Risks of Vinclozolin 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

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 FIFRA regulatory actions regarding use of vinclozolin on turf. 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 (USFWS) and National Marine Fisheries Service (NMFS) *Endangered Species Consultation Handbook* (USFWS/NMFS, 1998 and procedures outlined in the Agency's Overview Document (U.S. EPA, 2004).

The CRLF was listed as a threatened species by USFWS 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 (USFWS, 1996) in California.

Vinclozolin is a non-systemic fungicide currently registered in the United States for use on canola and turf (industrial lawns and golf courses). Applications to residential turf grass are prohibited. Labels also prohibit applications of vinclozolin to canola in California. Therefore, uses on turf grass (industrial lawns and golf courses) are considered as part of the federal action evaluated in this assessment. From 1999 to 2006, an annual average of 709 lbs of vinclozolin were applied for landscape maintenance in the state of California.

The product label relevant to vinclozolin use on turf grass indicates that single applications of vinclozolin should be made at a maximum of 1.35 lbs a.i./A with intervals of 10-28 days. The label indicates that the product should be applied at a maximum seasonal rate of 4 lbs a.i./A, which is equivalent to 3 applications of 1.35 lbs a.i./A. Applications are made by ground spray. Applications by air and by chemigation are prohibited.

Available laboratory studies for vinclozolin indicate that it degrades via hydrolysis quickly in neutral water (half-life = 1.3 d). In aerobic and anaerobic environments, vinclozolin breaks down via microbial degradation, with half-lives ranging 17.6-134 days. Vinclozolin can also be degraded via photolysis, with half-lives of 18.1 and 27.2 days in soil and aqueous environments, respectively. Vinclozolin has several major degradates, including metabolites B (N-3,5-dichlorophenyl)carbamic acid(1-carboxy-1-methyl)-2-propenyl ester), E (N-3,5-dichlorophenyl)-2-hydroxy-2-methyl-3-butenoic acid amide), S (N-(3,5-dichlorophenyl)-5-methyl-2,4-oxazolidinedione) and 3, 5-dichloroanaline (3,5-DCA). There are no data available on the persistence of metabolites B, E and S. A limited amount of data are available to characterize the environmental fate of 3,5-DCA.

According to the Reregistration Eligibility Decision (RED) for vinclozolin, there is evidence that vinclozolin binds fairly weakly to the androgen receptor but that metabolites B and E, which occur in mammals, plants, and soil are responsible for much of the antiandrogenic activity attributed to vinclozolin. The antiandrogenic mode of action of vinclozolin and several of its degradates can lead to reproductive effects across a range of taxa. Therefore, vinclozolin,

metabolite B, and metabolite E may have similar modes of action and the sum of their residues is considered when assessing exposures of non-target organisms to vinclozolin. The vinclozolin degradate 3,5-DCA is classified as a carcinogen. It is unknown whether 3,5-DCA would have a similar mode of action compared to vinclozolin, metabolite B, and metabolite E. In addition, it is unknown whether or not Metabolites F and S will have similar modes of action compared to vinclozolin and 3,5-DCA. For the purpose of this assessment, vinclozolin as well as 3,5-DCA are considered to be of concern for posing risks to non-target organisms. Because all other major degradates of vinclozolin contain the 3,5-DCA moiety, the other major degradates of vinclozolin (including Metabolites B, E, F and S) are also considered to be of concern. In this assessment, EECs are generated to represent vinclozolin's total residues of concern.

In regards to the transport of vinclozolin in the environment, based on available soil partitioning data, vinclozolin and its residues of concern have the potential to move from treatment sites to non-target areas via runoff and leaching. Volatilization and bioaccumulation are unlikely to be major routes of transport for vinclozolin and its degradates.

Since CRLFs exist within aquatic and terrestrial habitats, exposure of the CRLF, its prey and its habitats to vinclozolin and degradates of concern are assessed separately for the two habitats. Tier-II aquatic exposure models are used to estimate high-end exposures of vinclozolin in aquatic habitats resulting from runoff and spray drift from different uses. Peak model-estimated one-in-ten year environmental concentrations for vinclozolin use on turf grass are 9.75 µg/L for vinclozolin alone, 30.7 µg/L for vinclozolin + metabolite B + metabolite E and 13.2 µg/L for 3,5-DCA. No California-specific water monitoring data are available for vinclozolin or metabolites B, E, F or S; however, data are available for 3,5-DCA from the U. S. Geological Survey's (USGS) National Water Quality Assessment (NAWQA) program. The maximum concentration of 3,5-DCA reported by NAWQA for California surface waters is 0.03 µg/L. This value is approximately 3 orders of magnitude lower than the maximum model-estimated environmental concentration for 3,5-DCA. Also, there is uncertainty regarding the source of the measured 3,5-DCA as it could be attributed to use of vinclozolin or iprodione (a fungicide that is also used in California).

To estimate vinclozolin exposures to the terrestrial-phase CRLF, and its potential prey resulting from uses involving vinclozolin applications, the T-REX model is used. AgDRIFT is also used to estimate deposition of vinclozolin on terrestrial and aquatic habitats from spray drift. The T-HERPS model is used to allow for further characterization of dietary exposures of terrestrial-phase CRLFs relative to birds.

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 generally 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. Although indirect effects due to modification of the terrestrial habitat are generally characterized by available data for terrestrial monocots and dicots, a lack of data on the effects of vinclozolin on terrestrial plants prevents this characterization and as such, risk is presumed for terrestrial plants.

Vinclozolin is moderately toxic to freshwater fish and invertebrates on an acute exposure basis. The no observed adverse effect concentration (NOAEC) for chronic effects to the fathead minnow is 60 µg/L, with a lowest observed adverse affect concentration (LOAEC) of 255 µg/L based on an increase in plasma vitellogenin in females and decreased testicular weights relative to body weights, (*i.e.*, gonadal-somatic index) in males. Both of these effects can impact reproductive fitness/success. At higher concentrations female fish exposed to 450 µg/L in this study exhibited atresia of 90% of their oocytes and failed to reproduce. As such, exposure to vinclozolin may lead to a direct reduction in CRLF reproductive success and affect the availability of prey items. Available chronic toxicity data for aquatic invertebrates include a NOAEC of 790 µg/L, with a LOAEC of 1400 µg/L based on impaired reproduction and reductions in growth. The EC_{50} for algae exposed to vinclozolin is <1060 µg/L, based on stimulated growth. For aquatic vascular plants, the EC_{50} is >900 µg/L, based on stimulated growth. The increased growth of aquatic plants can lead to increased shading and compromised water quality.

Vinclozolin is practically non-toxic to birds on an acute oral and subacute dietary exposure basis, and practically non-toxic to mammals on an acute oral exposure basis. On an acute contact exposure basis, vinclozolin is practically non-toxic to honeybees. The NOAEC for chronic effects to the northern bobwhite quail is 50 mg/kg-diet, with a LOAEC of 125 mg/kg-diet based on reduced number of eggs laid, decreased eggshell thickness and reduced survival of hatchlings. The no observed adverse effect level (NOAEL) for the laboratory rat is 30 mg/kg/day. The lowest observed adverse effect level (LOAEL) of 96 mg/kg/day is based on genital/reproductive tract malformations and reproductive failure in male rats and is used to estimate potential chronic effects on small mammals that serve as prey for CRLF.

At this time, no toxicity data are available to characterize the effects of metabolites B, E, F or S, to non-target organisms. Therefore, it is assumed that data available for vinclozolin are representative of effects to non-target organisms that may be caused by these metabolites that have structures similar to the parent compound. Limited toxicity data are available for 3,5-DCA showing that it is less toxic than the parent compound, and it is assumed that this chemical has a different mode of action compared to vinclozolin.

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 vinclozolin 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 vinclozolin 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 and its critical habitat.

Based on the best available information, the Agency makes a May Affect, and Likely to Adversely Affect determination for the CRLF from the use of vinclozolin. Additionally, the Agency has determined that there is the potential for modification of CRLF designated critical habitat from the use of the chemical. Based on chronic direct effects on the terrestrial-phase CRLF and indirect effects on terrestrial-phase CRLF due to chronic effects on prey items, the use of vinclozolin on turf grass (specifically industrial lawns and golf courses) in California is considered likely to adversely affect the CRLF. Additionally, there is uncertainty (due to a lack of data) regarding the potential effects of vinclozolin on terrestrial plants and because of this uncertainty, the use of vinclozolin may result in habitat modification. It should be noted that the lack of terrestrial plant data renders the risk conclusions for habitat modification highly uncertain. A summary of the risk conclusions and effects determinations for the CRLF and its critical habitat is presented in

Table 1 and **Table 2.** Further information on the results of the effects determination is included as part of the Risk Description in Section 5.2. Given the LAA determination for the CRLF and potential modification of designated critical habitat, a description of the baseline status and cumulative effects for the CRLF is provided in **Attachment II**.

Table 1. Effects Determination Summary for Vinclozolin Use on Turf Grass and the CRLF.

Assessment Endpoint	Effects Determination	Basis for Determination
Survival, growth, and/or reproduction of CRLF individuals	May affect, likely to adversely affect (LAA)	**Potential for Direct Effects**
		Aquatic-phase (Eggs, Larvae, and Adults): Based on available data, both acute and chronic RQ values are below acute and chronic risk LOCs. As such, vinclozolin use on turf is determined to have no direct effect on aquatic-phase CRLF.
		Terrestrial-phase (Juveniles and Adults): Although vinclozolin is considered practically nontoxic to terrestrial-phase amphibians (based on avian data used as a surrogate) on an acute oral and sub-acute dietary exposure basis, chronic RQs based on impaired reproduction exceed the chronic risk LOC by a factor of 9X. EECs are also sufficient to exceed the level where reproductive effects were observed in birds (the LOAEC). As such, the use of vinclozolin on turf grass in California is determined to be likely to adversely affect terrestrial-phase CRLF due to direct chronic effects on reproduction.
		Potential for Indirect Effects
		Aquatic prey items, aquatic habitat, cover and/or primary productivity Acute and chronic RQ values are below the acute and chronic risk LOCs for freshwater invertebrates. RQ values for non-vascular and vascular aquatic plants are below the LOC and/or vinclozolin is not expected to adversely affect the aquatic plant community. Given that vinclozolin does not directly affect aquatic invertebrates or vertebrates, vinclozolin is determined to have no effect on fish and aquatic-phase amphibians that serve as prey for CRLF.
		Terrestrial prey items, riparian habitat Although vinclozolin is practically nontoxic to terrestrial-phase amphibians and mammals on an acute exposure basis, chronic RQs based on impaired reproduction exceed the chronic risk LOC by a factor of 9X for terrestrial-phase amphibians and factors as high as 12X for small mammals that serve as prey for CRLF. In addition, because of uncertainty regarding the potential effects of vinclozolin on terrestrial plants, risk is presumed for the riparian habitat on which CRLF depend. As such, the use of vinclozolin on turf in California is determined to be likely to adversely affect the CRLF through indirect effects on prey and habitat.

Table 2. Effects Determination Summary for Vinclozolin Use and CRLF Critical Habitat Impact Analysis.

Assessment Endpoint	Effects Determination	Basis for Determination
Modification of aquatic-phase PCE	Habitat Modification	Based on the weight of evidence, the use of vinclozolin on turf grass in California is determined to have no adverse effect on aquatic plants; however, there is uncertainty regarding the potential effects of vinclozolin on terrestrial plants. Because of this uncertainty, risk is presumed and there is a potential for habitat modification due to effects on riparian cover surrounding aquatic areas.
Modification of terrestrial-phase PCE		There is uncertainty regarding the potential effects of vinclozolin on terrestrial plants. Because of this uncertainty, risk is presumed and there is a potential for habitat modification due to effects on riparian cover.

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

When evaluating the significance of this risk assessment's direct/indirect and habitat modification effects determinations, it is important to note that pesticide exposures and predicted risks to the species and its resources (*i.e.*, food and habitat) are not expected to be uniform across the action area. 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. EPA's Guidance for Ecological Risk Assessment (U.S. EPA 1998), the Services' Endangered Species Consultation Handbook (USFWS/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 (USFWS/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 vinclozolin on turf grass (specifically: golf courses and industrial parks). In addition, this assessment evaluates whether use on turf grass 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. In addition, T-HERPS is used to characterize risks to the terrestrial-phase CRLF, using amphibian specific ingestion rates. 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 vinclozolin 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 vinclozolin 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 vinclozolin 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 vinclozolin 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 vinclozolin.

If a determination is made that use of vinclozolin 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 vinclozolin use sites) and further evaluation of the potential impact of vinclozolin 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 vinclozolin is expected to directly impact living organisms within the action area (defined in Section 2.7), critical habitat analysis for vinclozolin 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 vinclozolin 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

According to the reregistration eligibility decision (RED; USEPA 2000) on vinclozolin, the non-systemic fungicide was once registered in the United States for use on raspberries, chicory grown for Belgian endive, lettuce, kiwi, canola, succulent beans, and dry bulbs. Vinclozolin was also registered for use on ornamentals and turf grass. Trade names included Ronilar®, Curalan®, Vorlan® and Touche®. In 2000, the registrant, BASF, requested immediate cancellation of uses on onions, raspberries and ornamentals, a phase-out of the California Section 24c uses on kiwi and chicory by December 2001, and a phase-out of uses on lettuce and snap beans by July 2004. After 2005, only uses on canola and turf remained. Product reregistration was completed in July 2006. Use of vinclozolin on canola is prohibited in California. Use on turf is limited to golf courses and industrial park landscapes.

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

Although current registrations of vinclozolin allow for use nationwide, this ecological risk assessment and effects determination addresses currently registered uses of vinclozolin in portions of the action area that are reasonably assumed to be biologically relevant to the CRLF and its designated critical habitat. Further discussion of the action area for the CRLF and its critical habitat is provided in Section 2.7. At this time, the only vinclozolin use that is registered for use in California is turf grass.

According to the Reregistration Eligibility Decision (RED) for vinclozolin, there is evidence that vinclozolin binds fairly weakly to the androgen receptor but that metabolites B and E, which occur in mammals, plants, and soil are responsible for much of the antiandrogenic activity attributed to vinclozolin. The antiandrogenic mode of action of vinclozolin and several of its degradates can lead to reproductive effects across a range of taxa. Therefore, vinclozolin, metabolite B, and metabolite E may have similar modes of action and the sum of their residues is considered when assessing exposures of non-target organisms to vinclozolin. 3,5-DCA is classified as a carcinogen. It is unknown whether 3,5-DCA would have a similar mode of action compared to vinclozolin, metabolite B, and metabolite E. In addition, it is unknown whether or not Metabolites F and S will have similar modes of action compared to vinclozolin and 3,5-DCA. For the purpose of this assessment, vinclozolin as well as 3,5-DCA are considered to be of concern for posing risks to non-target organisms. Because all other major degradates of vinclozolin contain the 3,5-DCA moiety, the other major degradates of vinclozolin (including Metabolites B, E, F and S) are also considered to be of concern. In this assessment, EECs are generated to represent vinclozolin's total residues of concern.

Vinclozolin does not have any registered products that contain multiple active ingredients. 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; USFWS/NMFS 2004).

2.3 Previous Assessments

Vinclozolin was registered for use on ornamentals and turf in 1981, on stone fruits in 1982, on potatoes in 1994, and on snap beans on 1997. The 1997 assessment noted that chronic toxicity studies were unavailable for aquatic animals; however, chronic exposure to birds and mammals resulted in reproductive effects that were characterized as anti-androgenic and indicative of a chemical acting on endocrine-mediated processes. The RED was signed in October 2000 and product reregistration was completed in July 2006.

Several emergency (Section 18) exemptions have been granted for the use of vinclozolin on caneberries in Washington State (1985) and on canola (1997) in North Dakota and Minnesota. Section 18 actions have noted that vinclozolin and its degradation products could persist in the environment and be available for runoff for several weeks to months post-application. In terms of potential effects these assessments have stated that vinclozolin's use may pose a chronic risk to bird and mammalian species and that the chemical acts on endocrine-mediated processes in mammals

In the 2000 RED, vinclozolin was classified as a Group C chemical (possible human carcinogen). The terminal metabolite of vinclozolin, 3,5-DCA was considered to have a genotoxic mode of tumor induction based on its similarity to its structural analog parachloraniline, which is carcinogenic in mammals.

2.4 Stressor Source and Distribution

2.4.1 Environmental Fate and Transport Assessment

Available laboratory studies for vinclozolin indicate that it degrades via hydrolysis quickly in neutral water (half-life = 1.3 d). In aerobic and anaerobic environments, vinclozolin breaks down via microbial degradation, with half-lives ranging 17.6-352 days. Vinclozolin can also be degraded via photolysis, with half-lives of 18.1 and 27.2 days in soil and aqueous environments, respectively (**Table 3**). However, as described below, vinclozolin breaks down to several degradates that are of concern for this assessment.

According the available environmental fate studies, vinclozolin has several major (form ≥10% of applied) degradates (Table 3, Figure 1), including:

- metabolite B (N-3,5-dichlorophenyl)carbamic acid(1-carboxy-1-methyl)-2-propenyl ester),
- metabolite E (N-3,5-dichlorophenyl)-2-hydroxy-2-methyl-3-butenoic acid amide),

- metabolite S (N-(3,5-dichlorophenyl)-5-methyl-2,4-oxazolidinedione) and
- 3,5-DCA.

