treated bait. The CTS may be exposed to residues from secondary exposure from consumption of small prey that has consumed treated bait.

It is unlikely that a small CTS will consume mammals. However, larger ones may. CTS body weights are as high as approximately 80 grams. Using allometric equations previously described, a 20-gram mouse was estimated to be contaminated with approximately 1 mg a.i. after one daily feeding on contaminated bait ( $0.621 \times 20 \text{ g}^{0.564}$ ). The resulting dose in a CTS that consumed this mammal would be  $1 \text{ mg a.i.} / 0.8 \text{ kg bw} = 1.25 \text{ mg a.i./kg bw}$ . The resulting RQ would be 0.06 (EEC of 1.25 mg/kg-bw / LD50 of 20.75 mg/kg-bw = 0.06).

Therefore, the LOC is not exceeded under these conditions.

#### **5.1.3. Primary Toxicity to Mammals**

Based on the acute dietary toxicity of diphacinone and potential exposure to 0.005% ai bait, primary risk to mammals is expected. The dietary risk quotient (RQ), a comparison of potential exposure (bait, ppm ai) to dietary toxicity (LC50), does exceed ( $50 \text{ ppm ai} / \text{LC50 of } 2.3 \text{ ppm} = \text{an RQ of } 21.7$ ) the endangered mammal LOC of 0.1. Using a 0.01% bait, the exceedance is even greater ( $100 \text{ ppm ai} / 2.3 \text{ ppm} = \text{an RQ of } 43.5$ ). Thus there are direct effects to the SMHM and SJKF from consuming bait treated with diphacinone.

Diphacinone does have the potential to directly affect the SMHM and SJKF. Both of these species may feed on treated bait. Additionally, since the acute RQs are exceeded, there is a potential for indirect effects to those listed species that rely on mammals during at least some portion of their life-cycle (*i.e.*, SJKF).

#### **5.1.4. Secondary Toxicity to Mammals**

The available laboratory and/or field data indicate that ingestion of diphacinone baits and/or rodents poisoned with diphacinone can indeed kill mammalian primary and secondary consumers. Multiple mammalian species killed by diphacinone have been reported to the Agency. Incident reports available show that a SJKF has been killed by diphacinone. The dead adult kit fox (found in Bakersfield CA and weighing 5.75 lbs) was sampled by immunoassay and diphacinone was found in liver and blood at a concentration of 0.18 ppm. Also a yearling female coyote was found dead in Ventura CA and contained diphacinone residues of 1.3 ppm in liver, 0.1 ppm in blood and 0.16 ppm in stomach contents. Baits contain 50-100 ppm ai. Thus, the weight of evidence points to possible direct as well as secondary toxicity to SJKF. Since the SJKF eats live prey, the assumption is that it could consume a small mammal that has eaten treated bait.

The concentration of active ingredient in food was assumed simply to be the concentration of AI in the bait. The Agency does not have an approved standard method of predicting secondary exposure from terrestrial animals that eat other animals which have ingested bait. As a Tier 1 risk assessment, the amount of active ingredient ingested by the SJKF from secondary exposure was assumed to be equal to the amount of active ingredient that prey would ingest if it consumed

bait at its daily ingestion rate. The prey was assumed to be a mammal or mammals in the amount that the SJKF could ingest. The amount of bait these prey species could ingest was assumed based on their body weights. The weights of these prey species were the maximum percentage the consumer could ingest in one day or sitting (the SJKF could ingest approximately 10% of its BW per day) as estimated using the food intake allometric equations presented below for a 2300 gram SJKF. All of the diphacinone ingested by the prey was assumed to be available to and assimilated by the SJKF once prey is consumed.

Calculations for secondary exposure were conducted for a 2300 g SJKF that has fed on the appropriate amount of prey eaten per day. These prey species were assumed to have consumed treated bait as their daily ingestion rate. The prey species were assumed to have consumed rodenticide bait with a diphacinone concentration of 0.01%.

The average daily food intake rates of prey species were estimated using the following allometric equation for mammals as previously discussed:

$$\text{Food intake (g dry weight): } FI = 0.621 W^{0.564}$$

$$\text{Food intake (g, wet weight): } FI (\text{g wet-wt/day}) = FI (\text{g dry-wt/day}) / 0.32$$

Where FI is the food intake rate in g/d, and W is the bodyweight of the rodent in g. 0.32 is the water content for wet weight calculation.

The calculated FI for a 50 g mammal was 18.3 g/day wet weight.

To assess the maximum exposure that the SJKF could receive through secondary exposure, 100% of the active ingredient which was ingested by the mammalian prey source was assumed to be present in the animal when it was consumed by the fox. The amount of active ingredient the prey was assumed to ingest was also the dose, in mg, which the fox was assumed to ingest. This dose was then divided by the assumed body weight of the fox to convert the dose into units of mg ai/kg BW. Finally, the acute secondary exposure risk quotient was calculated by dividing the predicted dose of diphacinone (mg-ai/kg-bw) by the acute oral LD<sub>50</sub> value for the coyote (surrogate for the SJKF).

It was assumed that a 2300 g SJKF can eat approximately 10% of its bw at one sitting. If a 50 g mammal eats 18.3 g then a 2300 g Fox eating 230 g of small mammals per day = 84,180 mg/day. 84,180 x 0.01% bait = 8.418 mg ai/day.

$$\text{Dose based EEC} = 8.418 \text{ mg a.i. day} / 2.3 \text{ kg SJKF bw} = 3.66 \text{ mg ai/kg bw.}$$

$$\text{RQ} = \text{EEC } 3.66 / 0.6 \text{ Coyote LD}_{50} = 6.1$$

The RQ would be exceeded even if the Fox only consumed one 50 g mouse (RQ = 1.326). The calculated FI for a 50 g mammal was 18.3 g/day wet weight. The resulting dose based EEC can be calculated using the following equation: 1.83 mg ai per day / 2.3 kg fox bw = 0.795 mg ai/kg bw. The RQ can then be calculated using the following equation: EEC 0.795 mg ai/kg bw / fox 0.6 mg/kg bw LD<sub>50</sub> = 1.326 RQ.

**Table 5-4. RQs for Acute Effects to the AW from Consumption of a Small Mammal that Ingested Diphacinone Bait**

Bait Type	Prey Species	Assumed weight of consumer	%AI in Bait	Prey FI <sup>1</sup> (mg/d)	Dose (mg ai/kg BW)	Acute RQ <sup>2</sup>
Rodent bait	230g of mice	2300 g SJKF	0.01	84,180	3.66	6.1
Rodent bait	50g	2300 g SJKF	0.01	18,300	0.795	1.326

<sup>1</sup> Daily food ingestion rate.

<sup>2</sup> Based on the dose in the ingested prey and the acute oral LD<sub>50</sub> of 0.6 mg/kg-bw for the coyote

Because the acute RQs for secondary exposure exceed 0.1, the LOC for acute effects to listed species, use of diphacinone has the potential to directly affect the SJKF by way of secondary exposure.

#### **5.1.2.1 Terrestrial Invertebrates**

No toxicity data were submitted to assess risk to terrestrial invertebrates. Risk is assumed to be low due to the mode of action of diphacinone (an anticoagulant) and its relatively low exposure potential due to low application rates and relatively rapid consumption by other wildlife.

