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

Pesticide Effects Determination

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Washington, D.C. 20460

October 17, 2008

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1. 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 the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) regulatory actions regarding use of glyphosate and its salts on agricultural and non-agricultural sites. In addition, this assessment evaluates whether these actions can be expected to result in modification of the species' designated critical habitat. This assessment was completed in accordance with the U.S. Fish and Wildlife Service (USFWS) and National Marine Fisheries Service (NMFS) *Endangered Species Consultation Handbook* (USFWS/NMFS, 1998 and procedures outlined in the U.S. Environmental Protection Agency 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.

Glyphosate (*N*-(phosphonomethyl)glycine) is a non-selective, systemic herbicide widely used to control weeds in agricultural crops and non-agricultural sites. Both the parent acid and several of its salts are registered as active ingredients and all are considered in this assessment. As of the 1993 Re-registration Eligibility Decision (RED), labeled uses of glyphosate included over 100 terrestrial food crops. In addition, there are many other uses under the categories of terrestrial food, non-food and feed crop; forestry; aquatic food crop and non-food outdoor and industrial; greenhouse food and non-food crop; indoor non-food and outdoor residential. The following uses are considered as part of the federal action evaluated in this assessment: many agricultural crops, non-grass forage/fodder/straw/hay, rights-of-way/fence rows/hedgerows, farm structures/buildings and equipment, pastures, grasses grown for seed and Christmas tree plantations; ornamental shade trees, ground cover, herbaceous plants, non-flowering plants and lawns; nursery stock and turf; commercial, urban and residential outdoor buildings/structures, premises, path/patio, paved areas and recreational areas; rangeland and forestry conifer release, nursery plantings (fir transplant purposes), trees (all or unspecified) and aquatic uses on emergent plants.

Glyphosate is stable towards abiotic hydrolysis and direct photolysis in water. Its major route of transformation identified in laboratory studies and in the field is microbial degradation, where the major metabolite is **a**mino**m**ethyl **p**hosphonic **a**cid (AMPA). Glyphosate is very soluble in water. It has low potential to volatilize from soil or water, as suggested by its low vapor pressure and Henry's Law Constant. Glyphosate adsorbs strongly to soils and sediments. Based on its strong adsorption to soil/sediments alone, leaching to ground water or entering surface water dissolved in runoff would be minimized. However, surface water can be contaminated by transport of suspended soil particulates, followed by desorption from the soil particulates and/or from sediments.

Offsite exposure is also possible via spray drift, colloidal transport, inadvertent direct overspray and wind transport of soil particulates loaded with adsorbed glyphosate residues. Glyphosate is very hydrophilic and is unlikely to bioaccumulate in fish.

Glyphosate is an acid which can be associated with different counter cations to form salts. For comparison purposes in this assessment, each salt is considered in terms of its “glyphosate equivalent,” (acid equivalent; ae) as determined by multiplying by the acid equivalence ratio (the ratio of the molecular weight of *N*-(phosphonomethyl)glycine to the molecular weight of the salt). For the assessment of risk to technical glyphosate, both application rates and the toxicity endpoint values are expressed as acid equivalents.

Risks from exposure to glyphosate formulations are also assessed because some of the formulations are more toxic than the technical material. For aquatic organisms, exposures to glyphosate formulations following terrestrial and aquatic applications are considered separately. Terrestrial uses allow for application of formulations that contain a surfactant that is toxic to aquatic organisms (polyethoxylated tallow amines (POEA)), whereas the toxic surfactant is not allowed in formulations designated for aquatic use.

Since CRLFs exist within both aquatic and terrestrial habitats, exposure to glyphosate is assessed separately for the two habitats and for the CRLF and its prey in each habitat. The highest aquatic exposure to both glyphosate and its formulations is expected to result from uses with direct aquatic applications. Estimated environmental concentrations (EECs) for these uses were derived with simple dilution calculations based on the mass of the applied pesticide and the volume of the water body. For glyphosate and its formulations, peak EECs for aquatic uses were 210 µg ae/L and 1840 µg form./L, respectively. These estimates are supplemented with analysis of available California surface water monitoring data from U. S. Geological Survey’s National Water Quality Assessment (NAWQA) program and the California Department of Pesticide Regulation (DPR). These data sources included biweekly monitoring for glyphosate at three sites between 2002-2003. Both glyphosate and AMPA, its degradate, were detected at least once at all sites, with maximum reported concentrations of 7.46 µg/L for glyphosate and 1.07 µg/L for AMPA. Both peak concentrations were detected at an agricultural site in Stanislaus County.

To estimate glyphosate exposures to the terrestrial-phase CRLF, and its potential prey resulting from uses involving glyphosate applications, the T-REX model is used for foliar uses. The AgDRIFT model is also used to estimate deposition of glyphosate on terrestrial and aquatic habitats from spray drift. The TerrPlant model is used to estimate glyphosate exposures to terrestrial-phase CRLF habitat, including plants inhabiting semi-aquatic and dry areas, resulting from uses involving foliar glyphosate applications.

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 and on aquatic-phase amphibians. In the terrestrial habitat, direct effects are based on toxicity information for birds, which are used as a surrogate for terrestrial-phase amphibians. Given that the CRLF's prey items and designated critical habitat requirements in the aquatic habitat are dependant on the availability of freshwater aquatic invertebrates and aquatic plants, toxicity information for these taxonomic groups is also discussed. In the terrestrial habitat, indirect effects due to depletion of prey are assessed by considering effects to terrestrial insects, small terrestrial mammals, and frogs. Indirect effects due to modification of the terrestrial habitat are characterized by available data for terrestrial monocots and dicots.

Acute toxicity data are available for the degradate, AMPA, with freshwater fish, birds and aquatic invertebrates. Since AMPA appears to be less toxic than the parent, this degradate was not considered in exposure estimations.

Risk quotients (RQs) are derived as quantitative estimates of potential high-end risk. Acute and chronic RQs are compared to the U.S. Environmental Protection Agency (referred to as 'the Agency' in subsequent text) levels of concern (LOCs) to identify instances where glyphosate 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 a particular type of effect are below LOCs, the pesticide is determined to have "no effect" on the subject species. 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 glyphosate 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 glyphosate. Additionally, the Agency has determined that there is the potential for modification of CRLF designated critical habitat from the use of the chemical.

There are no direct effects on the aquatic-phase CRLF for any of the terrestrial or aquatic uses. The terrestrial-phase CRLF eating broadleaf plants, small insects and small herbivorous mammals on a dietary-basis may be at risk to direct effects following chronic exposure to glyphosate at application rates of 7.5 lb a.e./A and above (forestry, areas with impervious surfaces and rights of way). In addition, terrestrial phase amphibians may be at risk following acute exposure to one particular formulation (Registration No. 524-424), at application rates of 1.1 lbs formulation/A and above (ornamental lawns and turf and industrial outdoor uses). Indirect effects to the aquatic-phase CRLF, based on reduction in the prey base may occur with aquatic nonvascular plants with aquatic weed management uses at an application rate of 3.75 lb a.e./A. Indirect effects to the terrestrial-phase CRLF, based on reduction in the prey base may occur with small insects at any registered rate, large insects at an application rate of 7.95 lb a.e./A (forestry uses), terrestrial phase amphibians following chronic exposure at application rates of 7.5 lb a.e./A and above and following acute exposure to one formulation at application rates of 1.1 lbs formulation/A and above and mammals following chronic exposure at application rates of 3.84 lbs a.e./A and above (i.e., many crops, forestry, rights of way and areas with impervious surfaces).

Indirect effects to both the aquatic- and terrestrial-phase CRLF, based on habitat effects may occur with aquatic non-vascular plants following aquatic weed management use and with aquatic emergent plants and terrestrial plants exposed via spray drift with aerial application at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A.

A summary of the risk conclusions and effects determinations for the CRLF and its critical habitat is presented in **Tables 1.1 and 1.2**. Use-specific determinations for direct and indirect effects to the CRLF are provided in **Tables 1.3 and 1.4**. 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 2**.

