Risks of Diflubenzuron Use to Federally Threatened California Red-legged Frog
(*Rana aurora draytonii*)

Pesticide Effects Determination

Environmental Fate and Effects Division
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1.0 Executive Summary

1.1. Purpose

The purpose of this assessment is to evaluate potential direct and indirect effects on the California red-legged frog *(Rana aurora draytonii*) (CRLF) arising from Federal Insecticide, Fungicide, Rodenticide, Act (FIFRA) regulatory actions regarding use of diflubenzuron on agricultural and non-agricultural sites. In addition, this assessment evaluates whether these actions can be expected to result in modification of the species' designated critical habitat. This assessment was completed in accordance with the U.S. Fish and Wildlife Service (U.S. FWS) and National Marine Fisheries Service (NMFS) *Endangered Species Consultation Handbook* (U.S. FWS/NMFS 1998) and procedures outlined in the Agency's Overview Document (U.S. EPA 2004).

The CRLF was listed as a threatened species by U.S. FWS in 1996. The species is endemic to California and Baja California (Mexico) and inhabits both coastal and interior mountain ranges. A total of 243 streams or drainages are believed to be currently occupied by the species, with the greatest numbers in Monterey, San Luis Obispo, and Santa Barbara counties (U.S. FWS 1996) in California.

1.2. Chemical Assessed

Diflubenzuron is a benzoylphenylurea insecticide that kills immature insects by disrupting the molting process. Formulation types registered include liquid, water dispersible granules, wettable powder, effervescent tablets (for ornamental pond treatment), and termite bait (in bait stations).

1.3. Use Characterization

Labeled uses of diflubenzuron include citrus, cotton, forestry, mushrooms, ornamentals, pastures, soybeans, standing water, sewage systems, termite bait stations, and wide-area general outdoor treatment sites. All of these uses are considered as part of the federal action evaluated in this assessment with the exception of termite bait stations (in which a solidified form of the pesticide is placed in an enclosed bait station to minimize loss to the environment) and soybeans, which are not grown in California.

1.4. Environmental Fate and Exposure Summary

Diflubenzuron appears to be relatively non-persistent and immobile under normal use conditions. Diflubenzuron is relatively stable to hydrolysis and photolysis. The major route of dissipation appears to be biotic processes (half-life of approximately 2 days for aerobic soil metabolism). Available data indicate that it is unlikely that diflubenzuron will contaminate ground water or appreciably volatilize and be transported through long-range atmospheric transport processes. However, a degradate of diflubenzuron, 4-chlorophenylurea (CPU), has a much greater potential to leach through soil. Spray drift and transport with eroded soil in runoff are thought to be the main mechanisms of transport from the site of application.

Since CRLFs exist within aquatic and terrestrial habitats, exposure of the CRLF, its prey and its habitats to diflubenzuron are assessed separately for the two habitats. Tier-II aquatic exposure models are used to estimate high-end exposures of diflubenzuron in aquatic habitats resulting from runoff and spray drift from different uses. Peak model-estimated environmental concentrations resulting from different diflubenzuron uses range from 0.01 to 113 µg/L. Diflubenzuron is typically not measured by surface and groundwater monitoring programs, therefore, no monitoring data are available for comparison with the model estimated diflubenzuron concentrations.

1.4. Toxicity Summary

Diflubenzuron is very highly toxic to aquatic invertebrates. The most sensitive LC_{50} identified for diflubenzuron was 0.0028 µg/L (Ecotox no. 16591). Toxicity is influenced by species and life-stage of the test organism. The most sensitive NOAEC was 0.00025 µg/L (Ecotox No. 9397). Diflubenzuron is less toxic to fish, with a 96-hr LC_{50} of 129,000 µg/L (MRID 00056150) and a reproduction NOAEC of 110 µg/L (MRID 00099755). Diflubenzuron was not toxic to aquatic plants at up to 200 µg/L (MRID 45252205 and 42940103).

Diflubenzuron is also practically non-toxic to birds on an acute basis. The most sensitive study available produced an LD_{50} value of 3763 mg/kg-bw in the Red-Winged Blackbird (MRID 00038614).

Using the technical grade active ingredient (TGAI), diflubenzuron is categorized as practically non-toxic to avian species on a subacute dietary toxicity basis based on an LC_{50} value of >4640 ppm for the bobwhite quail and mallard duck (MRID 00039080). A 1 % Granular formulation was also categorized as practically non-toxic to bobwhite quail and mallard ducks based on an LC_{50} value of >20,000 ppm (MRID 00060381). The most sensitive NOAEC from a reliable reproduction study was 500 mg/kg-diet (MRID 4166800102). The NOAEC of 500 mg/kg-food was based on effects on eggshell thickness in mallard ducks and reduced egg production in bobwhite quail at 1000 mg/kg-diet.

The available mammalian acute toxicity data demonstrate that diflubenzuron is practically nontoxic to mammals (LD_{50} > 5000 mg/kg; MRID 00157103). In a 2-generation reproduction study no effects on reproductive performance were observed at any dose level in F0 or F1 males or females. Litter and mean pup weights decreased slightly from birth to 21 days postpartum in F1 offspring at 2500 mg/kg/day. The NOEL for reproductive performance in parental adults is 2500 mg/kg/day. The NOEL for developmental toxicity in progeny is 250 mg/kg/day and the LOAEL is 2500 mg/kg/day, based on decreased body weights in F1 pups from birth to 21 days postpartum. (MRID 43578301). A NOAEL of 250 mg/kg-bw is used in this assessment.

Currently, there are no guideline terrestrial plant toxicity data for diflubenzuron for use in this assessment. However, given the mode of toxicity of diflubenzuron as an insect growth regulator, the long history of use on numerous agricultural commodities without incidence of effects to terrestrial plants that are clearly associated with diflubenzuron, and the available efficacy data that reported minimal phytotoxic effects, the available data do not suggest that terrestrial plants are likely to be impacted to an extent that indirect effects will result to species that depend on them for survival or reproduction.

This assessment was based on potential risks from diflubenzuron. Several degradates have been identified that are of equal or greater toxicity than the parent chemical. These degradates were considered in this assessment; however, analysis of potential exposure to and toxicity of these degradates suggests that potential risks to these degradates is negligible relative to risks posed by parent diflubenzuron.

1.5. Effects Determination

The effects determination assessment endpoints for the CRLF include direct toxic effects on the survival, reproduction, and growth of the CRLF itself, as well as indirect effects, such as reduction of the prey base or modification of its habitat. Direct effects to the CRLF in the aquatic habitat are based on toxicity information for freshwater fish, which are used as a surrogate for aquatic-phase amphibians. In the terrestrial habitat, direct effects are based on toxicity information for birds, which are used as a surrogate for terrestrial-phase amphibians. Given that the CRLF's prey items and designated critical habitat requirements in the aquatic habitat are dependant on the availability of freshwater aquatic invertebrates and aquatic plants, toxicity information for these taxonomic groups is also discussed. In the terrestrial habitat, indirect effects due to depletion of prey are assessed by considering effects to terrestrial insects, small terrestrial mammals, and frogs. Indirect effects due to modification of the terrestrial habitat are characterized by available data for terrestrial monocots and dicots. However, given the lack of guideline toxicity studies on terrestrial plants, additional lines of evidence were used to determine if terrestrial plants may be impacted to an extent that could result in indirect effects to the CRLF.

Risk quotients (RQs) are derived as quantitative estimates of potential high-end risk. Acute and chronic RQs are compared to the Agency's levels of concern (LOCs) to identify instances where diflubenzuron use within the action area has the potential to adversely affect the CRLF and its designated critical habitat via direct toxicity or indirectly based on direct effects to its food supply (*i.e.*, freshwater invertebrates, algae, fish, frogs, terrestrial invertebrates, and mammals) or habitat (*i.e.*, aquatic plants and terrestrial upland and riparian vegetation). When RQs for each particular type of effect are below LOCs, the pesticide is determined to have "no effect" on the CRLF. Where RQs exceed LOCs, a potential to cause adverse effects is identified, leading to a conclusion of "may affect." If a determination is made that use of diflubenzuron use within the action area "may affect" the CRLF and its designated critical habitat, additional information is considered to refine the potential for exposure and effects, and the best available information is used to distinguish those actions that "may affect, but are not likely to adversely affect" (NLAA) from those actions that are "likely to adversely affect" (LAA) the CRLF. Similarly for critical habitat, additional information is considered to refine the potential for exposure and effects to distinguish those actions that do or do not result in modification of its critical habitat.

Based on the best available information, the Agency makes a Likely to Adversely Affect determination for the CRLF from the use of diflubenzuron. Additionally, the Agency has determined that there is a potential for modification of CRLF designated critical habitat from the use of the chemical. A summary of the risk conclusions and effects determinations for the CRLF and its critical habitat is presented in Table 1-1 and 1-2.

Table 1-1 Effects Determination Summary for Diflubenzuron Use and the CRLF

Assessment Endpoint	Effects Determination [1]	Basis for Determination
Survival, growth, and/or reproduction of CRLF individuals	LAA, all uses	**Potential for Direct Effects**
		Aquatic-phase (Eggs, Larvae, and Adults): There was an LOC exceedance for risk to fish (CRLF surrogate) from chronic exposures to diflubenzuron posed by the rice use. However, the risk was determined to be discountable because effects were considered to be unlikely to occur for reasons discussed in Section 5.2. LOCs were not exceeded for any other use. The effects determination for direct effects to aquatic phase CRLFs was NE for all uses except rice and NLAA for rice.
		Terrestrial-phase (Juveniles and Adults): Direct effects to CRLFs could occur from diflubenzuron's use on barn yards based on acute and chronic LOC exceedances for birds and refined analysis for frogs. Direct effects to CRLFs are not expected to occur for any other use. The effects determination is LAA for barnyard/mushroom use and NE for all other uses.
		Potential for Indirect Effects
		Aquatic prey items, aquatic habitat, cover and/or primary productivity Aquatic prey of the CRLF may be impacted based on potential impacts to aquatic invertebrate for all uses. Several lines of evidence support the conclusion that aquatic invertebrates may be impacted by labeled diflubenzuron uses including laboratory studies and field studies. The effects determination is LAA for all uses based on potential impacts to aquatic invertebrates.
		Terrestrial prey items, riparian habitat Terrestrial prey items of the CRLF are expected to be impacted based on potential impacts to terrestrial invertebrates. The effects determination is LAA for all uses based on potential impacts to terrestrial invertebrates.

[1] No effect (NE); May affect, but not likely to adversely affect (NLAA); May affect, likely to adversely affect (LAA)

Table 1-2. Effects Determination Summary for Diflubenzuron Use and CRLF Critical Habitat Impact Analysis

Assessment Endpoint	Effects Determination [1]	Basis for Determination
Modification of aquatic-phase PCE	HM	There are LOC exceedances for risk to terrestrial and aquatic invertebrate prey of the CRLF.
Modification of terrestrial-phase PCE		There are LOC exceedances for risk to terrestrial and aquatic invertebrate prey of the CRLF.

[1] Habitat Modification or No effect (NE)

Use-specific determinations for direct and indirect effects to the CRLF are provided in Table 1-3, 1-4, and 1-5. Further information on the results of the effects determination is included as part of the Risk Description in Section 5.2.

Table 1-3 Diflubenzuron Use-specific Direct Effects Determinations[1] for the CRLF

Use(s)	Aquatic Habitat		Terrestrial Habitat	
	Acute	Chronic	Acute	Chronic
Barnyard/Mushroom	NE	NE	LAA	LAA
Spray on manure, Manure to field	NE	NE	NE	NE
Beech Nut	NE	NE	NE	NE
Brassica (head and stem) vegetables	NE	NE	NE	NE
Citrus hybrids, grapefruit, orange, pummelo (shaddock), and tangerines	NE	NE	NE	NE
Broccoli raab, cabbage - Chinese, collards, and kale	NE	NE	NE	NE
Cotton (unspecified)	NE	NE	NE	NE
Christmas tree plantations and forest trees (softwoods - conifers)	NE	NE	NE	NE
Apricot, nectarine, peach, pear, plum, prune, and stone fruits	NE	NE	NE	NE
Almond, Brazil nut, cashew, chestnut, chinquapin, filbert (hazelnut), hickory nut, macadamia nut (bushnut), pecan, tree nuts, and walnut (English/black)	NE	NE	NE	NE
Barley, canola\rape, oats, triticale, and wheat	NE	NE	NE	NE
Forest nursery plantings (for transplant purposes), ornamental and/or shade trees, ornamental woody shrubs and vines, and shelterbelt plantings	NE	NE	NE	NE
Grass forage/fodder/hay, pastures, and rangeland	NE	NE	NE	NE
Pistachio	NE	NE	NE	NE
Household/domestic dwellings outdoor premises	NE	NE	NE	NE
Rice/Ornamental Pond	NE	NLAA	NE	NE
Agricultural rights-of-way/fencerows/hedgerows, fencerows/hedgerows, and nonagricultural rights-of-way/fencerows/hedgerows	NE	NE	NE	NE
Artichoke, mustard, peanuts (unspecified), and pepper	NE	NE	NE	NE
Commercial/institutional/industrial premises/equipment (outdoor), nonagricultural outdoor buildings/structures, paved areas (private roads/sidewalks), refuse/solid waste sites (outdoor), and wide area/general outdoor treatment (quarantine/eradication use)	NE	NE	NE	NE
Butternut	NE	NE	NE	NE
Agricultural fallow/idleland, golf course turf, and nonagricultural uncultivated areas/soils	NE	NE	NE	NE
Ornamental sod farm (turf) and recreational areas	NE	NE	NE	NE
Turnip (greens)	NE	NE	NE	NE

[1] NE = No effect; NLAA = May affect, not likely to adversely affect; LAA = Likely to adversely affect

Table 1-4 Diflubenzuron Use-specific Indirect Effects Determinations[1] Based on Effects to Aquatic Prey

Use(s)	Algae	Aquatic Invertebrates		Aquatic-phase frogs and fish	
		Acute	Chronic	Acute	Chronic
Barnyard/Mushroom	NE	LAA	LAA	NE	NE
Spray on manure, Manure to field	NE	LAA	LAA	NE	NE
Beech Nut	NE	LAA	LAA	NE	NE
Brassica (head and stem) vegetables	NE	LAA	LAA	NE	NE
Citrus hybrids, grapefruit, orange, pummelo (shaddock), and tangerines	NE	LAA	LAA	NE	NE
Broccoli raab, cabbage - Chinese, collards, and kale	NE	LAA	LAA	NE	NE
Cotton (unspecified)	NE	LAA	LAA	NE	NE
Christmas tree plantations and forest trees (softwoods - conifers)	NE	LAA	LAA	NE	NE
Apricot, nectarine, peach, pear, plum, prune, and stone fruits	NE	LAA	LAA	NE	NE
Almond, Brazil nut, cashew, chestnut, chinquapin, filbert (hazelnut), hickory nut, macadamia nut (bushnut), pecan, tree nuts, and walnut (English/black)	NE	LAA	LAA	NE	NE
Barley, canola\rape, oats, triticale, and wheat	NE	LAA	LAA	NE	NE
Forest nursery plantings (for transplant purposes), ornamental and/or shade trees, ornamental woody shrubs and vines, and shelterbelt plantings	NE	LAA	LAA	NE	NE
Grass forage/fodder/hay, pastures, and rangeland	NE	LAA	LAA	NE	NE
Pistachio	NE	LAA	LAA	NE	NE
Household/domestic dwellings outdoor premises	NE	LAA	LAA	NE	NE
Rice/Ornamental Pond	NE	LAA	LAA	NE	NLAA
Agricultural rights-of-way/fencerows/hedgerows, fencerows/hedgerows, and nonagricultural rights-of-way/fencerows/hedgerows	NE	LAA	LAA	NE	NE
Artichoke, mustard, peanuts (unspecified), and pepper	NE	LAA	LAA	NE	NE
Commercial/institutional/industrial premises/equipment (outdoor), nonagricultural outdoor buildings/structures, paved areas (private roads/sidewalks), refuse/solid waste sites (outdoor), and wide area/general outdoor treatment (quarantine/eradication use)	NE	LAA	LAA	NE	NE
Butternut	NE	LAA	LAA	NE	NE
Agricultural fallow/idleland, golf course turf, and nonagricultural uncultivated areas/soils	NE	LAA	LAA	NE	NE
Ornamental sod farm (turf) and recreational areas	NE	LAA	LAA	NE	NE
Turnip (greens)	NE	LAA	LAA	NE	NE

[1] NE = No effect; NLAA = May affect, not likely to adversely affect; LAA = Likely to adversely affect

Table 1-5. Diflubenzuron Use-specific Indirect Effects Determinations[1] Based on Effects to Terrestrial Prey

Use(s)	Terrestrial Inverts. (Acute)	Terrestrial-phase frogs		Small Mammals	
		Acute	Chronic	Acute	Chronic
Barnyard/Mushroom	LAA	LAA	LAA	NE	NE
Spray on manure, Manure to field	LAA	NE	NE	NE	NE
Beech Nut	LAA	NE	NE	NE	NE
Brassica (head and stem) vegetables	LAA	NE	NE	NE	NE
Citrus hybrids, grapefruit, orange, pummelo (shaddock), and tangerines	LAA	NE	NE	NE	NE
Broccoli raab, cabbage - Chinese, collards, and kale	LAA	NE	NE	NE	NE
Cotton (unspecified)	LAA	NE	NE	NE	NE
Christmas tree plantations and forest trees (softwoods - conifers)	LAA	NE	NE	NE	NE
Apricot, nectarine, peach, pear, plum, prune, and stone fruits	LAA	NE	NE	NE	NE
Almond, Brazil nut, cashew, chestnut, chinquapin, filbert (hazelnut), hickory nut, macadamia nut (bushnut), pecan, tree nuts, and walnut (English/black)	LAA	NE	NE	NE	NE
Barley, canola\rape, oats, triticale, and wheat	LAA	NE	NE	NE	NE
Forest nursery plantings (for transplant purposes), ornamental and/or shade trees, ornamental woody shrubs and vines, and shelterbelt plantings	LAA	NE	NE	NE	NE
Grass forage/fodder/hay, pastures, and rangeland	LAA	NE	NE	NE	NE
Pistachio	LAA	NE	NE	NE	NE
Household/domestic dwellings outdoor premises	LAA	NE	NE	NE	NE
Rice/Ornamental Pond	LAA	NE	NE	NE	NE
Agricultural rights-of-way/fencerows/hedgerows, fencerows/hedgerows, and nonagricultural rights-of-way/fencerows/hedgerows	LAA	NE	NE	NE	NE
Artichoke, mustard, peanuts (unspecified), and pepper	LAA	NE	NE	NE	NE
Commercial/institutional/industrial premises/equipment (outdoor), nonagricultural outdoor buildings/structures, paved areas (private roads/sidewalks), refuse/solid waste sites (outdoor), and wide area/general outdoor treatment (quarantine/eradication use)	LAA	NE	NE	NE	NE
Butternut	LAA	NE	NE	NE	NE
Agricultural fallow/idleland, golf course turf, and nonagricultural uncultivated areas/soils	LAA	NE	NE	NE	NE
Ornamental sod farm (turf) and recreational areas	LAA	NE	NE	NE	NE
Turnip (greens)	LAA	NE	NE	NE	NE

[1] NE = No effect; NLAA = May affect, not likely to adversely affect; LAA = Likely to adversely affect

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. 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 CRLF life stages within specific recovery units and/or designated critical habitat within the action area. 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 species.
- Quantitative information on prey base requirements for individual aquatic- and terrestrial-phase frogs. While existing information provides a preliminary picture of the types of food sources utilized by the frog, 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 frogs and potential modification to critical habitat.

2.0 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. Environmental Protection Agency's (EPA's) Guidance for Ecological Risk Assessment (U.S. EPA 1998), the Services' Endangered Species Consultation Handbook (U.S. FWS/NMFS 1998) and is consistent with procedures and methodology outlined in the Overview Document (U.S. EPA 2004) and reviewed by the U.S. Fish and Wildlife Service and National Marine Fisheries Service (U.S. FWS/NMFS 2004).

2.1 Purpose

The purpose of this endangered species assessment is to evaluate potential direct and indirect effects on individuals of the federally threatened California red-legged frog (*Rana aurora draytonii*) (CRLF) arising from FIFRA regulatory actions regarding use of diflubenzuron on agricultural uses (nuts, cotton, rice, row crops, citrus, fruits, etc.), forestry, nursery, residential, right-of-ways, and turf uses. In addition, this assessment evaluates whether use on these sites is expected to result in modification of the species' designated critical habitat. This ecological risk assessment has been prepared consistent with a settlement agreement in the case Center for Biological Diversity (CBD) vs. EPA *et al.* (Case No. 02-1580-JSW(JL) settlement entered in Federal District Court for the Northern District of California on October 20, 2006.

In this assessment, direct and indirect effects to the CRLF and potential modification to its designated critical habitat are evaluated in accordance with the methods described in the Agency's Overview Document (U.S. EPA 2004). Screening level methods include use of standard models such as PRZM-EXAMS, T-REX, and AgDRIFT, all of which are described at length in the Overview Document. Additional refinements were made to PRZM/EXAMS scenarios to account for heterogeneous land cover within treatment areas in order to more accurately model residential and right-of-way uses. Use of such information is consistent with the methodology described in the Overview Document (U.S. EPA 2004), which specifies that "the assessment process may, on a case-by-case basis, incorporate additional methods, models, and lines of evidence that EPA finds technically appropriate for risk management objectives" (Section V, page 31 of U.S. EPA 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 diflubenzuron 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 diflubenzuron 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 CRLF and its designated critical habitat within the state of California. As part of the "effects determination," one of the following three conclusions will be reached regarding the potential use of diflubenzuron on the CRLF in accordance with current labels:

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

Designated critical habitat identifies specific areas that have the physical and biological features, (known as primary constituent elements or PCEs) essential to the conservation of the listed species. The PCEs for CRLFs are aquatic and upland areas where suitable breeding and non-breeding aquatic habitat is located, interspersed with upland foraging and dispersal habitat.

If the results of initial screening-level assessment methods show no direct or indirect effects (no LOC exceedances) upon individual CRLFs or upon the PCEs of the species' designated critical habitat, a "no effect" determination is made for use of diflubenzuron as it relates to this species and its designated critical habitat. If, however, potential direct or indirect effects to individual CRLFs are anticipated or effects may impact the PCEs of the CRLF's designated critical habitat, a preliminary "may affect" determination is made for the FIFRA regulatory action regarding diflubenzuron.

If a determination is made that use of diflubenzuron within the action area(s) associated with the CRLF "may affect" this species or its designated critical habitat, additional information is considered to refine the potential for exposure and for effects to the CRLF and other taxonomic groups upon which these species depend (*e.g.*, aquatic and terrestrial vertebrates and invertebrates, aquatic plants, riparian vegetation, etc.). Additional information, including spatial analysis (to determine the geographical proximity of CRLF habitat and diflubenzuron use sites) and further evaluation of the potential impact of diflubenzuron on the PCEs is also used to determine whether modification of designated critical habitat may occur. Based on the refined information, the Agency uses the best available information to distinguish those actions that "may affect, but are not likely to adversely affect" from those actions that "may affect and are likely to adversely affect" the CRLF or the PCEs of its designated critical habitat. This information is presented as part of the Risk Characterization in Section 5 of this document.

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 diflubenzuron is expected to directly impact living organisms within the action area (defined in Section 2.7), critical habitat analysis for diflubenzuron 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 (*i.e.*, the biological resource requirements for the listed species associated with the critical habitat or important physical aspects of the habitat that may be reasonably influenced through biological processes). Activities that may modify critical habitat are those that alter the PCEs and appreciably diminish the value of the habitat. Evaluation of actions related to use of diflubenzuron that may alter the PCEs of the CRLF's critical habitat form the basis of the critical habitat impact analysis. Actions that may affect the CRLF's designated critical habitat have been identified by the Services and are discussed further in Section 2.6.

2.2 Scope

Diflubenzuron is an insecticide that is registered for both agricultural and non-agricultural uses in California and can be applied through ground, aerial, and airblast methods.

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 diflubenzuron in accordance with the approved product labels for California is "the action" relevant to this ecological risk assessment.

Although current registrations of diflubenzuron allow for use nationwide, this ecological risk assessment and effects determination addresses currently registered uses of diflubenzuron in portions of the action area that are reasonably assumed to be biologically relevant to the CRLF and its designated critical habitat.

There are two reasons for considering the action area of diflubenzuron use to include the entire state of California. First, because of the large variety of label uses for diflubenzuron in the state of California, it would be difficult to exclude any portions of this state from potential for diflubenzuron use. Second, a degradate of diflubenzon is a suspected carcinogen and is genotoxic. Because there is no concentration threshold defined that would result in no genotoxic effects to exposed organisms, it is not possible to define a threshold below which effects can occur. Therefore, the action area is assumed to be the entire state of California. Further discussion of the action area for the CRLF and its critical habitat is provided in Section 2.7.

2.2.1. Chemicals Assessed

This assessment evaluates the potential for diflubenzuron to adversely affect the CRLF. Several degradates of diflubenzuron have been identified and tested for toxicity. The available toxicity data are summarized below and are further described in Section 4 and Appendix A.

Degradates of diflubenzuron include 2,6-diflubenzoic acid (DFBA), 4-chlorophenylurea (CPU), 4-chloroaniline (PCA), and 2,6-diflubenzamide (DFBAM). The available data suggest that the degradates are several orders of magnitude less toxic than diflubenzuron to invertebrates. For example, LC_{50}s in midge for PCA and DFBA are 43 mg/L and >100 mg/L, respectively, compared with an LC_{50} of 0.07 mg/L for parent chemical in midge.

Lower toxicity to invertebrates of degradates is consistent with the specific mode of action of diflubenzuron as a chitin inhibitor.

Several degradates have been shown to be of similar toxicity to fish compared with parent difbluenzuron. In particular, PCA has been shown to be more toxic than diflubenzuron to fish with LC_{50} values ranging from 2 mg/L to 23 mg/L. DFBA and PCPU appear to have similar toxicity relative to parent diflubenzuron with 96-hr LC_{50} values of approximately 70 mg/L to >100 mg/L in fish. The most sensitive LC_{50} in fish was 127 mg/L for diflubenzuron.

Although PCA has been shown to be more toxic to fish, it has not formed more than 10% of parent compound in available degradation studies. Potential risks resulting from exposure to PCA or other degradates were not quantified. It was determined that quantifying risks from the degradates would not affect risk conclusions because comparing EECs for parent diflubenzuron (which would represent a highly conservative exposure estimate for any of the degradates) with the lowest toxicity value across all degradates still results in no LOC exceedances for fish. Therefore, degradates were not further considered.

One diflubenzuron formulated product toxicity study provided a lower LC_{50} than values from studies on the TGAI. The lowest LC_{50} for formulated products was 57 mg/L (see Appendix A). EFED evaluates potential risks from formulated products resulting from drift only exposure. The highest drift fraction estimated to be deposited into water bodies was approximately 4% from aerial spray. The highest application rate for diflubenzuron is 8.5 lbs a.i./Acre (barnyard/mushroom use). The resulting drift only EEC is 0.003 mg a.i./L assuming a volume of 20,000,000 L in the receiving water. Based on an LC_{50} of 57 mg/L, the resulting RQ would be orders of magnitude below concern levels (approximately 10^{-5}). Therefore, toxicity values from formulated products were not further considered as part of 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 (U.S. EPA 2004; U.S. FWS/NMFS 2004).

Diflubenzuron has one registered product that contains multiple active ingredients (diflubenzuron and permethrin). Analysis of the available open literature and acute oral mammalian LD_{50} data for the diflubenzuron and permethrin products relative to the single active ingredient is provided in Appendix B. There are no product LD_{50} values, with associated 95% Confidence Intervals (CIs) available for diflubenzuron.

As discussed in USEPA (2004) a quantitative component-based evaluation of mixture toxicity requires data of appropriate quality for each component of a mixture. In this mixture evaluation an LD_{50} with associated 95% CI is needed for the formulated product. The same quality of data is also required for each component of the mixture. Given that the single formulated product for diflubenzuron does not have a 95% CI associated with the oral LD_{50} value, it is not possible to undertake a quantitative or qualitative analysis for potential interactive effects (See appendix B). However, because the active ingredients are not expected to have similar mechanisms of action, metabolites, or toxicokinetic behavior, it is reasonable to conclude that an assumption of dose-addition would be inappropriate. Consequently, an assessment based on the toxicity of diflubenzuron is the only reasonable approach that employs the available data to address the potential acute risks of the formulated products. Studies on mixtures located in the open literature are included in Appendix B.

2.3 Previous Assessments

A large number of risk assessments have been performed on diflubenzuron by EFED. Two recent, comprehensive assessments are described below.

The Reregistration Eligibility Decision (RED) Science Chapter (6/30/95) identified risks to aquatic invertebrates as the major environmental threat of diflubenzuron. Acute and chronic levels of concern (LOCs) were exceeded for both freshwater and estuarine and marine aquatic invertebrates, for the use of diflubenzuron on cotton, citrus, forest trees and forest plantings.

In an assessment entitled “Section 3 Registration Request for Uses of Diflubenzuron on Pears, Stone Fruits, Tree Nuts, Peppers, and Pasture Grass (Chemical # 108201, DP Barcode D279732, D279735, D278770)” from 2/5/02, EFED identified acute concerns for freshwater invertebrates, but not for fish, birds, or mammals for the uses assessed. The document indicates concern for sediment dwelling aquatic organisms due to the expectation of diflubenzuron accumulation in sediments, but did not quantify this risk due to the lack of chronic toxicity data for these organisms. This document developed the critical life stage hypothesis for diflubenzuron effects which is discussed in Section 4.1.2 of this document.

A major difference between this assessment and the previous assessments concerns the inclusion of open literature toxicity values in the current assessment. This assessment is the first to include open literature toxicity data from the Ecotox database (See Appendix G). Inclusion of this data resulted in much lower acute and chronic freshwater invertebrate endpoints, which result in much greater risk estimates.