##### **5.1.2.1.1 Terrestrial Plants**

No data on terrestrial plants has been submitted. However, risk to terrestrial plants is assumed to be lower than concern levels since diphacinone is an anticoagulant, and its mode of action is not expected to affect plants, the application rates and resulting plant EECs are low, and drift potential is negligible. Diphacinone would need to be as toxic as a potent herbicide to pose potential risks to terrestrial plants. Therefore, the weight of evidence suggests that there is not a potential for indirect effects to those listed species that rely on terrestrial plants during at least some portion of their life-cycle.

#### **5.1.2.5 Use of Probit Slope Response Relationship to Provide Information on the Endangered Species Levels of Concern**

The Agency uses the probit dose response relationship as a tool for providing additional information on the potential for acute direct effects to individual listed species and aquatic animals that may indirectly affect the listed species of concern (USEPA, 2004). As part of the risk characterization, an interpretation of acute RQs for listed species is discussed. This interpretation is presented in terms of the chance of an individual event (*i.e.*, mortality or immobilization) should exposure at the EEC actually occur for a species with sensitivity to diphacinone 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. If no dose response information is available to estimate a slope for this analysis, a default slope assumption of 4.5 (with lower and upper bounds of 2 to 9) (Urban and Cook, 1986) is used.

Individual effect probabilities are calculated based on an Excel spreadsheet tool IECv1.1 (Individual Effect Chance Model Version 1.1) developed by the U.S. EPA (OPP, Environmental Fate and Effects Division, June 22, 2004). The spreadsheet performs these calculations by entering the mean slope estimate (and the 95% confidence bounds of that estimate) as the slope parameter for the spreadsheet. The desired threshold for the probability of an individual effect is entered as the listed species LOC. In addition, the probability of an individual effect is also derived based on the calculated acute RQ. For these calculations, bird and mammal RQs from the primary exposure to bait (direct consumption) were used.

<b>Summary of Diphacinone Probit Dose Response Analysis for Listed Species</b>			
<b>Taxa (study type)</b>	<b>Acute Effect Slope (95% C.I.)</b>	<b>Chance of Individual Effect at Listed Species LOC <sup>1</sup>(95% C.I.)</b>	<b>Chance of Individual Effect at Derived Acute RQ (95% C.I.)</b>
Snake oral dose (max RQ = 1.08)	Mortality Slope = 4.2	1 in 7.49E+04	Approximately 1 in 2 assuming a range of slopes from 2 to 9.
Bird dietary (max RQ = 0.06-0.11)	Mortality Slope = 4.5	1 in 2.94E+05	<1 in 2.9E5
Mammal dietary fox (max RQ = 6.1)	Mortality Slope = 4.5	1 in 2.94E+05	Approximately 1 in 1 assuming a range of slopes from 2 to 9.
Mammal dietary mouse (max RQ = 21.7-43.5)	Mortality Slope = 4.23	1 in 8.56E+04	Approximately 1 in 1 assuming a range of slopes from 2 to 9.
Freshwater fish (RQ <0.001)	Mortality Slope = 4.5	1 in 4E+08	< 1 in 4E8
<sup>1</sup> Listed Species LOC = 0.10 for terrestrials, 0.05 for aquatic species			

### 5.1.3 Primary Constituent Elements of Designated Critical Habitat

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

## 5.2 Risk Description

The risk description synthesizes overall conclusions regarding the likelihood of adverse impacts leading to a preliminary effects determination (*i.e.*, “no effect,” “may affect, but not likely to adversely affect,” or “likely to adversely affect”) for the assessed species and the potential for modification of their designated critical habitat based on analysis of risk quotients and a comparison to the Level of Concern. The final No Effect/May Affect determination is made after the spatial analysis is completed at the end of the risk description, Section 5.2.3. In Section 5.2.3, a discussion of any potential overlap between areas where potential usage may result in

LAA effects and areas where species are expected to occur (including any designated critical habitat) is presented. If there is no overlap of the species habitat and occurrence sections with the Potential Area of LAA Effects a No Effect determination is made.

**Table 5-5. Risk Estimation Summary for Diphacinone - Direct and Indirect Effects**

<b>Taxa</b>	<b>LOC Exceedances (Yes/No)</b>	<b>Description of Results of Risk Estimation</b>	<b>Assessed Species Potentially Affected</b>
Birds, Reptiles, and Terrestrial-Phase Amphibians	Non-listed Species (Yes; reptiles only)	LOC exceeded	<u>Indirect Effects</u> : None
	Listed Species (Yes)	LOC exceeded	<u>Direct Effects</u> : AW
Mammals	Non-listed Species (Yes)	LOC exceeded	<u>Indirect Effects</u> : AW and CTS
	Listed Species (Yes)	LOC exceeded	<u>Direct Effects</u> : SMHM & SJKF

**Table 5-6. Risk Estimation Summary for Diphacinone – Effects to Designated Critical Habitat. (PCEs)**

<b>Taxa</b>	<b>LOC Exceedances (Yes/No)</b>	<b>Description of Results of Risk Estimation</b>	<b>Species Associated with a Designated Critical Habitat that May Be Modified by the Assessed Action</b>
Birds, Reptiles, and Terrestrial-Phase Amphibians	Non-listed Species (Yes)	LOC exceeded	AW
	Listed Species (Yes)	LOC exceeded	
Mammals	Non-listed Species (Yes)	LOC exceeded	AW & CTS
	Listed Species (Yes)		

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 the following sections. Each section 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 diphacinone. 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 AW, CTS, SJKF and SMHM**

### **5.2.1.1 Direct Effects**

There have been many incidents of mortality to mammals reported after ingestion of diphacinone treated baits as well as from consuming prey that have eaten treated baits. In addition, there have been specific incidents of SJKF mortality associated with diphacinone use. Results from the current assessment suggest that consumption of diphacinone treated bait may adversely affect the SJKF as well as the SMHM. Acute RQs for mammals were exceeded for potential direct effects to the SMHM and SJKF. Secondary acute RQs for the AW and SJKF were also exceeded by consuming prey that has eaten treated bait. Based on the weight-of-evidence, there is a potential for direct effects to AW, SMHM, and SJKF. The effects determination is LAA for these species for direct effects potential. However, the analysis suggests that RQs for the CTS were below concern levels. There were no incidents of amphibian mortality associated with diphacinone. Therefore, the effects determination for the CTS is “No effect” for direct effects.

### **5.2.1.2 Indirect Effects**

#### **5.2.1.2.1 Potential Loss of Prey and Shelter**

For indirect effects, if RQs exceed the non-listed species LOCs, then diphacinone is likely to indirectly affect the AW and CTS. The acute RQs for potential terrestrial prey taxa (*i.e.*, mammals) were exceeded. Many mortality incidents for mammals have been reported. Small mammals are important prey items for the AW and SJKF. In addition, the terrestrial phase of the

CTS can use mammalian burrows for shelters. Mammal mortalities may result in a loss of burrows and thus can indirectly affect the CTS through habitat modification. Because the burrow density required to support California tiger salamanders in an area is not known, the loss of burrows as a result of mammal control programs and its effect on salamanders cannot be quantified at this time. Active ground squirrel colonies are needed to sustain tiger salamanders because inactive burrow systems become progressively unsuitable over time. Burrow systems can collapse following abandonment by or loss of the ground squirrels.