In addition to these degradates, metabolite F (N-(3,5-dichlorophenyl)-2-methyl-2,3,4-trihydroxybutanoic acid amide) was observed as a major degrade in an available bioconcentration study with the bluegill sunfish. There are no data available on the persistence of metabolites B, E, F and S. A limited amount of data are available to characterize the environmental fate of 3,5-DCA. **It is assumed that metabolites B, E, F and S are intermediate metabolites between vinclozolin and its ultimate degradation product, 3,5-DCA.**

Based on available soil partitioning data, vinclozolin, metabolite E and 3,5-DCA have the potential to move from treatment sites to non-target areas via runoff and leaching. Available data indicate that vinclozolin residues of concern have the potential to be transported off site of treatment areas via volatilization. The compound may also move off-site through spray drift. Bioaccumulation is unlikely to be a concern for vinclozolin residues of concern. The physical and chemical properties of vinclozolin and 3,5-DCA are provided in **Table 4**. The environmental fate and transport data relevant to vinclozolin are summarized below and in **Table 3**.

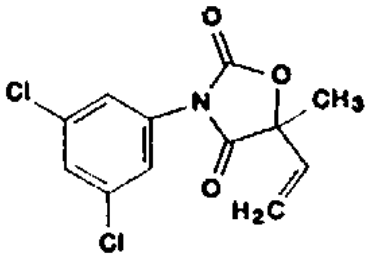
Table 3. Environmental fate half-lives relevant to vinclozolin and observed residues of concern.

Study	Vinclozolin Half-life (d)	Major degradates (≥10% of applied)	Minor degradates (<10% of applied)	Source (MRID)
Hydrolysis pH 5 pH 7 pH 9	 41.8 1.3 0.026 (38 min)	B & E	None reported	 41471006 44025301
Aqueous Photolysis	27.2	B & E	None reported	42394706
Soil Photolysis	18.1	B & S	E	41471008 44025302
Aerobic Soil Metabolism (loamy sand)	35	B	E and S	135376
	41	B	3,5-DCA, E and S	135376
	53	B	E and S	135376
	53	B	3,5-DCA, E	88288
	352	None reported	B, E, 3,5-DCA	43013001 44025303
Anaerobic Soil Metabolism	17.6	B	3,5-DCA, E	41471009
Aerobic aquatic metabolism	Not available			
Anaerobic aquatic metabolism	134	B, 3,5-DCA	E & S	43013002 43255801

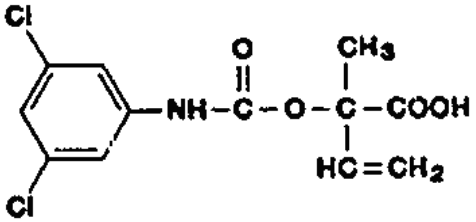
Table 4. Physical and chemical properties of vinclozolin and 3,5-DCA.

Parameter (units)	Vinclozolin[2]	3,5-DCA[3]
Molecular weight (g/mol)	286.11	162.02
Vapor Pressure (torr)	2.6×10^{-6}	2.12×10^{-2}
Henry's Law Constant (atm-m^3/mol)[1]	3.8×10^{-7}	5.8×10^{-6}
Solubility in Water (mg/L; @20^{o}C)	2.6	784
Octanol-water partition coefficient (Kow)	1054	794

[1] Calculated according to USEPA 2002*b* by: (VP *MW)÷(760*solubility).
[2] From registrant-submitted product chemistry data.
[3] Estimated using EPISuite



Vinclozolin



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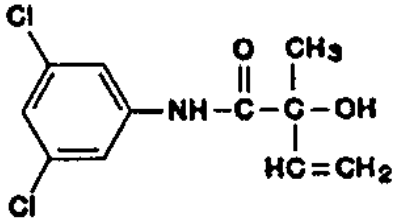

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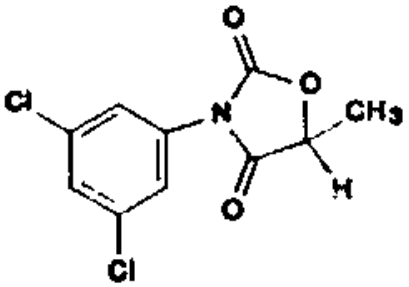
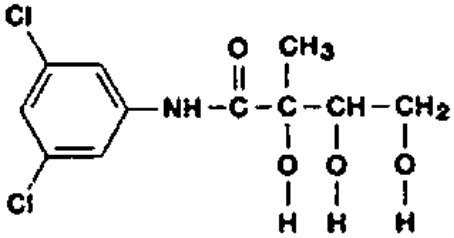
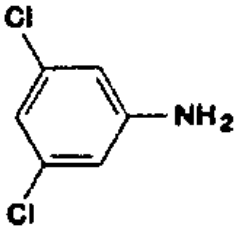


Metabolite S



Metabolite F



3,5-DCA

Figure 1. Structures of vinclozolin residues of concern.

Hydrolysis

Vinclozolin hydrolyzes quickly in neutral (pH 7) and basic (pH 9) conditions, with reported half-lives of 1.3 days and 38 minutes, respectively. Under acidic (pH 5) conditions, vinclozolin hydrolyzes more slowly, with a half-life of 41.8 days. In the available hydrolysis study for vinclozolin, metabolites B and E were observed as major degradates. No other degradates were considered as analytes in this study. Peak levels of metabolite B were observed in pH 5, 7 and 9 at 40.5, 76.4, and 68.7% of applied, respectively. Peak levels of metabolite E were observed in pH 5, 7 and 9 at 26.2, 16.8, and 20.9% of applied, respectively. In the 5-day study at pH 7,

vinclozolin was hydrolyzed almost completely (approximately 93% of total residues) to Metabolites B and E (MRIDs 41471006, 44025301). When metabolites B and E are considered as residues of concern, vinclozolin residues of concern are stable to hydrolysis.

Photolysis

Vinclozolin degraded in the aqueous environment via photolysis with a half-life of 27.2 days. As with the available hydrolysis study described above, metabolites B and E were observed as major degradates, with peak levels of 26.6% and 10.5% of applied observed in the light treatment. When considering the persistence of vinclozolin residues of concern, (this includes vinclozolin and metabolites B and E), the aqueous photolysis half-life of these residues is 75.5 d. Given that concentrations of metabolite E increased throughout the study, the 30 d study was not of sufficient duration to capture the full formation and decline of metabolite E. The study was also not of sufficient duration to capture the formation and decline of 3,5-DCA, which was not detected during this study (MRID 42394706).

In a soil photolysis study, vinclozolin had a half-life of 18.1 days. Metabolites B and S were observed as major degradates in the light treatment, with peak levels of 12.9% and 32.7% of applied, respectively. Metabolite E was observed as a minor degradate, with peak levels of 5.3% of applied. In the dark treatment, metabolite E was not detected (MRIDs 41471008, 44025302).

Microbial degradation (metabolism)

Under aerobic conditions, vinclozolin degraded slowly in soil (loamy sand), with a half-life of 352 days. Metabolites B and E and 3,5-DCA were observed during this study as minor degradates (i.e., % of applied was <10%) (MRID 43013001 and 44025302). In a second aerobic soil metabolism study, which was conducted using a German soil (loamy sand), vinclozolin degraded more quickly than the previously described study, with a half-life of 53 days. In this study, Metabolite B was observed as a major degradate, with a maximum observed level of 10.1% of applied. 3,5-DCA and Metabolite E were observed in the study; however their concentrations were not reported (MRID 88288). In a third aerobic soil metabolism study, vinclozolin degraded with half-lives of 35, 41, and 53 days on German soils (loamy sand). Metabolite B was observed as a major degradate in both soils. 3,5-DCA and Metabolites E and S were observed as minor degradates (MRID 136376).

An aerobic soil metabolism study of 3,5-DCA (on two different soils) showed little evidence that 3,5-DCA appreciably degraded over a 9-month period at 25ºC (MRID 45239201). Apparent dissipation was caused by a high level of unextracted residue. Unextracted residues accounted for 66% and 81% of the applied in the two systems. The only residues that were distinguishable from the parent amounted to only 4-5% of the applied ^{14}C.

Under anaerobic conditions, vinclozolin degraded more quickly in soil (loamy sand) compared to aerobic conditions. Available data indicate that vinclozolin has a half-life of 17.6 days in soil under anaerobic conditions. In this study, metabolite B was observed as a major degradate, with a peak observed level of 35.7%. 3,5-DCA and metabolite E were observed as minor degradates,

with maximum observed levels of 3.8 and 3.5% of applied. Concentrations of metabolite B increased throughout the study (MRID 41471009).

In an anaerobic aquatic metabolism study, vinclozolin degraded with a half-life of 134 days. 3,5-DCA and Metabolite B were observed in this study as major degradates, with a maximum of 10.5% and 57% of applied, respectively. Metabolites E and S were also observed in this study as minor degradates. When considering the persistence of vinclozolin residues of concern (vinclozolin + metabolites B , E, F + 3,5-DCA), the anaerobic aquatic metabolism half-life of these residues is 630 d. Given that concentrations of 3,5-DCA increased throughout the study, the 371 d study was not of sufficient duration to capture the full formation and decline of 3,5-DCA (MRIDs 43013002 and 43255801).

Volatilization

In a laboratory volatility study, a maximum of 7.1% of applied vinclozolin residues volatilized from sand over a 30 day period. This corresponded to a flux rate that ranged between 0.00159-0.0450 µg/cm^2*h (0.00341-0.0964 lb/A*d) for a 6 lb a.i./A application (which is higher than the currently allowed maximum application rate). The majority of volatile residues were identified as vinclozolin, with some residues identified as metabolites B and E (MRID 42513101). For a 1.35 lb a.i./A application, which is the maximum single application of vinclozolin relevant to CA, the corresponding flux rate would be 0.000767-0.0217 lb/A*d.

Based on this information, in combination with the vapor pressure and Henry's law constants of 3,5-DCA (Table 4), volatilization represents a potential transport pathway of vinclozolin residues of concern.

Sorption

Batch equilibrium studies indicate that vinclozolin, 3,5-DCA and metabolite E are moderately mobile in soil (according to the FAO classification scheme for K_{oc}) (**Table 5**).

Table 5. K_{oc} data for vinclozolin, 3,5-DCA and metabolite E in different soils.

Soil	Vinclozolin (MRID 41471010)	Metabolite E (MRID 41888904)	3,5-DCA	
			(MRID 41888904)	(MRID 45114101)
Sand	396	562	496	NA
Sandy loam	735	239	356	593
Silt Loam	NA	NA	NA	380
Loam	535	260	408	NA
Loamy sand	NA	NA	NA	626
Clay loam	476	611	908	NA
Clay	NA	NA	NA	932
Mean	536	418	542	633

NA = not available

In sand, sandy loam, loam and clay loam soils, K_{oc} values for vinclozolin were: 396 (Freundlich K_d = 0.46, 1/n = 0.76), 735 (K_d = 3.82, 1/n = 0.99), 535 (K_d = 3.4, 1/n = 0.92), and 476 (K_d = 5.27, 1/n = 0.82), respectively (MRID 41471010). Kd values for vinclozolin correlated with soil organic matter content (R^2 = 0.90), indicating that Koc is a representative measure of the soil partitioning of vinclozolin.

For Metabolite E, K_{oc} values were: 562 (K_d = 0.65, 1/n = 0.98), 239 (K_d = 1.24, 1/n = 0.84), 260 (K_d = 1.66, 1/n = 0.78) and 611 (K_d = 6.73, 1/n = 0.80) in sand, sandy loam, loam and clay loam soils, respectively (MRID 41888904). Kd values for metabolite E correlated with soil organic matter content (R^2 = 0.82), indicating that Koc is a representative measure of the soil partitioning of metabolite E.

For 3,5-DCA, K_{oc} values were: 496 (K_d = 0.58, 1/n = 0.74), 356(K_d = 1.86, 1/n = 0.82), 408 (K_d = 2.60, 1/n = 0.79) and 908 (K_d = 10.0, 1/n = 0.76) in sand, sandy loam, loam and clay loam soils, respectively (MRID 41888904). In an additional batch sorption study with 3,5-DCA, K_{oc} values were: 593 (K_d = 1.75, 1/n = 0.68), 626 (K_d = 7.17, 1/n = 0.634), 380 (K_d = 10.98, 1/n = 0.692) and 932 (K_d = 9.17, 1/n = 0.743) in sandy loam, loamy sand, silt loam and clay soils, respectively (MRID 45114101). Kd values for 3,5-DCA correlated with soil organic matter content (R^2 = 0.78), indicating that Koc is a representative measure of the soil partitioning of 3,5-DCA.

Based on this information, vinclozolin and its residues of concern have the potential to be transported from treatment sites through runoff to surface waters or leaching to ground water.

Bioaccumulation

In a bioconcentration study with bluegill sunfish, total radioactive residues of vinclozolin concentrated in fish tissues at a factor of 241X for whole fish. After a 14-day depuration period, total radioactive residues declined 97.7% (from maximum). Metabolite F was reported as a major degradate, representing as much as 24.7% and 9.3% of total radioactivity in edible and non-edible tissues, respectively (MRID 136387). In a second bioconcentration study with bluegill sunfish, total radioactive residues of vinclozolin concentrated in fish tissues at a factor of 279X for whole fish. 3,5-DCA and Metabolites B and S were observed in fish tissues. In the edible tissues, 6.9-17.1% of radioactivity was not identified. In the non-edible tissues, 27.5-34.5% of radioactivity was not identified (MRID 42847001). This leads to uncertainty in the identification and relative proportions of the vinclozolin degradates present in the fish.

The octanol-water partition coefficient (1054) along with the submitted BCF studies indicate that vinclozolin is not likely to bioaccumulate significantly in aquatic ecosystems.

Terrestrial Field Dissipation Studies

Four terrestrial field dissipation studies on multiple crops and locations are available for vinclozolin (MRIDs 41538301, 42687601, 42717401 and 43505907). In acceptable terrestrial field dissipation studies conducted in FL, NY, MO, and CA, vinclozolin dissipated with half-lives of 34 to 94 days. Half-lives for total residues (vinclozolin plus its dichloroaniline-

containing metabolites) were 179 to >1000 days. Dissipation half-lives of >2500 days for total residues were reported for bare ground and turf studies in MO and NY. Persistence of total residues appeared to be attributable to the resistance of 3,5-DCA to degradation and to the inclusion of soil-bound residues in the data. Intermittent detections of residues were reported at soil depths of 12-18, 18-24, and 24-30 inches. 3,5-DCA was detected regularly deeper than 6 inches. Residues may accumulate and be available for rotational crop uptake.

2.4.2 Mechanism of Action

Vinclozolin is a member of the carboximide fungicides used to control various blights and rots caused by fungal pathogens. In mammals, the principal toxic effects induced by vinclozolin and/or its metabolites are related to its antiandrogenic activity and its ability to act as a competitive antagonist at the androgen receptor (USEPA 2000). According to the RED, there is evidence that vinclozolin binds fairly weakly to the androgen receptor but that at least two vinclozolin metabolites (B and E) occurring in mammals, plants, and soil are responsible for much of the antiandrogenic activity attributed to vinclozolin.

2.4.3 Use Characterization

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

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

Currently, vinclozolin has two registered formulated products (registration #s: 7969-85 and 7969-224). Registration #7969-85 (Ronilan® EG fungicide) allows for vinclozolin use on canola in the U.S., with the exception of California and Florida. Therefore, use of vinclozolin on canola is not considered relevant to this assessment. Registration # 7969-224 (Curalan® EG fungicide) allows for vinclozolin use on turf grass in the U.S. Since there are no prohibitions of use in California on the label for Curalan® EG fungicide, use of vinclozolin on turf grass is considered relevant to this assessment.

Curalan® EG fungicide is formulated as a 50% extruded granule (EG) sold only in water-soluble packets. This product label indicates that it should be used as a preventative treatment. The label indicates that single applications of vinclozolin should be made at 1.35 lbs a.i./A with intervals of 10-28 days (the specific interval depends upon the disease being treated). According to the Use Verification Memo (**Appendix A**) issued by the EPA Office of Pesticide Programs (OPP) Pesticide Reregistration Division (formerly known as the Special Review and

Reregistration Division (SRRD)), the maximum number of applications per year is three (3). The label indicates that the product be applied at a maximum seasonal rate of 4 lbs a.i./A, and with a maximum of 3 applications per season, this is equivalent to 3 applications of 1.35 lbs a.i./A. Applications are made by ground spray. The label prohibits applications by air and by chemigation. The label also prohibits applications to residential turf. According to the Use Verification Memo (**Appendix A**), the technical registrant (BASF) restricted the area of the golf course to which product can applied to tee boxes, greens, and turf mowed at 1" or less. Additionally, sod was restricted to sod produced for golf course landscape only.

Figure 2 depicts total vinclozolin use in California from 1997 to 2007 and indicates that from 2006 – 2007, total use averaged 390 lbs based on California Pesticide Use Reports[1] (PUR). Compared to the peak use of 52,731 lbs reported for 1998, vinclozolin use in California has declined by roughly 99%. Where total acreage treated in 1998 was 69,067 acres, the acreage treated had declined to 258 acres in 2007 representing a 99.6% decline. This can be attributed to mitigation imposed by the 2000 RED with the majority of uses phased-out by 2004 (including use of lettuce that was ended in 2005). According to the California PUR use report in 2007, of the total pounds of vinclozolin applied, 82% was used in landscape maintenance. Of the remaining amount, the majority was used on ornamental flowers and plants in greenhouses and outdoors; approximately 4% was reportedly applied to peaches. If only use of vinclozolin in CA on "landscape maintenance" (assumed to be a surrogate for applications to turf grass) is considered from 1999 to 2006, applications ranged 289 to as high as 1898 lbs in a single year (Figure 3). This corresponds to an annual average of 709 lbs.