2.4 Stressor Source and Distribution

2.4.1 Environmental Fate Assessment

Diflubenzuron (DFB) is a member of a larger group of insecticides known as benzoylphenylureas. Based on acceptable and supplemental registrant-submitted data, diflubenzuron appears to be relatively non-persistent and relatively immobile under normal use conditions. The major route of dissipation for diflubenzuron appears to be biotic mediated processes ($t_{½}$ ≈2-14 days for aerobic soil metabolism). Anaerobic aquatic metabolism was reported to be slower (t½ = 34 days). Aerobic aquatic metabolism half-life in total water sediment system was 26 days. Other laboratory data indicate that diflubenzuron is stable to hydrolysis (t½ ≈30-433 days for pHs 5-9) and photolysis (t½ = 80 days for aquatic; t½ for control < light exposed for soil) and is relative immobile in soil (Rf values = 0.01, 0.07, 0.14, and 0.34 for silty clay loam, clay loam, and two sand loam soils, respectively). Supplemental and acceptable field data (including forestry dissipation data) confirm the laboratory data with reported half-lives of 5.8 to 60 days, and diflubenzuron detectable only in the 0-15 cm soil depth segments. However, calculated half-lives for CA and OR orchard applications were higher (t½ = 68.2-78 days). Diflubenzuron has not been detected in well monitoring data (National Summary-Pesticides in Ground Water Data base-A Compilation of Monitoring Studies: 1971-1991), as well.

Under aerobic conditions diflubenzuron appears to degrade to 4-chlorophenyl urea (CPU) which reached a maximum concentration of 37% of applied at 14 days post-treatment. The other major degradate, CO_2, was reported to reach a maximum concentration of 26.3% of applied by day 21 post-treatment. Three minor degradates, 2,6-difluorobenzoic acid, 2,6-difluorobenzamide, and p-chloroaniline, each reaching a maximum concentration of <10% of applied, were identified in the aerobic soil study. These metabolites were detectable in an anaerobic metabolism study, as well. Due to the stability of diflubenzuron to abiotic processes, limited data are available on the persistence and mobility of diflubenzuron metabolites. However, CPU was reported in leachate of a column leaching study (approximately 15 to 30% of applied in MRIDs 00039477 & 00040777). Even though CPU appears to be mobile under laboratory conditions, it has not been reported below the 0 to 15 cm soil depth segment in any field data.

Diflubenzuron does appear to accumulate at low levels and depurate rapidly in fish tissue. The reported bioconcentration factors ranged from 34 to 200X for fillet, 78 to 360X for whole fish, and 100 to 500X for viscera. In addition, the depuration rate indicates a rapid decrease (99%) of accumulated residues in tissue during the 14 day depuration period.

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

Table 2-1 Summary of Diflubenzuron Environmental Fate Properties

Study	Value (units)		Major Degradates *Minor Degradates*	MRID #	Study Status
Hydrolysis	^{14}C label	433 days @ pH 5 117 days @ pH 7 42 days @ pH 9	CPU – Not Quantified DFBA – Not Quantified	00143355	Supplemental
	^{3}H Label	187 days @ pH 5 158 days @ pH 7 40 days @ pH 9			
	Unlabeled	Stable @ pH 5 Stable @ pH 7 44 days @ pH 9			
	^{14}C and ^{3}H label	7.5 days @ pH 12			
	223.6 days @ pH 5 247 days @ pH 7 32.5 days @ pH 9		CPU – Not Quantified DFBA – Not Quantified	40859801 41087801	Acceptable
	Dark aqueous photolysis control: Stable @ pH 7		None Identified	40816301	Supplemental (15 days only)
Direct Aqueous Photolysis	40 days (continuous light) 80 days (assuming 12 hr. light/dark cycle		*CPU – 8%* *DFBA – 4%* *DFBAM – 1%* *2,6-Difluorobenzene – not quantified* *Unknown – 6%*	40816301 41087802	Acceptable
Soil Photolysis	144 days		Not Quantified	40941601	Supplemental
	11.3 days		CPU – 12.9 % (Day l0) *DFBA – 3.0% (Day 7)* *Unknown SP1 – 0.6% (Day 10)* *Unknown PK1 – 0.1% (Day 16)*	42251201	Acceptable
Aerobic Soil Metabolism	2 to 14 days		CPU – 37 % (Day l4) *DFBA – <10%* *DFBAM – <10%* *PCA – <10%*	00039473 00039474 00040154	Supplemental (39474 same as 40154)
				00099875	Supplemental
				41722801 Same as 46888708	Acceptable
Anaerobic Soil Metabolism	2 to 14 days		CPU – 37 % (Day 2-l4: depending on temperature) DFBA – 23% (Day 35)	00040782 41837601	Acceptable
Anaerobic Aquatic Metabolism	34 days		CPU – 31 % (Day 42) DFBA – 31% (Day 42) *PCA – 0.4% (Day 18)*	41837601	Acceptable
Aerobic Aquatic Metabolism	26 days		CPU – 30 % (Day l6) *DFBA – 9% (Day 1)* *DFBAM – 1.6% (Day 2)* *PCA – 2% (Day 16)*	44895001	Acceptable
	5.4 days 3.7 days		DFBA – 17% (Day 4) CPU – 48 % (Day l6)	46888707	Supplemental

Study	Value (units)	Major Degradates *Minor Degradates*	MRID #	Study Status
K_{oc}	2878 3008 3401 6918 6801 2780 1938	Not Applicable	00039476 00039477 00040777 00157842 46888704 46888705 46895401	Acceptable
Terrestrial Field Dissipation	The orchard and bare ground half-lives (5.8 to 13.2 days). CA citrus and the OR apple orchards half-life of approximately 68.2 to 78 days.	DFBA – up to 0.01 ppm CPU – up to 0.06 ppm	00040156 40660601 40660602 41816502 41816503 41821002 41826701 42290401 42290402 42441101	Acceptable
Forestry Field Dissipation	30 to 35 days	None Detected	41077201 41077202 41922201 42197701	Supplemental
Aquatic Field Dissipation	2 to 6 days	CPU	00040155 00161945 00161947 40598601 44399309 45009601 45191001 45197601	Acceptable
Accumulation	Bioconcentration factors of 34 to 200× for fillet, 78 to 360× for whole fish, and 100, to 550× for viscera. 99% depuration within 14 day	DFBAM – 16% (0.28,µg/g) in Fillet; 12% (0.37 µg/g) in Whole Fish; and 11% (0.55 µg/g) in Viscera. 6 unidentified minor metabolites	42258401 42258402	Acceptable

2.4.2 Environmental Transport Assessment

Surface water runoff and spray drift are expected to be the major routes of exposure for diflubenzuron.

In general, deposition of drifting pesticides is expected to be greatest close to the site of application. Computer models of spray drift (AgDRIFT) are used to determine potential exposures to aquatic and terrestrial organisms via spray drift. The distance of potential impact away from the use sites (action area) is determined by the distance required to fall below the LOC for aquatic invertebrates.

2.4.2 Mechanism of Action

Diflubenzuron is an insect growth regulator and works by preventing the formation of chitin, a molecule necessary to the formation of an insect's cuticle or outer shell. Insects exposed to a sufficient dose of diflubenzuron cannot form their protective outer shell and die during molting. It is most effective against insect larva, but also acts as an ovicide, killing insect eggs (U.S. EPA 1997).

2.4.3 Use Characterization

Currently, labeled uses of diflubenzuron include citrus, cotton, forestry, mushrooms, ornamentals, pastures, soybeans, standing water, sewage systems, termite bait stations, and wide-area general outdoor treatment sites. All of these uses are considered as part of the federal action evaluated in this assessment with the exception of termite bait stations (in which a solidified form of the pesticide is placed in an enclosed bait station to minimize loss to the environment) and soybeans, which are not grown in California (NASS 2002). If use patterns indicate that soybeans are grown in CA in the future, the conclusions of this assessment may need to be revisited.

Analysis of labeled use information is the critical first step in evaluating the federal action. The current label for diflubenzuron represents 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.

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

Table 2-2 Diflubenzuron Uses Assessed for the CRLF

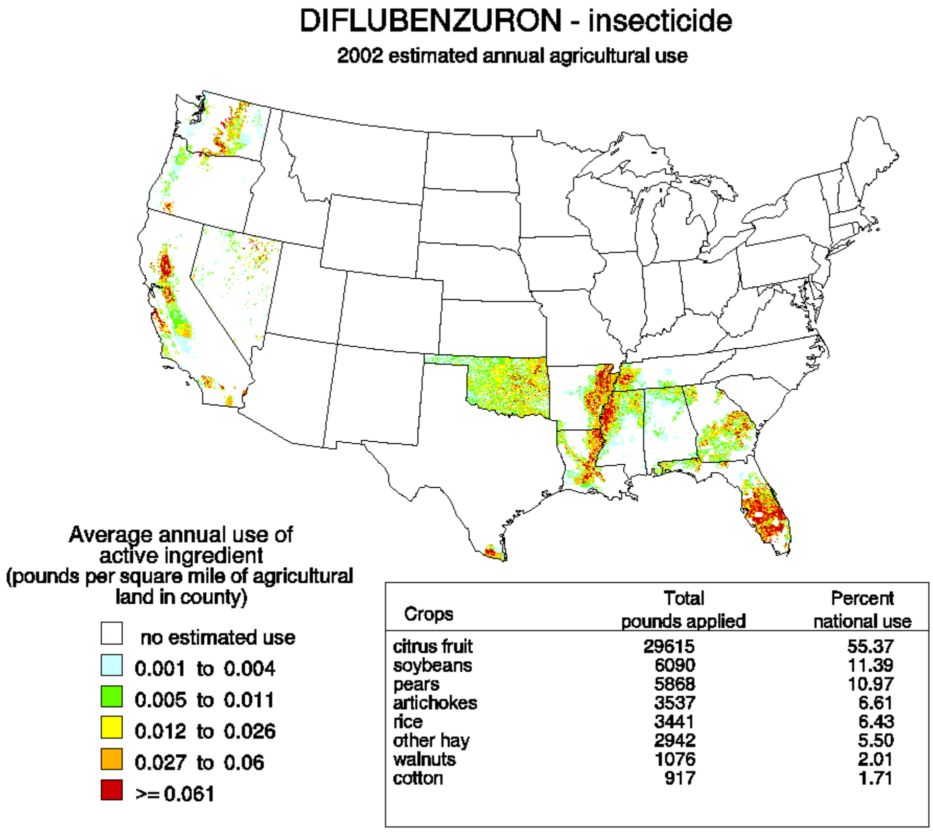
Use (Application Method – Ground (G), Airblast (AB), or Air (A))		Max. Single Appl. Rate (lb ai/A)	Max. Number of Application per Year	Application Interval
Spray on manure, Manure to field	G	8.508	NS (17)	NS (21)
Beech Nut	G	0.0408	3	7
	A	0.0408	3	7
Brassica (head and stem) vegetables	G	0.0313	1	NA
Citrus hybrids, grapefruit, orange, pummelo (shaddock), and tangerines	G	0.3125	3	21
	A	0.125	3	7
Broccoli raab, cabbage - Chinese, collards, and kale	G	0.25	4	21
Cotton (unspecified)	G	0.3125	3	7
	A	0.3125	3	90
Christmas tree plantations and forest trees (softwoods - conifers)	G	0.22048	NS (4)	14
	A	0.25	1	NA
Apricot, nectarine, peach, pear, plum, prune, and stone fruits	G	0.3125	3	7
	AB	0.3125	3	7
	A	0.3125	3	7
Almond, Brazil nut, cashew, chestnut, chinquapin, filbert (hazelnut), hickory nut, macadamia nut (bushnut), pecan, tree nuts, and walnut (English/black)	G	0.3125	3	7
	AB	0.3125	3	7
	A	0.3125	3	90
Barley, canola\rape, oats, triticale, and wheat	G	0.25	4	21
	A	0.125	3	NS (21)
Forest nursery plantings (for transplant purposes), ornamental and/or shade trees, ornamental woody shrubs and vines, and shelterbelt plantings	G	0.25	1	NA
	A	0.25	1	NA
Grass forage/fodder/hay, pastures, and rangeland	G	0.25	1	NA
	AB	0.25	1	NA
	A	0.25	1	NA
Pistachio	G	0.75	2	14
Household/domestic dwellings outdoor premises	G	0.25	1	NA
	A	0.25	6	10
Rice	A	0.625	6	5
Agricultural rights-of-way/fencerows/hedgerows, fencerows/hedgerows, and nonagricultural rights-of-way/fencerows/hedgerows	G	0.25	2	5
	AB	0.25	2	5
	A	0.25	2	5
Artichoke, mustard, peanuts (unspecified), and pepper	G	0.3125	3	21
	A	0.3125	3	14
Commercial/institutional/industrial premises/equipment (outdoor), nonagricultural outdoor buildings/structures, paved areas (private roads/sidewalks), refuse/solid waste sites (outdoor), and wide area/general outdoor treatment (quarantine/eradication use)	A	0.25	6	NS (10)
Butternut	G	0.25	4	15
	A	0.25	4	15
Agricultural fallow/idleland, golf course turf, and nonagricultural uncultivated areas/soils	G	0.25	2	14
	AB	0.25	2	14
	A	0.25	2	5
Ornamental sod farm (turf) and recreational areas	G	0.0313	3	7

Use (Application Method – Ground (G), Airblast (AB), or Air (A))		Max. Single Appl. Rate (lb ai/A)	Max. Number of Application per Year	Application Interval
	AB	0.0313	3	7
	A	0.0313	3	7
Turnip (greens)	G	0.25	2	21

NS = Not specified on label.

Several of the agricultural crop uses for diflubenzuron may have more than one crop cycle per year. Because many diflubenzuron labels specify application limits based on crop cycles rather than an annual limitation, the potential exists for more diflubenzuron to be applied than is reflected in Table2-2. However, because the persistence of diflubenzuron is relatively short compared to the length of a crop cycle, there would be little accumulation of the parent compound across crop cycles. Instead the greatest impact from multiple crop cycles would likely result from the extension of the time that diflubenzuron would be applied both earlier in the spring and later in the fall when more runoff would occur. This extension of the time that diflubenzuron may be used is addressed in this assessment in Section 3.2.1, which evaluates both early and late application dates on diflubenzuron exposure.

Figure 2-1 provides a visual depiction of the spatial distribution of diflubenzuron use across the United States in terms of pounds of diflubenzuron applied per square mile of agricultural land in each county. The map was downloaded from a U.S. Geological Survey (USGS), National Water Quality Assessment Program (NAWQA) website. Many simplifying assumptions were made in order to produce this map. Non-agricultural uses of diflubenzuron such as forestry, residential and right-of-way uses are not included in this depiction.



Crops	Total pounds applied	Percent national use
citrus fruit	29615	55.37
soybeans	6090	11.39
pears	5868	10.97
artichokes	3537	6.61
rice	3441	6.43
other hay	2942	5.50
walnuts	1076	2.01
cotton	917	1.71

Figure 2-1. Diflubenzuron use in total pounds per square mile of agricultural land in county.

The Agency's Biological and Economic Analysis Division (BEAD) provides an analysis of both national- and county-level usage information (U.S. EPA 2009) using state-level usage data obtained from USDA-NASS[1], Doane (www.doane.com; the full dataset is not provided due to its proprietary nature) and the California's Department of Pesticide Regulation Pesticide Use Reporting (CDPR PUR) database[2] . CDPR PUR is considered a more comprehensive source of usage data than USDA-NASS or EPA proprietary databases, and thus the usage data reported for diflubenzuron by county in this California-specific assessment were generated using CDPR PUR data. Eight years (1999-2006) of usage data were included in this analysis. Data from CDPR PUR were obtained for every pesticide application made on every use site at the section level (approximately one square mile) of the public land survey system. 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

[1] United States Depart of Agriculture (USDA), National Agricultural Statistics Service (NASS) Chemical Use Reports provide summary pesticide usage statistics for select agricultural use sites by chemical, crop and state. See http://www.usda.gov/nass/pubs/estindx1.htm#agchem.

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

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

A summary of diflubenzuron usage for all California use sites is provided below in Table 2-3.

Table 2-3 Summary of California Department of Pesticide Registration (CDPR) Pesticide Use Reporting (PUR) Data from 1999 to 2006 for Currently Registered Diflubenzuron Uses

Site Name, Application Method (Aerial, Ground, or Other), and Years of Data		Average Pounds Applied by Use	Average App. Rate by Use	Maximum App. Rate by Use
Almond	A (3)	988.30	0.24	4.05
	G (3)	8007.51	0.22	3.74
Apricot	A (1)	17.46	0.25	0.25
	G (3)	100.25	0.19	0.24
Aquatic Areas, Water Areas (All Or Unspec)	O (7)	15.82	0.22	0.25
Artichoke (Globe) (All Or Unspec)	A (8)	1737.13	0.14	2.23
	G (8)	1982.66	0.14	2.97
Beets, General	G (1)	3.50	5.00	5.00
Broccoli	A (1)	0.92	0.02	0.02
	G (1)	2.10	0.02	0.02
Brussels Sprouts	G (2)	1.36	0.41	0.70
Cherry	G (1)	3.75	0.25	0.25
Christmas Tree Plantations	G (6)	0.71	0.08	0.13
Cotton, General	A (3)	45.42	0.12	0.14
	G (2)	54.38	0.10	0.13
Endive (Escarole)	G (1)	26.64	0.19	0.19
Grapefruit	A (1)	2.44	0.13	0.13
	G (4)	73.25	0.17	0.78
Landscape Maintenance	O (8)	23.11	NR	NR
Nectarine	G (3)	38.51	0.09	0.25
N-Outdr Container/Fld Grwn Plants	A (3)	0.06	0.32	0.69
	G (8)	18.14	0.46	24.16
	O (1)	0.00	0.00	0.00
N-Outdr Grwn Cut Flwrs Or Greens	A (1)	0.03	0.00	0.00
	G (8)	2.82	0.29	3.57
N-Outdr Grwn Trnsplnt/Prpgtv Mtrl	A (1)	2.55	0.21	0.59
	G (6)	9.23	0.20	2.08
Orange (All Or Unspec)	A (2)	31.03	0.13	0.14
	G (4)	762.37	0.19	2.50
Pastures (All Or Unspec)	A (1)	0.95	0.02	0.02
Peach	G (4)	167.31	0.20	6.07
Pear	G (3)	75.48	0.22	0.26
Peppers (Chili Type) (Flavoring And Spice Crop)	G (1)	0.75	0.13	0.13
Peppers (Fruiting Vegetable), (Bell,Chili, Etc.)	G (1)	141.96	0.12	0.13
Pistachio (Pistache Nut)	G (2)	36.87	0.24	0.25

Site Name, Application Method (Aerial, Ground, or Other), and Years of Data		Average Pounds Applied by Use	Average App. Rate by Use	Maximum App. Rate by Use
Plum (Includes Wild Plums For Human Consumption)	A (1)	5.95	0.20	0.20
	G (3)	94.31	0.19	0.25
Prune	A (1)	5.56	0.25	0.25
	G (3)	66.94	0.25	0.95
Public Health Pest Control	O (8)	527.15	NR	NR
Rangeland (All Or Unspec)	A (2)	3.08	0.03	0.03
	G (1)	2.21	0.03	0.03
Regulatory Pest Control	O (5)	42.22	NR	NR
Rice (All Or Unspec)	A (8)	789.74	0.14	3.93
	G (1)	4.08	0.09	0.09
Rights Of Way	O (6)	9.98	NR	NR
Soil Application, Preplant-Outdoor (Seedbeds,Etc.)	G (3)	213.56	11.28	12.61
Strawberry (All Or Unspec)	G (1)	23.50	0.25	0.25
Structural Pest Control	O (8)	284.86	NR	NR
Tangerine (Mandarin, Satsuma, Murcott, Etc.)	G (2)	30.73	0.16	0.26
Uncultivated Agricultural Areas (All Or Unspec)	G (2)	1.39	0.05	0.07
Uncultivated Non-Ag Areas (All Or Unspec)	O (2)	8.15	0.06	0.13
Walnut (English Walnut, Persian Walnut)	A (4)	37.22	0.24	0.38
	G (8)	1406.79	0.26	2.02

2.5 Assessed Species

The CRLF was federally listed as a threatened species by U.S. FWS effective June 24, 1996 (U.S. FWS 1996). It is one of two subspecies of the red-legged frog and is the largest native frog in the western United States (U.S. FWS 2002). A brief summary of information regarding CRLF distribution, reproduction, diet, and habitat requirements is provided in Sections 2.5.1 through 2.5.4, respectively. Further information on the status, distribution, and life history of and specific threats to the CRLF is provided in Attachment I.

Final critical habitat for the CRLF was designated by U.S. FWS on April 13, 2006 (U.S. FWS 2006; 71 FR 19244-19346). Further information on designated critical habitat for the CRLF is provided in Section 2.6.

2.5.1 Distribution

The CRLF is endemic to California and Baja California (Mexico) and historically inhabited 46 counties in California including the Central Valley and both coastal and interior mountain ranges (U.S. FWS 1996). Its range has been reduced by about 70%, and the species currently resides in 22 counties in California (U.S. FWS 1996). The species has an elevational range of near sea level to 1,500 meters (5,200 feet) (Jennings and Hayes 1994); however, nearly all of the known CRLF populations have been documented below 1,050 meters (3,500 feet) (U.S. FWS 2002).

Populations currently exist along the northern California coast, northern Transverse Ranges (U.S. FWS 2002), foothills of the Sierra Nevada (5-6 populations), and in southern California south of Santa Barbara (two populations) (Fellers 2005a). Relatively larger numbers of CRLFs are located between Marin and Santa Barbara Counties (Jennings and Hayes 1994). A total of 243 streams or drainages are believed to be currently occupied by the species, with the greatest numbers in Monterey, San Luis Obispo, and Santa Barbara counties (U.S. FWS 1996). Occupied drainages or watersheds include all bodies of water that support CRLFs (*i.e.*, streams, creeks, tributaries, associated natural and artificial ponds, and adjacent drainages), and habitats through which CRLFs can move (*i.e.*, riparian vegetation, uplands) (U.S. FWS 2002).

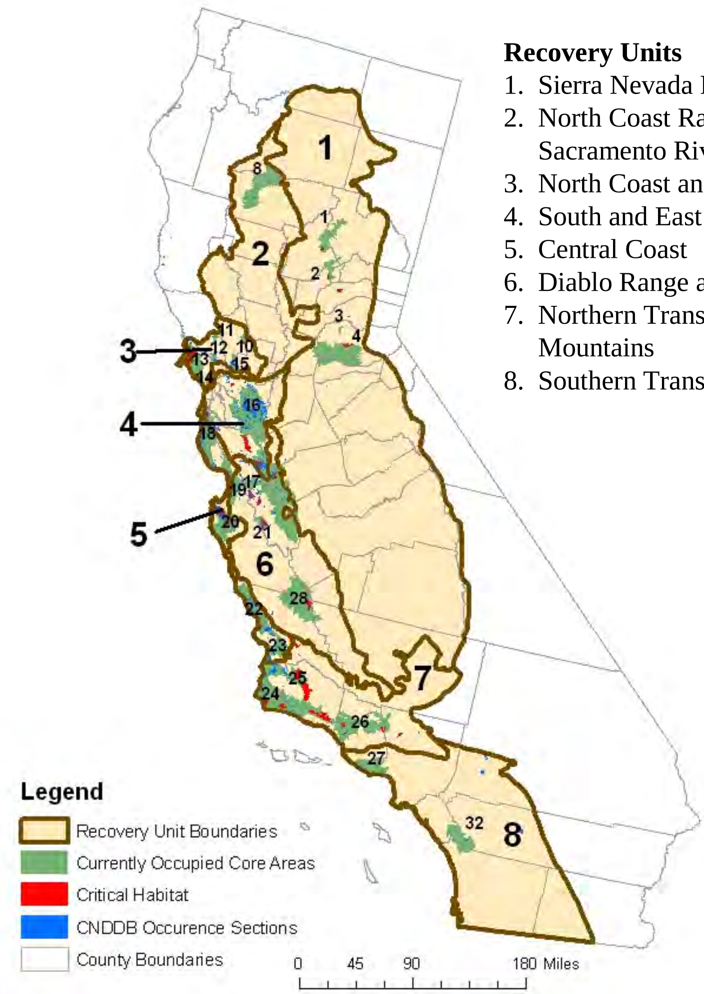
The distribution of CRLFs within California is addressed in this assessment using four categories of location including recovery units, core areas, designated critical habitat, and known occurrences of the CRLF reported in the California Natural Diversity Database (CNDDB) that are not included within core areas and/or designated critical habitat. Recovery units, core areas, and other known occurrences of the CRLF from the CNDDB are described in further detail in Attachment I, and designated critical habitat is addressed in Section 2.6. Recovery units are large areas defined at the watershed level that have similar conservation needs and management strategies. The recovery unit is primarily an administrative designation, and land area within the recovery unit boundary is not exclusively CRLF habitat. Core areas are smaller areas within the recovery units that comprise portions of the species' historic and current range and have been determined by U.S. FWS to be important in the preservation of the species. Designated critical habitat is generally contained within the core areas, although a number of critical habitat units are outside the boundaries of core areas, but within the boundaries of the recovery units. Additional information on CRLF occurrences from the CNDDB is used to cover the current range of the species not included in core areas and/or designated critical habitat, but within the recovery units.

Other Known Occurrences from the CNDBB

The CNDDB provides location and natural history information on species found in California. The CNDDB serves as a repository for historical and current species location sightings. Information regarding known occurrences of CRLFs outside of the currently occupied core areas and designated critical habitat is considered in defining the current range of the CRLF. See: http://www.dfg.ca.gov/bdb/html/cnddb_info.html for additional information on the CNDDB.

Recovery Units

1. Sierra Nevada Foothills and Central Valley
2. North Coast Range Foothills and Western Sacramento River Valley
3. North Coast and North San Francisco Bay
4. South and East San Francisco Bay
5. Central Coast
6. Diablo Range and Salinas Valley
7. Northern Transverse Ranges and Tehachapi Mountains
8. Southern Transverse and Peninsular Ranges



Core Areas

1. Feather River
2. Yuba River- S. Fork Feather River
3. Traverse Creek/ Middle Fork/ American R. Rubicon
4. Cosumnes River
5. South Fork Calaveras River*
6. Tuolumne River*
7. Piney Creek*
8. Cottonwood Creek
9. Putah Creek – Cache Creek*
10. Lake Berryessa Tributaries
11. Upper Sonoma Creek
12. Petaluma Creek – Sonoma Creek
13. Pt. Reyes Peninsula
14. Belvedere Lagoon
15. Jameson Canyon – Lower Napa River
16. East San Francisco Bay
17. Santa Clara Valley
18. South San Francisco Bay
19. Watsonville Slough-Elkhorn Slough
20. Carmel River – Santa Lucia
21. Gablan Range
22. Estero Bay
23. Arroyo Grange River
24. Santa Maria River – Santa Ynez River
25. Sisquoc River
26. Ventura River – Santa Clara River
27. Santa Monica Bay – Venura Coastal Streams
28. Estrella River
29. San Gabriel Mountain*
30. Forks of the Mojave*
31. Santa Ana Mountain*
32. Santa Rosa Plateau
33. San Luis Ray*
34. Sweetwater*
35. Laguna Mountain*

* Core areas that were historically occupied by the California red-legged frog are not included in the map

Figure 2-2 Recovery Unit, Core Area, Critical Habitat, and Occurrence Designations for CRLF

2.5.2 Reproduction

CRLFs breed primarily in ponds; however, they may also breed in quiescent streams, marshes, and lagoons (Fellers 2005a). According to the Recovery Plan (U.S. FWS 2002), CRLFs breed from November through late April. Peaks in spawning activity vary geographically; Fellers (2005b) reports peak spawning as early as January in parts of coastal central California. Eggs are fertilized as they are being laid. Egg masses are typically attached to emergent vegetation, such as bulrushes (*Scirpus* spp.) and cattails (*Typha* spp.) or roots and twigs, and float on or near the surface of the water (Hayes and Miyamoto 1984). Egg masses contain approximately 2000 to 6000 eggs ranging in size between 2 and 2.8 mm (Jennings and Hayes 1994). Embryos hatch 10 to 14 days after fertilization (Fellers 2005a) depending on water temperature. Egg predation is reported to be infrequent and most mortality is associated with the larval stage (particularly through predation by fish); however, predation on eggs by newts has also been reported (Rathburn 1998). Tadpoles require 11 to 28 weeks to metamorphose into juveniles (terrestrial-phase), typically between May and September (Jennings and Hayes 1994, U.S. FWS 2002); tadpoles have been observed to over-winter (delay metamorphosis until the following year) (Fellers 2005b; U.S. FWS 2002). Males reach sexual maturity at 2 years, and females reach sexual maturity at 3 years of age; adults have been reported to live 8 to 10 years (U.S. FWS 2002). Figure 2-3 depicts CRLF annual reproductive timing.

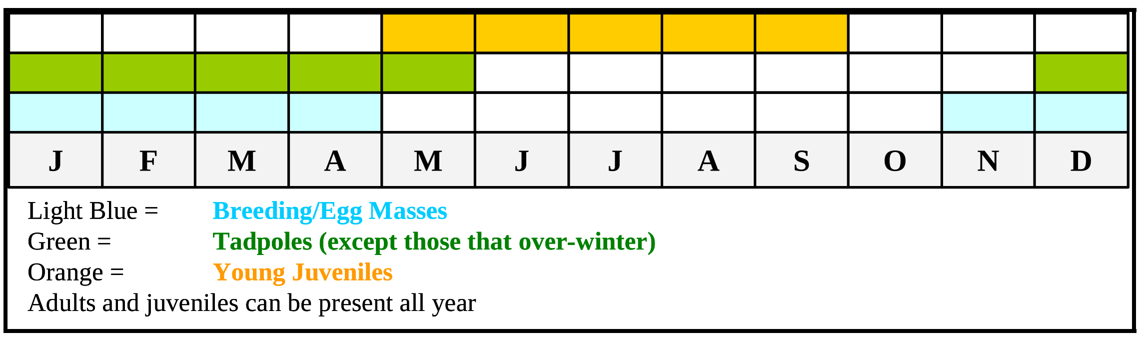


Figure 2-3 CRLF Reproductive Events by Month

2.5.3 Diet

Although the diet of CRLF aquatic-phase larvae (tadpoles) has not been studied specifically, it is assumed that their diet is similar to that of other frog species, with the aquatic phase feeding exclusively in water and consuming diatoms, algae, and detritus (U.S. FWS 2002). Tadpoles filter and entrap suspended algae (Seale and Beckvar 1980) via mouthparts designed for effective grazing of periphyton (Wassersug 1984; Kupferberg *et al.* 1994; Kupferberg 1997; Altig and McDiarmid 1999).