## **5.2.2 Modification of Designated Critical Habitat**

Designated critical habitat has been designated for the CTS and the AW. Based on the weight-of-evidence, there is a potential for the modification of designated critical habitat due to the potential for indirect effects to the CTS via reduction in mammal burrows and both direct and indirect effects to the the AW that could occur in designated critical habitat. If direct and/or indirect effects are expected, then modification to critical habitat may occur.

### **5.2.2.1 Spatial Extent of Potential Effects**

LAA determinations have been made for the SJKF, SMHM, CTS, and AW. Diphacinone can be used in many types of terrestrial habitats, including forestry, residential, and agricultural areas. Therefore, the spatial extent of potential effects could be substantial, depending on where the chemical is actually applied. Thus, effects to these species is possible wherever these species occur.

## **5.2.3 Effects Determinations**

### **5.2.3.1 Assessed Species**

LOCs were exceeded for the AW, SJKF and the SMHM for direct effects and the AW, SJKF, and the CTS for indirect effects (prey or habitat reductions primarily from reductions in mammals). Therefore, the Agency makes a “may affect, and likely to adversely affect” determination for the AW, SJKF, and SMHM and a habitat modification determination for the AW and terrestrial phase CTS for its designated critical habitat based on the potential for both direct and indirect effects and effects to the PCEs of critical habitat.

### **5.2.3.2 Addressing the Risk Hypotheses**

In order to conclude this risk assessment, it is necessary to address the risk hypotheses defined in Section 2.9.1. Based on the conclusions of this assessment, none of the hypotheses can be rejected, meaning that the stated hypotheses represent concerns in terms of direct and indirect effects of diphacinone on the AW, SMHM and SJKF and their designated critical habitat.

The labeled use of diphacinone may:

- ... indirectly affect the AW and SJKF and/or affect their designated critical habitat by reducing or changing the composition of the food supply (mostly small mammals).

- ...directly affect the SJKF and SWHM by direct mortality through ingestion of treated baits.
- ...directly affect the AW and SJKF by direct mortality through secondary toxicity by ingestion of treated prey (small mammals).
- ...indirectly affect the CTS and/or modify their designated critical habitat by reducing or changing terrestrial habitat in their current range (via reduction in small burrowing mammals leading to reduction in underground refugia/cover).

## **6.0 Uncertainties**

### **6.1 Exposure Assessment Uncertainties**

#### **6.1.1 Terrestrial Exposure Assessment Uncertainties**

Uncertainty in the exposure assessment stems mainly from assumption made in the assessment related to the consumption of bait by various types of animals. Animals were assumed to consume an amount of bait equal to their predicted daily food ingestion rate. Ingestion of bait is most certain for omnivorous mammals because the bait is designed to be attractive to rodents and other mammals. However, small mammals could eat less bait than their average daily ingestion rate, either because they are also feeding on other food sources, or because they exhibit bait shyness. Alternatively, if other food is scarce and they find the bait to be very attractive, then they could exhibit gorging behavior, consuming bait in excess of their daily average daily intake rate. Incidents have shown that species other than small mammals have been killed due to diphacinone poisoning. Animals which feed predominantly on live prey, including the AW, may not consume the bait. The use of allometric equations to estimate food daily food intake rate introduces additional uncertainty. The food intake rate was estimated from the body weights of the animals using allometric equations. How well the generic allometric equations used predict the specific food intake rate of the assessed species is an uncertainty. For example, the relationship for the AW was based on an equation developed for insectivores, whereas the AW consumes a wide variety of vertebrate prey in addition to terrestrial invertebrates.

The assessment of secondary exposure to the AW and SJKF involves additional uncertainties. A conservative assumption was made that the entire amount of active ingredient consumed by the prey is present in the prey animal when it is consumed by the snake and fox. In reality, the amount of active ingredient in the prey may decrease between the time it consumes the bait by the prey and the prey is consumed by the snake or fox as the result of elimination and detoxification. Also, the amount of and rate of assimilation of diphacinone from the consumed prey into the snake and fox is uncertain. Assimilation efficiency may be considerably less than the assumed 100%. Also, snakes typically digest large prey slowly over numerous days. Thus, the toxic residues in the ingested prey may be released and assimilated into the snake more slowly over numerous days. In addition, it would seem that the mode of action that produces mortality in the fox is different than that of the snake. Since diphacinone is an anticoagulant, mammals die through hemorrhaging. However, Brown Tree snakes dosed with diphacinone did not die of hemorrhage but by an unknown mechanism (Brooks et.al. 1998).

The dose of diphacinone from secondary exposure is dependent on the size of the prey and its intake rate. The size of prey that the AW was predicted to be able to consume (100% of its BW) is uncertain. Because the AW is a slender snake, these equations may overestimate the maximum size prey which it may consume.

Lastly, chronic risk is an uncertainty to the AW, SMHM and SJKF in part because no data is available but also because sublethal adverse effects to reproduction is not known. It is assumed that ingestion of enough diphacinone would induce mortality where reproduction would not be an issue. However, non-lethal effects to reproduction in these species is unknown. Foxes whelping pups do not hunt. Instead, the male fox will bring food to the female. In this case, the female may be killed if the prey brought by the male had ingested Diphacinone. In addition, since Diphacinone does not kill immediately, it is not known if Diphacinone may be excreted in mammalian milk, thus exposing whelping young.

Overall, the reported incident data indicates pathways are complete and includes at least one mortality of a species (kit fox) under the current assessment. Exposure assumptions are plausible and may result in mortality events.

### **6.1.2 Aquatic Exposure Modeling of Diphacinone**

Aquatic exposure from the use of diphacinone is expected to be negligible, and thus aquatic routes of exposure are not predicted to make significant contributions to the total exposure of the CTS, SMHM or the AW. Also, indirect risk from potential effects on aquatic plants was assumed to be discountable due to the mode of action of diphacinone as an anticoagulant. Uncertainties associated with these assumptions contribute to the uncertainty of the risk assessment.

## **6.2 Effects Assessment Uncertainties**

### **6.2.1 Data Gaps and Uncertainties**

There is an uncertainty regarding effects of diphacinone on avian and mammalian reproduction. Toxicity data are completely lacking on the effects of diphacinone to terrestrial invertebrates, terrestrial plants, and aquatic plants. However, effects to plants are unlikely due to diphacinone's anticoagulant mode of action.

### **6.2.2 Use of Surrogate Species Effects Data**

Guideline toxicity tests and open literature data on diphacinone are not available for aquatic-phase amphibian; therefore, freshwater fish are used as surrogate species for aquatic-phase amphibians and the CTS. Therefore, endpoints based on freshwater fish ecotoxicity data are assumed to be protective of potential direct effects to aquatic-phase amphibians including the CTS, and extrapolation of the risk conclusions from the most sensitive tested species to the aquatic-phase CTS is likely to overestimate the potential risks to those species. Efforts are made to select the organisms most likely to be affected by the type of compound and usage pattern; however, there is an inherent uncertainty in extrapolating across phyla. In addition, the

Agency's LOCs are intentionally set very low, and conservative estimates are made in the screening level risk assessment to account for these uncertainties.

### **6.2.3 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.

## **7.0 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 diphacinone to the CTS, AW, SMHM and SJKF and their designated critical habitat.