The uses considered in this risk assessment represent all currently registered uses according to a review of all current labels, relevant to the CRLF. No other uses are relevant to this assessment. Any reported use other than currently registered uses represent either historic uses that have been canceled, mis-reported uses, or mis-use. Historic uses, mis-reported uses, and misuse are not considered part of the federal action and, therefore, are not considered in this assessment.

[1] California Department of Pesticide Regulation. 2007. Summary of Pesticide Use Report Date 2007 Indexed by Chemical. http://www.cdpr.ca.gov/docs/pur/pur07rep/chmrpt07.pdf

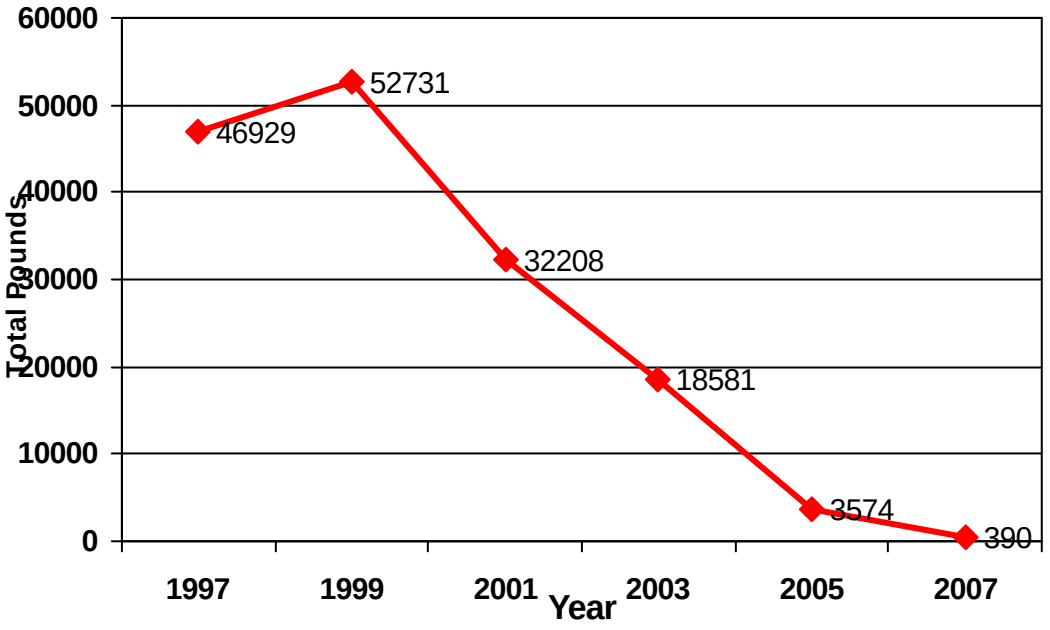


Figure 2. Total annual use of vinclozolin in California between 1996 - 2007. California Department of Pesticide Regulation.

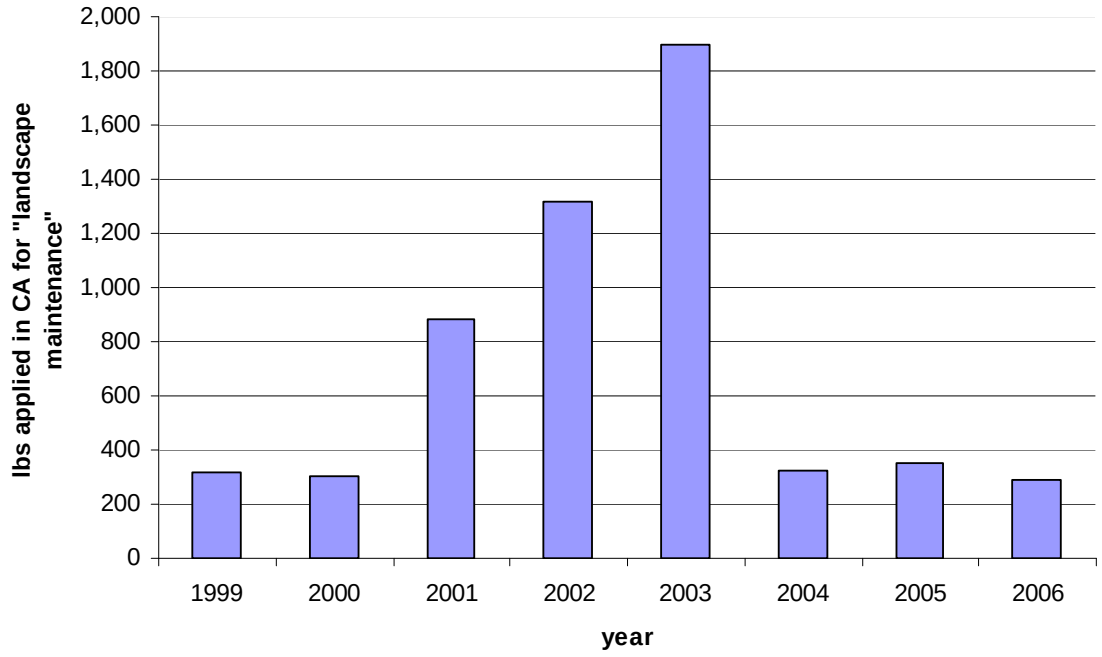


Figure 3. Pounds of vinclozolin applied in a single year for "landscape maintenance" in CA from 1999 to 2006. From CA PUR.

2.5 Assessed Species

The CRLF was federally listed as a threatened species by USFWS effective June 24, 1996 (USFWS 1996). It is one of two subspecies of the red-legged frog and is the largest native frog in the western United States (USFWS 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 USFWS on April 13, 2006 (USFWS 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 (USFWS 1996). Its range has been reduced by about 70%, and the species currently resides in 22 counties in California (USFWS 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) (USFWS 2002).

Populations currently exist along the northern California coast, northern Transverse Ranges (USFWS 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 (USFWS 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) (USFWS 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 (see Figure 4). 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 USFWS 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.

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 4. 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 (USFWS 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, USFWS 2002); tadpoles have been observed to over-winter (delay metamorphosis until the following year) (Fellers 2005b, USFWS 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 (USFWS 2002). Figure 5 depicts CRLF annual reproductive timing.

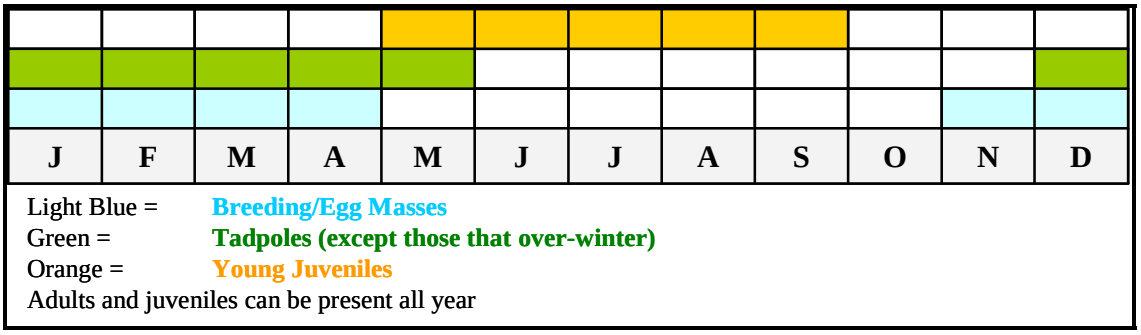


Figure 5. CRLF Reproductive Events by Month.

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 (USFWS 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 (USFWS 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 (USFWS 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 (USFWS 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://www.fws.gov/endangered/features/rl_frog/rlfrog.html#where).

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 (USFWS 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 (UWFWS 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 USFWS (USFWS 2006; FR 51 19244-19346). A summary of the 34 critical habitat units relative to USFWS-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, USFWS 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 FR listing notice in April 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.

USFWS has established adverse modification standards for designated critical habitat (USFWS 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 vinclozolin that may alter the PCEs of the CRLF's critical habitat form the basis of the critical habitat impact analysis. According to USFWS (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 vinclozolin is expected to directly impact living organisms within the action area, critical habitat analysis for vinclozolin 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 vinclozolin is likely to encompass considerable portions of the United States based on the use of vinclozolin on turf grass and canola (outside of California). 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 (USEPA 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 vinclozolin may be expected to have on the environment, the exposure levels to vinclozolin that are associated with those effects, and the best available information concerning the use of vinclozolin and its fate and transport within the state of California. Specific measures of ecological effect for the CRLF that define the action area include any direct and indirect toxic effect to the CRLF 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 vinclozolin. An analysis of labeled uses and review of available product labels was completed (see section 2.4.3). Use of vinclozolin on canola is permitted in the United States; however, since this use is prohibited in California (and also in Florida), it is not considered part of the federal action. For those uses relevant to the CRLF, the analysis indicates that, for vinclozolin, the following use is considered as part of the federal action evaluated in this assessment: turf grass (for golf courses and industrial park landscapes; this does not include residential areas).

Following a determination of the assessed uses, an evaluation of the potential "footprint" of vinclozolin use patterns (i.e., the area where pesticide application occurs) is usually determined. This "footprint" represents the initial area of concern, based on an analysis of available land cover data for the state of California. As indicated above, the federal action assessed here is the use of vinclozolin on golf course and industrial park landscape turf grass. Vinclozolin use is prohibited on residential turf. Available turf grass landcovers include residential areas; therefore, a landcover map representative of the footprint for vinclozolin cannot be derived.

In place of this initial footprint, an analysis of the pesticide use data for vinclozolin can provide useful information of where vinclozolin has been used in the past. From 1999-2006, vinclozolin was used in 26 counties in CA for "landscape maintenance." Several of these counties (Alameda, Butte, Contra Costa, Los Angeles, Marin, Merced, Monterey, Orange, Riverside, Sacramento, San Diego, San Joaquin, San Luis Obispo, San Mateo, Santa Barbara, Santa Clara,

Santa Cruz, Solano, Sonoma, Stanislaus, and Ventura) include core areas and critical habitat of the CRLF.

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. In this assessment, transport of vinclozolin through runoff and spray drift is considered in deriving quantitative estimates of vinclozolin exposure to CRLF, its prey and its habitats. Since this screening level risk assessment defines taxa that are predicted to be exposed through runoff and drift to vinclozolin at concentrations above the Agency's Levels of Concern (LOC), there is need to expand the action area to include areas that are affected indirectly by this federal action. Because vinclozolin was classified as a Group C chemical (possible human carcinogen) and the terminal metabolite of vinclozolin, 3,5-DCA was considered to have a genotoxic mode of tumor induction (based on its similarity to its structural analog parachloraniline which is carcinogenic in mammals), the action area for vinclozolin is established as 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."[2] 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 vinclozolin (*e.g.*, runoff, spray drift, etc.), and the routes by which ecological receptors are exposed to vinclozolin (*e.g.*, direct contact, *etc*.).

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 (USEPA 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.

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

A 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 vinclozolin is provided in **Table 6**.

Table 6. Assessment Endpoints and Measures of Ecological Effects.

Assessment Endpoint	Measures of Ecological Effects
Aquatic-Phase CRLF (Eggs, larvae, juveniles, and adults)[a]	
Direct Effects	
1. Survival, growth, and reproduction of CRLF	1a. Rainbow trout (*Oncorhynchus mykiss*) 96-hr LC_{50}, *i.e*., the most sensitive acute exposure data available for fish 1b. Fathead minnow (*Pimephales promelas*) NOAEC, *i.e*., the most sensitive chronic exposure data available for fish
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. Waterflea (*Daphnia magna*) EC_{50}, *i.e.*, most sensitive acute exposure data available for aquatic invertebrates 2b. Waterflea NOAEC, *i.e.*, most sensitive chronic exposure data available for aquatic invertebrates 2c. Rainbow trout LC_{50}, based on most sensitive acute exposure data available for fish 2d. Fathead minnow NOAEC, based on most sensitive chronic exposure data available for fish 2e. EC_{50}, based on available data for freshwater diatom *Navicula pelliculosa*.
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. EC_{50} based on most sensitive vascular plant, *i.e.*, duckweed (*Lemna gibba*). 3b. EC_{50} based on available data for freshwater diatom *Navicula pelliculosa*.
4. Survival, growth, and reproduction of CRLF individuals via effects to riparian vegetation	No data are available to quantify an endpoint to represent effects of vinclozolin exposures to terrestrial plants.
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. Northern bobwhite quail (*Colinus virginianus*) LD_{50}, based on most sensitive acute oral exposure data available for birds[2] 5b. Northern bobwhite quail LC_{50}, based on most sensitive subacute dietary exposure data available for birds[2] 5c. Northern bobwhite quail NOAEC, based on most sensitive chronic exposure data available for birds[2]
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. Honeybee (*Apis mellifera*) LD_{50}, based on most sensitive acute contact exposure data available for terrestrial invertebrates. 6b. Laboratory rat (*Rattus norvegicus*) LD_{50}, based on most sensitive acute oral exposure data available for mammals 6c. Laboratory rat NOAEC, based on most sensitive chronic exposure data available for mammals 6d. Northern bobwhite quail LD_{50}, based on most sensitive acute oral exposure data available for birds[2] 6e. Northern bobwhite quail LC_{50}, based on most sensitive subacute dietary exposure data available for birds[2] 6f. Northern bobwhite quail NOAEC, based on most sensitive chronic exposure data available for birds[2]
7. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat (*i.e.*, riparian and upland vegetation)	No data are available to quantify an endpoint to represent effects of vinclozolin exposures to terrestrial plants.

[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.

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 vinclozolin 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 vinclozolin 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 vinclozolin on critical habitat of the CRLF are described in **Table 7**. 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 USFWS (2006).

Table 7. Summary of Assessment Endpoints and Measures of Ecological Effect for Primary Constituent Elements of Designated Critical Habitat[a].

Assessment Endpoint	Measures of Ecological Effect
Aquatic-Phase CRLF 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.	3a. Duckweed EC_{50}, based on most sensitive vascular plant 3b. *N. pelliculosa* EC_{50} based on most sensitive non-vascular plant (freshwater diatom) No data are available to quantify an endpoint to represent effects of vinclozolin exposures to terrestrial 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.	*N. pelliculosa* EC_{50}, based on most sensitive non-vascular plant (freshwater diatom) No data are available to quantify an endpoint to represent effects of vinclozolin exposures to terrestrial plants.
Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.	LC_{50} = 2.84 mg/L, based on most sensitive acute exposure data available for fish EC_{50} = 4.0 mg/L, based on most sensitive acute exposure data available for aquatic invertebrates Fathead minnow NOAEC, based on most sensitive chronic exposure data available for fish Waterflea NOAEC, based on most sensitive chronic exposure data available for aquatic invertebrates
Reduction and/or modification of aquatic-based food sources for pre-metamorphs (*e.g.*, algae)	Duckweed EC_{50}, based on most sensitive vascular plant *N. pelliculosa* EC_{50}, based on most sensitive non-vascular plant (freshwater diatom)
Terrestrial-Phase CRLF 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 data are available to quantify an endpoint to represent effects of vinclozolin exposures to terrestrial plants. Honeybee LD_{50}, based on most sensitive acute exposure data available for terrestrial invertebrates Laboratory rat LD_{50}, based on most sensitive acute oral exposure data available for mammals Laboratory rat NOAEL, based on most sensitive chronic exposure data available for mammals Northern bobwhite quail LD_{50}, based on most sensitive acute oral exposure data available for birds[2] Northern bobwhite quail LC_{50}, based on most sensitive subacute dietary exposure data available for birds[2] Northern bobwhite quail NOAEC, based on most sensitive chronic exposure data available for birds[2]
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	
Reduction and/or modification of food sources for terrestrial phase juveniles and adults	
Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	

[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 vinclozolin to the environment. The following risk hypotheses are presumed for this endangered species assessment.

The labeled use of vinclozolin 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.
- 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 vinclozolin release mechanisms, biological receptor types, and effects endpoints of potential concern. The conceptual models for terrestrial and aquatic exposures are shown in **Figure 6** and **Figure 7**, respectively, which include the conceptual models for the aquatic and terrestrial PCE components of critical habitat.