Table 1.1 Effects Determination Summary for Glyphosate Use and the CRLF

Assessment Endpoint	Effects Determination [1]	Basis for Determination
Survival, growth, and/or reproduction of CRLF individuals	LAA[1]	**Potential for Direct Effects** ***Aquatic-phase (Eggs, Larvae, and Adults)***: The acute and chronic LOCs for freshwater fish and aquatic-phase amphibians are not exceeded for either glyphosate, its salts or its formulations. ***Terrestrial-phase (Juveniles and Adults)***: The chronic LOC for avian species (surrogate for CRLF) is exceeded at application rates of 7.5 lb a.e./A and above (forestry, areas with impervious surfaces and rights of way). The acute LOC for one particular formulation is exceeded for medium and large- and for medium-sized CRLF's eating small herbivorous mammals on a dose-basis at application rates of 5.5 (highest rate: industrial outdoor uses) and 1.1 (lowest rate: ornamental lawns and turf) lb formulation/A, respectively. For the formulation, the probability of an individual effect at the RQs for the highest and lowest application rates are 1 in 9.32 and 1 in 1.25E+05, respectively. Initial area of concern and action area are the entire state of California. Glyphosate is used in all 58 counties in California with landscape maintenance and rights of way among the highest usages in the counties which may have some currently CRLF occupied areas. **Potential for Indirect Effects** ***Aquatic prey items, aquatic habitat, cover and/or primary productivity*** The acute and chronic LOCs for freshwater invertebrates are not exceeded for glyphosate, its salts or formulations. In addition, the probit analysis indicates that the probability of an individual effect and the percentage effect to the

Table 1.1 Effects Determination Summary for Glyphosate Use and the CRLF		
Assessment Endpoint	**Effects Determination [1]**	**Basis for Determination**
		freshwater invertebrate population prey base would be very low, and the monitoring data are considerably lower than the modeled concentrations utilized in the risk assessment. For non-vascular plants, the LOC for aquatic plants is exceeded for formulations specified for aquatic uses. For vascular plants, the LOC for aquatic plants is not exceeded for either glyphosate, its salts or its formulations; however, for aquatic emergent plants, the terrestrial plant LOC is exceeded following spray drift with aerial applications at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A. The acute and chronic LOC for freshwater fish and aquatic-phase amphibians are not exceeded for either glyphosate, its salts or its formulations.
		Terrestrial prey items, riparian habitat For terrestrial invertebrates, the upper bound RQs for small insects exceed the LOC for listed terrestrial invertebrates for all uses and for non-listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above. The upper bound RQs for large insects exceed the LOC for listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above. At the highest upper bound RQ (<1.4 at 7.95 lbs a.e./A with uses on forestry and areas with impervious surfaces), the chance of an individual effect is <1 in 1.34 with a <75% percentage effect to the terrestrial invertebrate prey base. At the lowest upper bound RQ (<0.01 with 0.387 lbs a.e./A on rangeland), the chance of an individual effect is <8.86E+18 with a <1.13E-17 percentage effect to the terrestrial invertebrate prey base. The chronic RQs exceed the chronic LOC for small mammals on a dose-basis for application rates of 3.84 lbs/A and above (i.e., most crops, forestry, areas with impervious surfaces and rights of way). The chronic LOC for avian species (surrogate for CRLF) is exceeded at application rates of 7.5 lb a.e./A and above (forestry, areas with impervious surfaces and rights of way). The acute LOC for one particular formulation is exceeded for medium and large- and for medium-sized CRLF's eating small herbivorous mammals on a dose-basis at application rates of 5.5 (highest rate: industrial outdoor uses) and 1.1 (lowest rate: ornamental lawns and turf) lb formulation/A, respectively. For the formulation, the probability of an individual effect at the RQs for the highest and lowest application rates are 1 in 9.32 and 1 in 1.25E+05, respectively. For terrestrial plants, the LOC is exceeded following spray drift with aerial applications at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A. Initial area of concern and action area are the entire state of California.

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

Table 1.2 Effects Determination Summary for Glyphosate Use and CRLF Critical Habitat Impact Analysis		
Assessment Endpoint	**Effects Determination [1]**	**Basis for Determination**
Modification of aquatic-phase PCE	Habitat modification[1]	For terrestrial plants, the LOC is exceeded following spray drift with aerial applications at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A. For non-vascular plants, the LOC for aquatic plants is exceeded, only for formulations specified for aquatic uses. For vascular plants, the LOC for aquatic plants is not exceeded for either glyphosate, its salts or its formulations; however, for aquatic emergent plants, the terrestrial plant LOC is exceeded following spray drift with aerial applications at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A. The acute and chronic LOCs for freshwater fish and aquatic-phase amphibians are not exceeded for either glyphosate, its salts or its formulations. The acute and chronic LOCs for freshwater invertebrates are not exceeded for glyphosate, its salts or formulations. In addition, the probit analysis indicates that the probability of an individual effect and the percentage effect to the freshwater invertebrate population prey base would be very low.
Modification of terrestrial-phase PCE	Habitat modification[1]	For terrestrial plants, the LOC is exceeded following spray drift with aerial applications at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A. The chronic LOC for avian species (surrogate for CRLF) is exceeded at application rates of 7.5 lb a.e./A and above (forestry, areas with impervious surfaces and rights of way). The acute LOC for one particular formulation is exceeded for medium and large- and for medium-sized CRLF's eating small herbivorous mammals on a dose-basis at application rates of 5.5 (highest rate: industrial outdoor uses) and 1.1 (lowest rate: ornamental lawns and turf) lb formulation/A, respectively. For the formulation, the probability of an individual effect at the RQs for the highest and lowest application rates are 1 in 9.32 and 1 in 1.25E+05, respectively. For terrestrial invertebrates, the upper bound RQs for small insects exceed the LOC for listed terrestrial invertebrates for all uses and for non-listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above. The upper bound RQs for large insects exceed the LOC for listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above. At the highest upper bound RQ (<1.4 at 7.95 lbs a.e./A with uses on forestry and areas with impervious surfaces), the chance of an individual effect is <1 in 1.34 with a <75% percentage effect to the terrestrial invertebrate prey base. At the lowest upper bound RQ (<0.01 with 0.387 lbs a.e./A on rangeland), the chance of an individual effect is <8.86E+18 with a <1.13E-17 percentage effect to the terrestrial invertebrate prey base. The chronic RQs exceed the chronic LOC for small mammals on a dose-basis for application rates of 3.84 lbs/A and above (i.e., most crops, forestry, areas with impervious surfaces and rights of way).

[1] Habitat Modification or No effect (NE)

Table 1.3 Glyphosate Use-specific Direct Effects Determinations[1] for the CRLF				
Use(s)	Aquatic Habitat		Terrestrial Habitat	
	Acute	Chronic	Acute	Chronic
Forestry, areas with impervious surfaces and rights of way (application rates of 7.5 lbs a.e./A and above)	NE	NE	NE	LAA
One particular formulation (Reg No. 524-424): industrial sites, rights-of-way, ornamental lawns and turf at 1.1to 5.5 lbs formulation/A.	NA	NA	LAA	NA
All other uses at application rates of 3.85 lb a.e./A and below (all crops, forestry and impervious surfaces at lower rates, rangeland, residential, rights of way at lower rates and turf)	NE	NE	NE	NE
[1] NE = No effect; NLAA = May affect, but not likely to adversely affect; LAA = Likely to adversely affect; NA = data not available for this formulation.				

Table 1.4 Glyphosate Use-specific Indirect Effects Determinations[1] Based on Effects to Prey										
Use(s)	**Algae**	**Aquatic Invertebrates**		**Terrestrial Invertebrates (Acute)**	**Aquatic-phase frogs and fish**		**Terrestrial-phase frogs**		**Small Mammals**	
		Acute	**Chronic**		**Acute**	**Chronic**	**Acute**	**Chronic**	**Acute**	**Chronic**
Forestry, areas with impervious surfaces and rights of way (application rates of 7.5 lb a.e./A and above)	NE	NE	NE	LAA	NE	NE	NE	LAA	NE	LAA
One particular formulation (Reg No. 524-424): industrial sites, rights-of-way, ornamental lawns and turf at 1.1to 5.5 lbs formulation/A.	NA	NA	NA	LAA	NA	NA	LAA	NA	NLAA	NA
Most crops, forestry, rights of way and areas with impervious surfaces at application rates of 3.84 lbs a.e./A and above.	NE	NE	NE	LAA	NE	NE	NE	NE	NE	LAA
Aquatic uses at 3.75 lb a.e./A	LAA	NE	NE	NE	NE	NE	NE	NE	NE	NE

[1] NE = No effect; NLAA = May affect, not likely to adversely affect; LAA = Likely to adversely affect; NA = data not available for this formulation.

Based on the conclusions of this assessment, a formal consultation with the U. S. Fish and Wildlife Service under Section 7 of the Endangered Species Act should be initiated to determine whether there are reasonable and prudent alternatives and/or measures to reduce and/or eliminate potential incidental take.

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

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

2. 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 glyphosate on a large number of agricultural crops, non-grass forage/fodder/straw/hay, rights-of-way/fence rows/hedgerows, farm structures/buildings and equipment, pastures, grasses grown for seed and Christmas tree plantations; ornamental shade trees, ground cover, herbaceous plants, non-flowering plants and lawns; nursery stock and turf; commercial, urban and residential outdoor buildings/structures, premises, path/patio, paved areas and recreational areas; rangeland and forestry conifer release, nursery plantings (fir transplant purposes), trees (all or unspecified) and aquatic uses on emergent plants. In addition, this assessment evaluates whether use on these crops 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 GENEEC2, PRZM-EXAMS, T-REX, TerrPlant and AgDRIFT, all of which are described at length in the Overview Document. 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 Endangered Species Act (ESA), and the Services' *Endangered Species Consultation Handbook,* the assessment of effects associated with registrations of glyphosate is based on an action area. The action area is the area directly or indirectly affected by the federal action, as indicated by the exceedence 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 glyphosate 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 glyphosate 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 glyphosate 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 glyphosate.

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

Glyphosate is an herbicide approved for use on crops grown in California as well as for many non-agricultural and residential sites. These include the following categories: terrestrial non-food, food and feed crop; forestry; aquatic food crop and non-food outdoor and industrial; greenhouse food and non-food crop; indoor non-food and outdoor residential. Registered uses of glyphosate on crops that are not grown in California, including soybeans, will not be considered in this assessment. In addition to the parent acid, several salts of glyphosate can be used as active ingredients. All of these species are included in this assessment and will be referred to collectively as "glyphosate" throughout this document.