Based on the best available information, the Agency makes a Likely to Adversely Affect determination for the AW, SMHM and SJKF. Additionally, the Agency has determined that there is the potential for modification of the designated critical habitat for the AW and terrestrial phase CTS from the use of the chemical. Given the LAA determination for the AW, SJKF, SMHM and CTS and potential modification of designated critical habitat for the AW and CTS, a description of the baseline status and cumulative effects is provided in Attachment III.

A summary of the risk conclusions and effects determinations for the species assessed and their critical habitat, given the uncertainties discussed in Section 6.0 and Attachment I, is presented in Table 7- and Table 7-2. A use specific summary is presented in Table 7-3.

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

Species	Effects Determination	Basis for Determination
Alameda whipsnake ( <i>Masticophis lateralis euryxanthus</i> )	<i>May Affect and Likely to Adversely Affect (LAA)</i>	<b>Potential for Direct Effects</b>
		The Risk assessment indicates use of diphacinone potentially will result in direct effects to the AW from acute toxicity. Dietary exposure estimates and acute toxicity to reptiles (based on acute toxicity data for birds) result in acute RQs that exceed the LOC for both primary and secondary exposure. While adverse acute effects are possible for both primary and secondary exposure, secondary exposure is considered the primary threat to this species. Data were not available to assess chronic toxicity, but since risk is predicted for acute effects, risk is also assumed for chronic effects.
		<b>Potential for Indirect Effects</b>
		<p><b>Terrestrial prey items</b></p> <p>The Risk Assessment indicates use of diphacinone will likely reduce the abundance of terrestrial vertebrates which serve as prey for this species. This conclusion is based on acute RQs for birds and acute RQs for mammals, which exceed the LOC.</p> <p><b>Habitat Modifications</b></p> <p>The Risk Assessment indicates use of diphacinone may adversely modify the habitat of this species by reducing the availability of small mammal burrows. This conclusion is based on acute RQs for mammals that exceed the LOC. 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 and other prey eating reptiles.</p>
Salt marsh harvest mouse ( <i>Reithrodontomys ravivertis</i> )	<i>May Affect and Likely to Adversely Affect (LAA)</i>	<b>Potential for Direct Effects</b>
		The Risk Assessment indicates use of diphacinone will likely result in direct effects to the SMHM from acute toxicity. Dietary exposure estimates and data on acute toxicity to small mammals results in acute RQs that exceed the LOC for primary exposure. This species is predicted to be susceptible to primary exposure through direct contact with treated bait products. This contact may result in ingestion of the bait, which would likely result in acute and possibly chronic toxic effects.
		<b>Potential for Indirect Effects</b>
		<p><b>Habitat Modifications</b></p> <p>The Risk Assessment indicates use of diphacinone may adversely modify the habitat of this species by reducing the availability nest sites. This conclusion is based on acute RQs for mammals that exceed the LOC. Adverse effects to mammals may result in a reduction of abandoned mammal nests, which are used as nest sites by this species.</p>

Designated Critical Habitat for:	Effects Determination	Basis for Determination
San Joaquin Kit Fox ( <i>Vulpes macrotis mutica</i> )	<i>May Affect and Likely to Adversely Affect (LAA)</i>	<p><b>Potential for Direct Effects</b></p> <p>The Risk Assessment indicates use of diphacinone will likely result in direct effects to the SJKF from acute toxicity. Dietary exposure estimates and data on acute toxicity to small mammals results in acute RQs that exceed the LOC for primary exposure. This species is predicted to be susceptible to primary exposure through direct contact with treated bait products. This contact may result in ingestion of the bait, which would likely result in acute and possibly chronic toxic effects.</p>
		<p><b>Potential for Indirect Effects</b></p> <p>The Risk Assessment indicates use of diphacinone may adversely modify the habitat of this species by reducing the availability of prey items. This conclusion is based on acute RQs for mammals that exceed the LOC.</p>
California Tiger Salamander ( <i>Ambystoma californiense</i> )	<i>May Affect and Likely to Adversely Affect (LAA)</i>	<p><b>Potential for Direct Effects</b></p> <p><b><i>Terrestrial-phase (Juveniles and Adults):</i></b></p> <p>The Risk Assessment indicates use of diphacinone should not result in direct effects to the CTS from acute toxicity. Dietary exposure estimates and acute toxicity to terrestrial phase amphibians (based on acute toxicity data for snakes) result in acute RQs that do not exceed the LOC for primary or secondary exposure. However, it is unlikely that the CTS will ingest bait. Data were not available to assess chronic toxicity, but since risk is not predicted for acute effects, risk is also unlikely for chronic effects.</p>
		<p><b><i>Aquatic-phase (Eggs, Larvae, and Adults):</i></b></p> <p>The Risk Assessment indicates use of diphacinone should not result in direct effects to the CTS from acute toxicity. Dietary exposure estimates and acute toxicity to aquatic phase (based on acute toxicity data for fish) result in acute RQs that do not exceed the LOC for primary exposure. Data were not available to assess chronic toxicity, but since risk is not predicted for acute effects, risk is also unlikely for chronic effects.</p>
		<p><b>Potential for Indirect Effects</b></p> <p><b><i>Aquatic-phase (Eggs, Larvae, and Adults):</i></b></p> <p>The risk assessment indicates use of diphacinone should not result in indirect effects to the aquatic phase of the CTS.</p> <p><b><i>Terrestrial-phase (Juveniles and Adults):</i></b></p> <p>The Risk Assessment indicates use of diphacinone should not result in indirect effects to the CTS from acute toxicity. Dietary exposure estimates and acute toxicity to aquatic phase (based on acute toxicity data for fish) result in acute RQs that do not exceed the LOC for primary exposure. Data were not available to assess chronic toxicity, but since risk is not predicted for acute effects, risk is also unlikely for chronic effects. However, since the CTS can use small mammal burrows and diphacinone kills small mammals, a loss of burrows may result and may indirectly affect the CTS through habitat modification.</p>

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

<b>Designated Critical Habitat for:</b>	<b>Effects Determination</b>	<b>Basis for Determination</b>
AW	LAA and Habitat Modification	Loss of prey species
Terrestrial Phase CTS	LAA through Indirect Effects: Habitat Modification	Loss of mammalian burrows

**Table 7-3. Use Specific Summary of The Potential for Adverse Effects to Terrestrial Taxa**

Uses	SMHM and Small Mammals <sup>1</sup>		SJKF and Large Mammals <sup>2</sup>		CTS-CC, CTS-SC, CTS-SB and Amphibians <sup>3</sup>		AW, and Reptiles <sup>4</sup>	
	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic
Rodent Control	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

1 A yes in this column indicates a potential for direct effects to SMHM and indirect effects to SMHM, SJKF, AW, CTS-CC, CTS-SC, CTS, and CTS-SB.

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 CTS-CC, CTS-SC, CTS-SB and AW.

4 A yes in this column indicates the potential for direct and indirect effects to AW, and other reptiles.



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

When evaluating the significance of this risk assessment's direct/indirect and adverse habitat modification effects determinations, it is important to note that pesticide exposures and predicted risks to the listed species and its resources (*i.e.*, food and habitat) are not expected to be uniform across the action area. In fact, given the assumptions of drift and downstream transport (*i.e.*, attenuation with distance), pesticide exposure and associated risks to the species and its resources are expected to decrease with increasing distance away from the treated field or site of application. Evaluation of the implication of this non-uniform distribution of risk to the species would require information and assessment techniques that are not currently available.