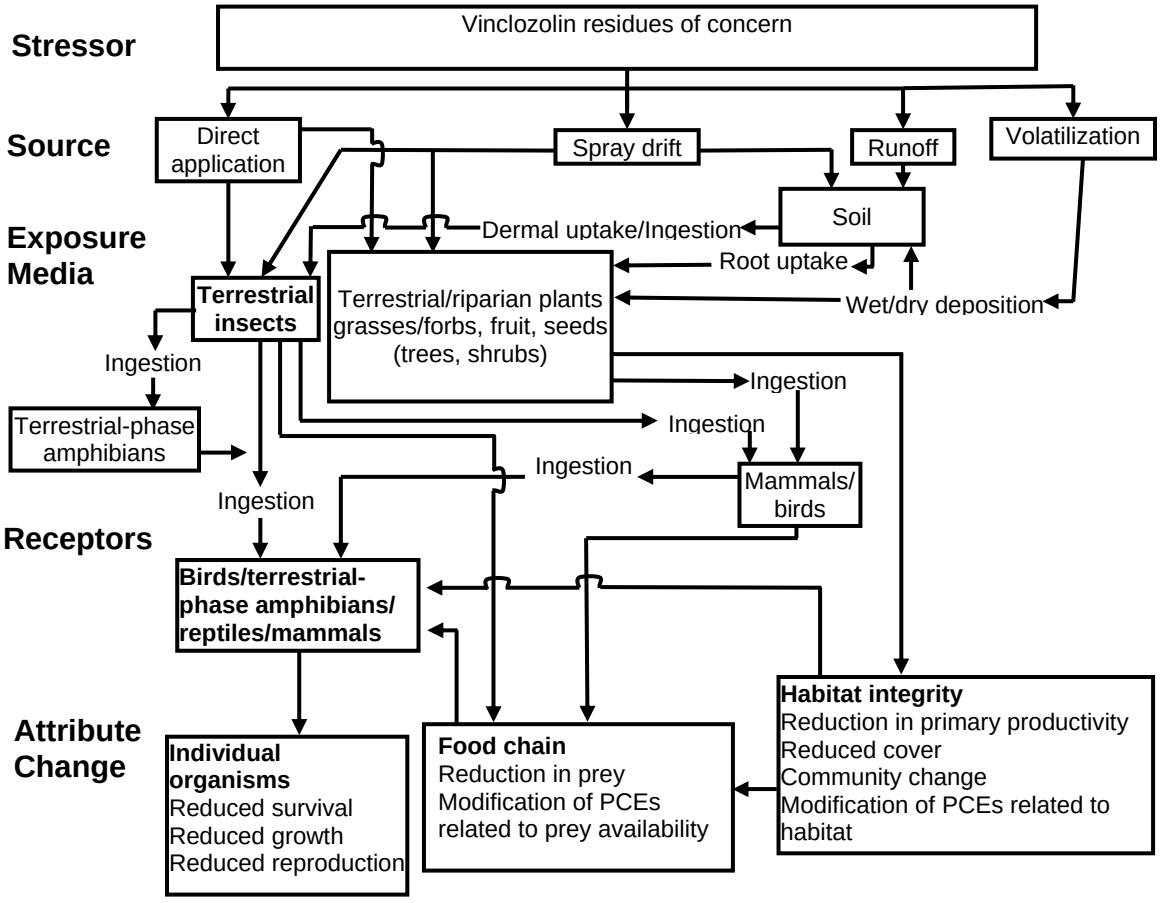


Figure 6. Conceptual Model for Vinclozolin Effects on Terrestrial Phase of the CRLF.

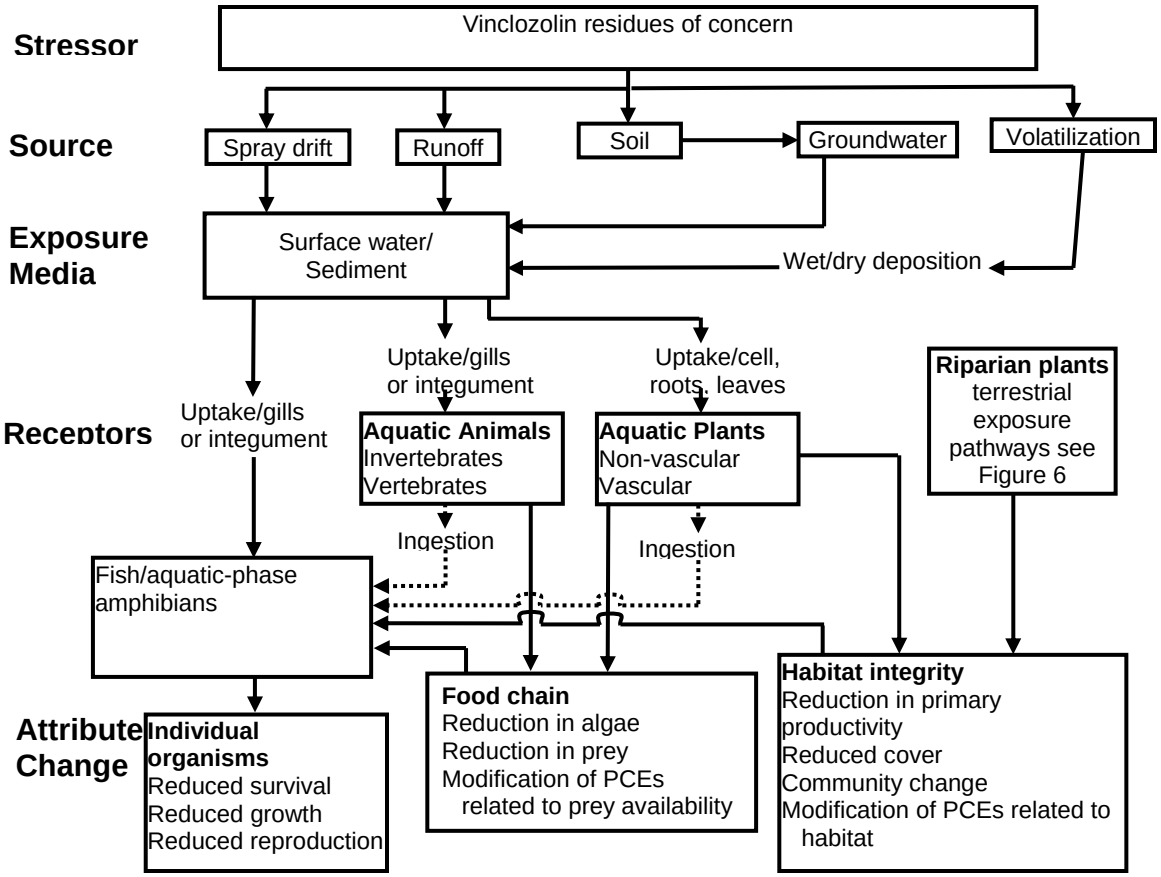


Figure 7. Conceptual Model for Vinclozolin Effects on Aquatic Phase of the CRLF.

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 vinclozolin 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 vinclozolin 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 vinclozolin indicate that runoff and spray drift are the principle potential transport mechanisms of vinclozolin to the aquatic and terrestrial habitats of the CRLF. In this assessment, transport of vinclozolin through runoff and spray drift is considered in deriving quantitative estimates of vinclozolin exposure to CRLF, its prey and its habitats.

Measures of exposure are based on aquatic and terrestrial models that predict estimated environmental concentrations (EECs) of vinclozolin and residues of concern 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). 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 vinclozolin residue that may occur in surface water bodies adjacent to application sites receiving loading 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 vinclozolin. 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.

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 1.4.1, 06/21/2008). 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 vinclozolin 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 vinclozolin 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 1.4.1) 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.

The spray drift model, AgDRIFT is used to assess exposures of terrestrial phase CRLF and its prey to vinclozolin deposited on terrestrial habitats by spray drift. In addition to the buffered area from the spray drift analysis, the downstream extent of vinclozolin that exceeds the LOC for the effects determination is also considered.

2.10.1.2 Measures of Effect

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 (USEPA 2009). 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 USEPA, 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 vinclozolin 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 vinclozolin, 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 vinclozolin 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) (USEPA, 2004) (see **Appendix B**).

For this endangered species assessment, listed species LOCs are used for comparing RQ values for acute and chronic exposures of vinclozolin directly to the CRLF. If estimated exposures directly to the CRLF of vinclozolin 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 vinclozolin 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 B**.

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

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

2.10.2 Data Gaps

At this time, there are no data available on the aerobic aquatic metabolism half-life for vinclozolin. Thus the extent to which vinclozolin is subject to biotic degradation in aerobic aquatic areas (*e.g.*, the water column of a pond) is uncertain. However, given the rapid rate of hydrolysis at environmentally relevant pHs, hydrolysis is expected to be the major degradation process in aquatic environments.

Additionally, there are no acceptable terrestrial plant toxicity data for vinclozolin. Since the EC_{50} has not been definitively established for freshwater diatoms (*N. pelliculosa*), it too represents a data gap.

There are also limited data available to characterize the environmental fate and effects of vinclozolin's major degradates.

3.0 Exposure Assessment

3.1 Label Application Rates and Intervals

As indicated in Section 2.4.3 (use characterization), the only use of vinclozolin that is relevant to California is applications to turf grass (specifically, golf courses and industrial park landscapes). The label indicates that single applications of vinclozolin should be made at 1.35 lbs a.i./A with intervals of 10-28 days (the specific interval depends upon the disease being treated). According to the Use Verification Memo (**Appendix A**), the maximum number of applications per year is three (3). The label indicates that the product be applied at a maximum seasonal rate of 4 lbs

a.i./A, and with a maximum of 3 applications per season, this is equivalent to 3 applications of 1.35 lbs a.i./A. Applications are made by ground spray. The label prohibits applications by air and by chemigation. The label also prohibits applications to residential turf.

3.2 Surface Water Exposure Assessment

3.2.1 Modeling Approach

Aquatic exposures are quantitatively estimated (using PRZM/EXAMS) for all of assessed uses using scenarios that represent high exposure sites for vinclozolin use. To model vinclozolin use on turf grass, the CA turf scenario was selected.

Each PRZM scenario 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.

3.2.2 Model Inputs for Vinclozolin Residues of Concern

The appropriate chemical-specific PRZM input parameters are selected from reviewed physical, chemical and environmental fate data submitted by the registrant (**Table 3** and **Table 4**) and in accordance with EFED water model input parameter selection guidance (U.S. EPA 2002). The input parameters for relevant to the fate of vinclozolin residues of concern used in PRZM and EXAMS are in **Table 8**. Outputs from PRZM/EXAMS are provided in **Appendix C**.

As noted in section 2.4.1, it is assumed that metabolites B, E, F and S are intermediate metabolites between vinclozolin and its ultimate degradation product, 3,5-DCA. There is some uncertainty associated with the half-lives obtained from the available laboratory degradation studies with vinclozolin in that they were not necessarily of sufficient duration to capture the full formation and decline of 3,5-DCA. Therefore, half-lives calculated using these laboratory studies may under predict the half-lives of the vinclozolin total residues of concern. In order to provide a conservative estimate of exposure of non-target aquatic organisms to vinclozolin total residues of concern, it was assumed that these total residues were stable to degradation. Uncertainties associated with this approach are further explored in section 6.

Table 8. PRZM/EXAMS input parameters relevant to the fate of vinclozolin.

Input Parameter	Value	Comments
Molecular Wt. (g/mol)	286.11	Value for vinclozolin; See **Table 4**.
Henry's Law Constant (atm-m^3/mol)	3.8×10^{-7}	Value for vinclozolin; See **Table 4**.
Vapor pressure (torr)	2.6×10^{-6}	Value for vinclozolin; See **Table 4**.
Solubility in water (mg/L @ pH 7, 20°C)	2.6	Value for vinclozolin; See **Table 4**.
Hydrolysis half-life (days)	0*	Vinclozolin residues of concern are stable to hydrolysis (MRID 41471006)
Aqueous photolysis half-life (days)	0*	An aqueous photolysis half-life of 75.5 days can be derived to represent vinclozolin + metabolites B and E. Given that concentrations of metabolite E increased throughout the study, the 30 d study was not necessarily of sufficient duration to capture the full formation and decline of metabolite E. The study was also not of sufficient duration to capture the formation and decline of 3,5-DCA, which was not detected during this study (MRID 42394706).
Aerobic Soil Metabolism Half-life (days)	0*	It is assumed that vinclozolin residues of concern are stable, based on an aerobic soil metabolism study indicating that 3,5-DCA (vinclozolin's terminal degradate) is stable (MRID 45239201)
Aerobic Aquatic Metabolism Half-life (days)	0*	No data are available for this half-life. Input parameter guidance indicates that in the case that a chemical is stable to hydrolysis, this parameter should be defined as 2x the aerobic soil metabolism half-life used in PRZM (which is 0).
Anaerobic Aquatic Metabolism Half-life (days)	0*	An anaerobic aquatic half-life of 630 days was derived to represent vinclozolin + metabolites B , E, F + 3,5-DCA. Given that concentrations of 3,5-DCA increased throughout the study, the 371 d study was not necessarily of sufficient duration to capture the full formation and decline of 3,5-DCA (MRID 43013002).
K_{oc} (L/kg_{OC})	532	Mean of K_{oc} values for vinclozolin, metabolite E and 3,5-DCA (**Table 5**).

*A value of 0 indicates that vinclozolin total residues of concern are stable to degradation.

Application methods, maximum rates per application and maximum number of applications per year are based on current label directions for use of vinclozolin on turf grass. Values relevant to these input parameters are provided in Table 9. The application date is not explicitly stated on the label. The application date used in this modeling approach was October 2, which was based on the date where the highest peak EEC was determined from the vinclozolin modeling that involved investigation of application date on EECs (see **Appendix D**).

Table 9. PRZM/EXAMS input parameters relevant to the use of vinclozolin.

Input Parameter	Value	Comments
# applications/year	3	Based on label
Maximum rate/application (kg a.i./ha)	1.51	Equivalent to 1.35 lbs a.i./A
CAM	2	For foliar application
IPSCND	1	In cases where CAM 2 is modeled, it is necessary to identify an IPSCND value, which represents the deposition of vinclozolin in the post-season. An IPSCND value of 1 represents conversion of vinclozolin remaining on foliage to surface application to the top soil layer.
Application date	October 2	Based on application date resulting in highest peak EEC observed for vinclozolin modeling.
Application interval (days)	10	Shortest interval indicated on label.
Spray drift fraction	0.01	Assumption relevant to ground application
Application efficiency	0.99	

3.2.3 Modeling Results

The peak one-in-ten year aquatic EEC for vinclozolin residues of concern is 52.0 µg/L. One-in-ten year 21-d and 60-d EECs were 51.1 and 49.9 µg/L, respectively.

3.2.4 Surface Water Monitoring Data

No California-specific water monitoring data are available for vinclozolin or metabolites B, E , F or S; however, data are available for its degradate of concern, 3,5-DCA, from the United States Geological Survey's (USGS) National Water Quality Assessment (NAWQA). These data are summarized below. No data are available in the CDPR Surface Water Database for vinclozolin or 3,5-DCA.

3,5-DCA was detected in 1.3% of 308 surface water samples collected from 2001-2009 in CA. The maximum reported concentration of 3,5-DCA was 0.0268 µg/L. The level of quantification of 3,5-DCA ranged 0.004-0.012 µg/L(USGS 2009).

It should be noted that available monitoring data are not necessarily targeted to detect maximum environmental concentrations of 3,5-DCA, and therefore may not be representative of peak chemical concentrations present in the field.

3.3 Ground Water Exposure Assessment

3.3.1 Modeling Approach

In order to estimate ground water EECs for vinclozolin residues of concern, Scigrow v2.3 was run with the input parameters provided in Table 10.

Table 10. Input parameters for Scigrow v.2.3 used to represent vinclozolin residues of concern.

Input Parameter	Value	Comments
Maximum rate/application (lbs a.i./A)	1.35	Based on label
# applications/year	3	Based on label
Koc (mL/g)	532	Mean of K_{oc} values for vinclozolin, metabolite E and 3,5-DCA (**Table 5**)
Soil metabolism half-life (days)	10,000	Selected large value to represent stable.

3.3.2 Modeling Results

The resulting ground water EEC was 6.71 µg/L. This value is an order of magnitude lower than the surface water EECs generated using PRZM/EXAMS, indicating that the surface water EECs represent more conservative values.

3.3.3 Ground Water Monitoring Data

No California-specific ground water monitoring data are available for vinclozolin or metabolites B, E , F or S; however, data are available for 3,5-DCA, from NAWQA. During 2001-2008, 3,5-DCA was detected in 5.7% of 229 ground water samples collected in CA. The maximum detected concentration of 3,5-DCA was 0.0983 µg/L (USGS 2009).

3.4 Terrestrial Exposure Assessment

T-REX (Version 1.4.1) is used to calculate dietary and dose-based EECs of vinclozolin for the CRLF and its potential prey inhabiting terrestrial areas (*i.e.*, small mammals, terrestrial-phase amphibians and terrestrial insects). EECs used to represent the CRLF are also used to represent exposure values for frogs serving as potential prey of CRLF adults. T-REX simulates a 1-year time period. For this assessment, spray applications of vinclozolin to turf grass are considered, as discussed in below.

Terrestrial EECs for foliar formulations of vinclozolin were derived for use on turf grass. Given that no data on interception and subsequent dissipation from foliar surfaces is available for vinclozolin, a default foliar dissipation half-life of 35 days is used based on the work of Willis and McDowell (1987). Vinclozolin use on turf grass was modeled as 3 applications of 1.35 lbs a.i./A with an application interval of 10 days. An output from T-REX is available in **Appendix E**.

For modeling purposes, exposures of the CRLF to vinclozolin 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 11**). Dietary-based EECs for small and large insects reported by T-REX as well as the resulting adjusted EECs are available in **Table 11**.

Table 11. Upper-bound Kenega Nomogram EECs for Dietary- and Dose-based Exposures of the CRLF and its Prey to Vinclozolin

EEC Description	Value	Unit
Dietary Based EEC for CRLF and terrestrial-phase amphibians (prey)	454	ppm
Dose Based for CRLF and terrestrial-phase amphibians (prey)	518	mg/kg-bw
Dietary based EEC for small mammals (prey)	808	ppm
Dose based EEC for small mammals (prey)	770	mg/kg-bw
Contact EEC for small insect (prey)	454	ppm
Contact EEC for large insect (prey)	50	ppm

Exposure estimates are not derived for terrestrial plants since no toxicity data are available for terrestrial plants with which to estimate potential risks from exposure.

3.5 Atmospheric Transport Assessment

Exposure of the CRLF to vinclozolin residues of concern through atmospheric transport and deposition cannot be precluded. At this time, an approved model for estimating atmospheric transport of pesticides and resulting exposure to organisms in areas receiving pesticide deposition from atmospheric transport is not available. Potential mechanisms of transport of vinclozolin residues of concern to the atmosphere via volatilization can only be discussed qualitatively; however, transport via spray drift is quantified in this assessment.