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. In addition, the labels usually specify application rates and frequency of application. Thus, the use or potential use of glyphosate in accordance with the approved product labels for California is "the action" relevant to this ecological risk assessment.

Although current registrations of glyphosate allow for use nationwide, this ecological risk assessment and effects determination addresses currently registered uses of glyphosate 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.

The primary degradate of glyphosate is AMPA, which can be formed through photolysis or through metabolism in both aerobic and anaerobic conditions. Acute ecotoxicity studies with freshwater fish and invertebrates and birds indicate that AMPA is not more toxic than the parent, glyphosate. Therefore, the degradate was not included in the assessment.

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).

Glyphosate has registered products that contain multiple active ingredients (**Table 2.1**). Analysis of the available acute oral mammalian toxicity data for multiple active ingredient products relative to the single active ingredient is provided in **Appendix A**. The results of this analysis show that an assessment based on the toxicity of the single active ingredient of glyphosate is appropriate. There are no currently registered product LD_{50} values, with associated 95% Confidence Intervals (CIs) available for mixtures containing glyphosate. As discussed in USEPA (2000), a quantitative component-based evaluation of mixture toxicity requires data of appropriate quality for each component of a mixture. In this mixture evaluation, an LD_{50} with associated 95% CI is needed for the formulated product. The same quality of data is also required for each component of the mixture. Given that the formulated products for mixtures containing glyphosate do not have LD_{50} data available, it is not possible to undertake a quantitative or qualitative analysis for potential interactive effects.

In some products, glyphosate is formulated with surfactants which have been shown to increase the toxicity of the parent compound. Therefore, risk from formulations containing surfactants is considered in this assessment as well as from glyphosate alone. Products containing surfactants will be referred to as "formulations" throughout this document and those containing only glyphosate are referred to as "glyphosate". Products containing the surfactant POEA are off-labeled for aquatic uses in California, so these products will only be assessed for terrestrial uses. This document only assesses a surfactant when it is included as part of the formulated product; it does not assess surfactant that may be included in the tank mix.

Table 2.1. Multiple Active-Ingredient Formulations for Glyphosate									
		Percent (%) Active Ingredient							
REG_NR	PROD_NAME	2,4-D	Dicamba	Diquat dibromide	Glyphosate	Imazethapyr	Oxyfluorfen	S-Metolachlor	Sulfuric acid, monourea adduct
00010001179	TOUCHDOWN DIQUAT HOME AND GARDEN CONCENTRATE			0.73	13.4				
00010001180	TOUCHDOWN DIQUAT HOME AND GARDEN READY TO USE			0.06	0.81				
00010001185	SEQUENCE HERBICIDE				21.8			29	
00010001186	TOUCHDOWN 008		0.6		43.5				
00023902694	ORTHO SEASON-LONG GRASS & WEED KILLER			0.1	8		1.5		
00024100404	STANDOUT HERBICIDE				21.9	2.7			
00035200675	ETK-2301 HERBICIDE				9.6				
00968800211	CHEMSICO HERBICIDE CONCENTRATE DT			1.9	14.6				
00968800213	CHEMSICO HERBICIDE RTU DT			0.1	0.81				
07136800030	NUFARM GLYKAMBA BROADSPECTRUM HERBICIDE		4.1		23.3				
07136800035	RECOIL BROAD SPECTRUM HERBICIDE	11.38			23.03				

2.3 Previous Assessments

The ecological risks associated with use of glyphosate as an herbicide have been assessed several times since 1974 when it was first registered for use in the United States. Findings from relevant ecological risk assessments are briefly summarized below.

- Glyphosate was assessed for the Reregistration Eligibility Decision in 1993. The Agency concluded that direct risks to birds, mammals, invertebrates and fish would be minimal. Under certain conditions, aquatic plants were expected to be at risk from glyphosate use. Additional data were needed for non-target terrestrial plants, including incident data and vegetative vigor testing on non-target terrestrial plants. The assessment stated that many endangered plants may be at risk from use of glyphosate with the registered use patterns. In addition, it was determined that the Houston Toad may be at risk from use of glyphosate on alfalfa.

- In 2003, the USDA Forest Service had a risk assessment conducted for glyphosate uses in Forest Service vegetation management programs (USDA, 2003). For forestry uses, all commercial formulations of glyphosate contained the isopropylamine salt of glyphosate (IPA). Application rates ranged from 0.5 lbs a.e./A to 7 lbs a.e./A with the most typical at 2 lb a.e./A. The USDA assessment did not conduct a separate assessment for amphibians. The document concluded that the amphibian data indicated that glyphosate is no more toxic to amphibians than it is to fish. The USDA risk assessment also used a "relative potency" method to estimate the chronic NOAEC for fish in more sensitive species. This appears to be similar to the Agency's acute to chronic ratio estimations. The NOAEC from a less sensitive fish study was divided by 10 to provide a NOAEC for a more sensitive fish. A similar approach was used for an estimation of a chronic NOAEC for glyphosate formulations on freshwater fish and invertebrates. Finally, as a note, some of the endpoints utilized in the USDA risk assessment were not the same endpoints as used in the Agency risk assessments. For example, the chronic mammal endpoint is also used as the acute endpoint for mammals (175 mg/kg from the developmental study in rabbits).

  Based on the available data, the USDA concluded that the risks were minimal to mammals, birds, fish, invertebrates and aquatic plants. Risks to fish following application of the more toxic formulations were not considered to be high; however, the assessment did state that at an application rate of 7 lb a.e./A, the acute exposures slightly exceeded the acute LC_{50} for a more tolerant freshwater fish and exceeded it by a factor of 2 for the less tolerant fish. These values were estimated from a worst-case scenario where there was a severe rainfall of about 7 inches over a 24-hour period in an area where runoff is favored. For terrestrial plants, the assessment concluded that for relatively tolerant plants, when a low-boom spray is utilized as the method of application, there is no indication that glyphosate would result in damage from spray drift at distances from the application site of 25 feet or greater. For more sensitive plants, the distance increased to approximately 100 feet. The applications requiring the use of backpack-directed spray, the distances would be less. No risks to terrestrial plants from runoff were expected.

- In 2004, the Agency assessed glyphosate's potential to affect 11 federally listed Pacific salmonids. That assessment determined that use of glyphosate "may affect, but is not likely to adversely affect" the species based on acute toxicity to fish for uses with application rates above 5 lb ai/A. For uses with application rates below 5 lb ai/A, the Agency determined glyphosate would have no effect on the 11 subject species.

- In 2006, the Agency assessed glyphosate for a new use on bentgrass (0.74 lb a.i./A) and for new uses on Indian mulberry (noni), dry peas, lentils, garbanzo, safflower and sunflower with the highest proposed ground application rate of 3.73 lbs ae/A. For all proposed new uses, the Agency concluded that there was minimal risk of direct acute effect to terrestrial animals (birds and mammals) and aquatic animals (fish, amphibians, and invertebrates) and minimal risk to terrestrial plants (both non-target and endangered plant species), aquatic non-vascular (algae and diatoms) and vascular (duckweed) plants from offtarget spray drift and runoff from ground-based application technology. In addition, there were no chronic risks to animals.

2.4 Stressor Source and Distribution

Glyphosate [*N*-(phosphonomethyl)glycine] is an acid, and it can also be associated with different counter cations to form salts. Several salts of glyphosate are currently marketed, as well as the acid, and are considered as the active ingredient in end-use products. The parent acid is the chemical species that exhibits herbicidal activity and so is the actual chemical stressor considered in this ecological risk assessment regardless of the salt, unless otherwise specified. In order to have comparable results, each salt is considered in terms of its glyphosate equivalent, (acid equivalent; ae), determined by multiplying the application rate by the acid equivalence ratio, defined as the ratio of the molecular weight of *N*-(phosphonomethyl)glycine to the molecular weight of the salt. Table 2.2 shows the salts of glyphosate that may be used as the source of the actual herbicide-active chemical species. Products that no longer have active registrations are included as well for reference purposes. For the purpose of this assessment, the acid and all salt species are referred to collectively as "glyphosate" throughout this document.

Table 2.2. Identification of Glyphosate and its Salts			
Counter Cation	**PC Code**	**CAS No.**	**Acid Equivalence Ratio**
Glyphosate acid (no counter cation)	417300	1071-83-6	1
Isopropyl amine	103601	38641-94-0	0.74
Monoammonium	103604	114370-14-8	0.94
Diammonium	103607	40465-66-5	0.83
N-methylmethanamine	103608	34494-07-7	0.79
Potassium	103613	39600-42-5; 70901-20-1	0.81
Sesquisodium	103603	70393-85-0	Inactive Registration
Ethanolamine	103605	--	Inactive Registration
Trimethyl sulfonium	128501	81591-81-3	Inactive Registration

Surfactants

In some end use products, the active ingredient is formulated with a surfactant to improve efficacy. Studies show that these formulated products can be more toxic than the active ingredient alone and so in this assessment, formulated products are considered independently of those containing only the active ingredient.