Examples of such information and methodology required for this type of analysis would include the following:

- Enhanced information on the density and distribution of life stages within the action area and/or applicable designated critical habitat. This information would allow for quantitative extrapolation of the present risk assessment's predictions of individual effects to the proportion of the population extant within geographical areas where those effects are predicted. Furthermore, such population information would allow for a more comprehensive evaluation of the significance of potential resource impairment to individuals of the assessed species.
- Quantitative information on prey base requirements for the assessed species. While existing information provides a preliminary picture of the types of food sources utilized by the assessed species, it does not establish minimal requirements to sustain healthy individuals at varying life stages. Such information could be used to establish biologically relevant thresholds of effects on the prey base, and ultimately establish geographical limits to those effects. This information could be used together with the density data discussed above to characterize the likelihood of adverse effects to individuals.
- Information on population responses of prey base organisms to the pesticide. Currently, methodologies are limited to predicting exposures and likely levels of direct mortality, growth or reproductive impairment immediately following exposure to the pesticide. The degree to which repeated exposure events and the inherent demographic characteristics of the prey population play into the extent to which prey resources may recover is not predictable. An enhanced understanding of long-term prey responses to pesticide exposure would allow for a more refined determination of the magnitude and duration of resource impairment, and together with the information described above, a more complete prediction of effects to individual species and potential modification to critical habitat.

## 8.0 References

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

Brooks, J.E., P.J. Savarie and J.J. Johnston. 1998. The oral and dermal toxicity of selected chemicals to brown tree snakes (*Boiga irregularis*). *Wildlife Research* 25:427-435.

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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.

## 9.0 MRID List

### 71-1 Avian Single Dose Oral Toxicity

MRID		Citation Reference
73753	See 128411	Fink, R. (1976) Final Report: Acute Oral LD 50 Mallard Duck: Project No. 107-122. (Unpublished study received Aug 21, 1978 under 876-EX-36; prepared by Truslow Farms, Inc., submitted by Velsicol Chemical Corp., Chicago, Ill.; CDL:234818-O)
106914	Letter- avian toxicity	Calo, C. (1975) Letter sent to R. Ostrowski dated Jul 28, 1975: Acute toxicity studies--Ramik mouse bait. (Unpublished study received Jun 28, 1977 under 876-124; submitted by Velsicol Chemical Corp., Chicago, IL; CDL:231295-AP)
128411	2028348 2028349	Fink, R. (1976) Acute Oral LD50--Mallard Duck--Technical Diphacinone: Project No. 107-122. Final rept. (Unpublished study received Jul 12, 1978 under 876-EX-35; prepared by Truslow Farms, Inc., submitted by Velsicol Chemical Corp., Chicago, IL; CDL:234422-K)
42245201	2028363	Campbell, S.; Hoxter, K.; Smith, G. (1991) Diphacinone Technical: An Acute Oral Toxicity Study with the Northern Bobwhite: Lab Project Number: 284-103. Unpublished study prepared by Wildlife International Ltd. 24 p.

### 71-2 Avian Dietary Toxicity

MRID		Citation Reference
73751	See 128413	Fink, R. (1976) Final Report: Eight-Day Dietary LC50--Mallard Duck: Project No. 107-121. (Unpublished study received Aug 21, 1978 under 876-EX-36; prepared by Truslow Farms, Inc., submitted by Velsicol Chemical Corp., Chicago, Ill.; CDL:234818-K)
73752	See 107432	Fink, R. (1976) Final Report: Eight-Day Dietary LC50--Bobwhite Quail: Project No. 107-120. (Unpublished study received Aug 21, 1978 under 876-EX-36; prepared by Truslow Farms, Inc., submitted by Velsicol Chemical Corp., Chicago, Ill.; CDL:234818-N)
79639	See 127697	Fink, R. (1975) Final Report: Eight-Day Dietary LC50--Bobwhite Quail: Project No. 107-117. (Unpublished study received Aug 21, 1978 under 876-EX-36; prepared by Truslow Farms, Inc., submitted by Velsicol Chemical Corp., Chicago, Ill.; CDL:234818-G)
88743	See 127698	Fink, R. (1975) Final Report: Eight-Day Dietary LC50-Mallard Duck: Project No. 107-118. (Unpublished study received Aug 21, 1978 under 876-EX-36; prepared by Truslow Farms, Inc., submitted by Velsicol Chemical Corp., Chicago, Ill.; CDL:234818-H)
107432	2028350	Fink, R. (1976) Final Report: Eight-day Dietary LC50--Bobwhite Quail: Technical Diphacinone: Project No. 107-120. (Unpublished study received May 12, 1976 under 876-124; prepared by Truslow Farms,

		Inc., submitted by Velsicol Chemical Corp., Chicago, IL; CDL:225250-A)
127697	2028353	Fink, R. (1975) Eight-day Dietary LC50: Bobwhite Quail: Diphacinone: Project No. 107-117. Final rept. (Unpublished study received Oct 29, 1975 under 876-184; prepared by Truslow Farms, Inc., submitted by Velsicol Chemical Corp., Chicago, IL; CDL: 222349-A)
127698	2028351	Fink, R. (1975) Eight-day Dietary LC50: Mallard Duck: Diphacinone: Project No. 107-118. Final rept. (Unpublished study received Oct 29, 1975 under 876-184; prepared by Truslow Farms, Inc., submitted by Velsicol Chemical Corp., Chicago, IL; CDL:222350-A)
128412	2028350 2028353	Fink, R. (1976) Eight-day Dietary LC50--Bobwhite Quail--Technical Diphacinone: Project No. 107-120. Final rept. (Unpublished study received Jul 12, 1978 under 876-EX-35; prepared by Truslow Farms, Inc., submitted by Velsicol Chemical Corp., Chicago, IL; CDL:234422-N)
128413	2028352	Fink, R. (1976) Eight-day Dietary LC50--Mallard Duck--Technical Diphacinone: Project No. 107-121. Final rept. (Unpublished study received Jul 12, 1978 under 876-EX-35; prepared by Truslow Farms, Inc., submitted by Velsicol Chemical Corp., Chicago, IL; CDL:234422-O)
42408801	2028365	Long, R.; Foster, J.; Hoxter, K. et al. (1992) Diphacinone Technical: A Dietary LC50 Study with the Northern Bobwhite: Lab Project Number: 284-101A. Unpublished study prepared by Bell Labs, Inc. 32 p.
42408802	2028366	Long, R.; Foster, J.; Hoxter, K. et al. (1992) Diphacinone Technical: A Dietary LC50 Study with the Mallard: Lab Project Number: 284-102B. Unpublished study prepared by Wildlife Intl. 36 p.