Juvenile and adult CRLFs forage in aquatic and terrestrial habitats, and their diet differs greatly from that of larvae. The main food source for juvenile aquatic- and terrestrial-phase CRLFs is thought to be aquatic and terrestrial invertebrates found along the shoreline and on the water surface. Hayes and Tennant (1985) report, based on a study examining the gut content of 35 juvenile and adult CRLFs, that the species feeds on as many as 42 different invertebrate taxa, including Arachnida, Amphipoda, Isopoda, Insecta, and Mollusca. The most commonly observed prey species were larval alderflies (*Sialis* cf. *californica*), pillbugs (*Armadilliadrium vulgare*), and water striders (*Gerris* sp). The preferred prey species, however, was the sowbug (Hayes and Tennant 1985). This study suggests that CRLFs forage primarily above water, although the authors note other data reporting that adults also feed under water, are cannibalistic, and consume fish. For larger CRLFs, over 50% of the prey mass may consists of vertebrates such as mice, frogs, and fish, although aquatic and terrestrial invertebrates were the most numerous food items (Hayes and Tennant 1985). For adults, feeding activity takes place primarily at night; for juveniles feeding occurs during the day and at night (Hayes and Tennant 1985).

2.5.4 Habitat

CRLFs require aquatic habitat for breeding, but also use other habitat types including riparian and upland areas throughout their life cycle. CRLF use of their environment varies; they may complete their entire life cycle in a particular habitat or they may utilize multiple habitat types. Overall, populations are most likely to exist where multiple breeding areas are embedded within varying habitats used for dispersal (U.S. FWS 2002). Generally, CRLFs utilize habitat with perennial or near-perennial water (Jennings *et al.* 1997). Dense vegetation close to water, shading, and water of moderate depth are habitat features that appear especially important for CRLF (Hayes and Jennings 1988). Breeding sites include streams, deep pools, backwaters within streams and creeks, ponds, marshes, sag ponds (land depressions between fault zones that have filled with water), dune ponds, and lagoons. Breeding adults have been found near deep (0.7 m) still or slow moving water surrounded by dense vegetation (U.S. FWS 2002); however, the largest number of tadpoles have been found in shallower pools (0.26 – 0.5 m) (Reis 1999). Data indicate that CRLFs do not frequently inhabit vernal pools, as conditions in these habitats generally are not suitable (Hayes and Jennings 1988).

CRLFs also frequently breed in artificial impoundments such as stock ponds, although additional research is needed to identify habitat requirements within artificial ponds (U.S. FWS 2002). Adult CRLFs use dense, shrubby, or emergent vegetation closely associated with deep-water pools bordered with cattails and dense stands of overhanging vegetation (http://ecos.fws.gov/speciesProfile/SpeciesReport.do?spcode=D02D).

In general, dispersal and habitat use depends on climatic conditions, habitat suitability, and life stage. Adults rely on riparian vegetation for resting, feeding, and dispersal. The foraging quality of the riparian habitat depends on moisture, composition of the plant community, and presence of pools and backwater aquatic areas for breeding. CRLFs can be found living within streams at distances up to 3 km (2 miles) from their breeding site and have been found up to 30 m (100 feet) from water in dense riparian vegetation for up to 77 days (U.S. FWS 2002).

During dry periods, the CRLF is rarely found far from water, although it will sometimes disperse from its breeding habitat to forage and seek other suitable habitat under downed trees or logs, industrial debris, and agricultural features (U.S. FWS 2002). According to Jennings and Hayes (1994), CRLFs also use small mammal burrows and moist leaf litter as habitat. In addition, CRLFs may also use large cracks in the bottom of dried ponds as refugia; these cracks may provide moisture for individuals avoiding predation and solar exposure (Alvarez 2000).

2.6 Designated Critical Habitat

In a final rule published on April 13, 2006, 34 separate units of critical habitat were designated for the CRLF by U.S. FWS (U.S. FWS 2006; FR 51 19244-19346). A summary of the 34 critical habitat units relative to U.S. FWS-designated recovery units and core areas (previously discussed in Section 2.5.1) is provided in Attachment I.

'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.' All designated critical habitat for the CRLF was occupied at the time of listing. Critical habitat receives protection under Section 7 of the ESA (Section 7) through prohibition against destruction or adverse modification with regard to actions carried out, funded, or authorized by a federal Agency. Section 7 requires consultation on federal actions that are likely to result in the destruction or adverse modification of critical habitat.

To be included in a critical habitat designation, the habitat must be '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)). PCEs include, but are not limited to, 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. The designated critical habitat areas for the CRLF are considered to have the following PCEs that justify critical habitat designation:

- Breeding aquatic habitat;
- Non-breeding aquatic habitat;
- Upland habitat; and
- Dispersal habitat.

Further description of these habitat types is provided in Attachment I.

Occupied habitat may be included in the critical habitat only if essential features within the habitat may require special management or protection. Therefore, U.S. FWS does not include areas where existing management is sufficient to conserve the species. Critical habitat is designated outside the geographic area presently occupied by the species only when a designation limited to its present range would be inadequate to ensure the conservation of the species. For the CRLF, all designated critical habitat units contain all four of the PCEs, and were occupied by the CRLF at the time of the Final Rule (FR) listing notice in April 2006 (71 FR 19243, 2006). The FR notice designating critical habitat for the CRLF includes a special rule exempting routine ranching activities associated with livestock ranching from incidental take prohibitions. The purpose of this exemption is to promote the conservation of rangelands, which could be beneficial to the CRLF, and to reduce the rate of conversion to other land uses that are incompatible with CRLF conservation. Please see Attachment I for a full explanation on this special rule.

U.S. FWS has established adverse modification standards for designated critical habitat (U.S. FWS 2006). 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 diflubenzuron that may alter the PCEs of the CRLF's critical habitat form the basis of the critical habitat impact analysis. According to U.S. FWS (2006), activities that may affect critical habitat and therefore result in adverse effects to the CRLF include, but are not limited to the following:

(1) Significant alteration of water chemistry or temperature to levels beyond the tolerances of the CRLF that result in direct or cumulative adverse effects to individuals and their life-cycles.
(2) Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs.
(3) Significant increase in sediment deposition within the stream channel or pond or disturbance of upland foraging and dispersal habitat that could result in elimination or reduction of habitat necessary for the growth and reproduction of the CRLF by increasing the sediment deposition to levels that would adversely affect their ability to complete their life cycles.
(4) Significant alteration of channel/pond morphology or geometry that may lead to changes to the hydrologic functioning of the stream or pond and alter the timing, duration, water flows, and levels that would degrade or eliminate the CRLF and/or its habitat. Such an effect could also lead to increased sedimentation and degradation in water quality to levels that are beyond the CRLF's tolerances.
(5) Elimination of upland foraging and/or aestivating habitat or dispersal habitat.
(6) Introduction, spread, or augmentation of non-native aquatic species in stream segments or ponds used by the CRLF.
(7) Alteration or elimination of the CRLF's food sources or prey base (also evaluated as indirect effects to the CRLF).

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 diflubenzuron is expected to directly impact living organisms within the action area, critical habitat analysis for diflubenzuron 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

For listed species assessment purposes, the action area is considered to be the area affected directly or indirectly by the federal action and not merely the immediate area involved in the action (50 CFR 402.02). It is recognized that the overall action area for the national registration of diflubenzuron is likely to encompass considerable portions of the United States based on the large array of 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 CRLF and its designated critical habitat within the state of California. The Agency's approach to defining the action area under the provisions of the Overview Document (U.S. EPA 2004) considers the results of the risk assessment process to establish boundaries for that action area with the understanding that exposures below the Agency's defined Levels of Concern (LOCs) constitute a no-effect threshold. For the purposes of this assessment, attention will be focused on the footprint of the action (*i.e.*, the area where pesticide application occurs), plus all areas where offsite transport (*i.e.*, spray drift, downstream dilution, etc.) may result in potential exposure within the state of California that exceeds the Agency's LOCs.

Deriving the geographical extent of this portion of the action area is based on consideration of the types of effects that diflubenzuron may be expected to have on the environment, the exposure levels to diflubenzuron that are associated with those effects, and the best available information concerning the use of diflubenzuron and its fate and transport within the state of California. Specific measures of ecological effect that define the action area include any direct and indirect toxic effect 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. Therefore, the action area extends to a point where environmental exposures are below any measured lethal or sublethal effect threshold for any biological entity at the whole organism, organ, tissue, and cellular level of organization. In situations where it is not possible to determine the threshold for an observed effect, the action area is not spatially limited and is assumed to be the entire state of California.

The definition of action area requires a stepwise approach that begins with an understanding of the federal action. The federal action is defined by the currently labeled uses for diflubenzuron. An analysis of labeled uses and review of available product labels was completed. Several of the currently labeled uses are special local needs (SLN) uses 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 CRLF, the analysis indicates that, for diflubenzuron, all uses are considered as part of the federal action evaluated in this assessment except soybeans and termite bait station uses.

Following a determination of the assessed uses, an evaluation of the potential "footprint" of diflubenzuron use patterns (*i.e.*, the area where pesticide application occurs) 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. Because diflubenzuron has a diverse set of uses that encompass all land cover classes used to define the initial "footprint", the initial area of concern is considered to be statewide.

Once the initial area of concern is defined, the next step is to define the potential boundaries of the action area by determining the extent of offsite transport via spray drift and runoff where exposure of one or more taxonomic groups to the pesticide exceeds the listed species LOCs.

As previously discussed, the action area is defined by the most sensitive measure of direct and indirect ecological toxic effects including reduction in survival, growth, reproduction, and the entire suite of sublethal effects from valid, peer-reviewed studies.

The action area is determined by the footprint of the action plus all offsite areas where exposure of one or more taxonomic groups to diflubenzuron exceeds the Agency's LOCs. The spatial extent at which the Agency's LOCs are not exceeded is based on the potential exposure level and the most sensitive effects endpoint. The most sensitive effects endpoint is the acute aquatic invertebrate endpoint. It is expected that invertebrates would be the most sensitive since this chemical is an insecticide. This endpoint and all other effects endpoints used in this assessment are discussed in Section 4.

Because diflubenzuron has a relatively short period of persistence relative to seasonal variations in rainfall in California, the major diflubenzuron exposure route to the organisms of concern (aquatic invertebrates) varies throughout the year. During the dry-season (late spring, summer, and early fall) when little runoff occurs, most of the exposure will occur via spray drift. During the wet season (late fall, winter, and early spring) when much more runoff occurs, runoff will be a major contributor to aquatic EECs and risks.

The initial footprint for diflubenzuron is the entire state of California given the large various types of uses (e.g., agriculture, residential, rights of ways, forestry). In addition, given the genotoxicity of a diflubenzuron degradates, defining a threshold below which effects can occur is not possible. Therefore, the action area is the entire state of California.

2.8 Assessment Endpoints and Measures of Ecological Effect

Assessment endpoints are defined as "explicit expressions of the actual environmental value that is to be protected."[3] Selection of the assessment endpoints is based on valued entities (*e.g.,* CRLF, organisms important in the life cycle of the CRLF, and the PCEs of its designated critical habitat), the ecosystems potentially at risk (*e.g.*, waterbodies, riparian vegetation, and upland and dispersal habitats), the migration pathways of diflubenzuron (*e.g.*, runoff, spray drift, etc.), and the routes by which ecological receptors are exposed to diflubenzuron (*e.g.*, direct contact, etc.).

[3] U.S. EPA (1992). *Framework for Ecological Risk Assessment.* EPA/630/R-92/001.

2.8.1 Assessment Endpoints for the CRLF

Assessment endpoints for the CRLF include direct toxic effects on the survival, reproduction, and growth of the CRLF, 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 potential effects to PCEs, which are components of the habitat areas that provide essential life cycle needs of the CRLF. Each assessment endpoint requires one or more "measures of ecological effect," defined as changes in the attributes of an assessment endpoint or changes in a surrogate entity or attribute in response to exposure to a pesticide. Specific measures of ecological effect are generally evaluated based on acute and chronic toxicity information from registrant-submitted guideline tests that are performed on a limited number of organisms. Additional ecological effects data from the open literature are also considered. It should be noted that assessment endpoints are limited to direct and indirect effects associated with survival, growth, and fecundity, and do not include the full suite of sublethal effects used to define the action area. According the Overview Document (U.S. EPA 2004), the Agency relies on acute and chronic effects endpoints that are either direct measures of impairment of survival, growth, or fecundity or endpoints for which there is a scientifically robust, peer reviewed relationship that can quantify the impact of the measured effect endpoint on the assessment endpoints of survival, growth, and fecundity.

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 4.0 of this document. A summary of the assessment endpoints and measures of ecological effect selected to characterize potential assessed direct and indirect CRLF risks associated with exposure to diflubenzuron is provided in Table 2-4.

Table 2-4. Assessment Endpoints and Measures of Ecological Effects

Assessment Endpoint	Measures of Ecological Effects[4]
Aquatic-Phase CRLF (Eggs, larvae, juveniles, and adults)[a]	
Direct Effects	
1. Survival, growth, and reproduction of CRLF	1a. Most sensitive fish acute LC_{50} (guideline study; test species: bluegill sunfish) 1b. Most sensitive fish chronic NOAEC (guideline; test species: fathead minnow)
Indirect Effects and Critical Habitat Effects	
2. Survival, growth, and reproduction of CRLF individuals via indirect effects on aquatic prey food supply (*i.e.*, fish, freshwater invertebrates, non-vascular plants)	2a. Most sensitive fish (guideline study; test species: bluegill sunfish), aquatic invertebrate (ECOTOX study; test species: mosquito larvae), and aquatic plant EC_{50} (guideline study; test species: Duck weed) or LC_{50} 2b. Most sensitive aquatic invertebrate and fish chronic NOAEC (ECOTOX study and guideline respectively)
3. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat, cover, food supply, and/or primary productivity (*i.e.*, aquatic plant community)	3a. Vascular plant acute EC_{50} (duckweed guideline test) 3b. Non-vascular plant acute EC_{50} (guideline study; test species: green algae)
4. Survival, growth, and reproduction of CRLF individuals via effects to riparian vegetation	Currently no acceptable, guideline terrestrial plant toxicity data are available.
Terrestrial-Phase CRLF (Juveniles and adults)	
Direct Effects	
5. Survival, growth, and reproduction of CRLF individuals via direct effects on terrestrial phase adults and juveniles	5a. Most sensitive bird[b] or terrestrial-phase amphibian acute LC_{50} or LD_{50} (guideline study; test species: Redwinged blackbird) 5b. Most sensitive bird[b] or terrestrial-phase amphibian chronic NOAEC (guideline study; test species: Bobwhite quail)
Indirect Effects and Critical Habitat Effects	
6. Survival, growth, and reproduction of CRLF individuals via effects on terrestrial prey (*i.e.*,terrestrial invertebrates, small mammals , and frogs)	6a. Most sensitive terrestrial invertebrate and vertebrate acute EC_{50} (guideline study; test species: Bobwhite quail)
7. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat (*i.e.*, riparian and upland vegetation)	Currently no acceptable, guideline terrestrial plant toxicity data are available.

[a] Adult frogs are no longer in the "aquatic phase" of the amphibian life cycle; however, submerged adult frogs are considered "aquatic" for the purposes of this assessment because exposure pathways in the water are considerably different that exposure pathways on land.
[b] Birds are used as surrogates for terrestrial phase amphibians.

[4] Citations for all registrant-submitted and open literature toxicity data reviewed for this assessment are included in Appendix A.

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 diflubenzuron that may alter the PCEs of the CRLF's critical habitat. PCEs for the CRLF 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 CRLF. 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 diflubenzuron effects data are available. Adverse modification to the critical habitat of the CRLF includes, but is not limited to, those listed in Section 2.6.

Measures of such possible effects by labeled use of diflubenzuron on critical habitat of the CRLF are described in Table 2-5. 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. Assessment endpoints used for the analysis of designated critical habitat are based on the adverse modification standard established by U.S. FWS (2006).

Table 2-5. Summary of Assessment Endpoints and Measures of Ecological Effect for Primary Constituent Elements of Designated Critical Habitat[a]

<table>
<tr><th>Assessment Endpoint</th><th>Measures of Ecological Effect</th></tr>
<tr><td colspan="2">Aquatic-Phase CRLF PCEs
(Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)</td></tr>
<tr><td>Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile and adult CRLFs.</td><td>a. Most sensitive aquatic plant EC_{50} (guideline study: test species: green algae)
b. Currently no guideline terrestrial plant toxicity data is available.</td></tr>
<tr><td>Alteration in water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs and their food source.</td><td>a. Most sensitive aquatic plant EC_{50}
b. Currently, no guideline terrestrial plant toxicity data is available.</td></tr>
<tr><td>Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.</td><td>a. Most sensitive EC_{50} or LC_{50} values for fish and aquatic invertebrates (guideline and ECOTOX studies respectively; respective test species: bluegill sunfish and mosquito larvae)
b. Most sensitive NOAEC values for fish and aquatic invertebrates (guideline and ECOTOX respectively; respective test species: fathead minnow and water flea)</td></tr>
<tr><td>Reduction and/or modification of aquatic-based food sources for pre-metamorphs (e.g., algae)</td><td>a. Most sensitive aquatic plant EC_{50} (guideline study; test species: green algae)</td></tr>
<tr><td colspan="2">Terrestrial-Phase CRLF PCEs
(Upland Habitat and Dispersal Habitat)</td></tr>
<tr><td>Elimination and/or disturbance of upland habitat; ability of habitat to support food source of CRLFs: Upland areas within 200 ft of the edge of the riparian vegetation or dripline surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance</td><td rowspan="4">a Currently, no guideline terrestrial plant toxicity data are available.
b. c. Most sensitive food source acute EC_{50}/LC_{50} and NOAEC values for terrestrial vertebrates (mammals) and invertebrates, birds or terrestrial-phase amphibians, and freshwater fish.</td></tr>
<tr><td>Elimination and/or disturbance of dispersal habitat: Upland or riparian dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal</td></tr>
<tr><td>Reduction and/or modification of food sources for terrestrial phase juveniles and adults</td></tr>
<tr><td>Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.</td></tr>
</table>

[a] Physico-chemical water quality parameters such as salinity, pH, and hardness are not evaluated because these processes are not biologically mediated and, therefore, are not relevant to the endpoints included in this assessment.

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 (U.S. EPA 1998). For this assessment, the risk is stressor-linked, where the stressor is the release of diflubenzuron to the environment. The following risk hypotheses are presumed for this endangered species assessment:

The labeled use of diflubenzuron within the action area may:

- directly affect the CRLF by causing mortality or by adversely affecting growth or fecundity;
- indirectly affect the CRLF by reducing or changing the composition of food supply;
- indirectly affect the CRLF or modify designated critical habitat by reducing or changing the composition of the aquatic plant community in the ponds and streams comprising the species' current range and designated critical habitat, thus affecting primary productivity and/or cover;
- indirectly affect the CRLF or modify designated critical habitat by reducing or changing the composition of the terrestrial plant community (*i.e.*, riparian habitat) required to maintain acceptable water quality and habitat in the ponds and streams comprising the species' current range and designated critical habitat;
- modify the designated critical habitat of the CRLF by reducing or changing breeding and non-breeding aquatic habitat (via modification of water quality parameters, habitat morphology, and/or sedimentation);
- modify the designated critical habitat of the CRLF by reducing the food supply required for normal growth and viability of juvenile and adult CRLFs;
- modify the designated critical habitat of the CRLF by reducing or changing upland habitat within 200 ft of the edge of the riparian vegetation necessary for shelter, foraging, and predator avoidance;
- modify the designated critical habitat of the CRLF by reducing or changing dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal; or
- modify the designated critical habitat of the CRLF by altering chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs.

2.9.2 Diagram

The conceptual model is a graphic representation of the structure of the risk assessment. It specifies the diflubenzuron release mechanisms, biological receptor types, and effects endpoints of potential concern. The conceptual models for all of the uses other than fly control at livestock operations for both terrestrial and aquatic exposures are shown in Figure 2-4 and Figure 2-5, respectively, which include the conceptual models for the aquatic and terrestrial PCE components of critical habitat. Exposure routes shown in dashed lines are not quantitatively considered because the contribution of those potential exposure routes to potential risks to the CRLF and modification to designated critical habitat is expected to be negligible.

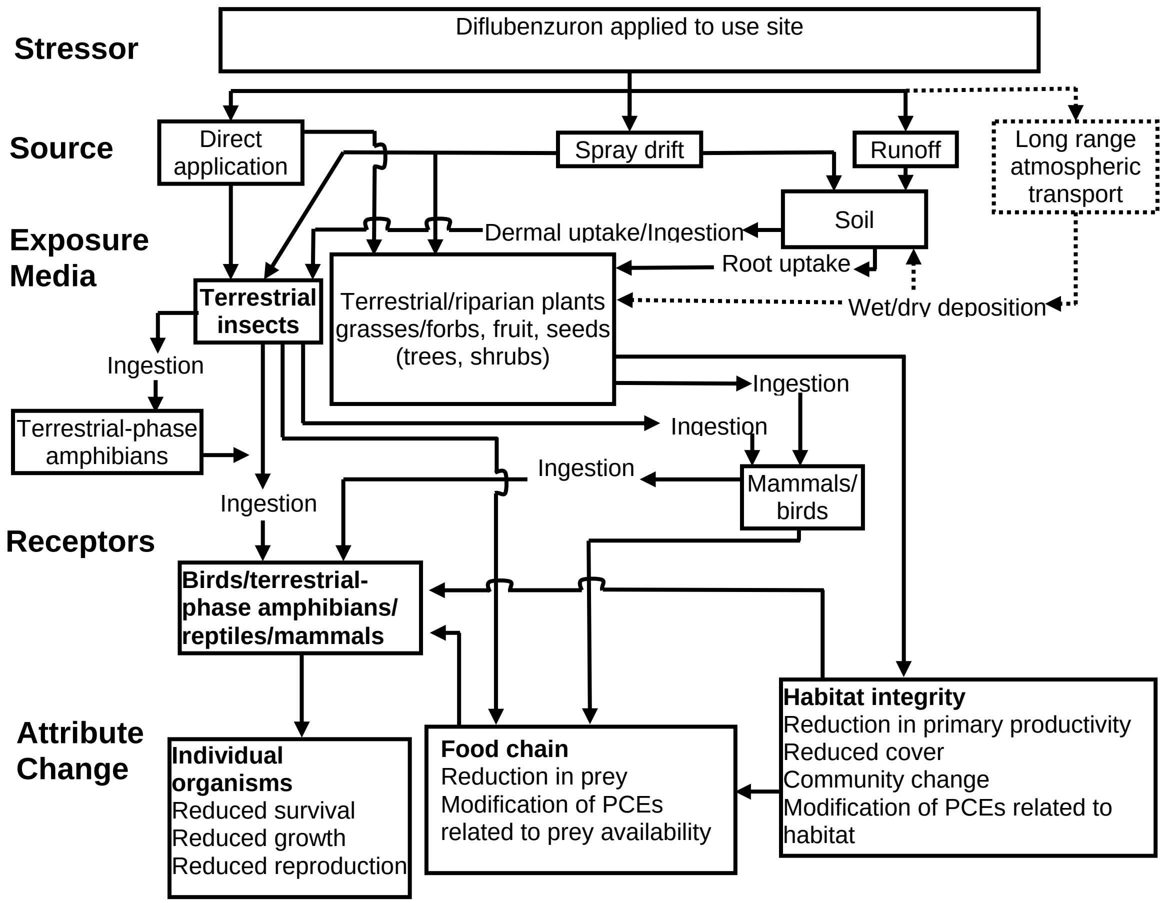


Figure 2-4 Conceptual Model for Pesticide Effects on Terrestrial Phase of the CRLF

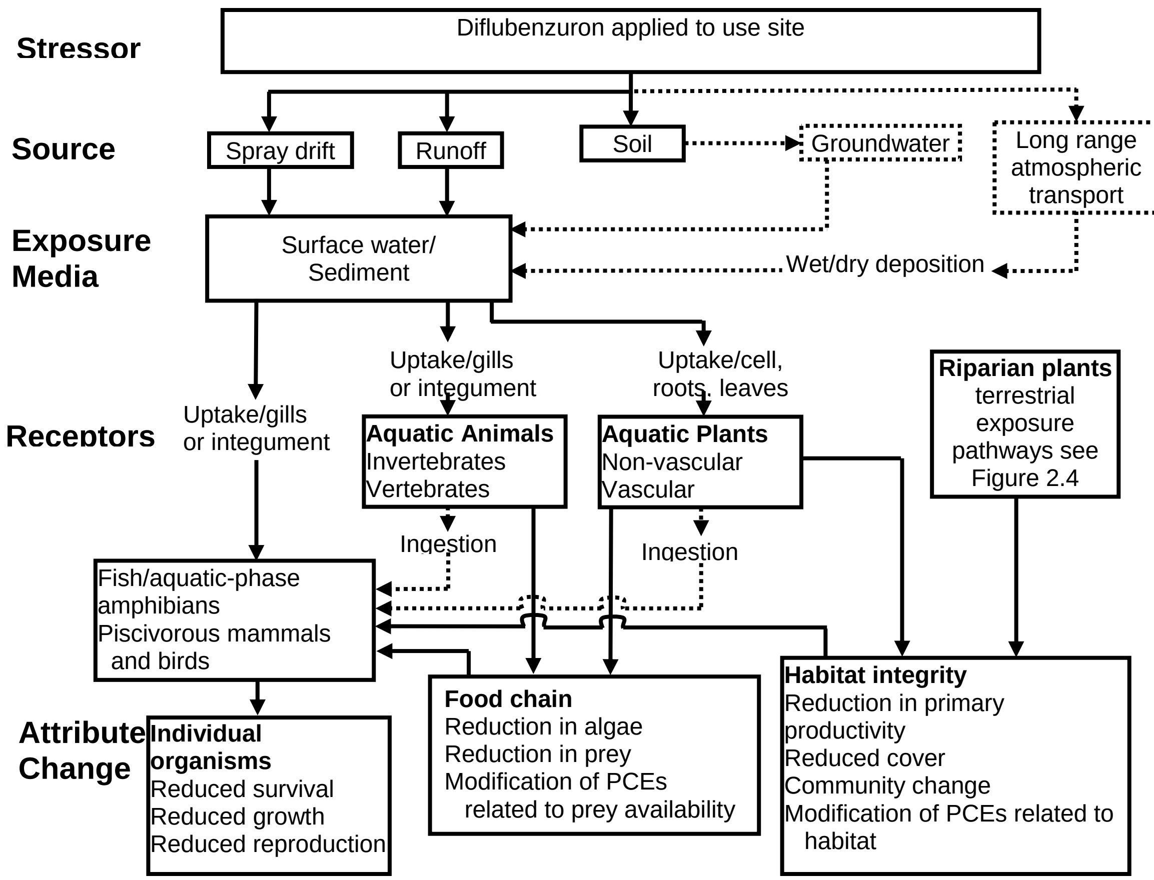


Figure 2-5. Conceptual Model for Pesticide Effects on Aquatic Phase of the CRLF.

Because the assessment methods are distinctly different for fly control at livestock operations, a separate conceptual model diagram is included along with the detailed description of these methods for this use in Appendix F. Other uses (right-of-ways in Appendix J and residential uses in Appendix K) have separate appendices to describe there unique assessment methods, but conceptually, are similar enough to use Figure 2-4 and 5 as the conceptual model diagram for these uses.

2.10 Analysis Plan

In order to address the risk hypothesis, the potential for direct and indirect effects to the CRLF, its prey, and its habitat is estimated. In the following sections, the use, environmental fate, and ecological effects of diflubenzuron 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 (U.S. EPA 2004), the likelihood of effects to individual organisms from particular uses of diflubenzuron 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 to Evaluate the Risk Hypothesis and Conceptual Model

2.10.1.1 Measures of Exposure

The environmental fate properties of diflubenzuron along with available monitoring data indicate that runoff and spray drift are the principle potential transport mechanisms of diflubenzuron to the aquatic and terrestrial habitats of the CRLF. In this assessment, transport of diflubenzuron through runoff and spray drift is considered in deriving quantitative estimates of diflubenzuron exposure to CRLF, its prey and its habitats. Long-range atmospheric transport of diflubenzuron is not considered due to this chemical's physical properties related to volatilization.

Measures of exposure are based on aquatic and terrestrial models that predict estimated environmental concentrations (EECs) of diflubenzuron using maximum labeled application rates and methods of application. The models used to predict aquatic EECs are the Pesticide Root Zone Model coupled with the Exposure Analysis Model System (PRZM/EXAMS) and GENeric Expected Environmental Concentration (GENEEC). The model used to predict terrestrial EECs on food items is T-REX. These models are parameterized using relevant reviewed registrant-submitted environmental fate data.

PRZM (v3.12.2, May 2005) and EXAMS (v2.98.4.6, April 2005) are screening simulation models coupled with the input shell pe5.pl (Aug 2007) to generate daily exposures and 1-in-10 year EECs of diflubenzuron that may occur in surface water bodies adjacent to application sites receiving diflubenzuron through runoff and spray drift. PRZM simulates pesticide application, movement and transformation on an agricultural field and the resultant pesticide loadings to a receiving water body via runoff, erosion and spray drift. EXAMS simulates the fate of the pesticide and resulting concentrations in the water body. The standard scenario used for ecological pesticide assessments assumes application to a 10-hectare agricultural field that drains into an adjacent 1-hectare water body, 2-meters deep (20,000 m^3 volume) with no outlet. PRZM/EXAMS was used to estimate screening-level exposure of aquatic organisms to diflubenzuron. The measure of exposure for aquatic species is the 1-in-10 year return peak or rolling mean concentration. The 1-in-10 year peak is used for estimating acute exposures of direct effects to the CRLF, as well as indirect effects to the CRLF through effects to potential prey items, including: algae, aquatic invertebrates, fish and frogs. The 1-in-10-year 60-day mean is used for assessing chronic exposure to the CRLF and fish and frogs serving as prey items; the 1-in-10-year 21-day mean is used for assessing chronic exposure for aquatic invertebrates, which are also potential prey items.