3.5.1 Spray Drift

In cases where RQs exceed the LOC for terrestrial animals, AgDRIFT was used to characterize the distance from the edge of the treated field where the risk extends. This was accomplished using the Tier 1 ground setting, assuming a high boom and ASAE very fine to fine droplet size distribution (90^{th} percentile of data). These parameter values were selected to represent the most conservative assumptions allowed by the Tier 1 ground setting of AgDRIFT. A terrestrial assessment was conducted to determine the distance from the edge of the field where the point deposition was below the lbs a.i./A rate that was required to result in no LOC exceedances for a taxa of concern (*i.e.*, terrestrial-phase CRLF and mammals). The results of this spray drift assessment are described in context of their relative RQ values in the risk description of this assessment.

3.5.2 Volatilization

As noted in section 2.4.1, in a laboratory volatility study, a maximum of 7.1% of applied vinclozolin residues volatilized from sand over a 30-day period (MRID 42513101). In addition, the vapor pressure of 3,5-DCA (2.12×10^{-2} torr, Table 4), indicates that vinclozolin residues of concern may volatilize from treatment sites and be transported to non-target areas.

3.5.3 Air Monitoring Data

Atmospheric monitoring conducted in Lompoc, California, in the summer of 2000 indicated quantifiable levels of vinclozolin at 16.2 ng/m^3 (Segawa 2003). The highest daily amount of vinclozolin used in Lompoc during 2000 was 119 lbs and the highest daily amount used during the monitoring period was 34.8 lbs. According to California pesticide use reporting data, use of vinclozolin in Santa Barbara County (where Lompoc is located) totaled 4,250 lbs in the year 2000.

4.0 Effects Assessment

This assessment evaluates the potential for vinclozolin 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, while terrestrial-phase effects are based on avian toxicity data, given that birds are generally used as a surrogate for terrestrial-phase amphibians. 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 vinclozolin.

Limited toxicity data are available to characterize the effects of vinclozolin degradates that have structures similar to the parent (metabolites B, E, S and F) to non-target organisms. As a conservative estimate, it is assumed that data available for vinclozolin are representative of effects to non-target organisms that may be caused by these metabolites. Based on the limited data available for 3,5-DCA, the degradate appears to be less toxic than the parent compound, and it is assumed that this chemical has a different mode of action compared to vinclozolin.

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). Open literature data presented in this assessment were obtained from ECOTOX information obtained on February 29, 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;
(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 vinclozolin.

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 F**. **Appendix F** also includes a rationale for rejection of those studies that did not pass the ECOTOX screen and those that were not evaluated as part of this endangered species risk assessment.

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

An open literature review was performed to ensure that all pertinent data were considered in this assessment. In the survey of ECOTOX studies conducted in February 2009, sixty-four studies were identified and citations are included in **Appendix F**. The studies were divided by their taxon of focus which resulted in the following distribution: 21 avian studies, 2 mammal studies, 20 freshwater fish studies, 5 freshwater invertebrate studies, 7 aquatic plant studies, 3 terrestrial plant study, 3 marine organism studies and 3 honey bee studies. Measurement endpoints from the studies in each of these groups were then compared to the lowest toxicity value identified in registrant-submitted studies presented earlier in this assessment to identify those studies which

contained more sensitive endpoints. Nineteen studies were found to have endpoints at or below the most sensitive registrant-submitted study endpoints. These 19 papers included 7 fish studies, 4 avian studies, 3 terrestrial plant studies, 2 aquatic invertebrate studies, 2 studies using both mammals and birds, and 1 aquatic plant study. Each of these studies then went through a primary assessment to determine their validity and relevance to the CRLF risk assessment. Studies deemed suitable underwent secondary review in which their statistical and research methods were scrutinized and evaluated.

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 vinclozolin. A summary of the available aquatic and terrestrial ecotoxicity information and the incident information for vinclozolin are provided in Sections 4.1 through 4.4.

In addition to vinclozolin, its degradates, principally Metabolites B and E and 3,5-DCA are known to be biologically active. The parent and its metabolites B and E can bind to the androgen receptor (USEPA 2000*a*) while 3,5-DCA is classified as a potential carcinogen. A detailed summary of the available ecotoxicity information for all vinclozolin degradates and formulated products is presented in **Appendix H**.

Vinclozolin is not formulated with any other actives and there are no additional toxicity data on vinclozolin [as a mixture] in either registrant-submitted and/or open literature sources.

4.1 Evaluation of Aquatic Ecotoxicity Studies for Vinclozolin

Table 12 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 H.**

Table 12. Freshwater Aquatic Toxicity Profile for Vinclozolin.

Assessment Endpoint	Species	Toxicity Value Used in Risk Assessment	Effect	Citation MRID (Author & Date)	Study Classification
Acute Direct Toxicity to Aquatic-Phase CRLF	Rainbow Trout (*Oncorhynchus mykiss*)	LC_{50} = 2.84 mg/L	Mortality	264302 (Gelbke 1980)	Supplemental
Chronic Direct Toxicity to Aquatic-Phase CRLF	Fathead Minnow (*Pimephales promelas*)	NOAEC 0.06 mg/L	Reduced number of spawns/female and reduced hatching success	Martinovic *et al.* 2008	Supplemental (open literature)

Indirect Toxicity to Aquatic-Phase CRLF via Acute Toxicity to Freshwater Invertebrates (*i.e.*, prey items)	*Daphnia magna*	EC_{50} = 4.0 mg/L	Immobilization	Union Carbide 1978	Acceptable
Indirect Toxicity to Aquatic-Phase CRLF via Chronic Toxicity to Freshwater Invertebrates (*i.e.*, prey items)	*Daphnia magna*	NOAEC =0.79 mg/L	Impaired reproduction and growth	452473-01 (Drottar *et al.* 1998)	Supplemental
Indirect Toxicity to Aquatic-Phase CRLF via Toxicity to Non-vascular Aquatic Plants	*Navicula pelliculosa*	EC_{50} <1.06 mg/L	Stimulated growth by 94%	423947-03 (Alexander and Hughes 1992)	Acceptable
Indirect Toxicity to Aquatic-Phase CRLF via Toxicity to Vascular Aquatic Plants	*Lemna gibba*	EC_{50} >0.90 mg/L	stimulated growth by 7.9%	423947-05 (Alexander and Hughes 1992)	Acceptable

Toxicity to aquatic fish and invertebrates is categorized using the system shown in **Table 13** (U.S. EPA, 2004). Toxicity categories for aquatic plants have not been defined.

Table 13. 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
> 10 - 100	Slightly toxic
> 100	Practically nontoxic

4.1.1 Toxicity to Freshwater Fish

Given that no vinclozolin 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 vinclozolin to the CRLF. Effects to freshwater fish resulting from exposure to vinclozolin may 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). A summary of acute and chronic freshwater fish data, including data from the open literature, is provided below.

4.1.1.1 Freshwater Fish: Acute Exposure (Mortality) Studies

The most sensitive freshwater fish species tested is the rainbow trout (*Oncorhynchus mykiss*) and resulted in a 96-hr LC_{50} of 2.84 mg a.i./L. Even with elevated co-solvent concentrations, precipitation of the test material was still a problem in the study. The study did not report whether water samples were centrifuged and/or filtered prior to analysis, so actual exposure concentrations may be uncertain. Based on the results of this study, vinclozolin is classified as moderately toxic to fish on an acute exposure basis. The mortality data from this study were analyzed using the moving average method to derive an LC_{50} value since the pattern of mortality across exposure concentrations did not support the use of probit analysis. As such, a probit dose-response slope is not available and OPP's default probit dose-response slope of 4.5 is use to estimate the likelihood of individual mortality.

No acceptable studies were identified in the open literature that are more sensitive that the available registrant-submitted data.

4.1.1.2 Freshwater Fish: Chronic Exposure Studies

In a study by Makynen *et al.* 2000 (MRID 452437-04), fathead minnows (*Pimephales promelas*) were exposed for 34 days beginning 6 hours post-hatch and then monitored the fish for 4 to 6 months post-exposure. This early life stage study resulted in a NOAEC and LOAEC of 600 and 1200 µg/L, respectively, based on 34-day (end of exposure period) body weights; this effect though appeared to be transitory as 90-day fish weights were not statistically different between vinclozolin-treated and control fish. The study also examined the effect of vinclozolin on adult fish in a 21-day exposure. Based on reductions (63% decrease) in the ratio of ovary weight to body weight, *i.e.* the gonadosomatic index (GSI), the NOAEC and the LOAEC are 200 and 700 µg/L; the reduction in GSI was attributed to a retarded maturation of oocytes. The GSI is considered an index of reproductive fitness and reductions in the GSI may reflect a reduction of the reproductive success of the test animal. Additionally, plasma β-estradiol concentrations were roughly 1.8X higher in males treated with 700 µg/L; however, histology did not indicate any qualitative differences between treated and control testes as all were well developed with mature spermatozoa.

In a fish early-life stage study (MRID 452437-04), embryonic (<6 hr old) fathead minnows were exposed to vinclozolin at mean measured concentrations ranging from 0.09 – 1.2 mg/L for 34 days and then monitored 4 – 6 months. Based on the results of this study, there was a significant reduction (33%) in growth (34-day body weight) at 1.2 mg/L; therefore, the NOAEC from this study is 0.54 mg/L.

A fish modified life-cycle study of the fathead minnow with vinclozolin in the presence of metabolites B and E was conducted where 8-month old fish were exposed for 112 days under flow-through conditions (MRID 452437-03). The mean measured concentration of total vinclozolin residues (parent plus metabolites B and E) was 0.12 mg/L. The number of spawns/female in the F_0 generation was statistically reduced by 54% compared to controls; however, the number of eggs per spawn was significantly higher (73% increase) in the vinclozolin-treated fish relative to controls. In the F_1 generation, hatch survival was statistically

reduced by roughly 35% in the vinclozolin-treated animals relative to controls. Since this study utilized only a single treatment group and control, it is not possible to establish a NOAEC; as such, the NOAEC<0.12 mg/L.

Of the three chronic toxicity studies of vinclozolin, the 112-day study with adult fish provides the most sensitive endpoint, *i.e.*, NOAEC<0.12 mg/L.

Open literature also contained supplemental information on the chronic effects of vinclozolin on fish. Bayley *et al*. 2003 reported that 10 to 14-wk exposures of male guppies (*Poecilia reticulate*) to vinclozolin in their diet at 1 and 10 µg/mg diet significantly ($p<0.05$) reduced sperm count and clutch size; however, there were significant uncertainties in actual exposure levels used in this study. In a study of Japanese medaka (*Oryzias latipe*), Kiparissis *et al*. 2003 exposed fish for 100 days starting at hatch; the study reported significant ($p<0.01$) effects on spermatogenesis at concentrations as low as 2.5 mg/L. In the Kiparissis study, there were no significant effects on sex ratio or the incidence of intersex; the results from this study provide qualitative evidence that chronic exposure to vinclozolin can result in reproductive effects in fish; however, the data cannot be used quantitatively since there was uncertainty regarding the study's exposure conditions.

In a study by Bayley *et al*. 2002, guppies were exposed to vinclozolin in their feed for 24 days. The intent of the study was to expose the fish during sexual development in their juvenile stage and then to track sexual development in males. According to the study exposure to vinclozolin at 0.1 and 10 µg/mg diet significantly altered sex ratios of offspring born to the treated fish resulting in more females; at the highest treatment concentration, offspring were 71% female while controls were 48% females. The study also reported significant ($p<0.01$) reductions in adult size (mm^2) and in sperm count at both treatment levels; however, the percent reductions could not be determined from the study. Gonadopodium length in males treated with 0.1 µg/mg diet was also reduced relative to controls. Because of uncertainties regarding the study's exposure conditions, these data cannot be used quantitatively; however, they do provide qualitative evidence that chronic exposure to vinclozolin effects both reproduction and growth.

In a 21-day reproductive study of fathead minnows (Matinovic *et al*. 2008), there was a concentration dependent decline in the cumulative number of eggs produced and female fish exposed to 450 µg/L failed to reproduce. Females exposed to vinclozolin had significantly higher ($p<0.05$) body weights at each of the vinclozolin concentrations tested; body weights were roughly 22% higher. The GSI of males exposed to vinclozolin at 255 and 450 µg/L was significantly different ($p<0.05$), by roughly a factor of 1.7X, compared to controls. Treatment with vinclozolin at 450 µg/L was also associated with significant reduction in secondary sexual characteristics such as skin tubercle development and dorsal pad development used in attracting mates. Also, at both 255 and 450 µg/L, plasma vitellogenin levels were significantly elevated in females. At 450 µg/L, the severity of oocyte atresia was significantly increased (90%) compared to controls (20%). The relevancy of the increased female body weight in this study at 60 µg/L is unclear however, the reproductive effects observed at 255 and 450 µg/L are consistent with effects discussed above. As such, the NOAEC for these effects would be 60 µg/L and the LOAEC would be 255 µg/L. The NOAEC of 60 µg/L is more sensitive than the value obtained

from registrant-submitted data and will serve as the chronic toxicity endpoint for this risk assessment.

4.1.2 Toxicity to Freshwater Invertebrates

Freshwater aquatic invertebrate toxicity data were used to assess potential indirect effects of vinclozolin to the CRLF. Effects to freshwater invertebrates resulting from exposure to vinclozolin may indirectly affect the CRLF via reduction in available food items. 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.

4.1.2.1 Freshwater Invertebrates: Acute Exposure (Mortality) Studies

Based on a 48-hr acute toxicity test with waterfleas (*Daphnia magna*), vinclozolin is classified as moderately toxic (EC_{50}=4.0 mg/L) on an acute exposure basis.

Open literature was reviewed to determine whether there were more sensitive measures of acute toxicity for aquatic invertebrates. In a study by Zavala-Aquirre et al. 2007 using the rotifer *Brachionus calyciflorus*, the 24-hr LC_{50} was 30.5 mg/L. Additionally, in a study by Haeba *et al*. 2008, the 48-hr acute toxicity of vinclozolin was evaluated using D. magna; the study reported an LC_{50}>3 mg/L with no mortality in any of the treatment groups. Both of these toxicity values however, are less sensitive that what is available through registrant-submitted data.

4.1.2.2 Freshwater Invertebrates: Chronic Exposure (Reproduction) Studies

In a 21-day flow-through full life-cycle study with *D. magna* (MRID 452473-01), growth (length and weight) was significantly reduced at 1.4 mg/L (LOAEC) and the NOAEC was determined to be 0.79 mg/L. At the LOAEC, mean body length was reduced by 3.8% and mean total dry weight was reduced by 11%. Reproduction was also affected with the mean number of young per adult reduced by 17% at the LOAEC.

Open literature was reviewed to determine whether there were more sensitive measures of chronic toxicity for aquatic invertebrates. In a study by Haeba *et al*. 2008, *D. magna* were exposed for 4 – 6 days under static renewal conditions. Vinclozolin significantly ($p<0.05$) altered sex ratio, reducing the number of males by a factor of 2; based on the results of this study, the NOAEC for sex ratio would be 0.1 mg/L or 100 µg/L. However, the study did not measure exposure concentrations and it relied on dimethylsulfoxide (DMSO) as a co-solvent that may have affected the uptake of vinclozolin. The study is considered supplemental and as providing useful qualitative information on the effects of vinclozolin exposure on freshwater aquatic invertebrates.

In a study of the ramshead snail (*Marisa cornuarietis*) Tillmann *et al*. 2001 reports that juvenile snails exposed to vinclozolin for 5 months showed a significant albeit transient effect on penis length and penis sheath at 0.03 and 1.0 µg/L during the first couple months of the study. By the

fourth month of the study, there was no difference between vinclozolin-treated and control animals. The study is confounded though by the fact that neither the treated nor control animals spawned and it is difficult to determine the relevancy of the decreased penis length and sheath to the reproductive success of the test animals.

4.1.3 Toxicity to Aquatic Plants

Aquatic plant toxicity studies were used as one of the measures of effect to evaluate whether vinclozolin 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.

Only Tier I studies of aquatic plants are available for vinclozolin. Only one nonvascular plant species *(Navicula pelliculosa*) exhibited an effect of greater than 50%. The remainder of the species tested exhibited less than a 10% at the highest concentration tested, *i.e.*, 1 mg/L.

The only nonvascular plant species tested to exhibit an effect greater than 50% was *N. pelliculosa*. In a 5-day study with a mean measured concentration of 1.06 mg a.i./L, the compound stimulated growth by 94.5% (MRID 423947-03). Growth stimulation like growth inhibition is considered an effect which can alter the number of plants in an aquatic community. Growth stimulation without sufficient nutrients to support such activity can result in aquatic plant blooms that cannot be sustained and may ultimately result in decreased water quality as the bloom rapidly subsides. Since this was a limit test, it is not possible to determine the EC_{50} from the available information. All that can be said is that the EC_{50} is likely less than 1.06 mg a.i/L; however, it is uncertain how much less. For the purposes of this assessment though, the EC_{50} is assumed to be <1.06 mg a.i./L. None of the other aquatic plants tested in registrant-submitted studies (*Pseudokirchneriella subcapitata, Anabaena flos-aquae*, and *Skeletonema costatum*) exhibited greater than a 8% effect at the maximum concentration tested (limit test concentrations ranging between 0.87 – 1.02 mg a.i./L).

For vascular aquatic plants, only a limit test is available for duckweed (*Lemna gibba*), and vinclozolin exposure resulted in a stimulation of growth (MRID 423947-05). At the maximum concentration tested (0.90 mg a.i./L) and following a 14-day exposure, plant growth was stimulated by 7.9%. For the purposes of this assessment the EC_{50} is assumed to be >0.90 mg a.i./L.