Surfactants (**surf**ace **act**ing **a**ge**nt**") are wetting agents that lower the surface tension of a liquid, allowing easier spreading, and lower the interfacial tension between two liquids. Usually they are organic chemicals that contain a hydrophobic group ("tail") and a hydrophilic group ("head") in the same molecule. For the most part, surfactants are mixtures of the same class with different length of the carbon chain. Usually, the mixture indicates the carbon-chain range in the surfactant (e.g., C10- C14 fraction).

Pesticides of high solubility in water, such as glyphosate, do not "wet" (cover) properly the waxy (hydrophobic) surfaces of plants. To attain proper coverage of plant surfaces and distribution of the herbicide, surfactants are added into the formulation of the pesticide. Proper coverage arises from hydrophobic interactions between the surfactant tail (usually long carbon chains) and the waxy surfaces of plants. Therefore, the ecological effects of the pesticide-surfactant combination may differ from that of the single pesticide or the single surfactant. Glyphosate labels also recommend using a nonionic surfactant in the tank mix to further enhance the "wettability" of glyphosate.

One class of surfactants used in glyphosate formulations are the polyethoxylated tallow amines (POEA). Use of POEA containing products is not allowed for aquatic uses in California. However, other formulations may contain a different class of surfactant. The nature of the surfactant included in the formulation is considered to be Confidential Business Information (CBI) and is not included on product labels.

2.4.1 Physical and Chemical Properties

The physical and chemical properties of glyphosate are shown in Table 2.3. Based on these physical and chemical properties alone, glyphosate has low potential to volatilize from soils (vapor pressure) or from water (Henry's Law Constant). It is also unlikely to bioaccumulate in fish given the low value of the Log *n*-octanol/water partition coefficient. **Appendix B** provides the structure and further chemical/molecular information on glyphosate. The molecular structure characteristics of glyphosate are important as they help understanding its mode of action at a molecular level as well as the binding of glyphosate to soil/sediment particulates**.**

Table 2.3 Physical and Chemical Properties of Glyphosate

Physical/Chemical Property	**Value**
Molecular Formula	$C_3H_8NO_5P$
Molecular Weight	170.8 g/mole
Melting Point	210-212° C (tech.) 215-219° C (pure)
Solubility in water, 25° C	12,000 mg L^{-1}
Vapor Pressure, Pa	1.3×10^{-7} (25° C)
Henry's Law Constant, Pa ·m^3·mol^{-1}	2.1×10^{-9}
Log K_{ow}	< -3
Dissociation Constants	$pKa_1 = 0.8$ $pKa_2 = 2.35$ $pKa_3 = 5.84$ $pKa_4 = 10.48$

2.4.2 Environmental Fate Properties

Table 2.4 summarizes the environmental fate behavior of glyphosate in different media. The environmental fate data shown in this Table are taken from required studies submitted in support of registration of glyphosate. These studies are conducted in a limited number of test systems (e.g., soils, water-sediments). These data are specific only for these systems. They may vary for other systems and may not be the same under actual use conditions.

The major route of transformation of glyphosate identified in laboratory studies is microbial degradation. In soils incubated under aerobic conditions, the half-life of glyphosate ranges from 1.8 to 5.4 days and in aerobic water-sediment systems is 7 days. However, anaerobic conditions limit the metabolism of glyphosate (half-life 8 to 199 days in anaerobic water-sediment systems). In laboratory studies, glyphosate was not observed to break down by abiotic processes in water, such as hydrolysis and direct aquatic photolysis, but soil photolysis occurred with a half-life of 6.6 days. In the field, dissipation half-lives were measured to be 2.4 to 160 days (n=6). Glyphosate dissipation appeared to correlate with climate, being more persistent in cold than in warm climates. Along with significant mineralization to carbon dioxide, the major metabolite of glyphosate is **a**mino **m**ethyl **p**hosphonic **a**cid (AMPA).

No data are available about the environmental fate behavior of glyphosate formulations.

Table 2.4. Summary of Glyphosate Environmental Fate Behavior

Transformation

Study	Value	Major Degradates[1], Comments	MRID #	Study Status
Abiotic Hydrolysis Half-life	Stable (at 25° C for at least 30 days)	None	00108192; 44320642	161-1 Satisfied
Direct Aqueous Photolysis	Stable (for at least 30 days)	None	41689101; 44320643	161-2 Satisfied
Soil Photolysis Half-life	Stable (for at least 30 days)	Degradation in dark control was equal to that in irradiated samples	44320645.	161-3 Satisfied
Aerobic Soil Metabolism Half-life	1.8 and 5.4 days (sandy loam) 2.6 days (silt loam)	AMPA (max 29% at 40 d) CO_2 (≥70% after 1 year)	42372501; 44320645	162-1 Satisfied
Anaerobic Aquatic Metabolism Half-life	208 days (Water- silty clay loam sediment system)	AMPA (max 25% at 15 d) CO_2 (≥ 35% after 1 year) Initial degradation was rapid but slowed considerably. Non-linear modeling predicts DT_{50} = 8.1 day and DT_{90} > 1 yr	41723701; 42372502	162-3 Satisfied
Aerobic Aquatic Metabolism Half-life	14.1 days (Water- silty clay loam sediment)	AMPA (19-25% at 7-30 d) CO_2 (≥ 23% after 30 d)	41723601; 42372503	162-4 Satisfied

Mobility

Study	Value						MRID #	Study Status
Batch Equilibrium (mL/g)	*Soil*	*Avg K_d*	*Avg K_{oc}*	*K_F*	*1/n*	*K_{Foc}*	44320646	163-1 Satisfied
	sand	170	58,000	64	0.75	22,000		
	sandy loam	18	3,100	9.4	0.72	1,600		
	sandy loam	230	13,000	90	0.76	5,000		
	silty clay loam	680	33,000	470	0.93	21,000		
	silty clay loam	1,000	47,000	700	0.94	33,000		

Field Dissipation				
Study	**Value**		**MRID #**	**Study Status**
Terrestrial Field Dissipation Half-life	Glyph. / AMPA 1.7 d / 131 d (TX) 7.3 d / 119 d (OH) 8.3 d / 958 d (GA) 13 d / 896 d (CA) 17 d / 142 d (AZ) 25 d / 302 d (MN) 114 d / 240 d (NY) 142 d / no data (IA)	Bare ground studies. Glyphosate and AMPA were found predominantly in the 0 to 6 inch layers	42607501; 42765001	
Aquatic Field Dissipation	7.5 days	In a farm pond in Missouri. At 3 sites (OR, GA, MI), half-lives could not be calculated due to recharging events.	40881601	
	Water: Dissipated rapidly immediately after treatment. Sediment: Glyphosate remained in pond sediments at ≥ 1 ppm at 1 year post treatment.	In ponds in Michigan and Oregon and a stream in Georgia Accumulation was higher in the pond than in the stream sediments	41552801.	
Forestry Dissipation	Foliage: < 1 day Ecosystem: Glyphosate: 100 d AMPA: 118 d	3.75 lb ae/A, aerial application	41552801.	

[1] Major degradates are defined as those which reach >10% of the applied.

2.4.3 Environmental Transport Mechanisms

The available field and laboratory data indicate that both glyphosate and AMPA adsorb strongly to soil. Soil partitioning coefficients (K_d) measured in batch equilibrium studies ranged from 18 to 1000 mL/g, with corresponding organic carbon partitioning coefficients (K_{oc}) of 3100 to 58000 mL/g_{oc}. The coefficient of variation for K_{oc} is less than the coefficient of variation for K_d, indicating that pesticide binding to the organic matter fraction of the soil explains some of the variability among the adsorption coefficients, and that K_{oc} is therefore the appropriate parameter to use in determining the soil mobility of the compound. Based on measured K_{oc} values, glyphosate is classified as slightly mobile to hardly mobile according to the FAO classification scheme and would not be expected to leach to groundwater or to move to surface water at high levels through dissolved runoff. However, glyphosate does have the potential to contaminate surface water from erosion via spray drift or transport of residues adsorbed to soil particles suspended in runoff, and transport of glyphosate with colloidal matter has been recognized as well.

The potential for volatilization from soil and water is expected to be low due to the low vapor pressure and low Henry's Law constant. Several studies conducted in use locations outside of California demonstrate that both glyphosate and AMPA can be found in rainwater near use locations. In most cases, these detections were found during the spraying season in the vicinity of local use areas and can be attributed to spray drift rather than to volatilization or long range transport (Baker et al., 2006; Quaghebeur et al., 2004). The highest concentrations were found in urban locations. At one site in Belgium that was 5 m from a spraying location in an urban parking lot, glyphosate was detected in rainwater for several months following an application (Quaghebeur et al., 2004). Deposition was measured to be 205 µg a.i./m^2 at one week after spraying and 0.829 µg/m^2 two months after spraying. These data suggest that volatilization of glyphosate from hard surfaces is possible despite its low vapor pressure, but detections at 5 m were low and so unlikely to have spread far or to have had an impact on exposure.

2.4.4 Mechanism of Action

Glyphosate is a foliar, non-selective, systemic herbicide widely used to control weeds in agricultural crops and non-agricultural sites. Glyphosate is a potent and specific inhibitor of the enzyme 5-enolpyruvylshikimate 3-phosphate (ESPS) synthase. This enzyme is the sixth enzyme on the shikimate pathway and it is essential for the biosynthesis of aromatic amino acids and other aromatic compounds in algae, higher plants, bacteria and fungi. Inhibition of this enzyme leads to plant cell death. The shikimate pathway is absent in mammals.