The EFED GENEEC (GENeric Expected Environmental Concentration) model (version 1.2) is used to estimate pesticide concentrations in a 1 hectare by 2 meter deep pond with no outlet draining an adjacent 10 hectare field. It provides an upper-bound screening concentration value for most types of surface water for up to 56 days after runoff. GENEEC is a single runoff event but accounts for spray drift from single or multiple applications. The pond receives a pesticide load from spray drift for each application plus loading from a single-runoff event, in this case, two days after the last application. The runoff event transports a maximum of 10% of the pesticide remaining in the top 2.5 cm of soil. This amount can be reduced through soil adsorption. The amount of pesticide remaining on the field in the top 2.5 cm of soil depends on the application rate, number of applications, interval between applications, incorporation depth, and degradation rate in the soil.

Exposure estimates for the terrestrial-phase CRLF and terrestrial invertebrates and mammals (serving as potential prey) assumed to be in the target area or in an area exposed to spray drift are derived using the T-REX model (version 1.3.1, 12/07/2006). This model incorporates the Kenega nomograph, as modified by Fletcher *et al.* (1994), which is based on a large set of actual field residue data. The upper limit values from the nomograph represented the 95th percentile of residue values from actual field measurements (Hoerger and Kenega 1972). For modeling purposes, direct exposures of the CRLF to diflubenzuron through contaminated food are estimated using the EECs for the small bird (20 g) which consumes small insects. Dietary-based and dose-based exposures of potential prey (small mammals) are assessed using the small mammal (15 g) which consumes short grass. The small bird (20g) consuming small insects and the small mammal (15g) consuming short grass are used because these categories represent the largest RQs of the size and dietary categories in T-REX that are appropriate surrogates for the CRLF and one of its prey items. Estimated exposures of terrestrial insects to diflubenzuron are bound by using the dietary based EECs for small insects and large insects.

Birds are currently used as surrogates for terrestrial-phase CRLF. However, amphibians are poikilotherms (body temperature varies with environmental temperature) while birds are homeotherms (temperature is regulated, constant, and largely independent of environmental temperatures). Therefore, amphibians tend to have much lower metabolic rates and lower caloric intake requirements than birds or mammals. As a consequence, birds are likely to consume more food than amphibians on a daily dietary intake basis, assuming similar caloric content of the food items. Therefore, the use of avian food intake allometric equation as a surrogate to amphibians is likely to result in an over-estimation of exposure and risk for reptiles and terrestrial-phase amphibians. Therefore, T-REX (version 1.3.1) has been refined to the T-HERPS model (v. 1.0), which allows for an estimation of food intake for poikilotherms using the same basic procedure as T-REX to estimate avian food intake.

AgDRIFT was used to assess aquatic exposures of aquatic phase CRLF and its prey to diflubenzuron deposited in aquatic habitats by spray drift and to determine the maximum distance from a treated area that effects may occur.

2.10.1.2 Measures of Effect

Diflubenzuron is a chitinase inhibitor, impairing the ability of insects to synthesize a critical component of their exoskeleton (U.S. EPA, 1997). Chitin synthesis is particularly important in the early life stages of insects, as they molt and form a new exoskeleton in various growth stages. Thus, aquatic guideline tests, which typically run for 48 hours for the aquatic invertebrate (*Daphnia magna*), may not capture a molting stage, and are not an appropriate "most sensitive" acute endpoint for assessments. Endpoints derived from studies that test the toxic effects of diflubenzuron on the larval/molting stages of freshwater invertebrates and that test the chronic exposure of freshwater invertebrates to diflubenzuron more appropriately assess the toxicity of this type of chemical. Diflubenzuron is fairly persistent in both aquatic and terrestrial environments. For this reason, data that evaluated the toxic effects of diflubenzuron at life stages (larval/molting stages) most vulnerable to this chemical were used as both acute and chronic endpoints for aquatic invertebrate RQs. Likewise, guideline tests on honeybees frequently run for only 48 hours and are conducted on adult bees, and, therefore, may not reflect the toxicity of diflubenzuron.

Data identified in Section 2.8 are used as measures of effect for direct and indirect effects to the CRLF. Data were obtained from registrant submitted studies or from literature studies identified by ECOTOX. The ECOTOXicology database (ECOTOX) was searched in order to provide more ecological effects data and in an attempt to bridge existing data gaps. ECOTOX is a source for locating single chemical toxicity data for aquatic life, terrestrial plants, and wildlife. ECOTOX was created and is maintained by the U.S. EPA, Office of Research and Development, and the National Health and Environmental Effects Research Laboratory's Mid-Continent Ecology Division.

The assessment of risk for direct effects to the terrestrial-phase CRLF makes the assumption that toxicity of diflubenzuron to birds is similar to or less than the toxicity to the terrestrial-phase CRLF. The same assumption is made for fish and aquatic-phase CRLF. Algae, aquatic invertebrates, fish, and amphibians represent potential prey of the CRLF in the aquatic habitat. Terrestrial invertebrates, small mammals, and terrestrial-phase amphibians represent potential prey of the CRLF in the terrestrial habitat. Aquatic, semi-aquatic, and terrestrial plants represent habitat of CRLF.

The acute measures of effect used for animals in this screening level assessment are the LD_{50}, LC_{50} and EC_{50}. LD stands for "Lethal Dose", and LD_{50} is the amount of a material, given all at once, that is estimated to cause the death of 50% of the test organisms. LC stands for "Lethal Concentration" and LC_{50} is the concentration of a chemical that is estimated to kill 50% of the test organisms. EC stands for "Effective Concentration" and the EC_{50} is the concentration of a chemical that is estimated to produce a specific effect in 50% of the test organisms. Endpoints for chronic measures of exposure for listed and non-listed animals are the NOAEL/NOAEC and NOEC. NOAEL stands for "No Observed-Adverse-Effect-Level" and refers to the highest tested dose of a substance that has been reported to have no harmful (adverse) effects on test organisms. The NOAEC (*i.e.*, "No-Observed-Adverse-Effect-Concentration") is the highest test concentration at which none of the observed effects were statistically different from the control. The NOEC is the No-Observed-Effects-Concentration. For non-listed plants, only acute exposures are assessed (*i.e.*, EC_{25} for terrestrial plants and EC_{50} for aquatic plants).

It is important to note that the measures of effect for direct and indirect effects to the CRLF and its designated critical habitat are associated with impacts to survival, growth, and fecundity, and do not include the full suite of sublethal effects used to define the action area. According the Overview Document (USEPA 2004), the Agency relies on effects endpoints that are either direct measures of impairment of survival, growth, or fecundity or endpoints for which there is a scientifically robust, peer reviewed relationship that can quantify the impact of the measured effect endpoint on the assessment endpoints of survival, growth, and fecundity.

2.10.1.3 Integration of Exposure and Effects

Risk characterization is the integration of exposure and ecological effects characterization to determine the potential ecological risk from agricultural and non-agricultural uses of diflubenzuron, and the likelihood of direct and indirect effects to CRLF 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. For the assessment of diflubenzuron risks, 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) (U.S. EPA 2004) (see Appendix C).

For this endangered species assessment, listed species LOCs are used for comparing RQ values for acute and chronic exposures of diflubenzuron directly to the CRLF. If estimated exposures directly to the CRLF of diflubenzuron resulting from a particular use are sufficient to exceed the listed species LOC, then the effects determination for that use is "may affect". When considering indirect effects to the CRLF due to effects to animal prey (aquatic and terrestrial invertebrates, fish, frogs, and mice), the listed species LOCs are also used. If estimated exposures to CRLF prey of diflubenzuron resulting from a particular use are sufficient to exceed the listed species LOC, then the effects determination for that use is a "may affect." If the RQ being considered also exceeds the non-listed species acute risk LOC, then the effects determination is a LAA. If the acute RQ is between the listed species LOC and the non-listed acute risk species LOC, then further lines of evidence (*i.e*., probability of individual effects, species sensitivity distributions) are considered in distinguishing between a determination of NLAA and a LAA. When considering indirect effects to the CRLF due to effects to algae as dietary items or plants as habitat, the non-listed species LOC for plants is used because the CRLF does not have an obligate relationship with any particular aquatic and/or terrestrial plant. If the RQ being considered for a particular use exceeds the non-listed species LOC for plants, the effects determination is "may affect". Further information on LOCs is provided in Appendix C.

2.10.1.4 Data Gaps

No guideline terrestrial plant toxicity data have been submitted to the Agency. However, open literature data and other lines of evidence will be used to evaluate the potential for diflubenzuron to indirectly affect CRLFs via impacts to terrestrial plants.

3.0 Exposure Assessment

Diflubenzuron formulations include liquid, water dispersible granules, wettable powder, effervescent tablets (for ornamental pond treatment), and termite bait (in bait stations). Application equipment includes ground, aerial, and airblast applicators and directed sprayers. Risks from ground boom and aerial applications are expected to result in the highest off-target levels of diflubenzuron due to generally higher spray drift levels. Ground boom and aerial modes of application tend to use lower volumes of application applied in finer sprays than applications coincident with sprayers and spreaders and thus have a higher potential for off-target movement via spray drift.

3.1. Label Application Rates and Intervals

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

Currently registered agricultural and non-agricultural uses of diflubenzuron within California include: almond, Brazil nut, cashew, chestnut, chinquapin, filbert (hazelnut), hickory nut, macadamia nut (bushnut), pecan, tree nuts, walnut (English/black), citrus hybrids other than tangelo, grapefruit, orange, pummelo (shaddock), tangerines, broccoli raab, cabbage - Chinese, collards, kale, soybeans (unspecified), cotton (unspecified), Christmas tree plantations, forest trees (softwoods - conifers), apricot, nectarine, peach, pear, plum, prune, stone fruits, commercial/institutional/industrial premises/equipment (outdoor), nonagricultural outdoor buildings/structures, paved areas (private roads/sidewalks), refuse/solid waste sites (outdoor), wide area/general outdoor treatment (quarantine/eradication use), butternut, forest nursery plantings (for transplant purposes), ornamental and/or shade trees, ornamental woody shrubs and vines, shelterbelt plantings, turnip (greens), household/domestic dwellings outdoor premises, artichoke, mustard, peanuts (unspecified), pepper, agricultural fallow/idleland, golf course turf, nonagricultural uncultivated areas/soils, barley, canola\rape, oats, triticale, wheat, grass forage/fodder/hay, pastures, rangeland, agricultural rights-of-way/fencerows/hedgerows, nonagricultural rights-of-way/fencerows/hedgerows, agricultural/farm structures/buildings and equipment, barns/barnyards/auction barns, manure, brassica (head and stem) vegetables, ornamental sod farm (turf), recreational areas, rice, drainage systems, forest plantings (reforestation programs)(tree farms - tree plantations - etc.), forest trees (all or unspecified), forest trees (softwoods - conifers), intermittently flooded areas/water, lakes/ponds/reservoirs (with human or wildlife use), lakes/ponds/reservoirs (without human or wildlife use), ornamental herbaceous plants, ornamental ponds/aquaria, ornamental woody shrubs and vines, shelterbelt plantings, streams/rivers/channeled water, swimming pool water systems, wide area/general outdoor treatment (quarantine/eradication use), beech nut, pistachio. The uses being assessed are summarized in Table 3-1.

Table 3-1 Diflubenzuron Uses, Scenarios, and Application Information for the CRLF risk assessment[1]

Scenario	Uses Represented by Scenario	Application Rate	Number of Applications	Application Interval	Application Method
1. Barnyard	Spray on manure, Manure to field	8.508	NS (17)	NS (21)	Ground
2. Beech Nut	Beech Nut	0.0408	3	7	Ground
		0.0408	3	7	Air
3. Brassica	Brassica (head and stem) vegetables	0.0313	1	NA	Ground
4. Citrus	Citrus hybrids, grapefruit, orange, pummelo (shaddock), and tangerines	0.3125	3	21	Ground
		0.125	3	7	Air
5. Cole Crop	Broccoli raab, cabbage - Chinese, collards, and kale	0.25	4	21	Ground
6. Cotton	Cotton (unspecified)	0.3125	3	7	Ground
		0.3125	3	90	Air
7. Forest	Christmas tree plantations and forest trees (softwoods - conifers)	0.22048	NS (4)	14	Ground
		0.25	1	NA	Air
8. Fruit	Apricot, nectarine, peach, pear, plum, prune, and stone fruits	0.3125	3	7	Ground
		0.3125	3	7	Airblast
		0.3125	3	7	Air
9. General Nuts	Almond, Brazil nut, cashew, chestnut, chinquapin, filbert (hazelnut), hickory nut, macadamia nut (bushnut), pecan, tree nuts, and walnut (English/black)	0.3125	3	7	Ground
		0.3125	3	7	Airblast
		0.3125	3	90	Air
10. Grains	Barley, canola\rape, oats, triticale, and wheat	0.25	4	21	Ground
		0.125	3	NS (21)	Air
11. Nursery	Forest nursery plantings (for transplant purposes), ornamental and/or shade trees, ornamental woody shrubs and vines, and shelterbelt plantings	0.25	1	NA	Ground
		0.25	1	NA	Air
12. Pasture	Grass forage/fodder/hay, pastures, and rangeland	0.25	1	NA	Ground
		0.25	1	NA	Airblast
		0.25	1	NA	Air
13. Pistachio	Pistachio	0.75	2	14	Ground
14. Residential	Household/domestic dwellings outdoor premises	0.25	1	NA	Ground
		0.25	6	10	Air
15. Rice	Rice	0.625	6	5	Air
16. Rights-of-way	Agricultural rights-of-way/fencerows/hedgerows, fencerows/hedgerows, and nonagricultural rights-of-way/fencerows/hedgerows	0.25	2	5	Ground
		0.25	2	5	Airblast
		0.25	2	5	Air
17. Row Crop	Artichoke, mustard, peanuts (unspecified), and pepper	0.3125	3	21	Ground
		0.3125	3	14	Air
18. Urban	Commercial/institutional/industrial premises/equipment (outdoor), nonagricultural outdoor buildings/structures, paved areas (private roads/sidewalks), refuse/solid waste sites (outdoor), and wide area/general outdoor treatment (quarantine/eradication use)	0.25	6	NS (10)	Air
19. Squash	Butternut	0.25	4	15	Ground
		0.25	4	15	Air

Scenario	Uses Represented by Scenario	Application Rate	Number of Applications	Application Interval	Application Method
20. Turf (High Appl. Rate)	Agricultural fallow/idleland, golf course turf, and nonagricultural uncultivated areas/soils	0.25	2	14	Ground
		0.25	2	14	Airblast
		0.25	2	5	Air
21. Turf (Low Appl. Rate)	Ornamental sod farm (turf) and recreational areas	0.0313	3	7	Ground
		0.0313	3	7	Airblast
		0.0313	3	7	Air
22. Turnip	Turnip (greens)	0.25	2	21	Ground

[1] Uses assessed based on memorandum from SRRD dated 2/24/09.
NS = Not specified on the label.

The large number of scenarios is mainly a reflection of the diverse uses of diflubenzuron. However, some uses were split from other similar uses because they had much higher or lower application rates.

Some of the scenarios require additional explanation. The barnyard scenario is a direct spray of diflubenzuron on manure. This scenario, methods of analysis, and resulting EECs are discussed in detail in Appendix F. Similarly, the rationales for the residential and right-of-way scenarios are described in appendices J and K.

In addition, diflubenzuron can be administered to cattle with a gun-like device that shoots a “bolus” impregnated with diflubenzuron down the throats of the cattle. This bolus slowly dissolves and releases diflubenzuron with the waste. One bolus treats an animal for as long as 21 weeks. Similarly, diflubenzuron can be administered through cattle and swine feed.

EFED does not have data indicating the diflubenzuron concentration in the manure resulting from all three methods (direct spray on manure, bolus, or feed-through treatments). However, the desired result from all three methods is to produce manure that has a concentration of diflubenzuron that inhibits the growth of the same species of nuisance flies. Therefore, EFED assumes for purposes of this assessment that the exposure levels from all three methods of manure treatment would be similar and considers the barnyard EECs to apply to all three manure treatment methods.

The urban scenario provides EECs for the quarantine/eradication use of diflubenzuron if used in urban areas. It assumes 100% impervious surfaces in the watershed treated.

Another non-standard diflubenzuron use is ornamental pond care. Because this use is a direct application to water (ornamental ponds) and results in EECs that are roughly equivalent to the EECS produced by the tier 1 rice model for the rice uses of diflubenzuron, the ornamental pond care use is assessed using the rice model EECs. However, an important difference between the rice use and ornamental pond care is ornamental ponds are required to not be connected to natural drainage-ways, while rice paddy water is assumed to be discharged to waters off of the use site.

As an example, the ornamental pond care EEC for label registration # 400-469 is calculated as:

$$EEC = \frac{pecent_{Diflubenzuron} \times Amount_{Applied}}{Volume_{Treated}}$$

$$= \frac{25\% \times 1g \times 10^6\ \mu g/g}{1000gal \times 3.8L/gal} = 66\,\mu g/L$$

where: *EEC* is the ornamental pond concentration after treatment in µg/L; $Amount_{Applied}$ is the amount of diflubenzuron applied to each 1000 gallons of pond water treated (converted to µg); and volume treated is 1000 gallons (converted to liters).

The last non-standard use is application of diflubenzuron to the material (often compost) in which mushrooms are grown. The compost is treated with diflubenzuron to keep nuisance flies from harming the mushroom crop. As mushrooms are grown, the ability of the compost material to grow mushrooms decreases. Therefore, the compost material that was treated with diflubenzuron must be periodically replaced and the old material discarded. Often this material is land applied on farm fields.

Mushrooms can be grown outdoors or in mushroom houses. When grown outdoors, there is potential for exposure to CRLFs while the diflubenzuron-treated compost is in use for growing mushrooms as well as after it has been land applied. Alternatively when mushrooms are grown indoors, exposure to the diflubenzuron-treated compost would only occur after the compost is land applied on farm fields.

The mushroom use of diflubenzuron is very similar to the barnyard or manure use described previously in terms of both exposure at the site of application and at the site of disposal through land application as well as the concentration in the compost and manure. For the barnyard or manure use (Appendix F), the resultant diflubenzuron concentration in the manure is approximately 10 to 60 ppm (Appendix F, Figure F3). For the mushroom use, the desired diflubenzuron concentration in the compost is approximately 30 to 50 ppm. Therefore, mushroom uses will be assessed using the EECs generated for the barnyard use.

An important assumption in the barnyard analysis (appendix F) is that degradation behavior and rate of diflubenzuron in manure is similar to the degradation behavior and rate of diflubenzuron in soil. This assumption is made because EFED does not have diflubenzuron fate data in manure. Similarly, the assumption is made that the behavior and rate of diflubenzuron degradation in mushroom compost is similar to the degradation behavior and rate of diflubenzuron in soil because EFED does not have diflubenzuron fate data in mushroom compost.

3.2. Aquatic Exposure Assessment

3.2.1. Modeling Approach

Aquatic exposures are quantitatively estimated for all of assessed uses using scenarios that represent high exposure sites for diflubenzuron use. Each of these sites represents a 10 hectare field that drains into a 1-hectare pond that is 2 meters deep and has no outlet. Exposure estimates generated using the standard pond are intended to represent a wide variety of vulnerable water bodies that occur at the top of watersheds including prairie pot holes, playa lakes, wetlands, vernal pools, man-made and natural ponds, and intermittent and first-order streams. As a group, there are factors that make these water bodies more or less vulnerable than the standard surrogate pond. Static water bodies that have larger ratios of drainage area to water body volume would be expected to have higher peak EECs than the standard pond. These water bodies will be either shallower or have large drainage areas (or both). Shallow water bodies tend to have limited additional storage capacity, and thus, tend to overflow and carry pesticide in the discharge whereas the standard pond has no discharge. As watershed size increases beyond 10 hectares, at some point, it becomes unlikely that the entire watershed is planted to a single crop, which is all treated with the pesticide. Headwater streams can also have peak concentrations higher than the standard pond, but they tend to persist for only short periods of time and are then carried downstream.

Crop-specific management practices for all of the assessed uses of diflubenzuron were used for modeling, including application rates, number of applications per year, application intervals, buffer widths and resulting spray drift values modeled from AgDRIFT, and the first application date for each crop. The date of first application was developed based on the use information provided by BEAD and a summary of individual applications by use from the CDPR PUR data. Because diflubenzuron persists in fields for only a short time after application (aerobic soil metabolism half-life of 2 to 14 days) and most runoff in the semi-arid portions of California occur mainly in the winter and early spring, application date will greatly affect the estimated exposure to diflubenzuron.

As an example of identifying scenario application dates based on PUR data, Figure 3-1 depicts variability in diflubenzuron application by date within a calendar year for ground citrus applications based on CDPR PUR data from 1990 through 2007. The curvy indigo (dark blue) line indicates the average pounds of diflubenzuron applied on each day. This average is calculated as a 15-day moving average of the average total pounds applied for each day of the year. The solid vertical line indicates the date on which this 15-day average is highest. The dashed vertical lines the first and last application dates which were calculated to bracket the highest daily average. Similar analyses for other uses are included in Appendix D, Figure D3. Because PUR data only identifies applications as ground or air, airblast application dates are based on the highest 15-day average of ground applications.

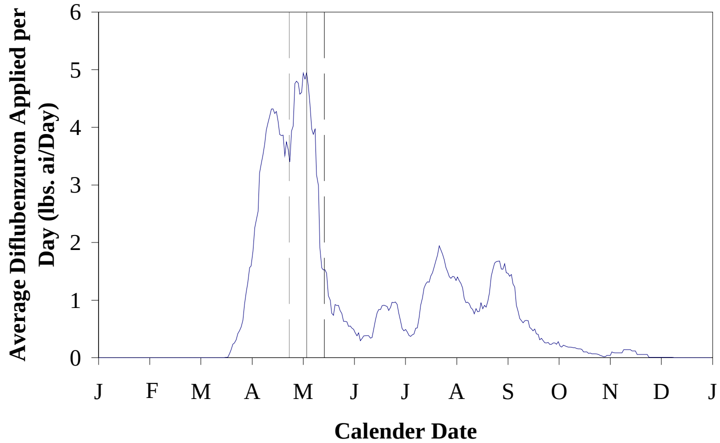


Figure 3-1. Identification of application dates based on analysis of the average pounds of diflubenzuron applied in California to citrus via ground application methods based on CDPR PUR data (1990-2007).

This method of selecting application dates works well for those uses that are well-represented in the PUR data set. However many of the uses that can be legally applied in California (and therefore, must be assessed in this document), do not appear in the PUR data set. For those uses that do not occur in the PUR, the model was run for each potential date of application and the date that resulted in the highest exposure was used in order to ensure a conservative exposure estimate. This method is described at the end of Section 3.2.3.

3.2.2. Model Inputs

Diflubenzuron is an insecticide used on a wide variety of food and non-food uses. Diflubenzuron environmental fate data used for generating model parameters is listed in Table 2-2. The input parameters for PRZM and EXAMS are in Table 3-2.

Table 3-2 Summary of PRZM/EZAMS Environmental Fate Data Used for Aquatic Exposure Inputs for Diflubenzuron Endangered Species Assessment for the CRLF [1]

Fate Property	Value (unit)	MRID (or source)
Molecular Weight	311 g/mol	Product Chemistry
Henry's constant	1.87×10^{-09} atm-m^3/mol	Product Chemistry
Vapor Pressure	9.00×10^{-10} torr	Product Chemistry
Solubility in Water	0.2 mg/L	Product Chemistry
Photolysis in Water	80 days	40816301 41087802
Aerobic Soil Metabolism Half-lives	4.7 days	00039473 00039474 41722801
Hydrolysis	Stable @ pH 5 119 days @ pH 7 32 days @ pH 9	00143355 40859801 41087801 40816301
Aerobic Aquatic Metabolism (water column)	23.4 days	Combined Data Mean = 11.70 days SD = 12.41 days, n=3 $t_{90, n-1}$ = 1.638 t_{input} = (mean of $t_{½}$) + ($t_{90,n-1}\times$SD)/$n^{½}$
Anaerobic Aquatic Metabolism (benthic)	102 days	34 days×3
Koc	3961	Mean from Table 2-1 Kocs
Application rate and frequency	(See Table 3-1)	Label or Maximum from LUIS Report
Application intervals	(See Table 3-1)	Label or Minimum from LUIS Report
Chemical Application Method (CAM)	2	Input Parameter Guidance[1]
Application Efficiency	0.99 (Ground/Airblast) or .95 (Air)	Input Parameter Guidance[1]
Spray Drift Fraction[2]	0.0007 (Ground), .015 (Airblast), or .039 (Air)	AgDRIFT Model

[1] Inputs determined in accordance with EFED "Guidance for Chemistry and Management Practice Input Parameters for Use in Modeling the Environmental Fate and Transport of Pesticides" dated February 28, 2002.

[2] Spray drift fraction assumed a buffer of 150 feet. The fraction was calculated using AgDrift and default Tier 1 parameters.

3.2.3. Results

The aquatic EECs for the various scenarios and application methods are listed in Table3-3 based on the first application date indicated in column three of this table. The highest EECs (113 µg/L) are for rice/ornamental pond (direct application to water). Because rice/ornamental pond EECs were calculated using the rice model which assumes no degradation, the highest peak, 1-in-10-year 21-day average, and 1-in-10-year 60-day average EECs are all the same. Excluding the rice/ornamental pond EECs: peak EECs varied by a factor of 5400 between uses from 0.01 to 54 µg/L; 1-in-10-year 21-day average EECs varied by a factor of 6900 between uses from 0.006 to 42 µg/L; and 1-in-10-year 60-day average EECs varied by a factor of 7500 between uses from 0.004 to 27 µg/L.

Table 3-3 Aquatic EECs (µg/L) for Diflubenzuron Uses in California

Scenario (Ground, Airblast, or Air)		Appl. Rate	1st – last Appl. Date[1]	Crops Represented	Peak EEC	21-day average EEC	60-day average EEC
1. Barnyard/ Mushroom	G	8.508	Year-round 17 appl.	Spray on manure	54.20	42.00	27.30
				Treated manure applied to field	7.29	5.63	3.65
2. Beech Nut	G	0.0408	NP (1/12)	Beech Nut	0.11	0.06	0.03
	A	0.0408	NP (1/12)		0.21	0.13	0.08
3. Brassica	G	0.0313	NP (12/30)	Brassica (head and stem) vegetables	0.07	0.04	0.02
4. Citrus	G	0.3125	4/23 – 5/14	Citrus hybrids, grapefruit, orange, pummelo (shaddock), and tangerines	0.24	0.10	0.05
	A	0.125	4/23 – 5/14		0.50	0.30	0.18
5. Cole Crop	G	0.25	9/11 – 12/4	Broccoli raab, cabbage - Chinese, collards, and kale	0.36	0.20	0.14
6. Cotton	G	0.3125	5/14 – 6/4	Cotton (unspecified)	0.15	0.11	0.06
	A	0.3125	3/14 – 12/9		0.82	0.47	0.30
7. Forest	G	0.22048	6/14 – 7/5	Christmas tree plantations and forest trees (softwoods - conifers)	0.58	0.31	0.19
	A	0.25	3/10		0.80	0.45	0.26
8. Fruit	G	0.3125	2/20 – 3/12	Apricot, nectarine, peach, pear, plum, prune, and stone fruits	0.26	0.14	0.08
	AB	0.3125	2/20 – 3/12		0.66	0.43	0.26
	A	0.3125	3/24 – 4/14		1.36	0.86	0.54
9. General Nuts	G	0.3125	1/13 – 4/6	Almond, Brazil nut, cashew, chestnut, chinquapin, filbert (hazelnut), hickory nut, macadamia nut (bushnut), pecan, tree nuts, and walnut (English/black)	0.90	0.46	0.25
	AB	0.3125	1/13 – 4/6		1.26	0.70	0.42
	A	0.3125	1/26 – 3/29		0.85	0.49	0.28
10. Grains	G	0.25	NP (1/19)	Barley, canola\rape, oats, triticale, and wheat	1.34	0.78	0.56
	A	0.125	NP (1/19)		0.93	0.52	0.41
11. Nursery	G	0.25	7/4	Forest nursery plantings (for transplant purposes), ornamental and/or shade trees, ornamental woody shrubs and vines, and shelterbelt plantings	0.20	0.11	0.08
	A	0.25	12/5		0.65	0.38	0.30
12. Pasture	G	0.25	2/28	Grass forage/fodder/hay, pastures, and rangeland	0.12	0.08	0.04
	AB	0.25	2/28		0.29	0.17	0.09
	A	0.25	6/6		0.55	0.25	0.13
13. Pistachio	G	0.75	3/30	Pistachio	0.15	0.08	0.06
14. Residential	G	0.25	NP (1/10)	Household/domestic dwellings outdoor premises	0.08	0.05	0.03
	A	0.25	NP (1/10)		0.40	0.30	0.25
15. Rice/ Ornamental Pond	A	0.625	5/14 – 6/13	Rice/Ornamental Pond	112.92	112.92	112.92
16. Rights-of-way	G	0.25	NP (1/12)	Agricultural rights-of-way/fencerows/hedgerows, fencerows/hedgerows, and nonagricultural rights-of-way/fencerows/hedgerows	5.55	3.04	1.61
	AB	0.25	NP (1/12)		8.13	4.39	2.32
	A	0.25	NP (1/12)		5.59	3.07	1.63
17. Row Crop	G	0.3125	8/14 - 10/16	Artichoke, mustard, peanuts (unspecified), and pepper	0.02	0.01	0.01

Scenario (Ground, Airblast, or Air)		Appl. Rate	1st – last Appl. Date[1]	Crops Represented	Peak EEC	21-day average EEC	60-day average EEC
	A	0.3125	2/1 – 3/14		1.15	0.77	0.58
18. Urban	A	0.25	NP (1/10)	Commercial/institutional/industrial premises/equipment (outdoor), nonagricultural outdoor buildings/structures, paved areas (private roads/sidewalks), refuse/solid waste sites (outdoor), and wide area/general outdoor treatment (quarantine/eradication use)	34.13	21.98	15.28
19. Squash	G	0.25	NP (1/10)	Butternut	1.15	0.65	0.38
	A	0.25	NP (1/10)		1.87	1.18	0.79
20. Turf (High Appl. Rate)	G	0.25	7/5 – 8/2	Agricultural fallow/idleland, golf course turf, and nonagricultural uncultivated areas/soils	0.01	0.01	0.00
	AB	0.25	7/5 – 8/2		0.21	0.13	0.08
	A	0.25	5/26 – 6/5		0.61	0.37	0.20
21. Turf (Low Appl. Rate)	G	0.0313	7/5 – 8/2	Ornamental sod farm (turf) and recreational areas	0.02	0.01	0.01
	AB	0.0313	7/5 – 8/2		0.07	0.04	0.02
	A	0.0313	5/26 – 6/5		0.13	0.08	0.05
22. Turnip	G	0.25	NP (1/18)	Turnip (greens)	0.72	0.35	0.19

[1]NP = Not in CDPR Pesticide Use Reporting data set. Typically, the date of first application that yielded the highest peak concentration (indicated in parentheses) was used as the first application date in the scenario modeled (see text for date selection for residential and right-of-way uses).