4.2 Toxicity of Vinclozolin to Terrestrial Organisms

Table 14 summarizes the most sensitive terrestrial toxicity endpoints for the CRLF, 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. Acute toxicity to terrestrial animals is categorized using the classification system shown in Table 15 (U.S. EPA, 2004). Toxicity categories for terrestrial plants have not been defined.

Table 14. Terrestrial Toxicity Profile for Vinclozolin.

Assessment Endpoint	Species	Toxicity Value Used in Risk Assessment	Effect	Citation MRID (Author & Date)	Study Classification
Acute Dose-based Direct Toxicity to Terrestrial-Phase CRLF	Northern Bobwhite Quail (*Colinus virginianus*)	LD_{50} >2,510 mg/kg	Mortality	92194-002 (Fink, 1978)	Acceptable
Acute Dietary-based Direct Toxicity to Terrestrial-Phase CRLF	Northern Bobwhite Quail	LC_{50} >5,620 mg/kg diet	Mortality	92194-003 (Fink, 1978)	Acceptable
Chronic Direct Toxicity to Terrestrial-Phase CRLF	Northern Bobwhite Quail	NOAEC = 50 mg/kg diet	Reduced numbers of eggs laid; eggshell thinning; reduced 14-day survival of hatchlings	428689-01 (Munk, 1993)	Supplemental
Indirect Toxicity to Terrestrial-Phase CRLF (via acute toxicity to mammalian prey items)	Laboratory Rat (*Rattus norvegicus*)	LD_{50} >10,000 mg/kg bw	Mortality	921940-10 O'Reilly	Acceptable
Indirect Toxicity to Terrestrial-Phase CRLF (via chronic toxicity to mammalian prey items)	Laboratory Rat	NOAEL=30 mg/kg/day	Reproductive tract malformations and reproductive failure.	425813-01 Hellwig 1993	Acceptable
Indirect Toxicity to Terrestrial-Phase CRLF (via acute toxicity to terrestrial invertebrate prey items)	Honey bee (*Apis mellifera*)	LD_{50} >100 µg/bee	Mortality	409928-01 (Hoxter 1988)	Acceptable

*although these endpoints are not typically used in ecological risk assessment, this study is used as a surrogate estimate of the NOAEC since the rat 2-generation reproduction study showing frank effects on rat reproductive organ relied on an estimated NOAEC (point of departure) of 3 mg/kg/day.

Table 15. 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 are available for vinclozolin; therefore, acute and chronic avian toxicity data are used to assess the potential direct effects of vinclozolin to terrestrial-phase CRLFs.

4.2.1.1 Birds: Acute Exposure (Mortality) Studies

Vinclozolin is classified as practically nontoxic to birds on both an acute oral and subacute dietary exposure basis. The acute oral LD_{50} and subacute dietary LC_{50} for bobwhite quail (*Colinus virginianus*) are 2,510 mg/kg bw and 5,620 mg/kg diet, respectively. In the acute oral toxicity study (MRID 92194-002), no mortality occurred in any of the treatment levels. In the subacute dietary toxicity study with bobwhite quail, while there was sporadic mortality across treatment groups, no dose-related mortality was reported. Sublethal effects were not reported in the acute oral and subacute dietary toxicity studies with quail.

Open literature was reviewed to determine whether any more sensitive acute toxicity endpoints are available for birds. In a study by Ronis *et al*. 1998, male bobwhite quail (200 g) were given three consecutive doses (gavage) of vinclozolin at 400 mg/kg bw/day, and the birds were sacrificed 48 hours after the last dose. Under the conditions tested, males exhibited statistically significant ($p<0.05$) induction in two markers for testosterone metabolism, i.e., testosterone 2β-hydroxylase (38% increase) and testosterone 15β-hydroxylase (67% increase), relative to controls. The authors also reported significantly induced cytochrome P450 enzymes. The dosing regime used in this study, i.e., three consecutive days of dosing, was selected because it was known to induce cytochrome P450 activity; however, its relevance to what may occur in the field is uncertain. While the information contained in this study indicates and effect that could potential impact steroidogenesis, the study is of limited utility as a measurement endpoint for acute and/or subacute toxicity.

In another study by Ronis *et al*. 1995, male bobwhite quail (200 g) were again dosed by gavage at a rate of 400 mg/kg bw per day for three consecutive days and were sacrificed 48 hours after treatment. Under the conditions tested liver cytochrome P450 enzyme activity was significantly ($p<0.005$) increased by an order of magnitude. Liver to body weight ratio, *i.e*., the hepatosomatic index (HSI), was also significantly ($p<0.05$) increased (30%) relative to controls and likely reflected the increase in enzymatic activity. Similar to the previous study, the exposure regime used in this study is relatively unusual and it is uncertain how similar exposure may occur under field conditions. Additionally, the endpoints identified in this study, *i.e.,* enzyme induction and increased HIS, are not typically used in evaluating acute toxicity.

In a third study by Ronis *et al*. 1994, male bobwhite quail (200 g) were again dosed via gavage using three consecutive treatments with 400 mg/kg bw/day and the birds were sacrificed 48 hours after the third treatment. Under the conditions tested, vinclozolin significantly and simultaneously affected different subfamilies of hepatic P450 enzyme activity in the birds. The

authors speculated that the increased cytochrome P450 activity could enhance the activation of other chemicals, *e.g*., the activation of organophosphate pesticides to their oxon, which may co-occur in the environment and in doing so result in the enhanced toxicity of the chemical mixture.

In a study by Riviere *et al.* 1983, Japanese quail (*Coturnix coturnix*) received diets of 2000 ppm for seven days at which time the birds were sacrificed. Vinclozolin-treated birds showed a significant ($p<0.05$) difference in liver weights, HIS, cytochrome P450 activity ($p<0.01$), NADPH-cytochrome c reductase ($p<0.01$), aniline hyroxylase ($p<0.05$), aldrin epoxidase ($p<0.01$) and 7-ethoxyresorufin dealkylase activity ($p<0.01$). Again though, the induction of hepatic enzyme activity is not typically used as a measure of acute toxicity.

4.2.1.2 Birds: Chronic Exposure (Growth, Reproduction) Studies

In an avian reproduction study of bobwhite quail, exposure to vinclozolin resulted in NOAEC of 50 mg/kg diet and a LOAEC of 125 mg/kg diet (MRID 428689-01). The LOAEC was based on significantly reduced number of eggs laid (13% reduction), decreased eggshell thickness (4.8% reduction), and reduced proportion of 14-day old survivors of chicks hatched (15% reduction). Also at the LOAEC, early embryonic mortalities increased by 37% relative to controls and total embryonic deaths were 33% higher than controls.

Open literature was reviewed to determine whether any more sensitive chronic toxicity endpoints are available for vinclozolin in birds. In a study by Niemann *et al*. 2004, Japanese quail received dietary exposures for 6 weeks at either 125 or 500 mg/kg diet. After 6 weeks of treatment, the number of spermatids per testes was statistically different ($p<0.05$) in birds treated with 500 ppm diet; on average, spermatids were 26% lower in the testes of birds treated with 500 ppm diet. The authors also report that sex ratio of chicks was significantly different ($p<0.05$) among offspring of quail feed at 500 ppm; treated animals had a ratio of 39:43 (male:female) while controls had a ratio of 45:24. Although the authors report that eggshell thickness (mm) of cracked eggs was significantly different ($p<0.05$) from vinclozolin-treated quail compared to controls; however, these differences existed prior to study initiation and likely reflect an artifact of the study. The authors reported that fertility and reproductive performance were not affected up to the highest dietary concentration tested, *i.e.*, 500 ppm; however, spermatid counts and histology provided evidence of an inhibition of spermatogenesis at both dietary concentrations. Based on shifts in sex ratio and reductions in spermatids, the NOAEC for this study is 125 ppm and the LOAEC is 500 ppm; however, these endpoints are not more sensitive than the registrant-submitted data.

4.2.1.3 Terrestrial-phase Amphibian Acute and Chronic Studies

No acute or chronic toxicity data on amphibians are available through registrant-submitted or open literature studies.

Toxicity to Mammals

Mammalian toxicity data are used to assess potential indirect effects of vinclozolin to the terrestrial-phase CRLF. Effects to small mammals resulting from exposure to vinclozolin 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).

4.2.1.4 Mammals: Acute Exposure (Mortality) Studies

With an LD_{50}>10,000 mg/kg bw (USEPA 2000*a*), vinclozolin is classified as practically nontoxic to rats on an acute oral exposure basis.

Two acute toxicity studies involving mammals were identified in the open literature and were by the same authors, *i.e.*, Ronis and Badger 1995 and Ronis *et al*. 1994 discussed for the acute toxicity of vinclozolin on birds. Each of these studies relied on three consecutive doses (by gavage) of vinclozolin at 400 mg/kg bw in 300 g Sprague-Dawley rats (*Rattus norvegicus*). In the Ronis *et al*. 1994 study, vinclozolin significantly (P<0.005) raised cytochrome P450 (Cyt-P450) levels by a factor of 3 - 4 fold in both rats and significantly (P<.0.05) elevated levels of cytochrome b_5 and Cyt-P450 reductase by factors of 2 - 4 fold. EROD and CYP 2B1/2-dependent BROD were increased 12- and 300-fold by vinclozolin treatment, respectively. Vinclozolin increased CYP 1A2-dependent MROD and BYP2B1/2-dependent PROD 30- to 40-fold, respectively. Androstendione, 2β- and 6β-hydroxytestosterone formation was significantly (P<0.05) increased in rats exposed to vinclozolin. In the Ronis and Badger 1995 study cytochrome P450 activity was significantly induced (2.9X increase) in rats and in the Ronis et al. 1998 study. As stated previously, the relevancy of the exposure conditions used in these studies to ecological risk assessment is uncertain; however, under the conditions tested, vinclozolin treatment resulted in a significant induction of hepatic microsomal enzyme activity. Since this measurement endpoint is not typically used to assess acute toxicity, it is not used in this assessment.

4.2.1.5 Mammals: Chronic Exposure (Growth, Reproduction) Studies

According to the RED (USEPA 2000), the principal toxic effects of vinclozolin and/or its metabolites in mammals are related to its anti-androgenic activity and its ability to act as a competitive antagonist at the androgen receptor. At low dose levels (>3 mg/kg/day), the most androgen sensitive effects are noted, such as decreased prostate weight, weight reductions in other sex organs; at higher concentrations, sex organ malformations are observed. Vinclozolin and its metabolites also cause Leydig cell (testicular) tumors in rats via its antiandrogenic mechanism of action. The extent to which these effects relate to marked impacts on reproduction are uncertain; however, this assessment is intentionally conservative to account for the uncertainty given the inter-related nature of endocrine-mediated processes. However, in the rat 2-generation study, vinclozolin was associated with decreased epididymal weights with a LOAEC of 30 mg/kg/day and an estimated NOAEC (point of departure) of 4.9 mg/kg/day (USEPA . Since the point of departure is an estimated value for a frank endpoint, *i.e.*, decreased reproductive organ weight, this assessment relies on the measured NOAEC from the chronic

dietary toxicity study. Vinclozolin is also classified as a possible human carcinogen based on Leydig (interstitial testicular) cell tumors in chronic and carcinogenicity studies. However, frank effects on apical endpoints were not observed at the NOAEL/NOAEC discussed above. Frank effects on reproduction were noted in the rat 2-generation reproduction study (Hellwig 1992) where adult male offspring exhibited genital and reproductive tract malformations and sire no offspring at dietary treatments of 1000 (LOAEL; 96 mg/kg/day) and 3000 ppm. Based on this study, the NOAEL is 300 ppm (30 mg/kg bw/day) and it is this value that will be used to assess risk to mammals serving as forage for terrestrial-phase CRLF..

No additional chronic mammalian toxicity data were obtained from the open literature that are more sensitive than the registrant-submitted data.

4.2.2 Toxicity to Terrestrial Invertebrates

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

An acute contact toxicity study with honeybees (*Apis mellifera*) resulted in an LD_{50} greater than the highest dose tested (100 µg/bee) (MRID 409928-01). As such, vinclozolin is classified as practically nontoxic to honeybees and an acute contact exposure basis, *i.e.*, LD_{50}>100 µg/bee.

No additional terrestrial invertebrate toxicity date were identified in the open literature.

4.2.3 Toxicity to Terrestrial Plants

Terrestrial plant toxicity data are used to evaluate the potential for vinclozolin 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 of upland and riparian habitat that provides shelter, foraging, predator avoidance and dispersal for juvenile and adult CRLFs.

No registrant-submitted data are available on the potential effects of vinclozolin on terrestrial plants. Several terrestrial plant studies were identified in the open literature for vinclozolin. In a study by Rouchard et al. 1984, the effects of formulated vinclozolin on carotenoid pigment in lettuce (*Lactuca sativa*) was evaluated. Ronilan® (vinclozolin 50 % a.i. treated at 10 g Ronilan®/acre) was applied to lettuce at the 12-leaf stage. Although vinclozolin did not have any significant effect on carotenoid pigment, treated plants were significantly larger (47% increase in fresh weight relative to control) 14 days after treatment; however, by 32 days after treatment there was not significant difference. Because of uncertainties regarding exposure conditions in the study, it cannot be used quantitatively; however, it provides qualitative information that vinclozolin can affect terrestrial plants.

In a study by Lorenzini *et al*. 1987, the ability of formulated vinclozolin to counteract the effects of ozone damage to tobacco plants (*Nicotiana tabacus*) were examined. In this study (Rovral®, 50% a.i. applied at 1500 g a.i./ha) did not prevent or combat ozone damage. In this case, at the application rate used, vinclozolin treatment did not appear to harm the plants; however, only a limited number of measurements were conducted and as such, the study provides little insight on the potential effects of vinclozolin on terrestrial plants.

In a study by Olien *et al*. 1995, the effect of vinclozolin alone and in combination with the fertilizer ammonium thiosulphate in controlling brown rot (*Monilinia fructicola*) on peach trees (*Prunus persica*) was examined. Vinclozolin was applied in Ronilan® DF (2.4 g/L) at a rate of 1.58 kg a.i./ha. The study showed that vinclozolin was effective in reducing *M. fructicola* blossom blight cankers per tree. The study examines a relatively specific endpoint and it is difficult to gauge the overall phytotoxicity potential of either vinclozolin from this study. The study is essentially measures efficacy relative to plant damage from brown rot fungus. Frost damaged 10-20% of the flowers; it's unclear how this may have impacted the study.

4.3 Toxicity of 3,5-DCA

Several studies were identified in the open literature for 3,5-DCA. The only data available for fish in the open literature was a 14-day LC_{50} value of 3900 µg/L for guppies (*Poecilia reticulate*) (Maas-Diepeveen and van Leeuwen 1986). These data suggest that guppies are considerably less sensitive to the 3,5-DCA degradate than other species tested against the parent compound. Rainbow trout exposed to vinclozolin had an LC_{50} of 2840 µg/L after 4 days of treatment compared to the LC_{50} of 3900 µg/L for guppies after exposure to the degradate for roughly 3.5 times longer.

In a 48-hr study with waterfleas (*D. magna*) the EC_{50} was 1120 µg/L (Maas-Diepeveen and van Leeuwen 1986) and is roughly 1.4 times less sensitive than the equivalent toxicity endpoint for waterfleas using the parent compound (48-hr EC_{50}=790 µg/L). A 96-hr study of 3,5-DCA with shrimp (*Crangon septemspinosa*) resulted in an LC_{50} value of 2500 µg/L (McLeese *et al.* 1979) and is considerably less toxic than the parent compound. Finally, in a 96-hr study with green algae (*Chlorella pyrenoidosa*), the EC_{50} was 7500 µg/L (*Maas-Diepeveen and van Leeuwen 1986*) and is seven times less toxic than the estimate for the most sensitive freshwater nonvascular plant (*N. pelliculosa*) where a 5-day study with a mean measured concentration of 1.06 mg a.i./L, the compound stimulated growth by 94.5% Therefore, based on the weight of evidence provided through toxicity values reported in the open literature, 3,5-DCA is considered at least 1.4 times less toxic to aquatic organisms than the parent compound. Based on measured and estimated toxicity values for 3,5-DCA, the compound would be classified as moderately toxic to aquatic animals on an acute exposure basis.

A single chronic toxicity value for 3,5-DCA is available through the open literature in which zebrafish (*Brachydario rerio*) were exposed for 28 days and resulted in a NOAEC of 1000 µg/L (1 mg/L) (van Leeuwen *et al*. 1990) based on survival, hatching and growth. Analytical measurements for 3,5-DCA were highly uncertain in the study and the extent of the effect on survival, hatching and growth is not discussed. No invertebrate chronic toxicity data were

available from the open literature for 3,5-DCA. With an measured NOAEC of 1000 µg/L, 3,5-DCA is less toxic on a chronic exposure basis compared to the most sensitive chronic toxicity estimate for the parent, *i.e.*, fathead minnow NOAEC=60 µg/L.

4.4 Incident Database Review

A review of the EIIS database for ecological incidents involving vinclozolin was completed on August 10, 2009. No incidents were reported in the EIIS involving vinclozolin. Additionally, the American Bird Conservancy's Avian Incident Monitoring System (AIMS) was also searched on August 10, 2009, and again, no incidents associated with vinclozolin are reported.