2.4.5 Use Characterization

2.4.5.1 Labeled Use Pattern

Analysis of labeled use information is the critical first step in evaluating the federal action. The current labels for glyphosate represent the FIFRA regulatory action; therefore, labeled use and application rates specified on the label form the basis of this assessment. The assessment of use information is critical to the development of the action area and selection of appropriate modeling scenarios and inputs.

Glyphosate (*N*-(phosphonomethyl)glycine) is a non-selective, systemic herbicide widely used to control weeds in agricultural crops and non-agricultural sites. **Table 2.5** presents a listing of all registered uses for crops grown in California, grouped into categories. In addition to terrestrial food (agricultural crop) uses, this assessment also considers non-agricultural uses such as rights of way, nurseries, Christmas tree plantations, and around buildings and paved areas, as detailed below. Glyphosate also has aquatic uses which allow direct application to water bodies for control of emergent plants.

Table 2.5. Glyphosate Uses Assessed for the CRLF	
Group Name	**Uses represented**
Aquatic uses on emergent plants	
Avocado	
Blueberry; Passion Fruit (Granadilla)	
Citrus	Grapefruit; Lemon; Orange; Tangelos; Tangerine; Kumquat
Cole crops	Broccoli; Cabbage; Cauliflower; Horseradish; Mustard
Corn	Corn- (Field-, Pop-, Sweet- [silage]); Millet – Proso (Broomcorn); Sunflower
Cotton	
Eggplant; Okra; Tomatillo; Tomato	
Fodder	Alfalfa; Clover; Non-grass forage/Fodder/Straw/Hay
Forestry	Christmas Tree Plantations; Conifer release; Forest Nursery Plantings (fir transplant purposes); Forest trees (all or unspecified)
Fruit	Apple; Apricot; Cherry; Fig; Nectarine; Peach; Pear; Pomegranate; Prune
Garlic; Leek	
Grains/Cereal	Barley; Oats; Rye; Safflower; Sorghum (including silage); Triticale; Wheat
Grapes	
Leafy Vegetables	Brussels Sprouts; Chicory; Endive (Escarole);Lettuce; Parsley
Melons	Melons (Cantaloupe, Honeydew, Mango, Musk Melons, Watermelons, Winter Melons [Casaba/Crenshaw/Honeydew/ Persian]), Pumpkins
Non-Crop Uses	Agricultural/Farm structures/Buildings and Equipment; Commercial Storages/Warehouses Premises; Household/Domestic Dwellings Outdoor Premises; Industrial Areas; Non-agricultural Outdoor Buildings/Structures; Path/Patios; Paved Areas (Private roads/Sidewalks); Urban Areas
Nuts	Almond; Pecan; Pistachio;Walnuts (English/Black)
Olive	
Onions	
Ornamentals	Ornamental and/or shade trees, groundcover, herbaceous plants, non-flowering plants, Nursery stock

Table 2.5. Glyphosate Uses Assessed for the CRLF	
Group Name	**Uses represented**
Residential	Ornamental lawns and turf; Recreational areas
Rangeland	Bermudagrass; Pastures; Rangeland
Rights of way	Agricultural rights-of-way/Fence Rows/Hedgerows
Root crops	Potato White/Irish; Rutabaga; Sweet Potato; Turnip (greens); Turnip (root)
Row crops	Artichoke; Artichoke- Jerusalem; Asparagus; Beans; Beets; Carrots (including tops); Celery; Pepper; Peas- Dried Type; Peas
Strawberry	
Sugar beet (including tops), Parsnip	
Turf	Ornamental sod farm (turf), Grasses grown for seed

Table 2.6 presents application rates and methods for the groups of uses considered in this assessment. The reported application rates represent the maximum application rate used in any crop/use site within each group. The information was extracted from existing product labels. When available, the number and frequency of applications were taken from the label. In some cases, the number of applications had to be estimated based on maximum seasonal application rates and maximum single application rates. For these uses, application intervals were assumed to be 14 days. All of the glyphosate application rates are in units of lb acid equivalents (ae)/A, regardless of the source of glyphosate in the end-use product.

Unlike for the active ingredient, labels only provide formulation application rates in terms of volume applied rather than in terms of mass applied, as is required for estimating exposure concentrations. For this assessment, application rates for formulations were back-calculated based on application rates for glyphosate and the fraction of active ingredient in the formulation. To calculate an application rate for the formulated product, the seasonal application rate of glyphosate acid was converted from acid equivalents to active ingredient, and this rate was then divided by the fraction of active ingredient in the formulated product, according to the following equation:

$$\textit{Seasonal application rate}\text{ (lb formulated product/A)} = \frac{[\textit{Seasonal application rate}\text{ (lb ae/A)} \div \textit{acid equivalence ratio}]}{[\textit{fraction of a.i. in formulated product}]}$$

The formulation rates have only been calculated for seasonal applications, and not separated out for single maximum application rates. Additionally, application methods corresponding to formulation application rates have not been extracted from the label. In order to be conservative, when quantitative estimations are necessary, calculations are based on the assumption of aerial application.

The uses considered in this risk assessment represent all currently registered uses according to a review of all current labels. Historical uses, mis-reported uses, and misuse that may have been listed in the California PUR data are not considered part of the federal action and, therefore, are not considered in this assessment.

Table 2.6 Maximum Application Rates Assessed for Glyphosate and Glyphosate Formulations

GROUP NAME	GLYPHOSATE		GLYPHOSATE FORMULATIONS
	Application Method	Max. Single App. Rate * Apps/season [1] (lb ae/A)	Max. Seasonal App. Rate [2] (lb formulation/A)
Aquatic uses on emergent plants	N/A	3.75 * 1	32.9
Avocado	Ground	3.75 * 2	28.2
Blueberry; Passion Fruit (Granadilla)	Aerial	3.85 (*1st app*), 2.3 (*2nd app*) [3]	8.7
Citrus	Ground	3.85; 2.3	28.2
Cole crops	Aerial	3.85; 2.3	23.9
Corn	Aerial	0.75 * 8	25.7
Cotton	Ground	3.75; 2.25	14.1
Eggplant; Okra; Tomatillo; Tomato	Aerial	3.75; 2.35	8.7
Fodder	Ground	3.75 * 2	20.1
Forestry	Aerial	7.95 * 1	32.1
Fruit	Ground	3.84 * 1	26.7
Garlic; Leek	Ground	3.75; 2.25	8.5
Grains/Cereal	Ground	3.75; 2.25	25.7
Grapes	Ground	3.84 * 2	11.5
Leafy Vegetables	Aerial	3.85; 2.3	21.3
Melons	Aerial	3.85; 2.3	8.7
Non-Crop Uses	Ground	7.95 * 1	34.0
Nuts	Ground	3.84 * 2	11.5
Olive	Ground	3.84 * 2	11.5

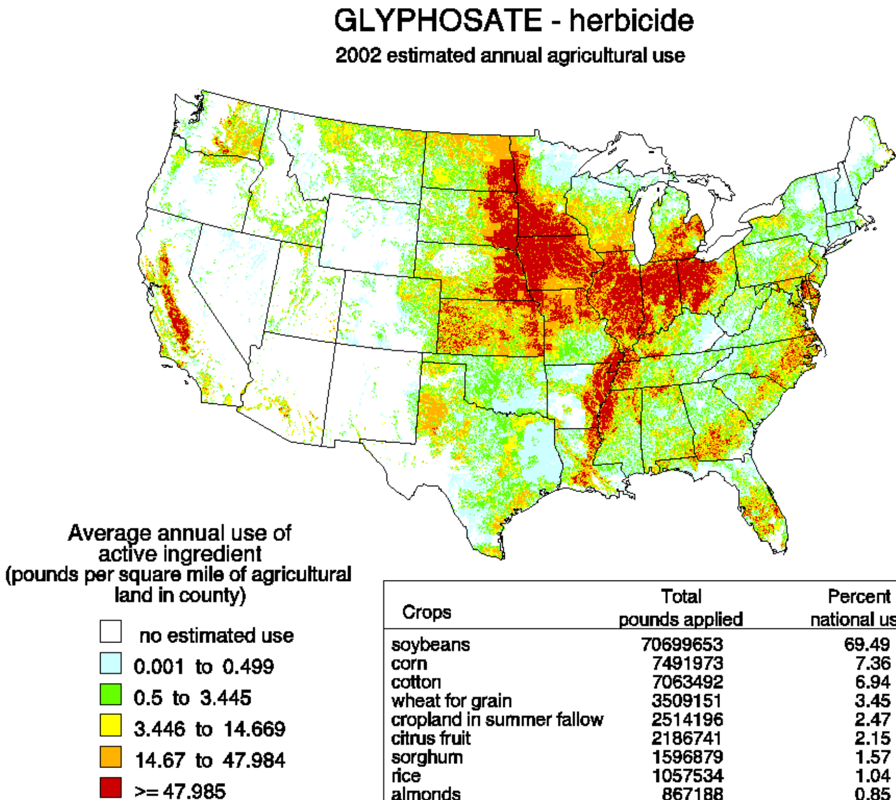
Table 2.6 Maximum Application Rates Assessed for Glyphosate and Glyphosate Formulations			
GROUP NAME	GLYPHOSATE		GLYPHOSATE FORMULATIONS
	Application Method	Max. Single App. Rate * Apps/season [1] (lb ae/A)	Max. Seasonal App. Rate [2] (lb formulation/A)
Onions	Aerial	3.85; 2.3	20.1
Ornamentals	Aerial	3.75 * 2	34.0
Residential	Ground	3.75 * 2	34.0
Rangeland	Aerial	3.75 * 2	34.0
Rights of way	Aerial	7.5 * 1	34.0
Root crops	Aerial	3.85; 2.3	25.7
Row crops	Ground	3.75; 2.25	25.7
Strawberry	Ground	3.75; 2.25	15.1
Sugar beet (including tops), Parsnip	Aerial	3.75; 2.35	18.4
Turf	Aerial	3.75 * 2	34.0

[1] Application intervals are 14 days.
[2] Application rates in lb formulation/A were calculated based on labeled application rates in lbs ae/A, fraction a.i. in the product, and the appropriate acid equivalent ratio for the salt in the active ingredient, as described above.
[3] Throughout table, when two application rates are listed consecutively, they represent different maximum application rates for the first and second single applications, with two applications allowed per season.