The EECs for several of the uses are based on application dates that were selected specifically because that application date would yield the highest EECs. These highest dates were used to produce a conservative estimate EECs because there was no PUR data available to better indicate when diflubenzuron would be applied for that use (Section 3.2.1). Figure 3-2 depicts the variation in PRZM/EXAMS EECs based on the day of the year chosen as the first application date. As shown in Figure 3.2a, using the date that yields the highest EECs (typically in the winter) could be extremely conservative (high EECs) if the actual application of diflubenzuron would only occur in the summer. This is not always the case though as is shown in Figure 3.2b. Typically, ground applications (Figure 3.2a) will show large variations between winter and summer diflubenzuron EECs because ground application EECs are dominated by runoff contributions which vary with rainfall. Aerial applications (Figure 3.2b) result in a much larger spray drift contribution, which does not vary with application date and therefore, results in less variation in EECs between summer and winter. Appendix D, Figure D4, provides similar graphs for all of the diflubenzuron uses for which PUR data was not available.

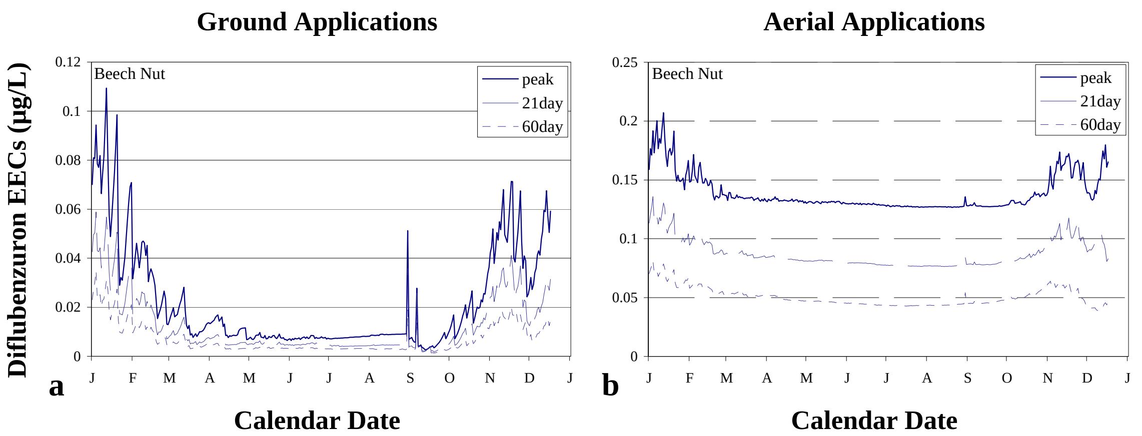


Figure 3-2. Variation in diflubenzuron EECs (peak, 21-day, and 60-day) as function of application date (first application date for scenarios with multiple applications).

However, even for those uses for which the PUR data suggest a specific date is typically used for diflubenzuron applications (as in Figure 3-1), it is important to consider that diflubenzuron is applied over a range of dates. In Figure 3-3, the distribution of PUR data application dates is compared to the diflubenzuron EECs for each first application date. According to the PUR data, March 2nd is the most typical application date, while January 25th generates the highest EECs. It is important to consider that diflubenzuron is applied on the date that yields the highest EECs, but because of the procedure used to select application dates, application dates surrounding March 2nd were used in this assessment. Appendix D, Figure D5, provides similar graphs for all of the diflubenzuron uses for which PUR data were available.

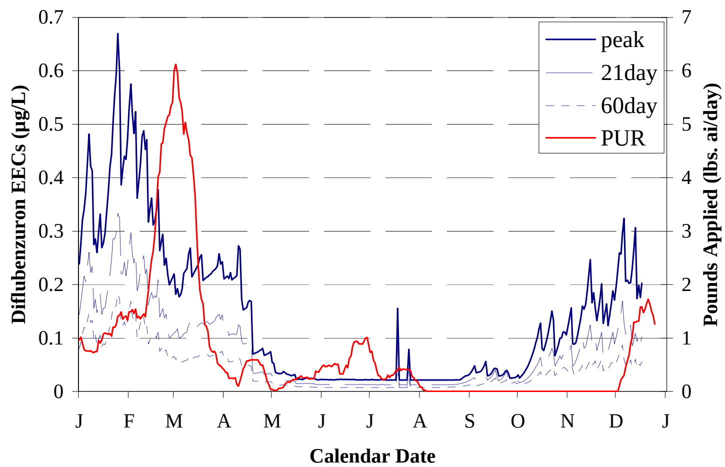


Figure 3-3. Variation in diflubenzuron EECs (peak, 21-day, and 60-day) as function of application date (first application date for scenarios with multiple applications) compared to variation in the amount of diflubenzuron applied (lbs. ai/day) per day in California for fruit use.

3.2.4. Existing Monitoring Data

A critical step in the process of characterizing EECs is comparing the modeled estimates with available surface water monitoring data. EFED was unable to locate diflubenzuron monitoring for surface and groundwater and atmospheric data (Fellers *et al* 2004, LeNoir *et al* 1999, and McConnell *et al* 1998). The sources checked included the USGS National Water-Quality Assessment (NAWQA) Program and California Department of Pesticide Regulation (CDPR) data sets.

3.3. Terrestrial Animal Exposure Assessment

T-REX (Version 1.4.1) is used to calculate dietary and dose-based EECs of diflubenzuron for the CRLF and its potential prey (*e.g*. small mammals and terrestrial insects) inhabiting terrestrial areas. EECs used to represent the CRLF are also used to represent exposure values for frogs serving as potential prey of CRLF adults.

Terrestrial EECs for foliar formulations of diflubenzuron were derived for the uses summarized in Table 3-4. Foliar dissipation half-lives were reported in Willis and McDowell (1987) for diflubenzuron of 12, 27, and 35 days. Therefore, a 35-day half-life was used in this assessment. Use specific input values, including number of applications, application rate and application interval are provided in Table 3-4.

Table 3-4 Input Parameters for Foliar Applications Used to Derive Terrestrial EECs for Diflubenzuron with T-REX

Scenario Name[a]	Maximum Application Rate (lbs/A)			Number of Applications (Re-treatment Interval in Days)		
	Aerial	Ground	Other	Aerial	Ground	Other
Barnyard/Mushroom			8.508			17 (21)
Beech nut	0.0408	0.0408		2.3 (7)	2.3 (7)	
Brassica		0.0313			1 (NA)	
Citrus	0.125	0.3125		3 (7)	3 (21)	
Cole Crop		0.25			4 (21)	
Cotton	0.3125	0.3125		3 (90)	3 (7)	
Forest	0.25	0.22048		1 (NA)	NS[1] (14)	
Fruit	0.3125	0.3125		3 (7)	3 (7)	
General Nuts	0.3125	0.3125		3 (90)	3 (7)	
Grains	0.125	0.25		3 (NS[2])	4 (21)	
Nursery	0.25	0.25		1 (NA)	1 (NA)	
Pasture	0.25	0.25		1 (NA)	1 (NA)	
Pistachio		0.75			1.333 (14)	
Residential	0.25	0.25		1 (NA)	6 (10)	
Rice/Ornamental Pond	0.0625			6 (5)		
Rights-of-way	0.25	0.25		1.5 (5)	1.5 (5)	
Row Crop	0.3125	0.3125		3 (14)	3 (21)	
Squash	0.25	0.25		NS[3] (15)	4 (15)	
Turf (High App. Rate)	0.25	0.25		1.5 (5)	1.5 (14)	
Turf (Low App. Rate)	0.0313	0.0313		3 (7)	3 (7)	
Turnip		0.25			2 (21)	

NA = Not applicable (there is no re-treatment interval if only 1 application is allowed).
NS = Not specified on any label.
[a]No terrestrial assessment was performed for *bait, pond,* and *soybean* exposure scenarios (see text).
[1]The number of applications was assumed to be 4 as a conservative assumption because 4 applications were the most applications allowed on any non-residential label.
[2]A re-treatment interval of 21 days is assumed because 21 days is the smallest re-treatment interval allowed on the ground application to grains label.
[3]The number of applications was assumed to be 4 as a conservative assumption because 4 applications were the most applications allowed on any non-residential label.

T-REX is also used to calculate EECs for terrestrial insects exposed to diflubenzuron. Dietary-based EECs calculated by T-REX for small and large insects (units of a.i./g) are used to bound an estimate of exposure to terrestrial insects. Available acute contact toxicity data for bees exposed to diflubenzuron (in units of µg a.i./bee), are converted to µg a.i./g (of bee) by multiplying by 1 bee/0.128 g. The EECs are later compared to the adjusted acute contact toxicity data for bees in order to derive RQs.

For modeling purposes, exposures of the CRLF to diflubenzuron through contaminated food are estimated using the EECs for the small bird (20 g) which consumes small insects. Dietary-based and dose-based exposures of potential prey are assessed using the small mammal (15 g) which consumes short grass. Upper-bound Kenega nomogram values reported by T-REX for these two organism types are used for derivation of EECs for the CRLF and its potential prey (Table 3-5). Dietary-based EECs for small and large insects reported by T-REX as well as the resulting adjusted EECs are available in Table 3-6.

Table 3-5. Upper-bound Kenega Nomogram EECs for Dietary- and Dose-based Exposures of the CRLF and its Prey to Diflubenzuron.

Use	EECs for CRLF (small insect food item)		EECs for Prey (short grass food item) (small mammals)	
	Dietary-based EEC (ppm)	Dose-based EEC (mg/kg-bw)	Dietary-based EEC (ppm)	Dose-based EEC (mg/kg-bw)
Barnyard/Mushroom	3369	3840.66	5990	5690.5
Beech nut	10.3	11.742	18.32	17.404
Brassica	4.2	4.788	7.5	7.125
Citrus	88	100.32	157	149.15
Cole Crop	80	91.2	143	135.85
Cotton	111	126.54	197	187.15
Forest	34	38.76	60	57
Fruit	111	126.54	197	187.15
General Nuts	111	126.54	197	187.15
Grains	80	91.2	143	135.85
Nursery	34	38.76	60	57
Pasture	34	38.76	60	57
Pistachio	101	115.14	180	171
Residential	131	149.34	232	220.4
Rice/Ornamental Pond	40	45.6	71	67.45
Rights-of-way	34	38.76	60	57
Row Crop	88	100.32	157	149.15
Squash	91	103.74	162	153.9
Turf (High App. Rate)	34	38.76	60	57
Turf (Low App. Rate)	11	12.54	20	19
Turnip	56	63.84	100	95

Table 3-6 EECs (ppm) for Indirect Effects to the Terrestrial-Phase CRLF via Effects to Terrestrial Invertebrate Prey Items

Use	Small Insect	Large Insect
Barnyard/Mushroom	3369	374
Beech nut	10.3	1.1
Brassica	4.2	0.47
Citrus	88	9.8
Cole Crop	80	8.9
Cotton, fruit, nuts	111	12
Forest	34	3.8
Grains	80	8.9
Nursery	34	3.8
Pasture	34	3.8
Pistachio	101	11
Residential	131	15
Rice/Ornamental Pond	40	4.4
Rights-of-way	34	3.8
Row Crop	88	9. 8
Squash	91	10
Turf (High App. Rate)	34	3.8
Turf (Low App. Rate)	11	1.2
Turnip	56	6.2

3.5 Terrestrial Plant Exposure Assessment

Exposures to terrestrial plants were not quantified because no guideline toxicity data are available for diflubenzuron. Therefore, RQs were not calculated for terrestrial plants. See Section 5.2 for discussion of potential risks to terrestrial plants in the context of this effects determination.

4.0 Effects Assessment

This assessment evaluates the potential for diflubenzuron to directly or indirectly affect the CRLF or modify its designated critical habitat. As previously discussed in Section 2.7, assessment endpoints for the CRLF effects determination include direct toxic effects on the survival, reproduction, and growth of CRLF, 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 the CRLF. Direct effects to the aquatic-phase of the CRLF are based on toxicity information for freshwater fish because no aquatic phase amphibian data have been submitted or were located in the open literature. Terrestrial-phase effects assessments are based on avian toxicity data because no open literature or registrant-submitted studies on terrestrial phase amphibians were located. Because the frog's prey items and habitat requirements are dependent on the availability of freshwater fish and invertebrates, small mammals, terrestrial invertebrates, and aquatic and terrestrial plants, toxicity information for these taxa are also discussed. 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 diflubenzuron.

As described in the Agency's Overview Document (U.S. EPA 2004), the most sensitive endpoint for each taxon is used for risk estimation. For this assessment, evaluated taxa include aquatic-phase amphibians, freshwater fish, freshwater invertebrates, aquatic plants, birds (surrogate for terrestrial-phase amphibians), mammals, terrestrial invertebrates, and terrestrial plants.

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) (U.S. EPA 2004). A complete list of all available registrant-submitted studies is in Appendix H. Open literature data presented in this assessment were obtained from the ECOTOX database searched on April 28, 2009. 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;[5]
(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.

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.*, maintenance of CRLF 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 to define the action area for diflubenzuron.

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 G1 to G4. Appendix G also includes a rationale for rejection of those studies that did not pass the ECOTOX screen

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[5] The studies that have information on mixtures are listed in the bibliography as rejected due to the presence of mixtures. These studies are evaluated by EFED when applicable to the assessment; however, the data is not used quantitatively in the assessment.

and those that were not evaluated as part of this endangered species risk assessment. Rejection Codes are described in Appendix I.

A detailed spreadsheet of the available ECOTOX open literature data, including the full suite of lethal and sublethal endpoints is presented in Appendix G1. Appendix E includes a summary of the human health effects data for diflubenzuron.

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 the Ecological Incident Information System (EIIS), are conducted to further refine the characterization of potential ecological effects associated with exposure to diflubenzuron. A summary of the available aquatic and terrestrial ecotoxicity information, use of the probit dose response relationship, and the incident information for diflubenzuron are provided in Sections 4.3 through 4.4 respectively. A detailed summary of the available registrant-submitted ecotoxicity information is presented in Appendix A, and open literature information is presented in Appendix G.

4.1. Evaluation of Aquatic Ecotoxicity Studies

Table 4-1 summarizes the most sensitive aquatic toxicity endpoints for the CRLF, 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 CRLF is presented below. Additional information is provided in **Appendix A and Appendix G.**

Table 4-1 Freshwater Aquatic Toxicity Profile for Diflubenzuron

Freshwater Aquatic Toxicity Profile for Diflubenzuron (TGAI)				
Assessment Endpoint	**Surrogate Species**	**Toxicity Value Used**	**Source Citation**	**Study Classification**
Acute Toxicity to Fish and Aquatic-phase Amphibians	Bluegill sunfish (*Lepomis macrochirus*)	129 mg/L (CI: 116 to 142) Slope: 4.7 (3.5 to 5.9)	MRID 00056150	Supplemental Note 1
Chronic Toxicity Fish and Aquatic-phase Amphibians	Fathead minnow	NOAEC = 0.100 mg/L a.i. LOAEC = >0.100 mg/L a.i.	MRID 00099755	Acceptable Note 2
Acute Toxicity to Aquatic Invertebrates	2nd instar *Aedes albopictus* (mosquito larvae)	EC_{50} was 0.0028 µg/L Slope: Not available	. Ho et al. (1987) (Open literature study:ECOTOX # 16591)	Quantitative Note 3
Chronic Toxicity to Aquatic Invertebrates	Water flea *(Daphnia magna)*	NOAEC = 0.00025 µg/L	Kashian et al (2002) (Open literature study; ECOTOX 93397)	Quantitative Note 4
Acute Toxicity to non-vascular Aquatic Plants	Green algae (*Selanustrum capricornutum*)	EC_{50} = > 0.20 mg/L a.i. NOAEL = 0.20 mg/L a.i.	45252205	Supplemental Note 5
Acute Toxicity to vascular Aquatic Plants	Duckweed	EC_{50} = >0.190 mg/L a.i. NOAEC = 0.190 mg/L	MRID 42940103	Supplemental Note 6

Note 1: The available data evaluation record does not specify whether the LC_{50} was based on the mean measured or nominal concentration. It is important to note that given the persistence of the compound, nominal concentrations could potentially underestimate the toxicity.
Note 2: Although the data evaluation record (DER) deems this study as acceptable, the DER does not specify whether the LC_{50} was based on the mean measured or nominal concentration. It is important to note that given the persistence of the compound, nominal concentrations could potentially underestimate the toxicity.
Note 3: The study Authors do not specify whether the EC_{50} was based on the mean measured or nominal concentration. It is important to note that given the persistence of the compound, nominal concentrations could potentially underestimate the toxicity.
Note 4: The NOAEC is based on the nominal concentration. It is important to note that given the persistence of the compound, nominal concentrations could potentially underestimate the toxicity.
Note 5: The study was deemed supplemental because the test procedures deviated from guidelines in the following manner: The study was not continued after three days because of an unexpected high growth of the algae, which caused the pH to increase to a value of 9 after 3 days. Additionally the NOAEC is based on the nominal concentration. It is important to note that given the limited persistence of the compound, nominal concentrations could potentially underestimate the toxicity.
Note 6: The study was deemed supplemental because 20% of the initial measured concentration. Based on the mean measured concentration of 190 µg/l, growth of *L. gibba* exposed to diflubenzuron was not reduced in comparison to the solvent control over the 14-day study period.

Toxicity to fish and aquatic invertebrates is categorized using the system shown in Table 4-2 (U.S. EPA 2004). Toxicity categories for aquatic plants have not been defined.

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

LC_{50} (ppm)	Toxicity Category
< 0.1	Very highly toxic
> 0.1 - 1	Highly toxic
> 1 - 10	Moderately toxic

LC_{50} (ppm)	Toxicity Category
> 10 - 100	Slightly toxic
> 100	Practically nontoxic

4.1.1. Toxicity to Freshwater Fish

Given that no diflubenzuron toxicity data are available for aquatic-phase amphibians, freshwater fish data were used as a surrogate to estimate direct acute and chronic risks to the CRLF. Freshwater fish toxicity data were also used to assess potential indirect effects of diflubenzuron to the CRLF from reduction in available food. As discussed in Section 2.5.3, over 50% of the prey mass of the CRLF may consist of vertebrates such as mice, frogs, and fish (Hayes and Tennant 1985).

A summary of acute and chronic freshwater fish data, including data from the open literature, is provided below in Sections 4.1.1.1 through 4.1.1.3.

4.1.1.1. Freshwater Fish: Acute Exposure (Mortality) Studies

There are numerous registrant submitted freshwater fish acute toxicity studies testing the technical grade active ingredient of diflubenzuron. These studies tested 7 different species of freshwater fish and demonstrate that the technical grade active ingredient of diflubenzuron is slightly to practically non-toxic to freshwater fish. The LC_{50} from these studies range from 129 ppm to >500 ppm. Further details about these studies are provided in Appendix A. The most sensitive, acceptable, freshwater fish LC_{50} of 129 ppm (MRID 00056150) will be use to assess the acute risk of the technical grade active ingredient to the aquatic-phase CRLF and to freshwater fish prey of the CRLF. Appendix A provides further details regarding all the fish acute toxicity studies of the TGAI.

There are 16 registrant submitted studies testing several formulated products of diflubenzuron. The toxicity results of these studies range from slightly to practically nontoxic (LC_{50}= 57 ppm to >1000 ppm), which suggests that the toxicity of formulated products are not dramatically greater than the toxicity of the technical grade material. Further details about these studies are also provided in Appendix A. Potential risks from exposure to formulated products were not quantified as discussed in Section 2. No studies were located in the open literature that reported LC_{50}s that were lower than those reported in the registrant-submitted studies (see Appendix G).

Degradate toxicity data are summarized in Appendix A. These data suggest that PCA is more toxic than diflubenzuron to fish with LC_{50} values ranging from 2 mg/L to 23 mg/L. DFBA and PCPU appear to have similar toxicity relative to parent diflubenzuron. As discussed in Section 2, potential risks from degradates were not quantified, and were considered to have negligible impact on conclusions of this assessment.

4.1.1.2. Freshwater Fish: Chronic Exposure (Early Life Stage and Reproduction) Studies

There is one available registrant submitted freshwater fish chronic toxicity study (Appendix A). Based on the results of this study, there were no effects to survival, reproduction, or growth at concentrations up to 0.1 mg/L in a full life-cycle study (egg to egg exposure). The LOAEC was > 0.1 mg a.i./L and the NOAEC was 0.1 mg a.i./L (MRID 00099755). The NOAEC of 0.1 mg/L will be used to assess the chronic risk of diflubenzuron to the aquatic-phase CRLF and to freshwater fish prey of the CRLF since this endpoint is the most sensitive chronic toxic endpoint available for freshwater fish. However, because a LOAEC was not established (no effects occurred at any test concentration), use of 0.1 mg a.i./L as a chronic endpoint is likely conservative.

4.1.1.3. Freshwater Fish: Sublethal Effects and Additional Open Literature Information

The available freshwater fish toxicity data do not indicate that diflubenzuron caused sublethal effects to freshwater fish at levels below those used to calculate RQs in this assessment.

Currently, there are no available guideline acute or chronic aquatic-phase amphibian toxicity studies testing diflubenzuron. However, one field study was located in ECOTOX in Pacific chorus frogs that did not observe any effects at 0.28 kg a.i./hectare (0.25 lbs a.i./Acre) (Ecotox No. 111232). No other test levels were evaluated, and a LOAEC was not established. Therefore, fish are used in this assessment as a surrogate for aquatic phase amphibians.

4.1.2. Toxicity to Freshwater Invertebrates

Freshwater aquatic invertebrate toxicity data were used to assess potential indirect effects of diflubenzuron to the CRLF via prey reduction. As discussed in Section 2.5.3, the main food source for juvenile aquatic- and terrestrial-phase CRLFs is thought to be aquatic invertebrates found along the shoreline and on the water surface, including aquatic sowbugs, larval alderflies, and water striders.

A summary of acute and chronic freshwater invertebrate data, including data published in the open literature, is provided below in Sections 4.1.2.1 through 4.1.2.3. Diflubenzuron is a chitinase inhibitor, impairing the ability of insects to synthesize a critical component of their exoskeleton (U.S. EPA, 1997). Chitin synthesis is particularly important in the early life stages of insects, as they molt and form a new exoskeleton in various growth stages. Thus, aquatic guideline tests, which typically run for 48 hours for the aquatic invertebrate (*Daphnia magna*), may not capture a molting stage, and are not an appropriate “most sensitive” acute endpoint for assessments. Endpoints derived from studies that test the toxic effects of diflubenzuron on the larval/molting stages of freshwater invertebrates and that test the chronic exposure of freshwater invertebrates to diflubenzuron more appropriately assess the toxicity of this type of chemical.

Diflubenzuron is fairly persistent in both aquatic and terrestrial environments. For this reason, data that evaluated the toxic effects of diflubenzuron at life stages (larval/molting stages) most vulnerable to this chemical were used as both acute and chronic endpoints for aquatic invertebrate RQs. Results from acute studies are also presented. Likewise, guideline tests on honeybees frequently run for only 48 hours and are conducted on adult bees, and, therefore, may not reflect the toxicity of diflubenzuron.

4.1.2.1. Freshwater Invertebrates: Acute Exposure (Mortality) Studies from Registrant-Submitted Studies

There are 8 registrant submitted freshwater invertebrate aquatic toxicity studies testing the technical grade diflubenzuron. Based on the registrant submitted freshwater invertebrate toxicity studies diflubenzuron is very highly toxic to slightly toxic to freshwater invertebrates (EC_{50} ranges from 0.0026 ppm to > 100 ppm). Further details about these studies are provided in Appendix A.

There are also several diflubenzuron formulated product studies (Appendix A). The toxicity values of these studies range from 1300 µg/L to >1,000,000 µg/L. These data suggest that the toxicity of the formulated products is not greater than toxicity of the technical grade material.

Degradate toxicity data are also summarized in Appendix A. The degradates were orders of magnitude less toxic to invertebrates than parent diflubenzuron. For example, LC_{50}s in midge for PCA and DFBA are 43 mg/L and >100 mg/L, respectively, compared with an LC_{50} of 0.07 mg/L for parent chemical in midge (See Appendix A for additional details).

4.1.2.2. Freshwater Invertebrates: Chronic Exposure (Reproduction) Studies from Registrant-Submitted Studies

Registrant-submitted chronic studies in aquatic invertebrates are summarized in Appendix A. The most sensitive endpoint produced in these studies was a NOAEL of 0.05 µg/L and a LOAEL of 0.075 µg/L. In registrant submitted studies, no sublethal effects were observed at levels lower than the NOAEC used to calculate RQs. There is uncertainty in the most sensitive NOAEC because diflubenzuron was not detected in 4 of the 7 test samples analyzed. Therefore, the mean measured values assumed a test concentration of the detection limit when measured values were below the detection limit. Appendix A contains more detail on registrant-submitted studies. Registrant-submitted toxicity studies were not used to calculate RQs because an open literature study was located that produced a lower toxicity value. See Section 4.1.2.3.

4.1.2.3. Freshwater Invertebrates: Sublethal Effects and Open Literature Data

Several open literature studies have been identified that produced more sensitive endpoints than any of the registrant submitted studies. Ho et al. (1987) reported 30% and 44% mortality in 2nd instar *Aedes albopictus* (mosquito larvae) at diflubenzuron concentrations of 0.00000025 mg/L (0.00025 µg/L) and 0.0000025 mg/L (0.0025 µg/L), respectively, in a 1 day study. Pairwise comparisons between control and treated groups were not made; however, no larval mortality occurred in controls. The EC_{50} was 0.0000028 mg/L (0.0028 µg/L) in this study as determined by probit analysis. A concentration dependent reduction in normal pupation and adult emergence was observed in this study in 2nd, 3rd, and 4th instar animals, with younger animals being more sensitive. A number of sublethal effects were observed in this study including physical abnormalities and biochemical changes. However, these sublethal effects were only observed at levels that were also associated with increased mortality. The EC_{50} of 0.0000028 mg/L will be used to calculate the acute risk of diflubenzuron to aquatic invertebrates because this value is based on a larval/molting stage of mosquito and because it is the most sensitive freshwater invertebrate acute toxicity value.
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In addition, Kashian et al (2002) observed significant ($p<0.05$) reduction in survival at diflubenzuron concentrations of 0.01 µg/L (NOAEC = 0.00001 mg/L) in a 6-day semi-static study in *daphnia magna* (40 per test level, replicated test). Test concentrations were not measured; however, the study was replicated. Mortality rate was approximately 30% at 0.01 µg/L (estimated from Figure 2).

The toxicity of 0.00025 µg/L from Ho et al. (1987) will be used to calculate the chronic risk of diflubenzuron to aquatic invertebrates because it is the most sensitive toxicity value available for freshwater invertebrates impacting vulnerable life stage that this chemical is expected to impact.

4.1.3. Toxicity to Aquatic Plants

Aquatic plant toxicity studies were used as one of the measures of effect to evaluate whether diflubenzuron may affect primary production and the availability of aquatic plants as food for CRLF tadpoles. Primary productivity is essential for indirectly supporting the growth and abundance of the CRLF.

Two types of studies were used to evaluate the potential of diflubenzuron to affect aquatic plants. Laboratory and field studies were used to determine whether diflubenzuron may cause direct effects to aquatic plants. A summary of the laboratory data and freshwater field studies for aquatic plants is provided in Sections 4.1.3.1 and 4.1.3.2

4.1.3.1. Aquatic Plants: Laboratory Data

There are four available aquatic plant toxicity studies including three nonvascular aquatic plant studies and 1 vascular plant study. In all of these studies the EC_{50} values were greater than the highest diflubenzuron concentrations tested. The most sensitive endpoints are summarized in the following table and produced an EC_{50} and NOAEL of >0.20 ppm and 0.20, respectively, in algae and duckweed (MRID 45252205 and 42940103). Since these are the most sensitive endpoints, these values will be used to assess the risk of diflubenzuron to aquatic plants. Appendix A provides further details regarding these studies.

Freshwater Aquatic Toxicity Profile for Diflubenzuron (TGAI)				
Assessment Endpoint	**Surrogate Species**	**Toxicity Value Used**	**Source Citation**	**Study Classification**
Acute Toxicity to non-vascular Aquatic Plants	Green algae (*Selanustrum capricornutum*)	EC_{50} = > 0.20 mg/L a.i. NOAEL = 0.20 mg/L a.i.	45252205	Supplemental [Note 1]
Acute Toxicity to vascular Aquatic Plants	Duckweed	EC_{50} = >0.190 mg/L a.i. NOAEC = 0.190 mg/L	42940103	Supplemental [Note 2]

Note 1: The study was deemed supplemental because the test procedures deviated from guidelines in the following manner: The study was not continued after three days because of an unexpected high growth of the algae, which caused the pH to increase to a value of 9 after 3 days. Additionally the NOAEC is based on the nominal concentration. It is important to note that given the limited persistence of the compound, nominal concentrations could potentially underestimate the toxicity.
Note 2: The study was deemed supplemental because 20% of the initial measured concentration. Based on the mean measured concentration of 190 ug/l, growth of L. gibba exposed to diflubenzuron was not reduced in comparison to the solvent control over the 14-day study period.

4.1.4. Freshwater Field Studies

The USEPA Mid-Continent Ecology Division, Duluth, MN conducted a study of the biological effects, persistence, and distribution of diflubenzuron in littoral enclosures (MRID 44386201). The Duluth study investigated the distribution of diflubenzuron (water, sediments and biota) and effects (plants, invertebrates, and fish) of diflubenzuron applied to oligotrophic littoral enclosures (2 applications 30 days apart). The following table (4-3) describes the observed effects in this study.

Table 4-3. Diflubenzuron Effects Reported in a Littoral Enclosure Study (MRID 443862-01).