4.5 Endocrine Disruptor Effects

Although the EPA has developed a process for determining whether a chemical acts on endocrine-mediated processes, the Tier I tests of the Endocrine Disruption Screening Program are only just being implemented. However, according to the RED (USEPA 2000), vinclozolin and some of its metabolites are already known to interfere with the endocrine system, exerting their effects most dramatically during the developmental stages of animals, resulting in reproductive effects in lab animals. Although vinclozolin binds weakly to the androgen receptor, metabolites B and E have higher binding affinity for the androgen receptor. According to the RED chapter, all androgen dependent functions are reduced; the more sensitive organs and functions are the male sex organ weight reductions, reduced fertility and abnormal or ambiguous sexual differentiation in the male rat (USEPA 2000).

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 vinclozolin 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 B**). 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 vinclozolin usage scenarios summarized in the use characterization and the appropriate aquatic toxicity endpoint from **Table 12**. 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 vinclozolin and the appropriate toxicity endpoint from **Table 14**. As discussed earlier, no toxicity data are available for terrestrial plants and as a result risk cannot be estimated for these taxa.

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 peak 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. Although vinclozolin is moderately toxic to fish on an acute exposure basis (LC_{50}=2840 µg/L**)**, the peak EEC (52.0 µg/L), representing vinclozolin residues of concern results in an acute RQ (0.018) that is below the endangered species LOC of 0.05 (**Table 16**). As such, risk of acute effects (mortality) to listed species from the use of vinclozolin on turf is presumed to be low. However, vinclozolin exposure resulted in effects on growth and reproduction in fathead minnows (NOAEC=0.06 mg/L) following 21-day exposure to residues of vinclozolin; the RQ value (0.83) is below the chronic risk LOC of 1.0 (**Table 16**). Based on acute and chronic RQ values below LOCs, vinclozolin's is not expected to affect the aquatic-phase of the CRLF.

Table 16. Summary of Direct Effect RQs for the Aquatic-phase CRLF.

Direct Effects to CRLF[a]	Surrogate Species	Toxicity Value (µg/L)	EEC (µg/L)[b]	RQ	Probability of Individual Effect at ES LOC	Probability of Individual Effect at RQ
Acute Direct Toxicity	Rainbow trout	LC_{50} = 2840	Peak: 52.0	0.018[d]	1 in 4.18 x 10^8 (1 in 216 to 1 in 1.75 x 10^{31})[c]	1 in 4.86x10^{14}
Chronic Direct Toxicity	Fathead minnow	NOAEC= 60 µg/L	60-day: 49.9	0.83[e]	Not calculated for chronic endpoints	

[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 foliar use of vinclozolin on turf at 1.35 lb a.i/A representing vinclozolin residues of concern
[c] A probit slope value for the acute fathead minnow toxicity test is not available; therefore, the effect probability was calculated based on a default slope assumption of 4.5 with upper and lower 95% confidence intervals of 2 and 9 (Urban and Cook, 1986).
[d] RQ < acute endangered species LOC of 0.05.
[e] RQ less than chronic risk 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 vinclozolin to the aquatic-phase CRLF (tadpoles) via reduction in non-vascular aquatic plants in its diet are based on peak EECs from the standard pond and the lowest toxicity value (EC_{50}) for aquatic non-vascular plants. The most sensitive nonvascular aquatic plant is *N. pelliculosa* with an EC_{50} value of <1060 µg/L. Based on a peak EEC of 52.0 µg/L representing vinclozolin residues of concern, the RQ is >0.05. Since the aquatic plant toxicity data are based on a limit test, the concentration required to result in an effect of 50% is uncertain. As noted previously, none on the other aquatic non-vascular plants tested had growth effects at treatment concentrations ranging between 0.87 – 1.02 mg a.i./L. The maximum effect based on either inhibition or stimulation of growth on these test species was less than 8%. Given that none of the other aquatic plant species tested exhibited growth effects greater 8% at concentrations roughly 28 times higher than the maximum total toxic residues estimated for vinclozolin and its degradates, vinclozolin is not likely to indirectly affect the CRLF via reduction in non-vascular plants.

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. For chronic risks, 21-day EECs and the lowest chronic toxicity value for invertebrates are used to derive RQs. Although vinclozolin is moderately toxic (EC_{50}=4.0 mg/L) on an acute exposure basis, the peak EEC based on total toxic residues is such that the resultant acute RQ (0.013) is below the acute risk to listed species LOC of 0.05. Further, the chronic toxicity endpoint (NOAEC=790 µg/L) is sufficiently greater than the 21-day EEC for vinclozolin residues of concern (51.1 µg/L) to yield an RQ (0.065) that is below the chronic risk LOC of 1; therefore, vinclozolin is not likely to indirectly affect the CRLF via reduction in freshwater invertebrates prey items.

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 16**) are used to assess potential indirect effects to the CRLF based on a reduction in freshwater fish and frogs as food items. Based on data indicating that vinclozolin is moderately toxic to fish and by extension to aquatic-phase amphibians, on an acute exposure basis and RQ values well below the acute risk LOC, the likelihood of acute effects on fish/amphibians serving as prey for adult aquatic-phase CRLF is considered low. Similarly, the chronic RQ for fish is below the chronic risk LOC for fish/amphibians and as such, the likelihood of chronic effects on fish/amphibians serving as prey for aquatic-phase CRLF is considered low. Based on the available data, vinclozolin is not likely to indirectly affect the CRLF via reduction in freshwater fish and frogs as food items.

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.

The toxicity value for vascular aquatic plants is based on a limit test using *L. gibba* and based on the results of that test, the EC_{50} is greater than the highest concentration tested, *i.e.*, >900 µg/L. With a peak EEC of 52.0 µg/L for vinclozolin residues of concern, the RQ is <0.058 and is below the LOC of 1.0. As such, vinclozolin is not likely to indirectly affect the CRLF via reduction in vascular plants. As discussed previously, none of the majority of aquatic nonvascular plant species tested exhibited growth effects greater 8% at concentrations roughly 28 times higher than the maximum total toxic residues estimated for vinclozolin and its degradates, vinclozolin is not likely to indirectly affect the CRLF via reduction in non-vascular plants.

5.1.2 Exposures in the Terrestrial Habitat

5.1.2.1 Direct Effects to Terrestrial-phase CRLF

As previously discussed in Section 3.3, potential direct effects to terrestrial-phase CRLFs are based on ground applications of vinclozolin to turf grass.

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 and acute oral and subacute dietary toxicity endpoints for avian species. RQ values were not calculated for acute and subacute exposures to the terrestrial-phase CRLF because: 1) vinclozolin is practically nontoxic to birds on an acute oral (LD_{50}>2,510 mg/kg bw) and subacute dietary (LC_{50}>5,620 mg/kg diet) toxicity basis; 2) there was no treatment related mortality and/or sublethal effects at the highest treatment levels, and 3) EECs for the small bird consuming small invertebrates are below the highest treatment levels of the acute oral and subacute dietary toxicity tests. Therefore, risk of direct effects to terrestrial-phase CRLF is presumed low.

Potential direct chronic effects of vinclozolin 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. The NOAEC for vinclozolin in bobwhite quail is 50 mg/kg diet and is based on reduced reproduction and based on dietary EECs for small birds feeding on small insects, the RQ (9.09) exceeds the chronic risk LOC (LOC>1.0).

Based on potential reproductive effects from chronic exposure, vinclozolin may affect the terrestrial-phase of the CRLF.

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

Terrestrial Invertebrates

In order to assess the risks of vinclozolin to terrestrial invertebrates, which are considered prey of CRLF in terrestrial habitats, the honeybee is used as a surrogate for terrestrial invertebrates. EECs (µg a.i./g of bee) calculated by T-REX for small and large insects are 454 and 51 ppm (µg a.i./g of bee), respectively. RQ values were not calculated for acute exposures to the terrestrial invertebrates because: 1) vinclozolin is practically nontoxic to honey bees on an acute contact basis (LD_{50}>100 µg a.i. / bee or >781 µg a.i. / g of bee[3]); 2) there was no treatment related mortality at the highest treatment level, and 3) EECs for terrestrial invertebrates are below the highest treatment level of the acute contact test (where no mortality was observed). Therefore, risk of effects to terrestrial invertebrates and subsequent indirect effects to the CRLF through decrease in terrestrial invertebrate prey is presumed low.

[3] Based on the assumption that an adult honey bee weighs 0.128 g.

Mammals

Risks associated with ingestion of small mammals by large terrestrial-phase CRLFs are derived for dietary-based and dose-based exposures modeled in T-REX for a small mammal (15g) consuming short grass. Acute and chronic effects are estimated using the most sensitive mammalian toxicity data. RQ values were not calculated for acute exposures to small mammals serving as prey to the CRLF because: 1) vinclozolin is practically nontoxic to mammals on an oral (LD_{50}>10,000 mg/kg -bw) toxicity basis; 2) there was no treatment related mortality at the highest treatment level, and 3) the acute, dose-based EEC for the small mammal consuming short grass (770 mg/kg-bw) is below the highest treatment level of the acute oral test. Therefore, risk of effects to small mammals exposed to vinclozolin on an acute oral basis and subsequent indirect effects to the CRLF through decrease in terrestrial mammals is presumed low.

Chronic EECs are divided by the chronic toxicity values to estimate dose-based RQs as well as dietary-based RQs. With a NOAEC of 30 mg/kg-day based on decreased reproduction, dose-based (RQ=12) and dietary-based (RQ=2.7) RQ values exceed the chronic risk LOC (RQ>1.0). Based on potential reproductive effects from chronic exposure, vinclozolin is likely to indirectly affect the CRLF via reduction in small mammal prey items.

Frogs

An additional prey item of the adult terrestrial-phase CRLF is other species of frogs. 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. Direct acute effects on aquatic-phase amphibians are not considered likely; however, direct chronic effects on aquatic-phase amphibians may occur. As described in Section 5.2.1.2.1, direct chronic effects on terrestrial-phase amphibians are also considered likely with a chronic RQ of 9.09. Based on the potential chronic effects of vinclozolin on aquatic and terrestrial-phase amphibians that may serve as prey for the CRLF, vinclozolin is likely to indirectly affect the CRLF via reduction in frogs as prey items.

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. However, since no terrestrial plant toxicity data are available to assess the potential effects of vinclozolin, RQ values could not be calculated. In the absence of data to the contrary, it is conservatively assumed that vinclozolin is likely to indirectly affect the CRLF via reduction in terrestrial plants.

5.1.3 Primary Constituent Elements of Designated Critical Habitat

For vinclozolin 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.

5.1.3.1 Aquatic-Phase (Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)

Three of the four assessment endpoints for the aquatic-phase primary constituent elements (PCEs) of designated critical habitat for the CRLF are related to potential effects to aquatic and/or terrestrial plants:

- 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.
- 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.
- Reduction and/or modification of aquatic-based food sources for pre-metamorphs (*e.g.*, algae).

Acute and chronic RQ values for freshwater fish and invertebrates are below LOCs and there is a low likelihood that vinclozolin may directly affect aquatic animals (Section 5.1.1.2). Although risk estimates for effects to aquatic plants (Section 5.1.1.3) are below the LOC, there is uncertainty regarding the potential effect of vinclozolin on riparian plants (Section 5.1.2.3). Because there are no terrestrial plant data (surrogate for riparian vegetation) available for vinclozolin the presumption is that the chemical is likely to affect aquatic-phase PCEs of designated habitat related to potential effects on terrestrial plants.

The remaining aquatic-phase PCE is "alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source." To assess the impact of vinclozolin 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. Acute and chronic RQ values for freshwater fish invertebrates are below LOCs and there is a low likelihood that vinclozolin will affect aquatic animals. As such, vinclozolin is not likely to affect aquatic-phase PCEs of designated habitat related to effects of alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.

5.1.3.2 Terrestrial-Phase (Upland Habitat and Dispersal Habitat)

The first two assessment endpoints for the terrestrial-phase PCEs of designated critical habitat for the CRLF are related to potential effects to terrestrial plants:

- 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

- 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

The risk estimation for terrestrial-phase PCEs of designated habitat related to potential effects on terrestrial plants is provided in Section 5.1.2.3. Since no data are available on the potential effects of vinclozolin on terrestrial plants, the presumption is that vinclozolin is likely to affect the first and second terrestrial - phase PCEs.

The third terrestrial-phase PCE is "reduction and/or modification of food sources for terrestrial phase juveniles and adults." To assess the impact of vinclozolin on this PCE, acute and chronic toxicity endpoints for birds, mammals, and terrestrial invertebrates are used as measures of effects. RQs for these endpoints were calculated in **Section 5.1.2.2**. Although RQ values for terrestrial invertebrates nominally exceed the LOC, no mortality occurred at the highest exposure concentration tested (LD_{50}>100 µg/bee) and as such, risks to terrestrial invertebrates from the use of vinclozolin on turf is considered low. Vinclozolin is practically nontoxic to birds and mammals on an acute exposure basis and the likelihood of acute adverse effects in these animals from the use of vinclozolin on turf is considered low; however, chronic reproductive effects in birds, terrestrial-phase amphibians, and mammals are possible. Based on the potential for chronic effects of vinclozolin on animals that may serve as prey for CRLF, vinclozolin is likely to affect the third terrestrial - phase PCEs.

The fourth terrestrial-phase PC is based on alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source. Direct acute and chronic RQs for terrestrial-phase CRLFs are presented in Section 5.2.1.21. Although the likelihood of direct acute effects on terrestrial-phase CRLFs is considered low, direct chronic effects are considered likely (RQ=9.09). Therefore, vinclozolin is likely to affect the forth terrestrial - phase PCEs.

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 critical habitat. The direct or indirect effect LOCs are exceeded or effects may modify the PCEs of the CRLF's critical habitat, the Agency concludes a preliminary "may affect" determination for the FIFRA regulatory action regarding vinclozolin. A summary of the results of the risk estimation results are provided in Table 17 for

direct and indirect effects to the CRLF and in **Table 18** for the PCEs of designated critical habitat for the CRLF.

Table 17. Risk Estimation Summary for Vinclozolin Direct and Indirect Effects to CRLF.

Assessment Endpoint	LOC Exceedances (Y/N)	Description of Results of Risk Estimation
Aquatic-Phase CRLF (eggs, larvae, tadpoles, juveniles, and adults)		
Direct Effects Survival, growth, and reproduction of CRLF individuals via direct effects on aquatic phases	N	Acute and chronic RQ values for fish are below LOCs.
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects to food supply (*i.e.*, freshwater invertebrates, non-vascular plants)	N	Acute and chronic RQ values for freshwater invertebrates are below the acute and chronic risk LOCs. Indirect effects on aquatic-phase CRLF from effects on non-vascular aquatic plants are not considered likely since RQ values for non-vascular plants are below the LOC.
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects on habitat, cover, and/or primary productivity (*i.e.*, aquatic plant community)	N	Available data indicate that vinclozolin is not likely to cause adverse effects on the aquatic plant community.
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.	Y	There is uncertainty [due to the lack of data] regarding the chemical's potential effect on terrestrial plants that provide [riparian] cover for aquatic environment; therefore, risk is presumed.
Terrestrial-Phase CRLF (Juveniles and adults)		
Direct Effects Survival, growth, and reproduction of CRLF individuals via direct effects on terrestrial phase adults and juveniles	Y	Although vinclozolin is practically nontoxic to terrestrial animals on an acute exposure basis, chronic exposure may adversely affect reproduction. The chronic RQ value exceeds the chronic risk LOC by a factor of 9X.
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects on prey (*i.e.*, terrestrial invertebrates, small terrestrial mammals and terrestrial-phase amphibians)	Y	Chronic RQ values for both small mammals and aquatic- and terrestrial-phase amphibians serving as prey for CRLF exceed the chronic risk LOC by factors as high as 12X (dose-based RQ).
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects on habitat (*i.e.*, riparian vegetation)	Y	There is uncertainty regarding the chemical's potential effect on terrestrial plants that provide [riparian] cover for aquatic environment; therefore, risk is presumed.

Table 18. Risk Estimation Summary for vinclozolin– PCEs of Designated Critical Habitat for the CRLF.

Assessment Endpoint	LOC Exceedances (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.	Y	There is uncertainty regarding the chemical's potential effect on terrestrial plants that provide [riparian] cover for aquatic environment; therefore, risk is presumed.
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.	Y	There is uncertainty regarding the chemical's potential effect on terrestrial plants that provide [riparian] cover for aquatic environment; therefore, risk is presumed.
Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.	N	Available data indicate that vinclozolin is not likely to cause adverse effects on the aquatic plant or animal community representing the prey of the aquatic-phase CRLF.
Reduction and/or modification of aquatic-based food sources for pre-metamorphs (e.g., algae)	N	Available data indicate that vinclozolin is not likely to cause adverse effects on the aquatic plant community.
Terrestrial-Phase CRLF 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	Y	There is uncertainty regarding the chemical's potential effect on terrestrial plants that provide cover for terrestrial environment; therefore, risk is presumed.
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	Y	There is uncertainty regarding the chemical's potential effect on terrestrial plants that provide cover for terrestrial environment; therefore, risk is presumed.
Reduction and/or modification of food sources for terrestrial-phase juveniles and adults	Y	RQs exceed the LOC for chronic exposures of small mammals and aquatic- and terrestrial-phase amphibians to vinclozolin.
Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	Y	RQs exceed the LOC for chronic exposures of small mammals and terrestrial-phase amphibians to vinclozolin.

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 vinclozolin.

Acute and chronic RQ values are below the LOCs for the aquatic-phase CRLF. The likelihood of individual acute mortality is 1 in 4.86 $x10^{14}$ and there are no incident data to indicate that the use of vinclozolin on turf in California is having a direct effect on aquatic-phase CRLF. Based on the available information and the weight of evidence the potential direct impact to the aquatic-phase of the CRLF based on acute mortality is considered low.