2.4.5.2 Use Statistics

As shown in **Figure 2.1**, glyphosate is used on agricultural crops across the country, with the highest usage concentrated in the Upper Midwest and Mississippi River basin. The use of glyphosate on soybeans represents about 70% of the national agricultural use. This map was downloaded from a U.S. Geological Survey (USGS) National Water Quality Assessment Program (NAWQA) website.[1]

[1] http://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=02&map=m1099



Crops	Total pounds applied	Percent national use
soybeans	70699653	69.49
corn	7491973	7.36
cotton	7063492	6.94
wheat for grain	3509151	3.45
cropland in summer fallow	2514196	2.47
citrus fruit	2186741	2.15
sorghum	1596879	1.57
rice	1057534	1.04
almonds	867188	0.85
grapes	711121	0.70

Figure 2.1 Glyphosate Use in Total Pounds per Square Mile

The Agency's Biological and Economic Analysis Division (BEAD) provides an analysis of both national- and county-level usage information (Kaul and Jones, 2006) using state-level usage data obtained from USDA-NASS[2], Doane (www.doane.com; the full dataset is not provided due to its proprietary nature) and the CDPR PUR database[3]. California State law requires that every pesticide application be reported to the state and made available to the public. Therefore, CDPR PUR is considered the most comprehensive source of pesticide usage data for the state and includes both agricultural and non-agricultural sites. It does not include home and garden use, industrial and institutional use, or any other uses by non-professional applicators. The usage data reported for glyphosate by county in this California-specific assessment were generated using CDPR PUR data.

Eight years (1999-2006) of usage data from CDPR PUR were obtained for every glyphosate application made on every use site at the field level. Usage data are available

[2] United States Depart of Agriculture (USDA), National Agricultural Statistics Service (NASS) Chemical Use Reports provide summary pesticide usage statistics for select agricultural use sites by chemical, crop and state. See http://www.usda.gov/nass/pubs/estindx1.htm#agchem.
[3] The California Department of Pesticide Regulation's Pesticide Use Reporting database provides a census of pesticide applications in the state. See http://www.cdpr.ca.gov/docs/pur/purmain.htm.

for glyphosate and several salts, including glyphosate-diammonium salt, glyphosate-isopropylamine salt, glyphosate-monoammonium salt, glyphosate-potassium salt, and glyphosate-trimesium. Total annual pounds applied and total annual area treated are calculated at the county level by site and pesticide active ingredient. Pesticide usage was also aggregated across all observations for eight years for each chemical-county-unit treated combination. Because pesticide applications are made in different area units, the units of area treated are provided where available. Years in which there is no reported use in a county are included as zeros in the calculation of the eight-year averages for pounds and area treated. Averages reflect years without use.

Between 1999 and 2006, glyphosate was reportedly used in all 58 counties in California. According to available information, the total amount of glyphosate active ingredients applied in California increased from about 4.4 million pounds (a.i.) in 1999 to about 7.8 million pounds (a.i.) in 2006 (CDPR PUR) (**Table 2.7**). The counties with the highest and lowest average total pounds from 1999-2006 were Fresno (56,868.9 lb a.i./year) and Alpine (6.2 lb a.i./year), respectively (**Table 2.8**). Glyphosate has a number of residential and industrial uses that are not represented in these data.

Table 2.7. Total Amount of Glyphosate Active Ingredients (lbs a.i.) Applied in California from 1999-2006 (Source: CDPR PUR)

Active Ingredient	Total Pounds Applied in California							
	1999	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**
GLYPHOSATE	30	843	55,486	157,014	116,168	113,383	307,172	523,482
GLYPHOSATE, DIAMMONIUM SALT	0	0	46	59,865	127,636	150,813	141,093	101,340
GLYPHOSATE, ISOPROPYLAMINE SALT	4,300,644	4,639,986	4,406,668	5,027,361	5,618,418	5,803,284	4,590,548	4,781,541
GLYPHOSATE, MONOAMMONIUM SALT	28,298	5,608	1,211	1,173	199,208	151,703	81,283	86,388
GLYPHOSATE, POTASSIUM SALT	0	0	0	0	79	95,034	1,861,410	2,247,232
GLYPHOSATE-TRIMESIUM	91,772	194,849	146,562	146,941	58,913	48,520	25,502	13,384
TOTAL	**4,420,744**	**4,841,286**	**4,609,973**	**5,392,354**	**6,120,422**	**6,362,738**	**7,007,008**	**7,753,367**

Table 2.8. Summary of County-Level Glyphosate Usage Information For California From 1999 to 2006 (Source: CDPR PUR)					
County	**AVG Annual Pounds Applied**	**AVG Application Rate**	**95 Percentile Application Rate**	**99 Percentile Application Rate**	**AVG MAX Application Rate**
ALAMEDA	5145.5	1.3	3.2	4.8	10.9
ALPINE	6.2	2.9	2.9	2.9	2.9
AMADOR	774.2	1.1	2.3	5.3	6.4
BUTTE	10777.8	0.9	1.7	2.8	21.4
CALAVERAS	766.3	1.7	4.5	5.1	9.0
COLUSA	6626.9	0.9	1.8	2.8	8.2
CONTRA COSTA	5930.4	1.0	2.1	3.2	7.7
DEL NORTE	229.9	1.2	1.5	1.7	1.8
EL DORADO	1319.5	1.0	2.2	10.5	21.2
FRESNO	56868.9	0.9	2.0	4.4	37.1
GLENN	9324.1	1.3	2.4	2.8	6.5
HUMBOLDT	566.1	0.5	1.2	2.1	4.8
IMPERIAL	15930.4	1.4	2.1	3.0	13.0
INYO	202.0	1.0	2.4	2.4	2.4
KERN	37356.2	1.2	2.2	3.3	57.5
KINGS	15437.9	0.9	1.7	2.3	26.3
LAKE	1288.9	3.8	7.2	7.6	12.9
LASSEN	505.6	2.4	3.3	4.0	5.3
LOS ANGELES	16188.7	2.5	5.3	6.8	26.1
MADERA	22289.5	1.0	2.2	4.5	15.2
MARIN	525.7	1.5	3.0	4.5	7.0
MARIPOSA	763.7	1.9	5.4	6.1	30.0
MENDOCINO	1741.1	1.2	2.6	3.5	7.0
MERCED	22682.8	2.1	5.2	8.0	25.4
MODOC	575.8	1.0	2.0	3.0	5.0
MONO	152.5	1.5	4.0	4.0	4.0
MONTEREY	7519.7	1.8	2.8	3.8	17.9
NAPA	3511.6	1.3	2.7	4.1	18.0
NEVADA	728.9	0.7	1.6	3.1	9.7
ORANGE	6976.5	2.2	5.7	6.9	9.6
PLACER	1198.4	1.9	5.6	7.9	11.2
PLUMAS	313.3	1.4	2.2	2.8	3.9
RIVERSIDE	11039.4	3.3	12.3	28.7	36.2
SACRAMENTO	7904.4	1.3	4.0	4.9	10.5
SAN BENITO	1435.3	4.1	6.0	8.5	13.0
SAN BERNARDINO	3419.1	1.4	2.8	3.9	8.7
SAN DIEGO	6801.2	0.6	1.8	4.0	26.3
SAN FRANCISCO	591.2	N/A	N/A	N/A	N/A
SAN JOAQUIN	16478.4	0.8	1.9	4.3	29.2