Endpoint	LOEC	NOEC	Comments
Aquatic plant Effects			
phytoplankton (chlorophyll a: numbers, and biovolume)	Not Applicable (highest dose tested produced no statistical effects)	30 µg/L	The littoral enclosures were determined by the study authors to be nutrient limited with respect to phytoplanktonic growth. Therefore, the experiment was suitable for measuring direct effects on phytoplankton by diflubenzuron only. It could not determine any indirect effects related to adverse impacts to herbivorous zooplankton, macroinvertebrates, insects, or fish.
periphyton (reduced biomass and chlorophyll a)	7 µg/L	2.5 µg/L	--
macrophytes (standing crop of submerged and floating plants, species composition)	Not Applicable (highest dose tested produced no statistical effects)	30 µg/L	--
decompos~t~ornat e of plant l~tter	Not Applicable (highest dose tested produced no statistical effects)	30 µg/L	--
Zooplankton Effects			
Cladocera (total numbers)	0.7 µg/L	0.6 µg/L	Though the effects observed at 0.7 µg/L were not statistically significant, they were very severe, with a reduction in total cladoceran population of >89%. 0 6 µg/L is the study authors' extrapolated MATC value
Copepoda (adults, juveniles, and total number)	0.7 µg/L	0.3 µg/L	At the lowest does tested, reductions in the numbers of adult, juvenile, and total copepod numbers reached 71%, 83%, and 81%, respectively. 0 3 µg/L is the study authors' extrapolated MATC value
Rotifera (total number)	Not Applicable (highest dose tested produced no statistical effects)		--
Ostracoda (total number)	7 µg/L	2.5 µg/L	The control variability was very large, thereby severely limiting the sensitivity of the test. Reductions in ostracods at the 7.0 and 30 µg/L doses were 59 to 98% lower than controls

Endpoint	LOEC	NOEC	Comments
Macroinvertebrate Effects			
Chironomidae abundance)	7 µg/L	2.5 µg/L	The high CV among treatment blocks severely limits the sensitivity of this endpoint. The LOEC for this endpoint equates to an approximate 50% reduction In Abundance.
Epherneroptera (abundance)	2.5 µg/L	0.7 µg/L	The high CV among treatment blocks severely limits the sensitivity of this endpoint. The LOEC for this endpoint equates to an approximate 50% reduction in Abundance
total adjusted macroinvertebrate community biomass	2.5 µg/L	0.7 µg/L	--
Mean macroinvertebrate taxa richness	0.7 µg/L	Not Applicable (lowest dose tested produced adverse effects)	--
Insect Emergence Effects			
Insect emergence (all insects)	0.7 µg/L	Not Applicable (lowest dose tested produced adverse effects)	The power of the test was severely limited by the number of replicates within treatments. Power analysis conducted by the study authors concluded that mean differences between treatments and control for insect emergence would have to be greater than 93% before the test would show statistically significant results. Emergence reductions of 17.7 to 29.9% were observed at the lowest concentration tested.
Chironomidae (emergence)	0.7 µg/L	Not Applicable (lowest dose tested produced adverse effects)	The power of the test was severely limited by the number of replicates within treatments. Power analysis conducted by the study authors concluded that mean differences between treatments and control for insect emergence would have to be greater than 93% before the test would show statistically significant Emergence reductions of 17. 4 to 31.1% were observed at the lowest concentration tested

Endpoint	LOEC	NOEC	Comments
Fish			
Bluegill sunfish young or the year (weight, length, growth rate)	2.5	0.7 µg/L	The authors concluded that young of the year bluegill growth rates were directly correlated to the density of several invertebrate taxa (notably Cladocera and Copepoda) that are preferred prey items for the species The authors concluded that the study clearly demonstrated the indirect effect of the pesticide on fish population without direct toxic effects on the fish
Indigenous fish (mean size, population numbers, biomass)	Not Applicable (highest dose tested produced no statistical effects)	30 µg/L	There is considerable uncertainty with respect to endpoints for indigenous fish. No measurements of effects variables were made immediately following treatments. Instead, measurements were made 70 days after application of the first treatment of pesticide and 37 days after the second application.

The results indicate that reductions in the abundance of zooplankton (particularly cladocerans and copepods) can be expected to occur at initial nominal concentrations lower than 0.7 µg/L. Macroinvertebrate biomass and abundance of selected insect families are reduced at initial nominal diflubenzuron concentrations greater than 0.7 µg/L. Aquatic insect emergence and the richness of macroinvertebrate taxa are reduced at concentrations of diflubenzuron at or below an initial nominal concentration 0.7 µg/L diflubenzuron. These impacts to the aquatic community were also observed to be correlated to reductions in the growth rate of introduced bluegill sunfish, with reductions in length, weight, and growth rate occurring at initial nominal concentrations of 2.5 µg/L and above. It is important to note that MRID 44386201 fails to empirically demonstrate a NOEC for a number of endpoints, namely effects on cladocerans, copepods, aquatic insect emergence and richness of macroinvertebrate taxa. Therefore, the study does not allow EFED to evaluate risks to these organisms for diflubenzuron concentrations below 0.7 µg/L with any certainty.

Results of several field studies and mesocosm studies were presented in MRID 44460702. The results of these other studies do not materially differ from results reported for the Duluth Study discussed above. Ali et al.(1 988) reported no observable effects on benthos and zooplankton in a pond adjacent to citrus treated with diflubenzuron. Concentrations were reported to range from a high of 0.197 pg/L immediately after diflubenzuron application to the orange groves to <0.027 pg/L by 14 days after pesticide application. Booth and Ferrell (1977) reported significant (up to 97%) reductions in aquatic invertebrates for up to 30 days in a pond with diflubenzuron at 36 µg/L immediately after treatment to trace (4 µg/L) after 23 days. Boyle et al. (1996) exposed mesocosms to 5 monthly or 9 biweekly treatments of diflubenzuron at a targeted rate of10 pg/L. The authors observed significant reductions in zooplankton and insect numbers, insect diversity, and growth of bass and bluegill recruit.

4.2. Toxicity of diflubenzuron to Terrestrial Organisms

Table 4-4 summarizes the most sensitive terrestrial toxicity endpoints used to evaluate toxicity of diflubenzuron to terrestrial phase CRLFs, 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 for the CRLF is presented below. Additional information can be found in Appendix A.

Table 4-4 Terrestrial Toxicity Profile for Diflubenzuron

Assessment Endpoint	Acute/ Chronic	Species	Toxicity Value Used in Risk Assessment	MRID	Classification
Acute Toxicity to Birds	Acute oral LD_{50}	Red-winged blackbird	LD_{50}= 3763 mg/kg a.i. bwt	MRID 00038614	Supplemental
	Acute dietary LC_{50}	Bobwhite quail	LC_{50} >4640 mg/kg a.i. bwt	MRID 00039080	Acceptable
Reproduction Toxicity to Birds	NOAEC:	Bobwhite quail	NOAEC= 500 mg/kg a.i. diet LOAEC= 1000 mg/kg a.i. diet	MRID 41668001	Supplemental
Acute Toxicity to Mammals	LD_{50}:	Rat	LD_{50}>5,000 mg/kg a.i. bwt	MRID 00157103	Acceptable
Reproduction Toxicity to Mammals	NOAEC:	Rat	NOAEC = 250 mg/kg-bw	MRID 43578301	Acceptable
Acute toxicity to Terrestrial Invertebrates	LD_{50}:	Honeybee	LD_{50} > 30 µg a.i./bee a.i.	MRID 05001991	Acceptable
Risk to Terrestrial Plants	No data submitted	N/A	N/A	N/A	N/A

Acute toxicity to terrestrial animals is categorized using the classification system shown in Table 4-5 (U.S. EPA 2004). Toxicity categories for terrestrial plants have not been defined.

Table 4-5 Categories of Acute Toxicity for Avian and Mammalian Studies

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

4.2.1. Toxicity to Birds

As specified in the Overview Document, the Agency uses birds as a surrogate for terrestrial-phase amphibians when amphibian toxicity data are not available (U.S. EPA

2004). No terrestrial-phase amphibian data have been submitted or were located in the open literature for diflubenzuron; therefore, acute and chronic avian toxicity data are used to assess the potential direct effects of diflubenzuron to terrestrial-phase CRLFs.

4.2.1.1. Birds: Acute Exposure (Mortality) Studies

Diflubenzuron is categorized as practically non-toxic to avian species on an acute oral toxicity basis. Studies in bobwhite quail and mallard duck did not produce toxic effects at any dose tested (MRID 00073935). The most sensitive study available produced an LD_{50} value of 3763 mg/kg-bw in the Red-Winged Blackbird (MRID 00038614). All three studies used technical grade material (Appendix A).

Using the TGAI, diflubnezuron is categorized as practically non-toxic to avian species on a subacute dietary toxicity basis based on an LC50 value of >4640 ppm for the bobwhite quail and mallard duck (MRID 00039080). A 1 % Granular formulation was also categorized as practically non-toxic to bobwhite quail and mallard ducks based on an LC50 value of >20,000 ppm (MRID 00060381). These studies were classified as "Acceptable". Appendix A provides further details regarding these studies.

4.2.1.2. Birds: Chronic Exposure (Growth, Reproduction) Studies

The most sensitive NOAEC from a reliable/verifiable study was 500 mg/kg-diet (MRID 4166800102). Other studies are available that produced lower NOAECs. However, these studies either did not produce a LOAEC (no effects were observed at any test concentration) or the NOAECs were considered unreliable. In particular, a NOAEC of <10 mg/kg-diet has been reported in a non-guideline study in mallard ducks (MRID 99862, Reinert ett al, 1975) based on a reduction in the number of eggs embryonated at 10 mg/kg-diet. However, this effect was not observed at higher test concentrations in the same study and has not been observed in more recent guideline studies. Therefore, a NOAEC of <10 mg/kg-diet was not considered reliable and was not chosen for use in risk estimation.

The NOAEC of 500 mg/kg-food was based on effects on eggshell thickness in mallard ducks and reduced egg production in bobwhite quail at 1000 mg/kg-diet. Further details about the avian reproduction studies are summarized in Appendix A.

4.2.2. Terrestrial-phase Amphibian Acute and Chronic Studies

No terrestrial-phase amphibian acute or chronic studies have been submitted by registrants or were located in the open literature.

4.2.3. Toxicity to Mammals

Mammalian toxicity data are used to assess potential indirect effects of diflubenzuron to the terrestrial-phase CRLF. Effects to small mammals resulting from exposure to diflubenzuron may also indirectly affect the CRLF via reduction in available food. As discussed in Section 2.5.3, over 50% of the prey mass of the CRLF may consist of vertebrates such as mice, frogs, and fish (Hayes and Tennant 1985).

4.2.3.1. Mammals: Acute Exposure (Mortality) Studies

The available mammalian acute toxicity data demonstrate that diflubenzuron is practically nontoxic to mammals (LD_{50} > 5000 mg/kg; MRID 00157103). Appendix A provides further details regarding this data.

4.2.3.2. Mammals: Chronic Exposure (Growth, Reproduction) Studies

In a 2-generation reproduction study technical grade diflubenzuron was administered in the diet to rats at dose levels of 0 (control), 500, 5000 or 50000 ppm (equivalent to about 0, 25, 250 or 2500 mg/kg/day).

No effects on reproductive performance were observed at any dose level in F0 or F1 males or females. Litter and mean pup weights decreased slightly from birth to 21 days postpartum in F1 offspring at 2500 mg/kg/day. The NOEL for reproductive performance in parental adults is 2500 mg/kg/day. The NOEL for developmental toxicity in progeny is 250 mg/kg/day and the LEL is 2500 mg/kg/day, based on decreased body weights in F1 pups from birth to 21 days postpartum. (MRID 43578301). A NOAEL of 250 mg/kg-bw is used in this assessment.

4.2.4. Toxicity to Terrestrial Invertebrates

Terrestrial invertebrate toxicity data are used to assess potential indirect effects of diflubenzuron to the terrestrial-phase CRLF. Effects to terrestrial invertebrates resulting from exposure to diflubenzuron may indirectly affect the CRLF via reduction in available food.

4.2.4.1. Terrestrial Invertebrates: Acute Exposure (Mortality) Studies

Guideline studies in honeybees have been submitted and accepted by the Agency. These tests produced acute oral and contact LD_{50}s of >30 µg/bee (MRID 05001991), resulting in a practically non-toxic classification. However, given the short duration of these tests (typically 48-72 hours), and the typical age of organisms used in the tests, it is unlikely that the contact / ingestion coincided with a molting stage, and, thus, the results may not accurately reflect the toxicity of diflubenzuron to terrestrial invertebrates.

A number of field studies regarding effects on terrestrial invertebrates were submitted to the Agency and reviewed in 1984 to 1985 (MRIDs 00099743, 000171816, 00071207, 00095416, 00071215, 00070179, 00071212, 00095407, and 00071210). The following information is based on the reviews of these studies, which evaluated a number of different organisms and exposure routes.

Three honey bee studies resulted in no effects on colonies near fields treated with application rates of up to 0.5 oz Dimilin W-25/A or 0.06 lbs a.i./A (MRIDs 0071816, 00099743, 00071212). Another study that exposed honey bees to 100 ppm ai in their water source determined that the exposure "almost eliminated production of a sealed brood" (MRID 0095407). A study on a stream receiving runoff from a treated field indicated that amphipod and aquatic beetle larvae populations were reduced and copepods and ostracods may also have been impacted" at an application rate of 0.08 lb ai/A (MRID 00071210). A study evaluating the effects of diflubenzuron (2.24 kg ai/ha) on soil arthropods when the chemical was incorporated into the soil showed some decrease in numbers of springtails at 2 months post-treatment and on soil mites at 6 weeks, but little effect on other soil organisms (MRID 0007121 5). Differences between control and treatment plots were not distinguishable at 4 months post-treatment. The Agency reviewer concluded diflubenzuron had "little adverse effect on the soil fauna." A study on beneficial arthropods showed some species were severely affected, while other species were not affected at all (MRID 00070179). Aerial application of granular Dimilin to salt marsh mosquito habitat significantly reduced numbers and diversity of aquatic invertebrates (MRID 00095416). Measured water concentrations of Dimilin (diflubenzuron) ranged from 0.24-1.8 µg/L. Based on a study on soil arthropods, Dimilin appears to have "little or no effect on soil microarthropods, but may adversely affect insect parasites" (MRID 007 1207).

Overall, studies on terrestrial invertebrates illustrate that the effect of diflubenzuron is highly dependent on the life stage of the organism when it is exposed. Diflubenzuron is very highly toxic to invertebrates that rely on chitin as an exoskeleton at the critical life stage (*i.e.*, a molting event). Available data are not sufficiently robust to estimate an assessment endpoint (e.g, LC50 or NOAEC) for this taxon, but adverse effects on non-target arthropods should be anticipated following use of diflubenzuron given its mode of action as an insecticide.

4.2.4.2. Terrestrial Invertebrates: Open Literature Studies

A number of open literature studies that evaluated effects to terrestrial invertebrates were identified in the ECOTOX database (Appendix G). These studies support the conclusions presented in Section 4.2.4.1 and do not add additional insight into the potential effects of diflubenzuron to terrestrial invertebrates (Ecotox Nos. 65290, 110971, and 111061).

4.2.5. Toxicity to Terrestrial Plants

Terrestrial plant toxicity data are used to evaluate the potential for diflubenzuron to affect riparian zone and upland vegetation within the action area for the CRLF. Impacts to riparian and upland (*i.e.*, grassland, woodland) vegetation may result in indirect effects to both aquatic- and terrestrial-phase CRLFs, as well as modification to designated critical habitat PCEs via increased sedimentation, alteration in water quality, and reduction in upland and riparian habitat that provides shelter, foraging, predator avoidance and dispersal for juvenile and adult CRLFs.

Plant toxicity data from both registrant-submitted studies and studies in the scientific literature are typically reviewed for this type of assessment. Registrant-submitted studies are conducted under conditions and with species defined in EPA toxicity test guidelines. Sub-lethal endpoints such as plant growth, dry weight, and biomass are evaluated for both monocots and dicots, and effects are evaluated at both seedling emergence and vegetative life stages. Guideline studies generally evaluate toxicity to ten crop species. A drawback to these tests is that they are conducted on herbaceous crop species only, and extrapolation of effects to other species, such as the woody shrubs and trees and wild herbaceous species, contributes uncertainty to risk conclusions.

Commercial crop species have been selectively bred, and may be more or less resistant to particular stressors than wild herbs and forbs. The direction of this uncertainty for specific plants and stressors, including diflubenzuron, is largely unknown. Homogenous test plant seed lots also lack the genetic variation that occurs in natural populations, so the range of effects seen from tests is likely to be smaller than would be expected from wild populations.

Currently, there are no available guideline terrestrial plant toxicity data for use in this assessment. However, Cooper et al. (1990) did not observe differences in characteristics of vegetation when comparing plots treated with diflubenzuron to untreated controls. The study evaluated 10 circular 0.04 ha plots (5 treated and 5 untreated). Vegetative characteristics evaluated included percent litter cover, litter depth, percent herbaceous cover, percent shrub cover, percent canopy cover in each of 5 canopy height classes, percent deciduous canopy cover, percent coniferous canopy cover, percent total canopy cover, and number of trees and number of snags in 6 dbh classes. Diflubenzuron was applied at 0.06 lbs a.i./Acre.

Similarly, Seidel and Whitmore did not observe differences in vegetative characteristics including litter depth, number of saplings, number of 2 classes of tree snags, and presence of herbaceous growth, shrubs. Diflubenzuron was applied to test plots at 140 g/ha by air (0.12 lbs a.i./Acre).

In addition, a number of efficacy studies have been submitted to the Agency that also evaluated phytotoxic effects to target species. These studies have typically noted no or minimal phytotoxicity (See Appendix H for citations). Last, diflubenzuron is labeled for use on numerous commodoties without reports of incidents that have been clearly associated with difblubenzuron toxicity.

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

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

Probit slopes used in this analysis are summarized in Table 4-6.

Table 4-6. Probit slopes used for taxonomic groups evaluated in this assessment

Taxonomic Group	Probit Slope	95% CI	MRID	Species
Fish	4.7	3.5 to 5.9	00056150	Bluegill
Aquatic Invertebrates	0.41	0.3 to 0.5	Ho et al., 1987 (Ecotox No. 16591)	Mosquito larvae
Birds	Default of 4.5: Data do not allow for calculation of reliable slope	2 to 9	00038614	Red-winged blackbird
Mammals	Default of 4.5: No mortality occurred in acute mammal studies	2 to 9	00157103	Rat

4.4. Incident Database Review

One incident (I009846-002) had been reported for diflubenzuron when the EIIS database was queried (10/2/2009). Damage to oranges in Polk County, Florida was reported following direct application. Legality of the application was classified as registered use, and certainty that diflubenzuron caused the damage was rated as possible. However, the incident was also associated with petroleum distillates, which have a number of other terrestrial plant incidents associates with its use. Given that diflubenzuron has a long history of use on citrus trees without other incidents and that petroleum distillates has been associated with several similar incidents on terrestrial plants, it is more likely that the damage to oranges was caused by use of petroleum distillates and not by diflubenzuron.

A lack of reported incidents should not be taken as an indicator of little or no ecological effects, especially in the case of a chemical like diflubenzuron, which acts primarily to inhibit growth of insects. Insects and other invertebrates that are vulnerable to chitinase inhibitors are generally not a highly visible component of the ecosystem, and even large-scale die-offs may go unreported.

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 CRLF or for modification to its designated critical habitat from the use of diflubenzuron 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 CRLF or its designated critical habitat (*i.e.*, “no effect,” “likely to adversely affect,” or “may affect, but not likely to adversely affect”).

5.1. Risk Estimation

Risk is estimated by calculating the ratio of exposure to toxicity. This ratio is the risk quotient (RQ), which is then compared to pre-established acute and chronic levels of concern (LOCs) for each category evaluated (Appendix C). For acute exposures to the CRLF and its animal prey in aquatic habitats, as well as terrestrial invertebrates, the LOC is 0.05. For acute exposures to the CRLF and mammals, the LOC is 0.1. The LOC for chronic exposures to CRLF and its prey, as well as acute exposures to plants is 1.0.

Risk to the aquatic-phase CRLF is estimated by calculating the ratio of exposure to toxicity using 1-in-10 year EECs based on the label-recommended diflubenzuron usage scenarios summarized in Table 3-3 and the appropriate aquatic toxicity endpoint from Section 4. Risks to the terrestrial-phase CRLF and its prey (*e.g.* terrestrial insects, small mammals and terrestrial-phase frogs) are estimated based on exposures resulting from applications of diflubenzuron (Section 3) and the appropriate toxicity endpoint from Section 4.

5.1.1. Exposures in the Aquatic Habitat

5.1.1.1 Direct Effects to Aquatic-Phase CRLF

Direct effects to the aquatic-phase CRLF are based on EECs in the standard pond and the lowest acute toxicity value for freshwater fish. In order to assess direct chronic risks to the CRLF, 60-day EECs and the lowest chronic toxicity value for freshwater fish are used. Based on the RQ calculations none of the LOCs were exceeded for risk to freshwater water fish or aquatic phase amphibians except for the aerial use of diflubenzuron on rice crops (See Table 5-1). The LOC was exceeded for chronic risk to freshwater fish or aquatic phase amphibians for this use. Based on these results only the diflubenzuron aerial use on rice “may affect” aquatic-phase CRLFs. No LOCs were exceeded for other uses. Therefore, all other registered uses are expected to have “no effect” on the aquatic-phase CRLF. RQs presented in Table 5-1 are further discussed in the context of an effects determination in Section 5.2.

Table 5-1 Summary of Direct Effect RQ LOCs for the Aquatic-phase CRLF

Direct Effects to CRLF[a]	Use	Surrogate Species	Toxicity Value (mg/L)	EEC (mg/L)[b]	RQ	Probability of Individual Effect at ES LOC	Probability of Individual Effect at RQ
Chronic Direct Toxicity	Direct Application to rice	Fathead minnow	NOAEC = 0.100	60-day: 0.112	1.12[c]	Not calculated for chronic endpoints	
	Manure			0.027	0.27		
	All other uses			<0.015	<0.15		
Acute direct toxicity	All uses	All species tested	129	<0.11	<0.01	1 in 3.7E20 (1 in 7.8E11 to 1 in 5E30)	

[a] RQs associated with acute and chronic direct toxicity to the CRLF are also used to assess potential indirect effects to the CRLF based on a reduction in freshwater fish and frogs as food items.
[b] The highest EEC based on aerial use of diflubenzuron on rice paddies (see Table 3-3).
[c] RQ > chronic LOC of 1.0.

5.1.1.2. Indirect Effects to Aquatic-Phase CRLF via Reduction in Prey (non-vascular aquatic plants, aquatic invertebrates, fish, and frogs)

Non-vascular Aquatic Plants

Indirect effects of diflubenzuron to the aquatic-phase CRLF (tadpoles) via reduction in non-vascular aquatic plants in its diet or via habitat modification are based on peak EECs and the lowest toxicity value (EC_{50}) for aquatic non-vascular plants. Based on the RQ calculations there were no LOC exceedances of diflubenzuron to aquatic plants. Therefore, diflubenzuron is expected to have "no effect" to CRLFs by affecting non-vascular aquatic plants. These RQs are further discussed in the context of an effects determination in Section 5.2.

Table 5-2 Summary of RQs Used to Estimate Indirect Effects to the CRLF via Effects to Non-Vascular Aquatic Plants (diet of CRLF in tadpole life stage and habitat of aquatic-phase CRLF)

Uses	Toxicity Value (µg/L)	Peak EEC (µg/L)	Indirect effects RQ (food and habitat)
All Uses	200 (MRID 45252205)	<113	<0.6

Aquatic Invertebrates

Indirect acute effects to the aquatic-phase CRLF via effects to prey (invertebrates) in aquatic habitats are based on peak EECs in the standard pond and the lowest acute toxicity value for freshwater invertebrates. Due to the mode of action of diflubenzuron, results from chronic toxicity studies are also considered to be acute effects. Therefore, chronic RQs are calculated using peak EECs. However, chronic RQs are also presented using 21-day EECs.

A summary of the acute and chronic RQ values for exposure to aquatic invertebrates (as prey items of aquatic-phase CRLFs) is provided in Table 5-3. Based on RQ calculations diflubenzuron exceeds either the acute or chronic LOC for risk to freshwater invertebrates for all the modeled uses of diflubenzuron. The acute RQs ranged from 4 to 40,000 and the chronic LOC exceedances range from 40 to 450,000. Based on these results, diflubenzuron "may affect" CRLFs via reduction in freshwater invertebrates prey items. These RQs are further discussed in the context of an effects determination in Section 5.2.

Table 5-3 Summary of Acute and Chronic RQs Used to Estimate Indirect Effects to the CRLF via Direct Effects on Aquatic Invertebrates as Dietary Food Items (prey of CRLF juveniles and adults in aquatic habitats)

Scenario Group	Application Type	Water Column Peak EEC (µg/L)	Acute RQ Based on a 6-day LC50 of 0.0028 µg/L)	Chronic RQ Based on a 6-Day NOAEC of 0.00025 µg/L and a 21-Day EEC	Chronic RQ Based on a 6-Day NOAEC of 0.00025 µg/L and a peak EEC
Beech Nut	Ground	0.11	39.07	227.21	437.56*
	Air	0.21	73.94	521.28	828.16*
Brassica	Ground	0.07	23.49	156.96	263.12*
Citrus	Ground	0.24	87.48	393.05	979.76*
	Air	0.50	178.06	1204.76	1994.32*
Cole Crop	Ground	0.36	128.46	817.76	1438.72*
Cotton	Ground	0.15	54.34	429.96	608.60*
	Air	0.82	293.71	1874.28	3289.56*
Forest	Ground	0.58	205.99	1221.32	2307.08*
	Air	0.80	285.44	1786.60	3196.96*
Fruit	Ground	0.26	94.15	556.48	1054.48*
	Airblast	0.66	236.88	1702.68	2653.00*
	Air	1.36	485.86	3424.92	5441.60*
General Nuts	Ground	0.90	322.81	1859.68	3615.44*
	Airblast	1.26	450.56	2794.92	5046.24*
	Air	0.85	303.31	1965.88	3397.04*
Grains	Ground	1.34	477.32	3117.32	5346.00*
	Air	0.93	333.08	2084.80	3730.48*
Nursery	Ground	0.20	71.60	449.60	801.96*
	Air	0.65	231.78	1504.92	2595.88*
Pasture	Ground	0.12	41.64	302.73	466.40*
	Airblast	0.29	102.95	665.00	1153.04*
	Air	0.55	195.64	1003.56	2191.20*
Pistachio	Ground	0.15	52.45	331.80	587.40*
Residential	Ground	0.08	29.37	184.23	328.96*
	Air	0.40	143.71	1207.47	1609.50*
Rice/Ornamental Pond	Air	112.92	40327.13	451663.90	451663.90*
Rights-of-way	Ground	5.55	1981.66	12178.53	22194.60*
	Airblast	8.13	2903.57	17541.57	32520.00*
	Air	5.59	1997.41	12268.73	22371.00*
Row Crop	Ground	0.02	6.86	59.72	76.82*
	Air	1.15	409.57	3098.08	4587.20*
Urban	Air	34	12187.86	87924.00	136504.00*
Squash	Ground	1.15	409.11	2610.32	4582.00*
	Air	1.87	666.75	4736.00	7467.60*
Turf (High Application Rate)	Ground	0.01	3.60	24.39	40.26*
	Airblast	0.21	76.57	506.32	857.60*
	Air	0.61	216.57	1489.40	2425.56*
Turf (Low Application Rate)	Ground	0.02	7.67	32.30	85.88*
	Airblast	0.07	23.89	146.85	267.52*
	Air	0.13	46.49	326.01	520.68*
Turnip	Ground	0.72	256.26	1402.12	2870.12*

***** Exceeds the chronic LOC of 1 for risk to aquatic invertebrates

Fish and Frogs

Fish and frogs also represent potential prey items of adult aquatic-phase CRLFs. RQs associated with acute and chronic direct toxicity to the CRLF (Table 5-1) are used to assess potential indirect effects to the CRLF based on a reduction in freshwater fish and frogs as food items. Based on the RQ calculations none of the LOCs were exceeded for risk to freshwater fish or aquatic phase amphibians except for the aerial use of diflubenzuron on rice (See Table 5-1). The LOC was exceeded for chronic risk to freshwater fish for this use. These RQs are further discussed in the context of an effects determination in Section 5.2.

5.1.1.3. Indirect Effects to CRLF via Reduction in Habitat and/or Primary Productivity (Freshwater Aquatic Plants)

Indirect effects to the CRLF via direct toxicity to aquatic plants are estimated using the most sensitive non-vascular and vascular plant toxicity endpoints. Because there are no obligate relationships between the CRLF and any aquatic plant species, the most sensitive EC_{50} values, rather than NOAEC values, were used to derive RQs. Based on the RQ calculations there were no LOC exceedances for risk of diflubenzuron to aquatic plants. Therefore, the effects determination is "no effect" for all uses. These RQs are further discussed in the context of an effects determination in Section 5.2.

Table 5-4 Summary of RQs Used to Estimate Indirect Effects to the CRLF via Effects to Vascular Aquatic Plants (habitat of aquatic-phase CRLF)[a]

Uses	Peak EEC (µg/L)	EC50 (µg/L)	Indirect effects RQ (habitat)
All Uses	<113	190	<0.6
[a] RQs used to estimate indirect effects to the CRLF via toxicity to non-vascular aquatic plants are summarized in Table 5-2			

5.1.2. Exposures in the Terrestrial Habitat

5.1.2.1. Direct Effects to Terrestrial-phase CRLF

Potential direct acute effects to the terrestrial-phase CRLF are derived by considering dose- and dietary-based EECs modeled in T-REX for a small bird (20 g) consuming small invertebrates (Table 5-5) and acute oral and subacute dietary toxicity endpoints for avian species.

Potential direct chronic effects of diflubenzuron to the terrestrial-phase CRLF are derived by considering dietary-based exposures modeled in T-REX for a small bird (20g) consuming small invertebrates. Chronic effects are estimated using the lowest available toxicity data for birds. EECs are divided by toxicity values to estimate chronic dietary-based RQs. Subacute dietary based RQs were not calculated because the dietary toxicity data indicated that no toxicological effects were observed at dietary concentrations that were equivalent to the limit concentration for acute dietary toxicity.