### 71-3 Wild mammal toxicity

MRID		Citation Reference
2467	Referenced in 2032283- no DER	Pank, L.F.; Hirata, D.N. (1976) Primary and Secondary Toxicity of Anticoagulant Rodenticides: Job Completion Report: Work Unit DF-103.7. (Unpublished study received on unknown date under unknown admin. no.; prepared by U.S. Fish and Wildlife Service, Denver Wildlife Research Center, Wildlife Damage Research Station, submitted by Velsicol Chemical Corp., Chicago, Ill.; CDL: 230307-B)
2468	Not located	U.S. Fish and Wildlife Service, Denver Wildlife Research Center (1973) Vertebrate Damage Control Research: 1973 Annual Report. By Denver Wildlife Research Center and U.S. Agency for International Development, Office of Agriculture and Fisheries. Denver, Colo.: U.S. Fish and Wildlife Service. (Available from: U.S. Fish and Wildlife Service, Denver Wildlife Research Center, Denver, CO; pp. 23-25 only; also In unpublished submission received on unknown date under unknown admin. no.; submitted by Velsicol Chemical Corp., Chicago, Ill.; CDL:230307-D)

## 71-5 Simulated or Actual Field Testing

MRID		Citation Reference
45855101	Efficacy study see 46117301	Salmon, T.; Whisson, D.; Gorenzel, W. (2001) Field Efficacy Studies Comparing 0.005% and 0.01% Diphacinone and Chlorophacinone Baits for Controlling California Ground Squirrels ( <i>Spermophilus beecheyi</i> ): Final Report: Lab Project Number: 00-0471: 29: 30. Unpublished study prepared by University of California, Davis. 131 p.
46911901	2056087	Mach, J. (2006) Secondary Hazard Study Using Diphacinone-Killed Rodents Fed to Domestic Ferrets ( <i>Mustela putorius furo</i> ): Final Report. Project Number: 05038, 06002. Unpublished study prepared by Genesis Laboratories, Inc. 32 p.

## 72-1 Acute Toxicity to Freshwater Fish

MRID		Citation Reference
60603 or 127699	2028354	Bentley, R.E. (1975) Acute Toxicity of Ramik Mouse Bait to Bluegill ( <i>Lepomis macrochirus</i> ), Rainbow Trout ( <i>Salmo gairdneri</i> ), and Channel Catfish ( <i>Ictalurus punctatus</i> ). (Unpublished study received Aug 21, 1978 under 876-EX-36; prepared by Bionomics, EG&G, submitted by Velsicol Chemical Corp., Chicago, Ill.; CDL:234818-E)
60607	2028355 2028356	Bentley, R.E. (1975) Acute Toxicity of Diphacinone Technical to Bluegill ( <i>Lepomis macrochirus</i> ), Channel Catfish ( <i>Ictalurus punctatus</i> ), and Rainbow Trout ( <i>Salmo gairdneri</i> ). (Unpublished study received Aug 21, 1978 under 876-EX-36; prepared by EG&G, Bionomics, submitted by Velsicol Chemical Corp., Chicago, Ill.; CDL:234818-L)
92049005	Registrant summary	Kelley, J. (1990) Haco Co. Phase 3 Summary of MRID 00060607. Acute Toxicity of Diphacinone Technical to Bluegill, Channel Catfish, and Rainbow Trout. Prepared by EG&G, Bionomics. 17 p.

## 72-2 Acute Toxicity to Freshwater Invertebrates

MRID		Citation Reference
42282201	2028364	Putt, A. (1992) Diphacinone Technical: Acute Toxicity to Daphnids ( <i>Daphnia magna</i> ) under Flow-through Conditions: Lab Project Number: 91-9-3938: 12455. 1290. 6100. 115: 90-10A. Unpublished study prepared by Springborn Labs, Inc. 66 p.
43138301	DER not located	Swigert, J.; Vukich, J. (1994) W-11, Supplement 1: (inert ingredient): A 48-Hour Flow-Through Acute Toxicity Test With the Cladoceran ( <i>Daphnia magna</i> ): Lab Project Number: 164-A-103B: 164A-103B. Unpublished study prepared by Wildlife International. 16 p.
43138302	DER not	Oblad, B.; Rossi, R. (1987) (inert ingredient): Acute Measured Static

located 48-Hour EC50 Test Using Water Flea (*Daphnia magna*) Exposed to GAF: Lab Project Number: L1802-03: L1802-04. Unpublished study prepared by Syracuse Research Corp. 11 p.

### 72-3 Acute Toxicity to Estuarine/Marine Organisms

MRID		Citation Reference
60604	2028359 2028360	Heitmuller, T. (1975) Acute Toxicity of Ramik to Pink Shrimp ( <i>Penaeus duorarum</i> ) and Fiddler Crabs ( <i>Uca pugilator</i> ). (Unpublished study received Aug 21, 1978 under 876-EX-36; prepared by Bionomics--EG&G, Inc., submitted by Velsicol Chemical Corp., Chicago, Ill.; CDL:234818-F)
60608	2028358 2028361	Heitmuller, T. (1975) Acute Toxicity of Diphacinone to Pink Shrimp ( <i>Penaeus duorarum</i> ) and Fiddler Crabs ( <i>Uca pugilator</i> ). (Unpublished study received Aug 21, 1978 under 876-EX-36; prepared by Bionomics--EG&G, Inc., submitted by Velsicol Chemical Corp., Chicago, Ill.; CDL:234818-M)

### 850.2500 Field testing for terrestrial wildlife

MRID		Citation Reference
45855102	DER not located	VerCauteren, K.; Pipas, M.; Bourassa, J. (2002) A Camera and Hook System for Viewing and Retrieving Rodent Carcasses From Burrows: (Diphacinone and Chlorophacinone Baits): Lab Project Number: QA-879. Unpublished study prepared by National Wildlife Research Center. 29 p. (OPPTS 850.2500)
46117301	2013053 summary	Salmon, T.; Whisson, D.; Gorenzel, W. (2001) Field Efficacy Studies Comparing 0.005% and 0.01% Diphacinone and Chlorophacinone Baits for Controlling California Ground Squirrels ( <i>Spermophilus beecheyi</i> ). Unpublished study prepared by University of California, Davis. 61 p.
46117302	2013053 summary	VerCauteren, K.; Pipas, M.; Bourassa, J. (2002) A Camera and Hook System for Viewing and Retrieving Rodent Carcasses from Burrows. Unpublished study prepared by U.S. Dept. of Agriculture, APHIS, ADC. 22 p.
46117303	2013053 Summary	Goodall, M.; Primus, T.; Johnston, J. (2002) Determination of Chlorophacinone (sic) and Diphacinone Residues in California Ground Squirrels and Non-Target Animals. Unpublished study prepared by U.S. Dept. of Agriculture, APHIS, ADC. 19 p.