Table 2.8. Summary of County-Level Glyphosate Usage Information For California From 1999 to 2006 (Source: CDPR PUR)					
County	**AVG Annual Pounds Applied**	**AVG Application Rate**	**95 Percentile Application Rate**	**99 Percentile Application Rate**	**AVG MAX Application Rate**
SAN LUIS OBISPO	4267.4	2.2	4.6	8.9	15.8
SAN MATEO	1223.4	3.2	8.3	11.9	23.4
SANTA BARBARA	5757.9	2.1	8.7	12.3	19.8
SANTA CLARA	8142.1	1.0	2.1	4.6	13.8
SANTA CRUZ	621.1	3.2	9.2	15.3	19.0
SHASTA	2085.4	1.5	3.0	3.9	8.4
SIERRA	188.3	1.6	4.4	6.7	6.7
SISKIYOU	860.1	0.9	2.2	3.5	7.3
SOLANO	4389.3	0.8	2.1	3.2	10.6
SONOMA	5286.9	1.0	2.3	2.9	9.3
STANISLAUS	16380.9	2.4	4.1	5.5	18.3
SUTTER	5268.7	1.2	2.0	3.3	7.8
TEHAMA	3271.7	2.8	7.3	7.5	11.5
TRINITY	1097.2	0.7	2.0	2.8	10.3
TULARE	37981.1	1.7	7.9	10.1	25.4
TUOLUMNE	2714.2	2.1	2.8	3.2	7.3
VENTURA	9031.7	1.6	3.2	8.1	37.3
YOLO	6917.5	0.7	1.4	2.1	6.3
YUBA	1668.7	1.1	2.1	2.8	5.4

Table 2.9 summarizes the five highest uses for each active ingredient in California in 2006. The highest use was a non-agricultural use in Santa Clara county; about 460,000 pounds of glyphosate isopropylamine was used for landscape maintenance. For agricultural crops in California, glyphosate was most heavily used on oranges, with about 182,000 pounds of glyphosate isopropylamine used in Tulare county. The next highest usage in an agricultural setting was on tree nuts (almonds, pistachios), cotton, corn, nectarines, and peaches.

Table 2.9. Top 5 Uses For Glyphosate and Its Salts in California in 2006.				
Active Ingredient	**County**	**Site Name**	**Total Pounds 2006**	**Total Area 2006 (acres)**
GLYPHOSATE	KERN	ALMOND	92,655	114,828
	FRESNO	ALMOND	51,378	45,125
	KERN	PISTACHIO	34,452	46,226
	MERCED	ALMOND	32,093	32,964
	KINGS	RIGHTS OF WAY	26,884	N/A
		Total	**237,462**	
GLYPHOSATE, DIAMMONIUM SALT	KERN	ALMOND	31,775	58,739
	COLUSA	ALMOND	9,893	16,950
	FRESNO	ALMOND	9,550	11,035
	MERCED	ALMOND	5,149	3,667
	COLUSA	TOMATO, PROCESSING	4,204	5,536
		Total	**60,570**	
GLYPHOSATE, ISOPROPYLAMINE SALT	SANTA CLARA	LANDSCAPE MAINTENANCE	460,113	N/A
	LOS ANGELES	LANDSCAPE MAINTENANCE	141,647	N/A
	LOS ANGELES	RIGHTS OF WAY	135,505	N/A
	TULARE	ORANGE	114,639	117,980
	IMPERIAL	RIGHTS OF WAY	105,572	N/A
		Total	**957,477**	
GLYPHOSATE, MONOAMMONIUM SALT	LOS ANGELES	LANDSCAPE MAINTENANCE	9,882	N/A
	LOS ANGELES	RIGHTS OF WAY	6,328	N/A
	SAN JOAQUIN	RIGHTS OF WAY	5,168	N/A
	SANTA CLARA	RIGHTS OF WAY	4,719	N/A
	SANTA CLARA	LANDSCAPE MAINTENANCE	4,419	N/A
		Total	**30,517**	
GLYPHOSATE, POTASSIUM SALT	KERN	ALMOND	181,668	164,038
	FRESNO	ALMOND	95,304	70,535
	FRESNO	COTTON	73,668	68,945
	KINGS	COTTON	58,394	62,818
	TULARE	CORN (FORAGE - FODDER)	65,124	57,999
		Total	**474,159**	
GLYPHOSATE-TRIMESIUM	FRESNO	NECTARINE	2,179	923
	SAN JOAQUIN	SOIL FUMIGATION/PREPLANT	2,067	1,933
	GLENN	ALMOND	2,052	817
	FRESNO	PEACH	1,003	368
	SUTTER	UNCULTIVATED AG	849	881
		Total	**8,150**	

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 1**.

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 2.2**). Recovery units, core areas, and other known occurrences of the CRLF from the CNDDB are described in further detail in this section, 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.

Recovery Units

Eight recovery units have been established by USFWS for the CRLF. These areas are considered essential to the recovery of the species, and the status of the CRLF "may be considered within the smaller scale of the recovery units, as opposed to the statewide range" (USFWS 2002). Recovery units reflect areas with similar conservation needs and population statuses, and therefore, similar recovery goals. The eight units described for the CRLF are delineated by watershed boundaries defined by US Geological Survey (USGS) hydrologic units and are limited to the elevational maximum for the species of 1,500 m above sea level. The eight recovery units for the CRLF are listed in **Table 2.10** and shown in **Figure 2.2**.

Core Areas

USFWS has designated 35 core areas across the eight recovery units to focus their recovery efforts for the CRLF (see **Figure 2.2**). **Table 2.10** summarizes the geographical relationship among recovery units, core areas, and designated critical habitat. The core areas, which are distributed throughout portions of the historic and current range of the species, represent areas that allow for long-term viability of existing populations and reestablishment of populations within their historic range. These areas were selected because they: 1) contain existing viable populations; or 2) they contribute to the connectivity of other habitat areas (USFWS 2002). Core area protection and enhancement are vital for maintenance and expansion of the CRLF's distribution and population throughout its range.

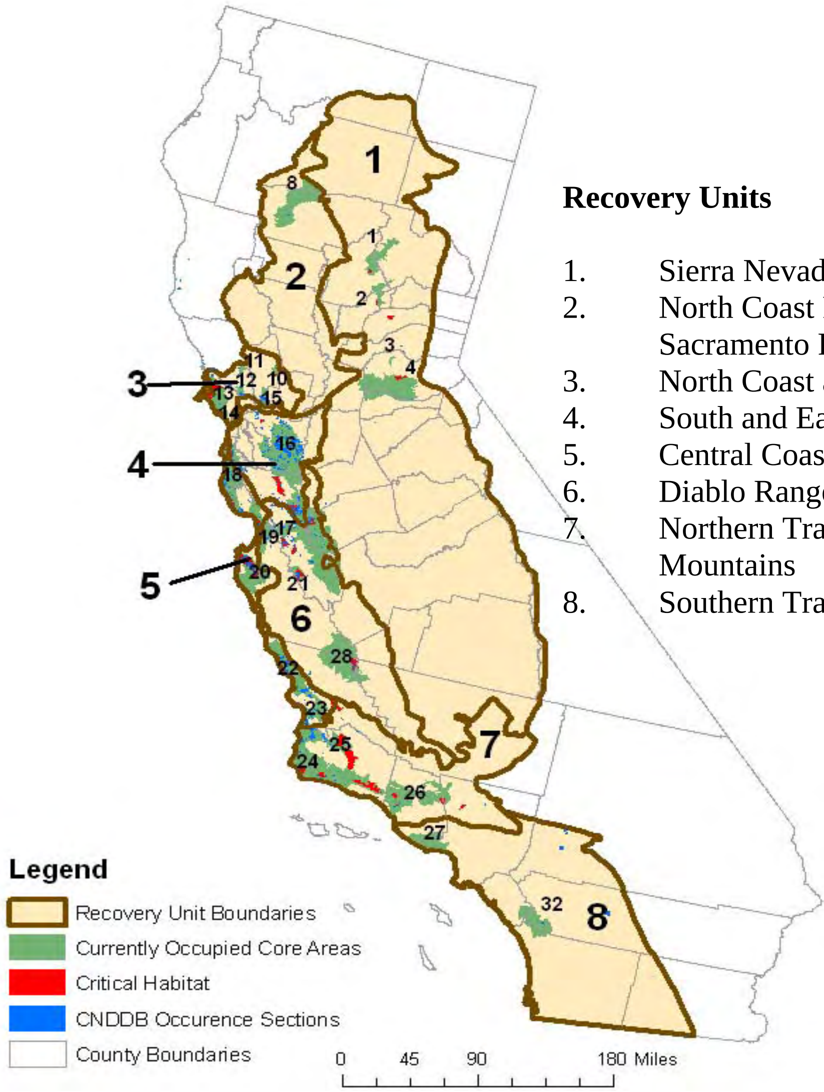
For purposes of this assessment, designated critical habitat, currently occupied (post-1985) core areas, and additional known occurrences of the CRLF from the CNDDB are considered. Historically occupied sections of the core areas are not evaluated as part of this assessment because the USFWS Recovery Plan (USFWS 2002) indicates that CRLFs are extirpated from these areas. A summary of currently and historically occupied core areas is provided in **Table 2.10** (currently occupied core areas are bolded). While core areas are considered essential for recovery of the CRLF, core areas are not federally-designated critical habitat, although designated critical habitat is generally contained within these core recovery areas. It should be noted, however, that several critical habitat units are located outside of the core areas, but within the recovery units. The focus of this assessment is currently occupied core areas, designated critical habitat, and other known CNDDB CRLF occurrences within the recovery units. Federally-designated critical habitat for the CRLF is further explained in Section 2.6.