Based on the acute and chronic RQ calculations there are only LOC exceedances for risks to the terrestrial-phase of the CRLF for use of diflubenzuron on barnyard manure. Thus, the preliminary determination is a "may effect" only for the use on barnyard manure and "no effect" for all other modeled uses. Table 5-5 lists only dose based acute RQs because no dietary LC_{50} study produced a definitive toxicity value (all LC_{50}s were above the limit dose).

Table 5-5 Summary of RQs Used to Estimate Direct Effects to the Terrestrial-phase CRLF

Model Use	Chronic RQ		Acute RQ	
	EEC	RQ	Dose-based EEC (based on 20-gram bird)	RQ
Barnyard (Manure)	3369	6.738*	3840.66	1.2**
Beech nut	10.3	0.0206	11.742	<0.01
Brassica	4.2	0.0084	4.788	0.00
Citrus	88	0.176	100.32	0.03
Cole Crop	80	0.16	91.2	0.03
Cotton	111	0.222	126.54	0.04
Forest	34	0.068	38.76	0.01
Fruit	111	0.222	126.54	0.04
General Nuts	111	0.222	126.54	0.04
Grains	80	0.16	91.2	0.03
Nursery	34	0.068	38.76	0.01
Pasture	34	0.068	38.76	0.01
Pistachio	101	0.202	115.14	0.04
Residential	131	0.262	149.34	0.05
Rice	40	0.08	45.6	0.01
Rights-of-way	34	0.068	38.76	0.01
Row Crop	88	0.176	100.32	0.03
Squash	91	0.182	103.74	0.03
Turf (High App. Rate)	34	0.068	38.76	0.01
Turf (Low App. Rate)	11	0.022	12.54	<0.01
Turnip	56	0.112	63.84	0.02

* Exceeds the chronic of LOC of 1.
** Exceeds the acute LOC of 0.5.

5.1.2.2. Indirect Effects to Terrestrial-Phase CRLF via Reduction in Prey (terrestrial invertebrates, mammals, and frogs)

a) <u>Terrestrial Invertebrates</u>

In order to assess the potential risks of diflubenzuron to terrestrial invertebrates, which are considered prey of CRLF in terrestrial habitats, the honey bee is used as a surrogate for terrestrial invertebrates. The toxicity value for terrestrial invertebrates is calculated by multiplying the lowest available acute contact LD_{50} of 30 µg a.i./bee by 1 bee/0.128g, which is based on the weight of an adult honey bee. EECs (µg a.i./g of bee) calculated by T-REX for small and large insects are divided by the calculated toxicity value for terrestrial invertebrates, which is 30 µg a.i./g of bee. Based on the RQ calculations, the modeled diflubenzuron uses except beechnut and brassica exceed the LOC for risks to terrestrial invertebrate (Table 5-6). Based on these results, the preliminary effects determination is a "may effect" for the potential of diflubenzuron to indirectly affect the CRLF by reducing terrestrial invertebrate prey. Section 5.2 further discusses these RQs and additional terrestrial invertebrate toxicity information in the context of this effects determination.

Table 5-6 Summary of RQs Used to Estimate Indirect Effects to the Terrestrial-phase CRLF via Direct Effects on Terrestrial Invertebrates as Dietary Food Items based on an LC50 of 234 ppm (30 µg per bee / 0.128 grams per bee)

Uses	Small Insects EEC	Small Insect RQ	Large Insects EEC	Large Insect RQ
Barnyard/Mushroom	3369	14.37	374	1.60
Beechnut	10.3	0.04	1.14	0.00
Brassica	4.2	0.02	0.47	0.00
Citrus	88	0.38	9.78	0.04
Cole	80	0.34	8.89	0.04
Cotton	111	0.47	12.33	0.05
Forest	34	0.15	3.78	0.02
Fruit	111	0.47	12.33	0.05
General Nut Crop	111	0.47	12.33	0.05
Grain	80	0.34	8.89	0.04
Impervious	34	0.15	3.78	0.02
Nursery	34	0.15	3.78	0.02
Pistachio	101	0.43	11.22	0.05
Residential	131	0.56	14.56	0.06
Rice/Ornamental Pond	40	0.17	4.44	0.02
Rights away	34	0.15	3.78	0.02
Row crops	88	0.38	9.78	0.04
Squash	91	0.39	10.11	0.04
Turf (high application rate)	34	0.15	3.78	0.02
Turf (low application rate)	11	0.05	1.22	0.01
Turnip	56	0.24	6.22	0.03

b) Mammals

Risks associated with reduction of small mammals as prey by large terrestrial-phase CRLFs are derived for dietary-based and dose-based exposures modeled in T-REX for a small mammal (15g) herbivore and insectivore. Acute and chronic effects are estimated using the most sensitive mammalian toxicity data. EECs are divided by the toxicity value to estimate acute and chronic dose-based RQs as well as chronic dietary-based RQs. There were no mortalities in the mammalian acute toxicity studies at the limit dose of 5000 mg/kg-bw; therefore, acute RQs were not calculated and risk was presumed to be below levels that could result in indirect effects to CRLFs. Based on the chronic RQ calculations, there are no chronic LOC exceedances for risk to small mammal prey items except for the barnyard/mushroom use (Table 5-7). Based on these results, the preliminary effects determination is "no effect" regarding potential risks to the CRLF from reduction in mammalian prey except for the barnyard/mushroom use. The chronic RQ is 10 for the barnyard/mushroom use. Therefore, the preliminary effects determination for this use is “may effect.” These RQs are further discussed in the context of an effects determination in Section 5.2.

Table 5-7 Summary of Chronic RQs Used to Estimate Indirect Effects to the Terrestrial-phase CRLF via Direct Effects on Small Mammals as Dietary Food Items

Model Use	Chronic RQ	
	EEC	**RQ**
Barnyard/Mushroom	5690.5	**10.37 (exceeds LOC of 1.0)**
Beech nut	17.404	0.03
Brassica	7.125	0.01
Citrus	149.15	0.27
Cole Crop	135.85	0.25
Cotton	187.15	0.34
Forest	57	0.10
Fruit	187.15	0.34
General Nuts	187.15	0.34
Grains	135.85	0.25
Nursery	57	0.10
Pasture	57	0.10
Pistachio	171	0.31
Residential	220.4	0.40
Rice/Ornamental Pond	67.45	0.12
Rights-of-way	57	0.10
Row Crop	149.15	0.27
Squash	153.9	0.28
Turf (High App. Rate)	57	0.10
Turf (Low App. Rate)	19	0.03
Turnip	95	0.17

c) Amphibians

An additional prey item of the adult terrestrial-phase CRLF is other amphibians. In order to assess risks to these organisms, dietary-based and dose-based exposures modeled in T-REX for a small bird (20g) consuming small invertebrates are used. See Section 5.1.2.1 and associated table (Table 5-5) for results. Based on LOC exceedances for the barnyard/mushroom use, this labeled use of diflubenzuron may affect the CRLF. Avian LOCs were not exceeded for any other use; therefore, the preliminary effects determination for diflubenzuron for all other uses is “no effect.” These RQs are further discussed in the context of an effects determination in Section 5.2.

5.1.2.3. Indirect Effects to CRLF via Reduction in Terrestrial Plant Community (Riparian and Upland Habitat)

Potential indirect effects to the CRLF resulting from direct effects on riparian and upland vegetation are assessed using RQs from terrestrial plant seedling emergence and vegetative vigor EC_{25} data as a screen. Currently there are no available guideline terrestrial plant toxicity data. Therefore, RQs were not calculated. Additional lines of evidence including open literature studies and non-guideline registrant-submitted studies regarding potential risks to terrestrial plants in the context of an effects determination for the CRLF are described in Section 5.2.

5.1.3. Primary Constituent Elements of Designated Critical Habitat

For diflubenzuron use, the assessment endpoints for designated critical habitat PCEs involve a reduction and/or modification of food sources necessary for normal growth and viability of aquatic-phase CRLFs, and/or a reduction and/or modification of food sources for terrestrial-phase juveniles and adults. Because these endpoints are also being assessed relative to the potential for indirect effects to aquatic- and terrestrial-phase CRLF, the effects determinations for indirect effects from the potential loss of food items are used as the basis of the effects determination for potential modification to designated critical habitat. As discussed in Sections 5.1.3 and 5.2.4, effects to aquatic and terrestrial plants are not expected to occur.

However, one PCE is "alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source." To assess the impact of diflubenzuron on this PCE (*i.e.*, alteration of food sources), acute and chronic freshwater fish and invertebrate toxicity endpoints, as well endpoints for aquatic non-vascular plants, are used as measures of effects. RQs for these endpoints were calculated in Sections 5.1.1.1 and 5.1.1.2. Based on LOC exceedances for aquatic invertebrates, diflubenzuron may modify aquatic-phase PCEs of designated critical habitat related to effects of alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.

Two terrestrial phase PCEs are not evaluated using solely terrestrial plant toxicity data. The PCE of "reduction and/or modification of food sources for terrestrial phase juveniles and adults" is evaluated using acute and chronic toxicity endpoints for birds, mammals, and terrestrial invertebrates as measures of effects. RQs for these endpoints were calculated in Section 5.1.2.2. Diflubenzuron has the potential for modifying critical habitat via this PCE because as an insecticide, terrestrial invertebrates are likely to be affected if diflubenzuron is used near critical habitat.

The fourth terrestrial-phase PCE is based on alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source. Based on potential impacts to terrestrial invertebrates for all assessed uses, diflubenzuron may impact critical habitat by altering chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs for all uses assessed.

5.2. Risk Description

The risk description synthesizes an overall conclusion regarding the likelihood of adverse impacts leading to an effects determination (*i.e.*, "no effect," "may affect, but not likely to adversely affect," or "likely to adversely affect") for the CRLF and its designated critical habitat.

Based on the RQs presented in the Risk Estimation (Section 5.1) a preliminary effects determination is "may affect" for the CRLF and modification of critical habitat.

A summary of the results of the risk estimation results are provided in Table 5-8 for direct and indirect effects to the CRLF and in Table 5-9 for the PCEs of designated critical habitat for the CRLF.

Table 5-8 Risk Estimation Summary for Diflubenzuron - Direct and Indirect Effects to CRLF

Assessment Endpoint	LOC Exceedances (Y/N)	Description of Results of Risk Estimation
Aquatic Phase (eggs, larvae, tadpoles, juveniles, and adults)		
Direct Effects Survival, growth, and reproduction of CRLF individuals via direct effects on aquatic phases	Yes Direct application to rice only	The chronic fish RQ was 1.12 for direct application to rice. All other chronic RQs were lower than the LOC of 1.0. Acute RQs were <0.01. The probability of an individual mortality at the acute RQs of 0.01 is <1 in 1,000,000 based on a slope of 4.7 (95% CI of 3.5 to 5.9 (MRID 56150).
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects to food supply (*i.e.*, freshwater invertebrates, non-vascular plants)	Yes All uses	Acute RQs ranged from approximately 4 to 40,000. The probability of an individual effect at these RQs approaches 100% for reasonable lower and upper bound slopes of 2 to 9. Chronic RQs also exceeded LOCs and were 25 to 450,000.
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects on habitat, cover, and/or primary productivity (*i.e.*, aquatic plant community)	No	No LOCs were exceeded for vascular or non-vascular aquatic plants. RQs were less than 0.6.
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects to riparian vegetation, required to maintain acceptable water quality and habitat in ponds and streams comprising the species' current range.	No	RQs were not calculated due to the lack of guideline terrestrial plant toxicity data; further discussion of potential risks to CRLFs resulting from impacts to terrestrial plants is presented in Section 5.2.
Terrestrial Phase (Juveniles and adults)		

Assessment Endpoint	LOC Exceedances (Y/N)	Description of Results of Risk Estimation
Direct Effects Survival, growth, and reproduction of CRLF individuals via direct effects on terrestrial phase adults and juveniles	Yes Barnyard/mushroom use only	Acute and chronic RQs exceeded LOCs. The acute RQ was 1.2 and the chronic RQ was 6.7. At an RQ of 1.2, the probability of an individual effect is 1 in 1.6 based on the default probit slope of 4.5. The range of probabilities of an individual effect is 1 in 1.4 to 1 in 1.8 based on lower and upper bound probit slopes of 2 to 8. LOCs were not exceeded for any other use.
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects on prey (*i.e.*, terrestrial invertebrates, small terrestrial mammals and terrestrial phase amphibians)	Yes All uses	Diflubenzuron is an insecticide. Therefore, there is potential for terrestrial invertebrate abundance to be impacted. In addition, LOCs were exceeded for all uses based on submitted data in the honey bee.
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects on habitat (*i.e.*, riparian vegetation)	No	RQs were not calculated for terrestrial plants based on the lack of terrestrial plants toxicity data available for diflubenzuron.

Table 5-9 Risk Estimation Summary for Diflubenzuron – PCEs of Designated Critical Habitat for the CRLF

Assessment Endpoint	Habitat Modification (Y/N)	Description of Results of Risk Estimation
Aquatic Phase PCEs (Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)		
Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile and adult CRLFs.	No	There are no LOC exceedances for risk to aquatic plants.
Alteration in water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	No	--
Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.	Yes	There are acute and chronic LOC exceedances for risk of diflubenzuron to aquatic invertebrate prey of the CRLF for all uses.
Reduction and/or modification of aquatic-based food sources for pre-metamorphs (*e.g.*, algae)	No	There were no LOC exceedances for risk to Algae for any use evaluated.

Assessment Endpoint	Habitat Modification (Y/N)	Description of Results of Risk Estimation
Terrestrial Phase PCEs (Upland Habitat and Dispersal Habitat)		
Elimination and/or disturbance of upland habitat; ability of habitat to support food source of CRLFs: Upland areas within 200 ft of the edge of the riparian vegetation or dripline surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance	No	Currently, there are no guideline terrestrial plant toxicity data available to assess this risk. See Section 5.2 for additional description of potential risks to CRLFs resulting from impacts to terrestrial vegetation.
Elimination and/or disturbance of dispersal habitat: Upland or riparian dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal	No	Currently, there are no guideline terrestrial plant toxicity data available to assess this risk. See Section 5.2 for additional description of potential risks to CRLFs resulting from impacts to terrestrial vegetation.
Reduction and/or modification of food sources for terrestrial phase juveniles and adults	Yes	There are LOC exceedances for risk terrestrial invertebrate prey for all uses, and potential magnitude of effect could be substantial such that CRLFs could be impacted.
Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	Yes	There are LOC exceedances for risk terrestrial invertebrate prey, and potential magnitude of effect could be substantial such that CRLFs could be impacted.

Following a "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 CRLF. 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 CRLF and its designated critical habitat.

The criteria used to make determinations that the effects of an action are "not likely to adversely affect" the CRLF and 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 CRLF and its designated critical habitat is provided in Sections 5.2.1 through 5.2.3.

5.2.1. Direct Effects

5.2.1.1. Aquatic-Phase CRLF

The aquatic-phase considers life stages of the frog that are obligatory aquatic organisms, including eggs and larvae. It also considers submerged terrestrial-phase juveniles and adults, which spend a portion of their time in water bodies that may receive runoff and spray drift containing diflubenzuron.

No acute LOCs were exceeded for fish based on the most sensitive species tested. No open literature studies were located that reported lower toxicity values in either fish or amphibians than those used to calculate risk quotients. No incidents have been reported for diflubenzuron that associate its use with effects to aquatic animals. The probability of an individual mortality at the acute RQs of <0.01 is < 1 in 3.7E20 (<1 in 7.8E11 to <5.1E31) based on a slope of 4.7 (95% CI of 3.5 to 5.9, MRID 56150).

Therefore, the effect determination for potential acute direct effects to the CRLF is "no effect".

The chronic LOC was exceeded only for direct application to rice. The RQ for this use was 1.12. RQs for other uses were <0.3. However, the RQ was based on results from a full life-cycle study that did not achieve a LOAEC (no effects were observed in this study at any concentration tested). In addition, the 60-day EEC used to calculate the rice exposure values did not allow for any degradation over time. Because diflubenzuron degradates somewhat rapidly in the standard pond, similar rates of degradation would be expected to occur in rice paddies; therefore, the EEC is likely an over-estimation of exposure. For these reasons (the close proximity of the RQ to the LOC, the lack of a LOAEC in the available study, and the lack of degradation in the 60-day EEC calculation), the effects determination for direct application to rice is "not likely to adversely affect" because the potential for a direct effect is discountable (highly unlikely to occur).

The effects determination for all other uses is "no effect" based on the lack of LOC exceedances and the low probability of an individual mortality.

5.2.1.2. Terrestrial-Phase CRLF

Barnyard/Mushroom Use

Acute and chronic RQs were exceeded for CRLFs based on the avian assessment used as a surrogate species. The acute RQ was 1.2. Assuming a probit slope of 4.5, the probability of an individual effect is approximately 1 in 2. The reproduction RQ was 6.7. The reproduction RQ was based on reduced egg production at 1000 mg/kg-food (NOAEC was 500 mg/kg-food). At an RQ of 6.7, exposure values were estimated to be approximately 3 times higher than levels associated with reduced egg production in birds (surrogate for CRLF). This assessment also assumed applications at the highest labeled application rate for 17 applications per year (21 days between applications). However, even assuming a single application results in LOC exceedances (acute RQ = 0.74; chronic RQ = 2).

Potential effects to CRLFs depend on the proportion of its diet that may occur from contaminated food located at or near the use site. The barnyard/mushroom use is a spot treatment; therefore, the likelihood that a CRLF will consume contaminated food depends on the number and size of "spots" applied to any given field, which may vary considerably from field to field. In addition, manure use is typically associated with feed lots, which are not attractive habitats for CRLFs due to high density of animals and resulting lack of habitat such as clean water and vegetation.

This assessment was based on food intake equations for birds. Birds are currently used as surrogates for reptiles and terrestrial-phase amphibians. However, reptiles and amphibians are poikilotherms (body temperature varies with environmental temperature) while birds are homeotherms (temperature is regulated, constant, and largely independent of environmental temperatures). Therefore, reptiles and amphibians (collectively referred to as herptiles) tend to have much lower metabolic rates and lower caloric intake requirements than birds or mammals. As a consequence, birds are likely to consume more food than amphibians or reptiles on a daily dietary intake basis, assuming similar caloric content of the food items. This can be seen when comparing the estimated caloric requirements for free living iguanid lizards (Iguanidae) (**EQ 1**) to passerines (song birds) (**EQ 2**) (U.S. EPA, 1993):

$$\textbf{iguanid FMR (kcal/day)} = 0.0535 * (\text{bw in g})^{0.799} \qquad \textbf{(EQ 1)}$$

$$\textbf{passerine FMR (kcal/day)} = 2.123 * (\text{bw in g})^{0.749} \qquad \textbf{(EQ 2)}$$

With relatively comparable exponents (slopes) to the allometric functions, one can see that, given a comparable body weight, the free living metabolic rate of birds can be 40 times higher than reptiles, though the requirement differences narrow with high body weights. Consequently, birds are conservative surrogates for terrestrial phase CRLFs because birds are expected to have higher potential exposures relative to terrestrial phase amphibians.

The potential differences in exposure of CRLFs and birds were quantified using a modified version of T-REX, called T-HERPS (version 1.0). T-HERPs incorporates food intake equations that are specific for terrestrial phase amphibians and includes two additional food items of the CRLF that are not included in T-REX (CRLFs that consume small mammals and other amphibians).

The results of this analysis for the barnyard/mushroom use are in Table 5-10. RQs remained above the endangered species LOC for the small mammal food item (CRLFs that consume small mammals). However, LOCs were not exceeded for other food items.

Table 5-10. Results from T-HERPS for the Barnyard/Mushroom Use.

Dose-based RQs (Dose-based EEC/adjusted LD50)	Amphibian/Reptile Acute RQs for Small, Medium, and Large Species (grams)		
	1.4	37	238
Short Grass	0.06	0.06	0.04
Tall Grass	0.03	0.03	0.02
Broadleaf plants/sm insects	0.03	0.03	0.02
Fruits/pods/seeds/lg insects	0.00	0.00	0.00
Small herbivore mammals	N/A	**0.97**	**0.15**
Small insectivore mammals	N/A	0.06	0.01
Small terrestrial phase amphibian	N/A	0.00	0.00

Although barnyards and feedlots are not likely to typically provide suitable habitat for CRLFs, the presence of CRLFs in such habitats cannot be precluded. Therefore, based on acute and chronic LOC exceedances and the likelihood of potential effects at the estimated exposure levels, the effects determination for direct effects to the CRLF is "likely to adversely affect" for the barnyard/mushroom use.

All Other Uses

No LOCs were exceeded. Acute RQs ranged from <0.01 to 0.04. At these RQs, the probability of an individual effect is 1 in 6E9 based on the default probit slope of 4.5 (see Section 4). Based on reasonable lower and upper bounds on the slope of 2 to 8, the probability of an individual effect ranged from 1 in 4E2 to 1 in 7E35.

No incidents associating diflubenzuron with effects to terrestrial animals have been reported to the Agency.

Also, as previously discussed, birds were used as a surrogate for the CRLF, which results in a conservative estimate of exposure and risk due to the greater energetic requirements and resulting greater food intake (and resulting potential exposures) of birds relative to terrestrial phase CRLFs.

Based on the lack of LOC exceedances in birds, the low probability of an individual mortality, and the lack of reported incidents, diflubenzuron is expected to have "no effect" via direct toxic effects on terrestrial phase CRLFs.

5.2.2. Indirect Effects (via Reductions in Prey Base)

5.2.2.1. Algae (non-vascular plants)

As discussed in Section 2.5.3, the diet of CRLF tadpoles is composed primarily of unicellular aquatic plants (*i.e.*, algae and diatoms) and detritus. LOCs were not exceeded for aquatic plants. No effects were observed in any of the available toxicity studies in aquatic plants, and all RQs were less than 1 (the highest RQ was 0.6). Therefore, estimated diflubenzuron concentrations were lower than NOAEC values in all aquatic plant species tested.

Therefore, the effects determination for CRLFs based on potential indirect effects from impacts to aquatic plants is "no effect."

5.2.2.2. Aquatic Invertebrates

The potential for diflubenzuron to elicit indirect effects to the CRLF via effects on freshwater invertebrate food items is dependent on several factors including: (1) the potential magnitude of effect on freshwater invertebrate individuals and populations; and (2) the number of prey species potentially affected relative to the expected number of species needed to maintain the dietary needs of the CRLF. Together, these data provide a basis to evaluate whether the number of individuals within a prey species is likely to be reduced such that it may indirectly affect the CRLF.

Based on the most sensitive species tested (mosquito), RQs exceeded LOCs and ranged from 4 to 40,000 (acute) and 25 to 450,000 (chronic). At these acute RQs, the probability of an individual mortality approaches 100%, and potential impacts to abundance of aquatic invertebrates could be sufficient to reduce available food of the CRLF.

As shown in Figure 5-1 below, based on open literature studies (Appendix G), the lowest EEC across all uses of diflubenzuron is above the average acute toxicity value for a majority of species tested (RQ >1). These values represent short-term toxicity studies, and may, therefore, underestimate potential impacts to aquatic invertebrates as discussed in Section 4.

The life stage of the exposed invertebrate is likely to influence potential effects. Adults are less sensitive to diflubenzuron than larvae and juveniles. The results of littoral enclosure studies discussed in Section 4 suggested that some taxonomic groups of aquatic invertebrates could be impacted at diflubenzuron levels lower than 0.7 µg/L (NOEC not achieved). These impacts to the aquatic community were also observed to be correlated to reductions in the growth rate of introduced bluegill sunfish, with reductions in length, weight, and growth rate occurring at initial nominal concentrations of 2.5 µg/L and above. Growth reductions in fish were attributed to reductions in food source (indirect effect). It is important to note that MRID 44386201 fails to empirically demonstrate a NOEC for a number of endpoints, namely effects on cladocerans, copepods, aquatic insect emergence and richness of macroinvertebrate taxa.

Based on the number of potential species that may be affected and the potential magnitude of effect, diflubenzuron is likely to adversely affect the CRLF by reducing available aquatic prey. This effects determination applied to all labeled uses included in this assessment.

Based on AgDRIFT analysis (version 2.1.0.3, Tier 3 analysis using aerial and ground spray), spray drift could be sufficient to result in indirect effects at applications greater than 5000 feet from treated sites as evidenced by LOC exceedances for the most sensitive species tested.

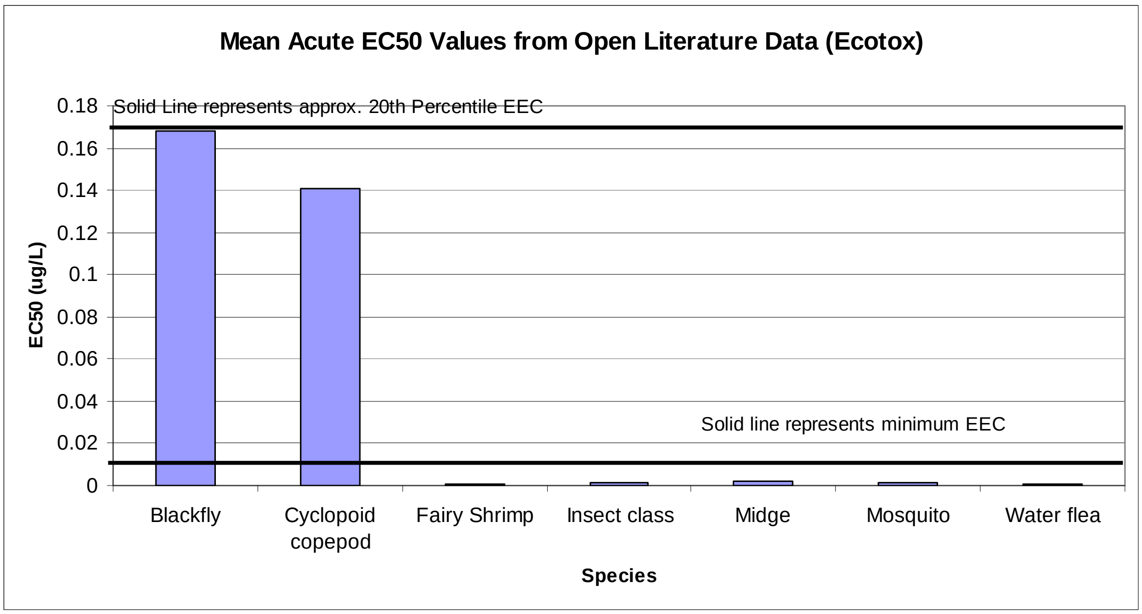

Figure 5-1. Mean acute EC50 values from open literature data according to the Ecotox data base.

Figure note: Values for fairy shrimp and water flea are low such that they are not visible on the graph. EC50s for two species were not included in this figure due to the scale of the graph. A single EC50 for parasitic nematode (EC50: 4.4 µg/L) and a single EC_{50} for backswimmer (EC_{50}: 1.9 µg/L).

5.2.2.3. Fish and Aquatic-phase Frogs

As described in Section 5.2.1.1, the effects determination for potential direct effects to the CRLF is “no effect” for acute effects and chronic effects for all uses except rice and is “not likely to adversely affect” for chronic effects for the rice use. These conclusions are based on potential effects to the most sensitive fish species tested. The same conclusions are reached for the potential for diflubenzuron to adversely affect the CRLF based on reduction in fish or frogs as food. See Section 5.2.1.1 for additional detail.

5.2.2.4. Terrestrial Invertebrates

When the terrestrial-phase CRLF reaches juvenile and adult stages, its diet is mainly composed of terrestrial invertebrates. RQs were based on acute studies in adult bees. Although RQs suggest that there is a potential for impacts to terrestrial invertebrates, these RQs were based on a study that did not produce mortality at any concentration tested (30 µg/bee). However, as discussed in Section 4, acute studies in adult bees are not considered to represent sensitive invertebrate life stages due to the mode of action of diflubenzuron as an insect growth regulator, which affects insects at molting. Results of field studies have been inconsistent as discussed in Section 4; however, several field studies have shown reduced abundance in invertebrates at currently approved application rates. Also, given the use of diflubenzuron as an insecticide, many insect species are presumably at risk if exposed at a sensitive life stage. Therefore, the effects determination from labeled use of diflubenzuron is "likely to adversely affect." The potential impact to the diet of the CRLF will depend on the timing of application and predominance of invertebrates in sensitive life stages.

The distance from treated fields that effects may occur has not been defined because the available data do not allow for quantitative measures of effects.

5.2.2.5. Mammals

Life history data for terrestrial-phase CRLFs indicate that large adult frogs consume terrestrial vertebrates, including mice. No acute LOCs were exceeded for mammals. No effects were observed in the available acute oral toxicity study at doses up to 5000 mg/kg-bw. No reproduction RQs were exceeded for any use except the barnyard/mushroom use. Therefore, the effects determination is "no effect" for all uses except barnyard/mushroom use based on the lack of LOC exceedances and the resulting low magnitude of any potential effects to mammals.

Reproduction LOCs were exceeded for barnyard/mushroom use (RQ = 10). There is a 10-fold dose spacing in the available 2-generation reproduction toxicity study; therefore, at an RQ of 10, estimated exposures are equivalent to the LOAEC in mammals. At the LOAEC, there was a reduction in body weight in F1 pups. Frank reproductive effects were not observed. The EECs were based on the short grass food item and assumed that small mammal prey consume only contaminated short grass at high-end exposure levels. However, the barnyard/mushroom use is a spot treatment (directed spray at manure). Therefore, it is unlikely that a small mammal will obtain 100% of its diet as contaminated short grass in environments consistent with the barnyard/mushroom use. Nonetheless, such effects could not be precluded. Therefore, the effects determination is "likely to adversely affect" based on potential reductions in mammalian prey. These conclusions are highly uncertain.

5.2.2.6. Terrestrial-phase Amphibians

Terrestrial-phase adult CRLFs also consume other amphibians. RQ values representing direct exposures of diflubenzuron to terrestrial-phase CRLFs are used to represent exposures of diflubenzuron to frogs in terrestrial habitats. The direct effects assessment was "no effect" for all uses except the barnyard/mushroom use. Therefore, the effects determination is also "no effect" via reduction in amphibian prey.

For the barnyard/mushroom use, the effects determination was "likely to adversely affect" based on potential direct effects to the CRLF. However, the potential magnitude of effect is expected to be relatively low when considering dietary factors of terrestrial phase amphibians relative to the surrogate avian species.