5.2.1.2 Terrestrial-Phase CRLF

Based on the use of avian surrogate toxicity data, vinclozolin is characterized as practically nontoxic to terrestrial-phase CRLF on an acute oral and sub-acute dietary exposure basis. However, based on potential reproductive effects observed in bird studies following chronic exposure, vinclozolin is likely to represent a chronic risk to terrestrial-phase amphibians. The chronic RQ value exceeds the chronic risk LOC by a factor of 9X. In addition, dietary-based chronic EECs generated by T-REX for the small bird consuming small insects exceed the LOAEC of 125 mg/kg-diet, indicating that EECs are sufficient to exceed a level where reproductive effects to birds were observed in the laboratory.

Evaluation of potential direct risk to the terrestrial-phase CRLF using the T-HERPS model indicates that chronic, dietary-based RQs for CRLF consuming small and large insects and small herbivore mammals exceed the LOC (1.0; **Table 19**).

Table 19. Chronic, dietary-based RQs for terrestrial-phase CRLF. RQs generated using T-HERPS.

Food item	RQ
Small Insects	9.09
Large Insects	1.01
Small herbivore mammals	10.65
Small insectivore mammals	0.67
Small terrestrial phase amphibian	0.32

Even if vinclozolin use on turf was limited to a single application, the resulting RQ would exceed the LOC by a factor of roughly 3.6X. The maximum single application rate would have to be reduced by roughly 75% to 0.36 lbs a.i./A for the RQ value to drop below the chronic risk LOC for direct effects on the terrestrial-phase CRLF. Based on a single ground application of vinclozolin at 1.35 lbs a.i./A, the LOC for chronic effects to the terrestrial-phase CRLF is exceeded up to 10 feet from the edge of the treatment field (determined using AgDRIFT Tier 1 ground model[4], terrestrial assessment).

[4] Assuming a high boom and ASAE very fine to fine droplet size distribution.

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. As discussed previously, the most sensitive nonvascular aquatic plant is *N. pelliculosa* with an EC_{50} < 1060 µg/L. Based on a peak EEC of 52.0 µg/L representing vinclozolin residues of concern, the RQ is >0.05. Since the aquatic plant toxicity data are based on a limit test, the concentration required to result in an effect of 50% is uncertain. As noted previously, none on the other aquatic non-vascular plants tested had effects at treatment concentrations ranging between 0.87 – 1.02 mg a.i./L. The maximum effect on these test species was less than 8%. Given that none of the other aquatic plant species tested exhibited much of an effect at concentrations roughly 28 times higher than the maximum residues estimated for vinclozolin and its degradates, vinclozolin is not likely to indirectly affect the CRLF via reduction in non-vascular plants.

5.2.2.2 Aquatic Invertebrates

The potential for vinclozolin 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 endpoints for acute (48-hr EC_{50}=4,000 µg/L) and chronic (790 µg/L), neither acute nor chronic RQ values exceed acute or chronic LOCs. As such, vinclozolin use on turf in California is not expected to directly affect freshwater invertebrates that serve as forage for aquatic-phase CRLF.

5.2.2.3 Fish and Aquatic-phase Frogs

Acute and chronic RQ values for fish and aquatic-phase frogs serving as prey for CRLF are below the acute and chronic risk LOCs. No incidents have been reported for fish involving either vinclozolin or its 3,5-DCA degradate. Although no monitoring data are available for vinclozolin, there are data for the 3,5-DCA degradate. The peak value reported in NAWQA for 3,5-DCA in surface water is 0.0268 µg/L. If the DCA degradate is assumed to be as toxic as the parent, *i.e.*, acute LC_{50}=2840 µg/L and chronic NOAEC=200 µg/L, then the acute and chronic RQs based on monitoring data would be orders of magnitude <0.01. The weight-of-evidence suggests that vinclozolin use on turf in California will have no effects on fish and aquatic-phase amphibians serving as prey.

5.2.2.4 Terrestrial Invertebrates

When the terrestrial-phase CRLF reaches juvenile and adult stages, its diet is mainly composed of terrestrial invertebrates. As indicated above, risk of effects to terrestrial invertebrates and subsequent indirect effects to the CRLF through decrease in terrestrial invertebrate prey is presumed low.

5.2.2.5 Mammals

Life history data for terrestrial-phase CRLFs indicate that large adult frogs consume terrestrial vertebrates, including mice. Vinclozolin is practically nontoxic to mammals on an acute exposure basis. Chronic exposure of mammals to vinclozolin at levels >30 mg/kg/day resulted in decreased genital and reproductive tract malformations that in turn resulted in reproductive failure (failure to sire offspring and these effects are believed to be consistent with the chemical's ability to bind to the androgen receptor. These effects have served as a basis for the endpoint (NOAEL=30 mg/kg/day) used to assess the potential chronic effects of vinclozolin on mammalian prey items.

In order to not exceed the NOAEL of 30 mg/kg/day, the maximum single application rate of 1.35 lbs a.i./A would have to be reduced to 0.25 lbs a.i./A, which is a reduction of 81.5%. Based on a single ground application of vinclozolin at 1.35 lbs a.i./A, the LOC for chronic (dose based) effects to small mammals consuming short grass is exceeded up to 12 feet from the edge of the treatment field (determined using AgDRIFT Tier 1 ground model, terrestrial assessment).

Based on the above information, vinclozolin is likely to indirectly affect the CRLF through a decrease in mammalian prey.

5.2.2.6 Terrestrial-phase Amphibians

Terrestrial-phase adult CRLFs also consume frogs. RQ values representing direct exposures of vinclozolin to terrestrial-phase CRLFs are used to represent exposures of vinclozolin to frogs in terrestrial habitats. Given the potential for effects to the terrestrial-phase CRLF resulting from chronic exposures to vinclozolin, there is also potential for reproductive effects to terrestrial-phase amphibians serving as prey to CRLF. Therefore, vinclozolin is likely to indirectly affect the CRLF through a decrease in amphibian prey.

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 near-shore areas and lower stream banks. 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. Based on a lack of LOC exceedances for nonvascular and vascular aquatic plants, indirect effects are not expected to the aquatic-phase CRLF due to effects to aquatic plants in its habitat.

Due to terrestrial plant data (surrogate for riparian vegetation) exposed to vinclozolin, there is uncertainty regarding the chemical's potential effect on riparian plants that provide cover for the aquatic environment; therefore, risk is presumed. As a result, there is potential for indirect effects to the CRLF due to effects to plants in its aquatic habitat.

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.

Due to a lack of effects data for terrestrial plants exposed to vinclozolin, there is uncertainty regarding the chemical's potential effect on terrestrial plants that provide cover for terrestrial environment; therefore, risk is presumed. As a result, there is potential for indirect effects to the CRLF due to effects to plants in its terrestrial habitat.

5.2.4 Modification to Designated Critical Habitat

5.2.4.1 Aquatic-Phase PCEs

Three of the four assessment endpoints for the aquatic-phase primary constituent elements (PCEs) of designated critical habitat for the CRLF are related to potential effects to aquatic and/or terrestrial plants:

- 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.
- 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.
- Reduction and/or modification of aquatic-based food sources for pre-metamorphs (*e.g.,* algae).

Conclusions for potential indirect effects to the CRLF via direct effects to aquatic and riparian plants are used to determine whether modification to critical habitat may occur. Although effects are not expected for nonvascular and vascular plants within the aquatic environment, due to lack of terrestrial plant data (surrogate for riparian vegetation) exposed to vinclozolin, there is uncertainty regarding the chemical's potential effect on riparian plants that are considered part of the aquatic environment; therefore, risk is presumed. As a result, there is potential for there is potential for effects to the aquatic-phase PCEs of the CRLF.

The remaining aquatic-phase PCE is "alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source." Other than impacts to algae as food items for tadpoles, this PCE is assessed by considering direct and indirect effects to the aquatic-phase CRLF via acute and chronic freshwater fish and invertebrate toxicity endpoints as measures of effects. Available data indicate that vinclozolin is not likely to cause adverse effects on non-vascular plants, aquatic invertebrates or fish representing the prey of the aquatic-phase CRLF.

5.2.4.2 Terrestrial-Phase PCEs

Two of the four assessment endpoints for the terrestrial-phase PCEs of designated critical habitat for the CRLF are related to potential effects to terrestrial plants:

- 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 drip line 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.

- 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.

There is uncertainty regarding the chemical's potential effect on terrestrial plants that provide cover in the terrestrial habitat; therefore, risk is presumed. As a result, there is potential for there is potential for effects to the terrestrial-phase PCEs of the CRLF.

The third terrestrial-phase PCE is "reduction and/or modification of food sources for terrestrial phase juveniles and adults." To assess the impact of vinclozolin on this PCE, acute and chronic toxicity endpoints for terrestrial invertebrates, mammals, and terrestrial-phase frogs are used as measures of effects. Due to LOC exceedances by chronic RQs for small mammals and terrestrial-phase amphibians, there is potential for effects to this PCE.

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. Due to LOC exceedances by chronic RQs for small mammals, terrestrial-phase amphibians, and terrestrial-phase CRLFs, there is potential for effects to this PCE.

5.2.5 Addressing the Risk Hypotheses

In order to conclude this risk assessment, it is necessary to address the risk hypotheses defined in Section 2.9.1. Based on the conclusions of this assessment, not all of the hypotheses can be rejected, meaning that some of the hypotheses represent concerns in terms of direct and indirect effects of vinclozolin on the CRLF and its designated critical habitat.

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.

6.1.2 Aquatic Exposure Modeling of Vinclozolin

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 be a surrogate for 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 (USFWS/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.

6.1.3 Total residues of concern

Metabolites B and E and 3,5-DCA, which are major degradates of vinclozolin, are considered to be residues of concern. Metabolite S also appears in 1 study (soil photolysis) as a major degradate. This degradate is similar in structure to vinclozolin and may be assumed to share a mode of action with vinclozolin, metabolite B and metabolite E; however, since this degradate appears as a major degradate only in the available soil photolysis study, which is not used to parameterize PRZM/EXAMS, this metabolite is not does not affect half-lives to represent total residues of concern for vinclozolin. In addition, Metabolite F, which appears in the fish bioconcentration study as a major degradate is not considered in calculating half-lives to represent total residues of concern for vinclozolin. This is also because the fish bioconcentration study is not used to parameterize PRZM/EXAMS. Since Metabolites S and F appear in environmental fate studies that do not affect the parameterization of the environmental fate models (i.e., PRZM/EXAMS), their presence does not alter the estimation of environmental concentrations of vinclozolin residues of concern.

The total residue of concern method is used to provide a conservative estimate of exposure of aquatic organisms to vinclozolin's residues of concern. There is some uncertainty in this approach, since conservative half-lives were used to represent the persistence of vinclozolin residues of concern in the environment. Also, it was assumed that the toxicities of vinclozolin's residues are equivalent to that of the parent. In order to characterize the uncertainty associated with these assumptions, aquatic EECs were derived for vinclozolin alone using half-lives specific to vinclozolin. The resulting EECs for vinclozolin (Appendix D) are an order of magnitude lower than the EECs for vinclozolin residues of concern.

6.1.3 Measured concentrations of 3,5-DCA in surface water

The maximum concentration of 3,5-DCA reported by NAWQA for California surface waters is 0.03 µg/L. This value is approximately 3 orders of magnitude lower the maximum model-estimated environmental concentration for 3,5-DCA (**Appendix D**). There is uncertainty regarding the source of the measured 3,5-DCA as it could be attributed to use of vinclozolin or iprodione.

6.1.5 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. 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.

6.1.6 Terrestrial Exposure Modeling of Vinclozolin

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. Environmental Protection Agency, 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.

6.1.7 Spray Drift Modeling

Although there may be multiple vinclozolin 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 vinclozolin from

multiple applications, each application of vinclozolin 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 made regarding the droplet size distributions being modeled ('ASAE Very Fine to Fine'), the application method, release heights (high boom) 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.

6.2.2 Use of Surrogate Species Effects Data

Guideline toxicity tests and open literature data on vinclozolin and its degradates 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 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.

Since 3,5-DCA is classified as a potential carcinogen and given the androgen receptor binding capacity of vinclozolin Metabolite B and E, there are a broad range of sublethal effects that could be associated with vinclozolin. The reproductive effects exhibited across a broad range of taxa indicate that the effects of vinclozolin on endocrine-mediated process are not limited to specific animals. This assessment has attempted to account for sublethal effects by setting the initial area of concern as the entire State of California. To the extent to which sublethal effects are not considered in this assessment, the potential direct and indirect effects of vinclozolin 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.

6.2.5 Potential Effects to Terrestrial and Riparian Plants

As indicated above, no data are available to characterize the effects of vinclozolin on terrestrial and riparian plants. Therefore, the risks to these plants from the use of vinclozolin on turf grass is unknown. Since risk cannot be precluded, this assessment concludes that use of vinclozolin on turf can result in modification of the CRLF's critical habitat. It should be noted that the lack of terrestrial plant data renders the risk conclusions for habitat modification highly uncertain.

6.2.6 Toxicities of Vinclozolin Degradates

At this time, limited toxicity data are available to characterize the effects of metabolites with structures similar to that of the parent or 3,5-DCA to non-target organisms. Therefore, it is assumed that data available for vinclozolin are representative of effects to non-target organisms

that may be caused by metabolites similarly structured degradates. If the toxicity of vinclozolin is different than that of metabolites B and E, the effects of vinclozolin may be under- or over-estimated. However, the extent to which this may be the case is unknown.

Limited toxicity data are available for 3,5-DCA that suggest that the compound is less toxic than the parent and it is assumed that this chemical has a different mode of action compared to vinclozolin; however, because only limited data are available, this assessment conservatively assumes that 3,5-DCA is as toxic as the parent to non-target organisms.

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 vinclozolin 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 vinclozolin on turf in California. The Agency has determined that there is the potential for modification of CRLF designated critical habitat from the single use of the chemical on turf. Based on chronic direct effects on the terrestrial-phase CRLF and indirect effects on both the aquatic-phase and terrestrial-phase CRLF due to chronic effects on prey items, the use of vinclozolin on turf in California is considered likely to adversely affect the CRLF. Additionally, there is uncertainty regarding the potential effects of vinclozolin on terrestrial plants because of the lack of terrestrial plant toxicity data and because of this uncertainty, the use of vinclozolin may result in habitat modification. Given the LAA determination for the CRLF and uncertainty regarding the potential modification of designated critical habitat, a description of the baseline status and cumulative effects for the CRLF is provided in Attachment II.

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 vinclozolin's use pattern is identified, using land cover data that correspond to vinclozolin's use on turf grass. 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 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 12 feet from its boundary (based on risks of chronic exposures to small mammals serving as prey to the terrestrial-phase CRLF; refer to Section 5.2.2.5).

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 20** and **Table 21.**

Table 20. Effects Determination Summary for Vinclozolin Use and the CRLF.

Assessment Endpoint	Effects Determination	Basis for Determination
Survival, growth, and/or reproduction of CRLF individuals	May affect, likely to adversely affect (LAA)	**Potential for Direct Effects** ***Aquatic-phase (Eggs, Larvae, and Adults)***: Based on available data, both acute and chronic RQ values are below acute and chronic risk LOCs. As such, vinclozolin use on turf is determined to have no direct effect on aquatic-phase CRLF. ***Terrestrial-phase (Juveniles and Adults)***: Although vinclozolin is considered practically nontoxic to terrestrial-phase amphibians (based on avian data used as a surrogate) on an acute oral and sub-acute dietary exposure basis, chronic RQs based on impaired reproduction exceed the chronic risk LOC by a factor of 9X. EECs are also sufficient to exceed the level where reproductive effects were observed in birds (the LOAEC). As such, the use of vinclozolin on turf grass in California is determined to be likely to adversely affect terrestrial-phase CRLF due to direct chronic effects on reproduction. **Potential for Indirect Effects** ***Aquatic prey items, aquatic habitat, cover and/or primary productivity*** Acute and chronic RQ values are below the acute and chronic risk LOCs for freshwater invertebrates. RQ values for non-vascular and vascular aquatic plants are below the LOC and/or vinclozolin is not expected to adversely affect the aquatic plant community. Given that vinclozolin does not directly affect aquatic vertebrates, vinclozolin is determined to have no effect on fish and aquatic-phase amphibians that serve as prey for CRLF. ***Terrestrial prey items, riparian habitat*** Although vinclozolin is practically nontoxic to terrestrial-phase amphibians and mammals on an acute exposure basis, chronic RQs based on impaired reproduction exceed the chronic risk LOC by a factor of 9X for terrestrial-phase amphibians and factors as high as 12X for small mammals that serve as prey for CRLF. In addition, because of uncertainty regarding the potential effects of vinclozolin on terrestrial plants, risk is presumed for the riparian habitat on which CRLF depend. As such, the use of vinclozolin on turf in California is determined to be likely to adversely affect the CRLF through indirect effects on prey and habitat.

Table 21. Effects Determination Summary for Vinclozolin Use and CRLF Critical Habitat Impact Analysis.

Assessment Endpoint	Effects Determination	Basis for Determination
Modification of aquatic-phase PCE	Habitat Modification	Based on the weight of evidence, the use of vinclozolin on turf grass in California is determined to have no adverse effect on aquatic plants; however, there is uncertainty regarding the potential effects of vinclozolin on terrestrial plants because of the absence of terrestrial plant toxicity data. Because of this uncertainty, risk is presumed and there is a potential for habitat modification due to effects on riparian cover surrounding aquatic areas.
Modification of terrestrial-phase PCE		There is uncertainty regarding the potential effects of vinclozolin on terrestrial plants because of the absence of data. Because of this uncertainty, risk is presumed and there is a potential for habitat modification due to effects on riparian cover.

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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