### Non Guideline Selections

2466	FWS-published	Evans, L. (1972) Diphacinone Hazards: Forest-Animal Unit, Olympia, Washington. (Unpublished study received May 30, 1972 under unknown admin. no.; prepared by U.S. Fish and Wildlife Service, Forest-Animal Damage Unit, submitted by Velsicol Chemical Corp., Chicago,
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		III.; CDL:230307-A)
2469	Open lit – Summary in 2028371 pg 14	Evans, J.; Ward, A.L. (1967) Secondary poisoning associated with anticoagulant-killed nutria. Journal of the American Veterinary Medical Association 151(7):856-861. (Also In unpublished submission received May 30, 1972 under unknown admin. no.; submitted by Velsicol Chemical Corp., Chicago, Ill.; CDL:230307-E)
5988 or 82540	Open lit	Brock, E.M. (1965) Toxicological feeding trials to evaluate the hazard of secondary poisoning to gopher snakes, <i>Pituophis catenifer</i> . Copeia (2):244-245. (Also In unpublished submission received Apr 23, 1976 under 6704-78; submitted by U.S. Dept. of Interior, Fish and Wildlife Service, Washington, D.C.; CDL:224031-L)
35806	2028362	Mendenhall, V.M.; Pank, L.F. (1979) Secondary Poisoning of Owls by Anticoagulant Rodenticides. (U.S. Fish and Wildlife Service, Patuxent and Denver Wildlife Research Centers, unpublished study)
35808	Presentation	Peacock, D.B.; Palmateer, S.D. (1979) Comparison of EPA animal biology laboratory and company laboratory efficacy data for federally registered rat and mouse baits. Pages 11-19, In Vertebrate Pest Control and Management Materials. Philadelphia, Pa.: American Society for Testing and Materials. (Special technical publication 680, published study)
60609 or 124410	2028372 pg 2	Fink, R. (1976) Eight-Week Secondary Poisoning Study with Sparrow Hawks Using Ramik: Project No. 107-130. (Unpublished study received Aug 21, 1978 under 876-EX-36; prepared by Truslow Farms, Inc., submitted by Velsicol Chemical Corp., Chicago, Ill.; CDL:234818-P)
79312	Open lit referenced but no DER located	Savarie, P.J.; Hayes, D.J.; McBride, R.T.; et al. (1979) Efficacy and safety of diphacinone as a predacide. Pages 69-79, In Avian and Mammalian Wildlife Toxicology. Edited by E.E. Kenaga. Philadelphia, Pa.: American Society for Testing and Materials. (ASTM special technical publication 693; also In unpublished submission received Aug 3, 1981 under 876-191; submitted by Velsicol Chemical Corp., Chicago, Ill.; CDL:245646-AH)
40565501	See 2032278	Keith, J.; Hirata, D. (1987) Determination of an Acute, Oral LD50 for Diphacinone against Mongooses ( <i>Herpestes auropunctatus</i> ): Mongoose Project (963). Unpublished study prepared by Denver Wildlife Research Center. 6 p.
40565502	See 2032278	Keith, J.; Hirata, D. (1986) Laboratory Trials To Determine Minimum Levels of Diphacinone in Bait that are Lethal to Mongooses ( <i>Herpestes auropunctatus</i> ): Mongoose Project (963). Unpublished study prepared by Denver Wildlife Research Center. 21 p.
40565503	See 2032278	Keith, J.; Hirata, D. (1987) Laboratory Trial To Determine Mortality of Mongooses ( <i>Herpestes auropunctatus</i> ) Fed 0.00025% Diphacinone Bait: Mongoose Project (963). Unpublished study prepared by Denver Wildlife Research Center. 6 p.
40565504	2032278 pg 7	Keith, J.; Espy, D.; Hirata, D. (1987) Small Field Efficacy Trials using Diphacinone Bait against Mongooses ( <i>Herpestes auropunctatus</i> ): Mongoose Project (963). Unpublished study prepared by Denver Wildlife Research Center. 8 p.



40565505	See 2032278	Keith, J.; Espy, D.; Hirata, D. (1987) Summary of Experience with Radiocollars on Mongooses ( <i>Herpestes auropunctatus</i> ): Mongoose Project (963). Unpublished study prepared by Denver Wildlife Research Center. 7 p.
40565506	See 2032278	Keith, J. (1988?) Data on the Acute Oral LD50 of Diphacinone against Laboratory Rats ( <i>Rattus rattus</i> ): Mongoose Project (963). Unpublished study prepared by Denver Wildlife Research Center. 6 p.
41177301 or 41375801	2032282	Keith, J.; Hirata, D.; Espy, D.; et al. (1989) Field Evaluation of 0.00025(percent) Diphacinone Bait for Mongoose Control in Hawaii: Project ID: QA-16. Unpublished study prepared by Denver Wildlife Research Center. 35 p.
43000400		Bell Laboratories, Inc. (1993) Submission of Toxicity in Support of Technical Diphacinone FIFRA 6(a)(2) Requirements. Transmittal of 1 study.
43038600	No EFED review located	U.S. Fish and Wildlife Service (1993) Submission of risk to non-target organisms data in response to EPA's request. Transmittal of 1 study.
43038601	See Endangered Species folder in SAN	U.S. Fish and Wildlife Service (1993) Effects of 16 Vertebrate Control Agents on Threatened and Endangered Species: Biological Opinion. 183 p.
45016803	See 45017202 below	Marsh, R.; Howard, W.; Cole, R. (1999) The Toxicity of Chlorophacinone and Diphacinone to Deer Mice. Unpublished study prepared by California Department of Food and Agriculture. 8 p.
45017202	Open lit	Marsh, R.; Howard, W.; Cole, R. (1977) The Toxicity of Chlorophacinone and Diphacinone to Deer Mice. <i>Journal of Wildlife Management</i> 41(2):298-301.

46750931	Open lit	Mendenhall, V.; Pank, L. (1980) Secondary Poisoning of Owls by Anticoagulant Rodenticides: (Difenacoum, Bromadiolone, Brodifacoum, Diphacinone, Fumarin, Chlorophacinone). The Wildlife Society Bulletin 8(4): 311-315.
46884101	Open lit	Eisemann, J. (2003) Studies Conducted to Assess the Nontarget Hazards of Aerial Broadcast of Diphacinone Rodenticide Baits to Control Rats in Hawaii for Conservation Purposes. Project Number: QA/1192, QA/1077, QA/770. Unpublished study prepared by U.S. Fish and Wildlife Services, Ecological Services, U.S. Dept. of Agriculture, APHIS, WS and Central Science Laboratory. 205 p.
TNM 051	TNM 051	Beltsville Laboratory Rat Dietary Study with 99.9% Diphacinone
TNM 075	TNM 075	Beltsville Laboratory Rat Dietary Study with 99.9% Diphacinone
N.A.	2028371	Effects to Insectivorous Bats, USFWS report 1973 on pg 11

## 161-1 Hydrolysis

MRID		Citation Reference
95000 or 136281	Summary in 2032264	Atallah, Y.H.; Diaz, L.I.; McGhee, M.E.; et al. (1977) Aqueous Hydrolysis of Diphacinone: Report 408398, No. 3. (Un- published study received Feb 17, 1981 under 876-EX-35; submitted by Velsicol Chemical Corp., Chicago, Ill.; CDL:244385-A)
43582401	2028341	Riekena, C. (1995) Hydrolysis of Technical Diphacinone (2-(Diphenylacetyl)-1H-indene-1,3(2H)-dione): Lab Project Number: BEL/0894/C143. Unpublished study prepared by Bell Labs, Inc. 40 p.
92049010	Registrant summary	Prasad, S. (1990) Haco, Co Phase 3 Summary of MRID 00095000. Diphacinone - Hydrolysis Study: Project No. 408398. Prepared by Velsicol Chemical Corp. 18 p.