When incorporating food intake equations specific for amphibians and reptiles using the T-HERPS model, LOCs were not exceeded for terrestrial phase amphibians that consume plants or insects. See Section 5.2.1.2 for additional information. Therefore, the effects determination for potential effects to the CRLF via reductions in available amphibian prey is "not likely to adversely affect."

5.2.3. Indirect Effects (via Habitat Effects)

5.2.3.1. Aquatic Plants (Vascular and Non-vascular)

Aquatic plants serve several important functions in aquatic ecosystems. Non-vascular aquatic plants are primary producers and provide the autochthonous energy base for aquatic ecosystems. Vascular plants provide structure as attachment sites and refugia for many aquatic invertebrates, fish, and juvenile organisms, such as fish and frogs. In addition, vascular plants also provide primary productivity and oxygen to the aquatic ecosystem. Rooted plants help reduce sediment loading and provide stability to nearshore areas and lower streambanks. In addition, vascular aquatic plants are important as attachment sites for egg masses of CRLFs.

Potential indirect effects to the CRLF based on impacts to habitat and/or primary production were assessed using RQs from freshwater aquatic vascular and non-vascular plant data. No LOCs were exceeded for vascular aquatic plants. No effects were observed in the available toxicity studies in aquatic plants at any test level. EECs were lower than all vascular plant NOAECs. Therefore, effects to vascular plants are not expected to occur. For this reason, the effects determination for the CRLF based on indirect effects resulting from impacts to vascular plants is "no effect."

5.2.3.2. Terrestrial Plants

Terrestrial plants serve several important habitat-related functions for the CRLF. In addition to providing habitat and cover for invertebrate and vertebrate prey items of the

CRLF, terrestrial vegetation also provides shelter for the CRLF and cover from predators while foraging. Terrestrial plants also provide energy to the terrestrial ecosystem through primary production. Upland vegetation including grassland and woodlands provides cover during dispersal. Riparian vegetation helps to maintain the integrity of aquatic systems by providing bank and thermal stability, serving as a buffer to filter out sediment, nutrients, and contaminants before they reach the watershed, and serving as an energy source.

Impacts to terrestrial plants are not expected to occur at levels that may result in indirect effects to CRLFs. Although no suitable toxicity data are available in terrestrial plants, diflubenzuron is an insecticide that has been used to treat numerous crops and non-crop plants for many years typically without incident. However, one incident has associated diflubenzuron with damage to oranges. This incident may or may not have been caused by diflubenzuron. It was assigned a certainty index of "possible".

Although the data do not allow for quantification of potential risks to terrestrial plants, the weight of evidence suggests that effects to plants from labeled uses of diflubenzuron are minimal.

No guideline studies have been submitted that evaluated effects to terrestrial plants. Therefore, additional lines of evidence were used to determine if diflubenzuron may affect terrestrial plants at a magnitude that may result in indirect effects to the CRLF.

Diflubenzuron has a long history of use on numerous types of crops including grasses, trees, vines, and numerous fruit and vegetable crops without clear evidence of any adverse effects to terrestrial plants. Two field studies were located in the open literature study that evaluated impacts to vegetation from use of diflubenzuron that did not observe impacts to vegetation (discussed in Section 4). No incidents have been reported that definitively linked use of diflubenzuron use to damage to terrestrial plants. One incident was associated with damage to orange trees; however, that incident is more likely associated with use of petroleum distillates as discussed in Section 4. In addition, numerous studies have been submitted to the Agency that evaluated efficacy of diflubenzuron, and no evidence of plant damage was reported in these studies.

For these reasons, use of diflubenzuron is not likely to affect the CRLF by impacting terrestrial vegetation.

The effects determination for the CRLF resulting from indirect effects from impacts to terrestrial plants is "no effect."

5.2.4. Modification to Designated Critical Habitat

Risk conclusions for designated critical habitat of the CRLF are the same as those for indirect effects. Critical habitat may be modified by impacting availability of prey (terrestrial and aquatic invertebrates) as follows:

- PCE: "reduction and/or modification of food sources for terrestrial phase juveniles and adults." This PCE may be modified by diflubenzuron by potentially impacting invertebrate food sources. See Section 5.2.2.4.
- PCE: "alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source." This PCE may be impacted by effects to food sources such as terrestrial invertebrates. See Section 5.2.2.4.

5.2.5. Spatial Extent of Potential Effects

Direct effects to the CRLF are limited to the barnyard/mushroom use. Locations of cattle feed lots (locations where diflubenzuron may be used on cattle manure) are depicted in Figure 5.2. For this use, diflubenzuron is applied as a directed spray to manure. Based on the low magnitude of the RQ for this use and the low potential for spray drift, it is assumed that potential risks are limited to treated sites.

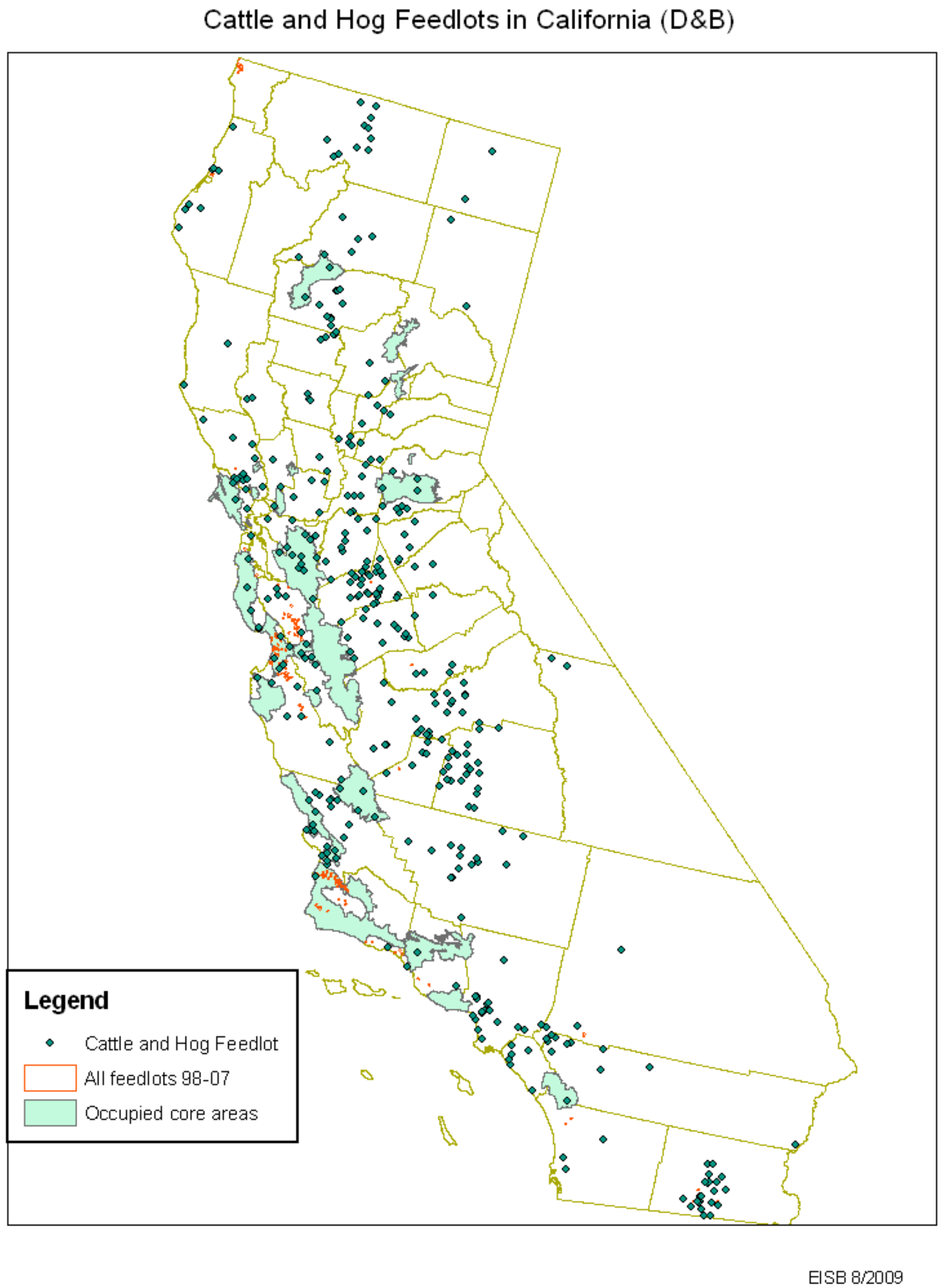

Figure 5-2. Comparison of location of sites likely to use diflubenzuron for control of flies at livestock facilities (see text) and CRLF occupied core areas.

RQs used to estimate potential for indirect effects to aquatic phase CRLFs were as high as approximately 500,000. The distance needed to reduce potential exposures to levels that do not exceed LOCs is greater than distances that can be predicted with any reliability. Potential indirect effects could occur at distances >5000 feet from the treated sites as described in Section 5.2.5.1

Indirect effects to the CRLF may also occur via impacts to terrestrial invertebrates. However, the data do not allow for quantification of the potential distance from treated sites to which risks extend primarily because the toxicity data used to estimate potential impacts to terrestrial invertebrates are based on adult honey bee studies. Sensitivity of adult honey bees to diflubenzuron is expected to be considerably lower than sensitivity of other insects, particularly invertebrates in early life stages. Therefore, potential impacts to terrestrial invertebrates are assumed to extend to distances greater than 1000 feet from treated fields.

An LAA effects determination applies to those areas where it is expected that the pesticide's use will directly or indirectly affect the CRLF or its designated critical habitat. To determine this area, the footprint of diflubenzuron's use pattern is identified, using land cover data that correspond to diflubenzuron's use pattern. Areas of potential exposure also include areas beyond the initial area of concern (*e.g.*, use footprint) that may be impacted by runoff and/or spray drift. The identified direct and indirect effects and modification to critical habitat are anticipated to occur only for those currently occupied core habitat areas, CNDDB occurrence sections, and designated critical habitat for the CRLF that overlap with the initial area of concern plus >5000 feet from its boundary. It is assumed that non-flowing waterbodies (or potential CRLF habitat) are included within this area. Direct effects are only expected to occur for the barnyard/mushroom use. For this use, risks are expected to be predominantly restricted to the treated site because of the mode of application (directed spray to manure).

The determination of the buffer distance and downstream dilution for spatial extent of the effects determination is described below.

5.2.5.1. Spray Drift

In order to determine terrestrial and aquatic habitats of concern due to diflubenzuron exposures through spray drift, it is necessary to estimate the distance that spray applications can drift from the treated area and still be present at concentrations that exceed levels of concern. An analysis of spray drift distances was completed using AgDRIFT (version 2.01). AgDRIFT was run using default parameters. Tier 3 was used to expand the distance evaluated to 5000 feet.

Table 5-11. Summary of AgDRIFT* Predicted Terrestrial Spray Drift Distances

Landcover	Uses Represented	Highest RQ	Distance to reduce spray drift to level that is <LOC
Forest	Forestry	3200	>5000 feet
Non-cropland agriculture	Turf, nursery	2600	>5000 feet
Residential	Residential, urban, rights of ways	137,000	>5000 feet
Orchard	Fruit, nuts, pistachio, Beech nut	5400	>5000 feet
Pasture/Rangeland	Barnyards, pastures+	217,000 (barnyard) 2200 (pasture)	>5000 feet
Cropland	Turnip, squash, row crops, rice, grains, nuts, cotton, cole crops, brassica,	452,000	>5000 feet

* AgDRIFT was run in Tier 3, with a maximum distance of 5000 feet and RQs for aquatic invertebrates. AgDRIFT has not been validated at distances >1000 feet; therefore, there is uncertainty in the estimations presented in Table 5-11.

+.Minimal drift is expected from the barnyard uses because of the mode of application (downward spray directed at manure); therefore, the drift distance was based on the pastureland RQ of 2200.

5.2.6.2. Downstream Dilution Analysis

The downstream extent of exposure in streams and rivers where the EEC could potentially be above levels that would exceed the most sensitive LOC was not completed because of the large number of uses and large corresponding landcover area. The large geographic extent of potential use sites precludes quantification of dilution potential based on landcover data representing non-use sites.

5.2.6.3. Overlap between CRLF habitat and Spatial Extent of Potential Effects

An LAA effects determination is made to those areas where it is expected that the pesticide's use will directly or indirectly affect the CRLF or its designated critical habitat and the area overlaps with the core areas, critical habitat and available occurrence data for CRLF.

For diflubenzuron, the use pattern encompasses all land cover classes. Therefore, the initial area of concern is the entire state of CA. As described in Section 5, diflubenzuron could potentially impact the CRLF via indirect effects to aquatic and terrestrial invertebrates. Effects could occur at distances >5000 feet from the site of application. Potential overlap between the CRLF and diflubenzuron uses is graphically represented in Figure 5.3.

Recovery Units

1. Sierra Nevada Foothills and Central Valley
2. North Coast Range Foothills and Western Sacramento River Valley
3. North Coast and North San Francisco Bay
4. South and East San Francisco Bay
5. Central Coast
6. Diablo Range and Salinas Valley
7. Northern Transverse Ranges and Tehachapi Mountains
8. Southern Transverse and Peninsular Ranges

Core Areas

1. Feather River
2. Yuba River- S. Fork Feather River
3. Traverse Creek/ Middle Fork/ American R. Rubicon
4. Cosumnes River
5. South Fork Calaveras River*
6. Tuolumne River*
7. Piney Creek*
8. Cottonwood Creek
9. Putah Creek – Cache Creek*
10. Lake Berryessa Tributaries
11. Upper Sonoma Creek
12. Petaluma Creek – Sonoma Creek
13. Pt. Reyes Peninsula
14. Belvedere Lagoon
15. Jameson Canyon – Lower Napa River
16. East San Francisco Bay
17. Santa Clara Valley
18. South San Francisco Bay
19. Watsonville Slough-Elkhorn Slough
20. Carmel River – Santa Lucia
21. Gablan Range
22. Estero Bay
23. Arroyo Grange River
24. Santa Maria River – Santa Ynez River
25. Sisquoc River
26. Ventura River – Santa Clara River
27. Santa Monica Bay – Venura Coastal Streams
28. Estrella River
29. San Gabriel Mountain*
30. Forks of the Mojave*
31. Santa Ana Mountain*
32. Santa Rosa Plateau
33. San Luis Ray*
34. Sweetwater*
35. Laguna Mountain*

* Core areas that were historically occupied by the California red-legged frog are not included in the map

Figure 5-3 Recovery Unit, Core Area, Critical Habitat, and Occurrence Designations for CRLF

6.0 Uncertainties

6.1 Exposure Assessment Uncertainties

6.1.1 Maximum Use Scenario

The screening-level risk assessment focuses on characterizing potential ecological risks resulting from a maximum use scenario, which is determined from labeled statements of maximum application rate and number of applications with the shortest time interval between applications. The frequency at which actual uses approach this maximum use scenario may be dependant on pest resistance, timing of applications, cultural practices, and market forces.

Several fast-maturing crops may have multiple crop cycles within a single year. For these crops, it is often the case that labels do not specify a maximum annual application rate, but rather a maximum crop cycle rate (maximum pounds per acre per crop cycle). Because diflubenzuron dissipates rapidly in the environment (aerobic soil metabolism rate is ~2 days), additional applications of diflubenzuron during following crop cycles are not expected to greatly increase terrestrial EECs within the field where it is applied or in the terrestrial spray drift zone. However, aquatic EECs can be affected because the multiple crop cycles extend the growing season into those parts of the year when runoff is more likely (fall through early spring). To address this uncertainty, aquatic EECs were modeled across a large range of application dates (Section 3.2.3 and Appendix tables D4 and D5).

6.1.2 Crops Not Grown in California (include if you had crops that are not grown in CA)

Soybeans are the only crop listed on diflubenzuron labels that is not grown in California according to National Agricultural Statistical Service data. Because these crops are not currently grown in California and are not expected to be grown in California in the future, use of diflubenzuron was not assessed for these crops. If use patterns indicate soybeans are grown in California in the future, the conclusions of this assessment may need to be revisited.

6.1.3 Aquatic Exposure Modeling of Diflubenzuron

The standard ecological water body scenario (EXAMS pond) used to calculate potential aquatic exposure to pesticides is intended to represent conservative estimates, and to avoid underestimations of the actual exposure. The standard scenario consists of application to a 10-hectare field bordering a 1-hectare, 2-meter deep (20,000 m^3) pond with no outlet. Exposure estimates generated using the EXAMS pond are intended to represent a wide variety of vulnerable water bodies that occur at the top of watersheds including prairie pot holes, playa lakes, wetlands, vernal pools, man-made and natural ponds, and intermittent and lower order streams. As a group, there are factors that make these water bodies more or less vulnerable than the EXAMS pond. Static water bodies that have larger ratios of pesticide-treated drainage area to water body volume would be expected to have higher peak EECs than the EXAMS pond. These water bodies will be either smaller in size or have larger drainage areas. Smaller water bodies have limited storage capacity and thus may overflow and carry pesticide in the discharge, whereas the EXAMS pond has no discharge. As watershed size increases beyond 10-hectares, it becomes increasingly unlikely that the entire watershed is planted with a single crop that is all treated simultaneously with the pesticide. Headwater streams can also have peak concentrations higher than the EXAMS pond, but they likely persist for only short periods of time and are then carried and dissipated downstream.

The Agency acknowledges that there are some unique aquatic habitats that are not accurately captured by this modeling scenario and modeling results may, therefore, under- or over-estimate exposure, depending on a number of variables. For example, aquatic-phase CRLFs may inhabit water bodies of different size and depth and/or are located adjacent to larger or smaller drainage areas than the EXAMS pond. The Agency does not currently have sufficient information regarding the hydrology of these aquatic habitats to develop a specific alternate scenario for the CRLF. CRLFs prefer habitat with perennial (present year-round) or near-perennial water and do not frequently inhabit vernal (temporary) pools because conditions in these habitats are generally not suitable (Hayes and Jennings 1988). Therefore, the EXAMS pond is assumed to be representative of exposure to aquatic-phase CRLFs. In addition, the Services agree that the existing EXAMS pond represents the best currently available approach for estimating aquatic exposure to pesticides (U.S. FWS/NMFS 2004).

In general, the linked PRZM/EXAMS model produces estimated aquatic concentrations that are expected to be exceeded once within a ten-year period. The Pesticide Root Zone Model is a process or “simulation” model that calculates what happens to a pesticide in an agricultural field on a day-to-day basis. It considers factors such as rainfall and plant transpiration of water, as well as how and when the pesticide is applied. It has two major components: hydrology and chemical transport. Water movement is simulated by the use of generalized soil parameters, including field capacity, wilting point, and saturation water content. The chemical transport component can simulate pesticide application on the soil or on the plant foliage. Dissolved, adsorbed, and vapor-phase concentrations in the soil are estimated by simultaneously considering the processes of pesticide uptake by plants, surface runoff, erosion, decay, volatilization, foliar wash-off, advection, dispersion, and retardation.

Uncertainties associated with each of these individual components add to the overall uncertainty of the modeled concentrations. Additionally, model inputs from the environmental fate degradation studies are chosen to represent the upper confidence bound on the mean values that are not expected to be exceeded in the environment approximately 90 percent of the time. Mobility input values are chosen to be representative of conditions in the environment. The natural variation in soils adds to the uncertainty of modeled values. Factors such as application date, crop emergence date, and canopy cover can also affect estimated concentrations, adding to the uncertainty of modeled values. Factors within the ambient environment such as soil temperatures, sunlight intensity, antecedent soil moisture, and surface water temperatures can cause actual aquatic concentrations to differ for the modeled values.

Unlike spray drift, tools are currently not available to evaluate the effectiveness of a vegetative setback on runoff and loadings. The effectiveness of vegetative setbacks is highly dependent on the condition of the vegetative strip. For example, a well-established, healthy vegetative setback can be a very effective means of reducing runoff and erosion from agricultural fields. Alternatively, a setback of poor vegetative quality or a setback that is channelized can be ineffective at reducing loadings. Until such time as a quantitative method to estimate the effect of vegetative setbacks on various conditions on pesticide loadings becomes available, the aquatic exposure predictions are likely to overestimate exposure where healthy vegetative setbacks exist and underestimate exposure where poorly developed, channelized, or bare setbacks exist.

6.1.4 Usage Uncertainties

County-level usage data were obtained from California's Department of Pesticide Regulation Pesticide Use Reporting (CDPR PUR) database. Eight years of data (1999 – 2006) were included in this analysis because statistical methodology for identifying outliers, in terms of area treated and pounds applied, was provided by CDPR for these years only. No methodology for removing outliers was provided by CDPR for 1998 and earlier pesticide data; therefore, this information was not included in the analysis because it may misrepresent actual usage patterns. CDPR PUR documentation indicates that errors in the data may include the following: a misplaced decimal; incorrect measures, area treated, or units; and reports of diluted pesticide concentrations. In addition, it is possible that the data may contain reports for pesticide uses that have been cancelled. The CPDR PUR data does not include home owner applied pesticides; therefore, residential uses are not likely to be reported. As with all pesticide usage data, there may be instances of misuse and misreporting. The Agency made use of the most current, verifiable information; in cases where there were discrepancies, the most conservative information was used.

6.1.5 Terrestrial Exposure Modeling of Diflubenzuron

The Agency relies on the work of Fletcher *et al.* (1994) for setting the assumed pesticide residues in wildlife dietary items. These residue assumptions are believed to reflect a realistic upper-bound residue estimate, although the degree to which this assumption reflects a specific percentile estimate is difficult to quantify. It is important to note that the field measurement efforts used to develop the Fletcher estimates of exposure involve highly varied sampling techniques. It is entirely possible that much of these data reflect residues averaged over entire above ground plants in the case of grass and forage sampling.

It was assumed that ingestion of food items in the field occurs at rates commensurate with those in the laboratory. Although the screening assessment process adjusts dry-weight estimates of food intake to reflect the increased mass in fresh-weight wildlife food intake estimates, it does not allow for gross energy differences. Direct comparison of a laboratory dietary concentration- based effects threshold to a fresh-weight pesticide residue estimate would result in an underestimation of field exposure by food consumption by a factor of 1.25 – 2.5 for most food items.

Differences in assimilative efficiency between laboratory and wild diets suggest that current screening assessment methods do not account for a potentially important aspect of food requirements. Depending upon species and dietary matrix, bird assimilation of wild diet energy ranges from 23 – 80%, and mammal's assimilation ranges from 41 – 85% (U.S. EPA 1993). If it is assumed that laboratory chow is formulated to maximize assimilative efficiency (*e.g.*, a value of 85%), a potential for underestimation of exposure may exist by assuming that consumption of food in the wild is comparable with consumption during laboratory testing. In the screening process, exposure may be underestimated because metabolic rates are not related to food consumption.

For the terrestrial exposure analysis of this risk assessment, a generic bird or mammal was assumed to occupy either the treated field or adjacent areas receiving a treatment rate on the field. Actual habitat requirements of any particular terrestrial species were not considered, and it was assumed that species occupy, exclusively and permanently, the modeled treatment area. Spray drift model predictions suggest that this assumption leads to an overestimation of exposure to species that do not occupy the treated field exclusively and permanently.

6.1.6 Spray Drift Modeling

Although there may be multiple diflubenzuron applications at a single site, it is unlikely that the same organism would be exposed to the maximum amount of spray drift from every application made. In order for an organism to receive the maximum concentration of diflubenzuron from multiple applications, each application of diflubenzuron would have to occur under identical atmospheric conditions (*e.g.*, same wind speed and – for plants – same wind direction) and (if it is an animal) the animal being exposed would have to be present directly downwind at the same distance after each application. Although there may be sites where the dominant wind direction is fairly consistent (at least during the relatively quiescent conditions that are most favorable for aerial spray applications), it is nevertheless highly unlikely that plants in any specific area would receive the maximum amount of spray drift repeatedly. It appears that in most areas (based upon available meteorological data) wind direction is temporally very changeable, even within the same day. Additionally, other factors, including variations in topography, cover, and meteorological conditions over the transport distance are not accounted for by the AgDRIFT model (*i.e.*, it models spray drift from aerial and ground applications in a flat area with little to no ground cover and a steady, constant wind speed and direction). Therefore, in most cases, the drift estimates from AgDRIFT may overestimate exposure even from single applications, especially as the distance increases from the site of application, since the model does not account for potential obstructions (*e.g.*, large hills, berms, buildings, trees, *etc*.). Furthermore, conservative assumptions are often made regarding the droplet size distributions being modeled ('ASAE Very Fine to Fine' for orchard uses and 'ASAE Very Fine' for agricultural uses), the application method (*e.g.*, aerial), release heights and wind speeds. Alterations in any of these inputs would change the area of potential effect.

6.2 Effects Assessment Uncertainties

6.2.1 Age Class and Sensitivity of Effects Thresholds

It is generally recognized that test organism age may have a significant impact on the observed sensitivity to a toxicant. The acute toxicity data for fish are collected on juvenile fish between 0.1 and 5 grams. Aquatic invertebrate acute testing is performed on recommended immature age classes (*e.g.*, first instar for daphnids, second instar for amphipods, stoneflies, mayflies, and third instar for midges).

Testing of juveniles may overestimate toxicity at older age classes for pesticide active ingredients that act directly without metabolic transformation because younger age classes may not have the enzymatic systems associated with detoxifying xenobiotics. In so far as the available toxicity data may provide ranges of sensitivity information with respect to age class, this assessment uses the most sensitive life-stage information as measures of effect for surrogate aquatic animals, and is therefore, considered as protective of the CRLF.

For diflubenzuron, the most sensitive endpoints are for immature invertebrates that are molting. However, acute toxicity test procedures typically select organisms that are not molting during the test. This may result in an underestimation of risk to molting individuals of the same species.

6.2.2 Use of Surrogate Species Effects Data

Guideline toxicity tests and open literature data on diflubenzuron are not available for frogs or any other aquatic-phase amphibian; therefore, freshwater fish are used as surrogate species for aquatic-phase amphibians. Therefore, endpoints based on freshwater fish ecotoxicity data are assumed to be protective of potential direct effects to aquatic-phase amphibians including the CRLF, and extrapolation of the risk conclusions from the most sensitive tested species to the aquatic-phase CRLF 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 t 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 purposes of defining the action area.

No open literature or registrant studies on sublethal effects associated with exposure to diflubenzuron were identified. To the extent to which sublethal effects are not considered in this assessment, the potential direct and indirect effects of diflubenzuron on CRLF may be underestimated.

6.2.4 Location of Wildlife Species

For the terrestrial exposure analysis of this risk assessment, a generic bird or mammal was assumed to occupy either the treated field or adjacent areas receiving a treatment rate on the field. Actual habitat requirements of any particular terrestrial species were not considered, and it was assumed that species occupy, exclusively and permanently, the modeled treatment area. Spray drift model predictions suggest that this assumption leads to an overestimation of exposure to species that do not occupy the treated field exclusively and permanently.

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 diflubenzuron to the CRLF and its designated critical habitat.

Based on the best available information, the Agency makes a Likely to Adversely Affect determination for the CRLF from the use of diflubenzuron. The Agency has determined that there is no potential for modification of CRLF designated critical habitat from the use of the chemical. All the uses of diflubenzuron are likely to adversely affect the CRLF primarily because of a there is a significant risk to terrestrial and aquatic invertebrate prey of the CRLF. There is a LAA for risk of direct effect to the aquatic phase CRLF only for the diflubenzuron use on rice and there is a LAA for risk of direct effect to the terrestrial phase CRLF for diflubenzuron use in barnyard/mushroom sites.

The LAA effects determination applies to those areas where it is expected that the pesticide's use will directly or indirectly affect the CRLF or its designated critical habitat. To determine this area, the footprint of diflubenzuron's use pattern is identified, using land cover data that correspond to diflubenzuron's use pattern. The spatial extent of the LAA effects determination also includes areas beyond the initial area of concern that may be impacted by runoff and/or spray drift. The identified indirect effects are anticipated to occur only for those currently occupied core habitat areas, CNDDB occurrence sections, and designated critical habitat for the CRLF that overlap with the initial area of concern + 5000 feet from its boundary (refer to analysis in Section 5.1.4). It is assumed that non-flowing waterbodies (or potential CRLF habitat) are included within this area.

A summary of the risk conclusions and effects determinations for the CRLF and its critical habitat, given the uncertainties discussed in Section 6, is presented in Table 7-1 and 7-2.

Table 7-1. Effects Determination Summary for Diflubenzuron Use and the CRLF

Assessment Endpoint	Effects Determination [1]	Basis for Determination
Survival, growth, and/or reproduction of CRLF individuals	LAA, all uses	**Potential for Direct Effects**
		Aquatic-phase (Eggs, Larvae, and Adults): Direct effects are not expected for any labeled use. All effects determinations are either NE or NLAA.
		Terrestrial-phase (Juveniles and Adults): Direct effects are only expected for diflubenzuron's use on barn yards based on acute and chronic LOC's for birds.
		Potential for Indirect Effects
		Aquatic prey items, aquatic habitat, cover and/or primary productivity

Assessment Endpoint	Effects Determination [1]	Basis for Determination
		Aquatic prey of the CRLF are expected to be impacted to an extent that could adversely affect CRLFs. ***Terrestrial prey items, riparian habitat*** Terrestrial prey items of the CRLF are expected to be impacted based on LOC exceedances for risk to terrestrial invertebrates.

[1] No effect (NE); May affect, but not likely to adversely affect (NLAA); May affect, likely to adversely affect (LAA)

Table 7-2. Effects Determination Summary for Diflubenzuron Use and CRLF Critical Habitat Impact Analysis

Assessment Endpoint	Effects Determination [1]	Basis for Determination
Modification of aquatic-phase PCE	**Habitat Modification**	There are LOC exceedances for risk to terrestrial and aquatic invertebrate prey of the CRLF.
Modification of terrestrial-phase PCE		There are LOC exceedances for risk to terrestrial and aquatic invertebrate prey of the CRLF.

[1] Habitat Modification or No effect (NE)

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. 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 CRLF life stages within specific recovery units and/or designated critical habitat within the action area. 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 species.
- Quantitative information on prey base requirements for individual aquatic- and terrestrial-phase frogs. While existing information provides a preliminary picture of the types of food sources utilized by the frog, 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 frogs and potential modification to critical habitat.

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