## 161-2 Photodegradation-water

MRID		Citation Reference
92049011	Registrant summary	Prasad, S. (1990) Haco, Co Phase 3 Summary of MRID 00127709. Diphacinone - Photodegradation in Water Study: Project No. 408398. Prepared by Velsicol Chemical Corp. 22 p.
127709	Summary in 2032264	Diaz, L.; Schwemmer, B.; Atallah, Y.; et al. (1976) Photolysis of 14C-diphacinone in Aqueous Solution: Report 40839, No. 5. (Un- published study received Aug 21, 1978 under 876-EX-36; submitted by Velsicol Chemical Corp., Chicago, IL; CDL:234819-D)

## 162-1 Aerobic soil metabolism

MRID		Citation Reference
128415	Summary in 2032264	Atallah, Y.; Diaz, L.; Yu, C.; et al. (1978) Soil Degradation of the Rodenticide Diphacinone: Report 408398, No. 6. (Unpublished study received Aug 21, 1978 under 876-EX-36; submitted by Velsicol Chemical Corp., Chicago, IL; CDL:234819-E)
42035001		Nomura, N.; Hilton, H. (1979) The Metabolism of ?Carbon 14]-Diphacinone to Carbon Dioxide in Two Hawaiian Sugarcane Soils Under Aerobic conditions. Unpublished study prepared by Hawaiian Sugar Planters. 27 p.
44260501		Riekena, C. (1997) Aerobic Soil Metabolism of (carbon 14)-Diphacinone: (Final Report): Lab Project Number: 42430: BEL/0497/C192. Unpublished study prepared by Bell Labs, Inc. 116 p.
92049012	Registrant summary	Prasad, S. (1990) Haco, Co Phase 3 Summary of MRID 00128415. Diphacinone - Aerobic Soil Metabolism Study: Project No. 408398-6. Prepared by Velsicol Chemical Corp. 22 p.

## 162-2 Anaerobic soil metabolism

MRID		Citation Reference
92049013	Registrant summary	Prasad, S. (1990) Haco, Co Phase 3 Summary of MRID 00128415. Diphacinone - Anaerobic Soil Metabolism Study: Project 408398-6. Prepared by Velsicol Chemical Corp. 24 p.
128415	Summary in 2032264	Atallah, Y.; Diaz, L.; Yu, C.; et al. (1978) Soil Degradation of the Rodenticide Diphacinone: Report 408398, No. 6. (Unpublished study received Aug 21, 1978 under 876-EX-36; submitted by Velsicol Chemical Corp., Chicago, IL; CDL:234819-E)

## 163-1 Leach/adsorp/desorption

MRID		Citation Reference
127711	Summary in 2032264	Weber, J. (19??) Leachability of Atrazine, Buthidazole, Bromacil, Diuron, Diphacinone, and Prometon through a Lakeland Sand. (Unpublished study received Aug 21, 1978 under 876-EX-36; prepared by North Carolina State Univ., submitted by Velsicol Chemical Corp., Chicago, IL; CDL:234819-G)
127712	Summary in 2032264	Nomura, N. (1978) Adsorption and Mobility of VEL 5026-14C in Five Hawaiian Sugarcane Soils. (Unpublished study received Aug 21, 1978 under 876-EX-36; prepared by Hawaiian Sugar Planters' As- soc., submitted by Velsicol Chemical Corp., Chicago, IL; CDL: 234819-H)

127713	Summary in 2032264	Weber, J. (19??) Adsorption and Characterization of Diphacinone and Prometon on Lakeland Sand, Alamance Silt Loam, Ca-montmorillonite, and Acidic Soil Organic Matter. (Unpublished study received Aug 21, 1978 under 876-EX-36; prepared by North Carolina State Univ., submitted by Velsicol Chemical Corp., Chicago, IL; CDL:234819-I)
134841	See 127711	Weber, J.; Swain, L. (19??) Report to ...: Leachability of Atzrazine, Buthiazole, Bromacil, Diuron, Diphacinone and Prometon through a Lakeland Sand. (Unpublished study received Jul 12, 1978 under 876-EX-35; prepared by N.C. State Univ., submitted by Velsicol Chemical, Corp., Chicago, IL; CDL:234423-I)
134842	See 127712	Nomura, N. (1978) Adsorption and Mobility of VEL 5026-14C in Five Hawaiian Sugarcane Soils. (Unpublished study received Jul 12, 1978 under 876-EX-35; prepared by Hawaiian Sugar Planters' Assoc., submitted by Velsicol Chemical, Corp., Chicago, IL; CDL: 234423-J)
134843	See 138841	Weber, J.; Swain, L. (19??) Report to ...: Adsorption and Characterization of Diphacinone and Prometon on Lakeland Sand, Alamance Silt Loam, Ca-montmorillonite and Acidic Soil Organic Matter. (Unpublished study received Jul 12, 1978 under 876-EX-35; prepared by N.C. State Univ., submitted by Velsicol Chemical, Corp., Chicago, IL; CDL:234423-K)
43565801	See 43582402	Riekena, C. (1995) Leaching Through Soil of Technical Diphacinone (2-(Diphenylacetyl)-1H-indene-1,2(2H)-dione): Lab Project Number: BEL/1194/C146. Unpublished study prepared by Bell Labs, Inc. 28 p.
43582402	2028342	Riekena, C. (1995) Leaching Through Soil of Technical Diphacinone (2-(Diphenylacetyl)-1H-indene-1,3(2H)-dione): Lab Project Number: BEL/1194/C146. Unpublished study prepared by Bell Labs, Inc. 28 p.
92049014	Registrant summary	Prasad, S. (1990) Haco, Co Phase 3 Summary of MRID 00127711. Leaching Study: [Diphacinone]. Prepared by North Carolina State University. 19 p.
92049015	Registrant summary	Prasad, S. (1990) Haco, Co Phase 3 Summary of MRID 00127712. Diphacinone - Leaching/Absorption Study. Prepared by Hawaiian Sugar Planters Assn. 28 p.
92049016	Registrant summary	Prasad, S. (1990) Haco, Co Phase 3 Summary of MRID 00127713. Diphacinone - Adsorption Study. Prepared by North Carolina State University. 26 p.

## 165-0 Accumulation Studies -- General

MRID	Citation Reference
127715	Summary in 2032264 Ells, S. (1976) Kinetics of Aged Diphacinone in a Model Aquatic Ecosystem. (Unpublished study received Aug 21, 1978 under 876-EX-36; prepared by EG & G, Bionomics, submitted by Velsicol Chemical Corp., Chicago, IL; CDL:234819-K)

### Non Guideline Selections

127710	Summary in 2032264	Rieck, C. (19??) Effect of Diphenadione on Soil Microbe Populations. (Unpublished study received Aug 21, 1978 under 876-EX- 36; prepared by Univ. of Kentucky, Agronomy Dept., submitted by Velsicol Chemical Corp., Chicago, IL; CDL:234819-F)
127714	Summary in 2032264	Nomura, N. (1977) Release Rate of 14C-diphacinone from Bait to Soil under High Rainfall Conditions. (Unpublished study received Aug 21, 1978 under 876-EX-36; prepared by Hawaiian Sugar Planters' Assoc., submitted by Velsicol Chemical Corp., Chicago, IL; CDL: 234819-J)
127716	Summary in 2032264	Velsicol Chemical Corp. (1977) Residue Analysis of Diphacinone in Soil: AM 0642. (Unpublished study received Aug 21, 1978 under 876-EX-36; CDL:234819-L)
127706	compilation	Velsicol Chemical Corp. (1978) Chemical & Physical Properties: ?Diphacinone . (Compilation; unpublished study received Aug 21, 1978 under 876-EX-36; CDL:234817-A)
134837	compilation	Velsicol Chemical, Corp. (1978) Chemical & Physical Properties: Diphacinone . (Compilation; unpublished study received Jul 12, 1978 under 876-EX-35; CDL:234419-A)