Table 2.10 California Red-legged Frog Recovery Units with Overlapping Core Areas and Designated Critical Habitat

Recovery Unit [1] (Figure 2.a)	Core Areas [2,7] (Figure 2.a)	Critical Habitat Units [3]	Currently Occupied (post-1985) [4]	Historically Occupied [4]
Sierra Nevada Foothills and Central Valley (1) (eastern boundary is the 1,500m elevation line)	**Cottonwood Creek (partial) (8)**	--	✓	
	Feather River (1)	BUT-1A-B	✓	
	Yuba River-S. Fork Feather River (2)	YUB-1	✓	
	--	NEV-1[6]		
	Traverse Creek/Middle Fork American River/Rubicon (3)	--	✓	
	Consumnes River (4)	ELD-1	✓	
	S. Fork Calaveras River (5)	--		✓
	Tuolumne River (6)	--		✓
	Piney Creek (7)	--		✓
	East San Francisco Bay (partial)(16)	--	✓	
North Coast Range Foothills and Western Sacramento River Valley (2)	**Cottonwood Creek (8)**	--	✓	
	Putah Creek-Cache Creek (9)	--		✓
	Jameson Canyon – Lower Napa Valley (partial) (15)	--	✓	
	Belvedere Lagoon (partial) (14)	--	✓	
	Pt. Reyes Peninsula (partial) (13)	--	✓	
North Coast and North San Francisco Bay (3)	Putah Creek-Cache Creek (partial) (9)	--		✓
	Lake Berryessa Tributaries (10)	NAP-1	✓	
	Upper Sonoma Creek (11)	--	✓	
	Petaluma Creek-Sonoma Creek (12)	--	✓	
	Pt. Reyes Peninsula (13)	MRN-1, MRN-2	✓	
	Belvedere Lagoon (14)	--	✓	
	Jameson Canyon-Lower Napa River (15)	SOL-1	✓	
South and East San Francisco Bay (4)	--	CCS-1A[6]		
	East San Francisco Bay (partial) (16)	ALA-1A, ALA-1B, STC-1B	✓	
	--	STC-1A[6]		
	South San Francisco Bay (partial) (18)	SNM-1A	✓	
Central Coast (5)	**South San Francisco Bay (partial) (18)**	SNM-1A, SNM-2C, SCZ-1	✓	
	Watsonville Slough- Elkhorn Slough (partial) (19)	SCZ-2 [5]	✓	
	Carmel River-Santa Lucia (20)	MNT-2	✓	

Table 2.10 California Red-legged Frog Recovery Units with Overlapping Core Areas and Designated Critical Habitat				
Recovery Unit [1] (Figure 2.a)	**Core Areas [2,7] (Figure 2.a)**	**Critical Habitat Units [3]**	**Currently Occupied (post-1985) [4]**	**Historically Occupied [4]**
	Estero Bay (22)	--	✓	
	--	SLO-8[6]		
	Arroyo Grande Creek (23)	--	✓	
	Santa Maria River-Santa Ynez River (24)	--	✓	
Diablo Range and Salinas Valley (6)	**East San Francisco Bay (partial) (16)**	MER-1A-B, STC-1B	✓	
	--	SNB-1[6], SNB-2[6]		
	Santa Clara Valley (17)	--	✓	
	Watsonville Slough- Elkhorn Slough (partial)(19)	MNT-1	✓	
	Carmel River-Santa Lucia (partial)(20)	--	✓	
	Gablan Range (21)	SNB-3	✓	
	Estrella River (28)	SLO-1A-B	✓	
Northern Transverse Ranges and Tehachapi Mountains (7)	--	SLO-8[6]		
	Santa Maria River-Santa Ynez River (24)	STB-4, STB-5, STB-7	✓	
	Sisquoc River (25)	STB-1, STB-3	✓	
	Ventura River-Santa Clara River (26)	VEN-1, VEN-2, VEN-3	✓	
	--	LOS-1[6]		
Southern Transverse and Peninsular Ranges (8)	**Santa Monica Bay-Ventura Coastal Streams (27)**	--	✓	
	San Gabriel Mountain (29)	--		✓
	Forks of the Mojave (30)	--		✓
	Santa Ana Mountain (31)	--		✓
	Santa Rosa Plateau (32)	--	✓	
	San Luis Rey (33)	--		✓
	Sweetwater (34)	--		✓
	Laguna Mountain (35)	--		✓

[1] Recovery units designated by the USFWS (USFWS 2000, pg 49).
[2] Core areas designated by the USFWS (USFWS 2000, pg 51).
[3] Critical habitat units designated by the USFWS on April 13, 2006 (USFWS 2006, 71 FR 19244-19346).
[4] Currently occupied (post-1985) and historically occupied core areas as designated by the USFWS (USFWS 2002, pg 54).
[5] Critical habitat unit where identified threats specifically included pesticides or agricultural runoff (USFWS 2002).
[6] Critical habitat units that are outside of core areas, but within recovery units.
[7] Currently occupied core areas that are included in this effects determination are bolded.



Recovery Units

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

Figure 2.2 Recovery Unit, Core Area, Critical Habitat, and Occurrence Designations for CRLF

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

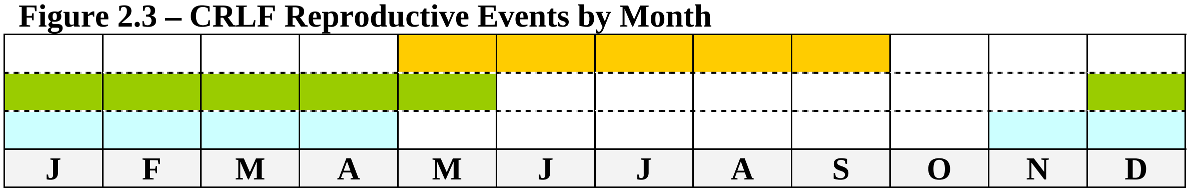
Other Known Occurrences from the CNDBB

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.

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 2.3** depicts CRLF annual reproductive timing.

Figure 2.3 – CRLF Reproductive Events by Month



Light Blue = **Breeding/Egg Masses**
Green = **Tadpoles (except those that over-winter)**
Orange = **Young Juveniles**
Adults and juveniles can be present all year

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 (USFWS 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 Table 2.10.

'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 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 1**.

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 1** 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 glyphosate 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) 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.
(3) 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.
(4) Elimination of upland foraging and/or aestivating habitat or dispersal habitat.
(5) Introduction, spread, or augmentation of non-native aquatic species in stream segments or ponds used by the CRLF.
(6) 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 glyphosate is expected to directly impact living organisms within the action area, critical habitat analysis for glyphosate 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 glyphosate is likely to encompass considerable portions of the United States based on the large array of agricultural uses. However, the scope of this assessment limits consideration of the overall action area to those portions that may be applicable to the protection of the CRLF and its designated critical habitat within the state of California. The Agency's approach to defining the action area under the provisions of the Overview Document (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 glyphosate may be expected to have on the environment, the exposure levels to glyphosate that are associated with those effects, and the best available information concerning the use of glyphosate 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 glyphosate. An analysis of labeled uses and review of available product labels was completed. Several of the currently labeled uses are classified as special local needs (SLNs) or are restricted to specific states and are consequently excluded from this assessment. In addition, a distinction has been made between food use crops and those that are non-food/non-agricultural uses. For those uses relevant to the CRLF, the analysis indicates that, for glyphosate, the following agricultural uses are considered as part of the federal action evaluated in this assessment:

- alfalfa, clover, non-grass forage/fodder/straw/hay, almond, pecan, pistachio, walnuts (english/black), avocado, grapefruit, lemon, orange, tangelos, tangerine, kumquat, broccoli, cabbage, cauliflower, horseradish, mustard, corn- field, corn-pop, corn- sweet corn, (silage), corn (unspecified), millet – proso (broomcorn), sunflower, cotton, cotton (unspecified), apple, apricot, cherry, fig, nectarine, peach, pear, pomegranate, prune, garlic, leek, grapes, brussels sprouts, chicory, endive (escarole), lettuce, parsley, melons, melons- cantaloupe, melons-honeydew, melons- mango, melons- musk, melons- water, melons- winter, casaba/crenshaw/honeydew/persian), pumpkins, olive, onions, potato white/irish, rutabaga sweet potato, turnip (greens), turnip (root), artichoke, artichoke-Jerusalem, asparagus, beans, beets, carrots (including tops), celery, pepper, peas-dried type, peas, strawberry, sugar beet, sugar beet (including tops), parsnip, eggplant, okra, tomatillo, tomato, barley, oats, rye, safflower, sorghum, sorghum (silage), sorghum (unspecified), triticale, wheat, blueberry and passion fruit (granadilla).

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

- Christmas tree plantations, conifer release, forest nursery plantings (fir transplant purposes), forest trees (all or unspecified), emergent aquatic plants, agricultural/farm structures/buildings and equipment, commercial storages/warehouses premises, household/domestic dwellings outdoor premises, industrial areas, non-agricultural outdoor buildings/structures, path/patios, paved areas (private roads/sidewalks), urban areas, ornamental and/or shade trees, ground cover, herbaceous plants, non-flowering plants, nursery stock, bermudagrass, pastures, rangeland, ornamental lawns and turf, recreational areas, agricultural rights-of-way/fence rows/hedgerows, ornamental sod farm (turf), grasses grown for seed and aquatic weed control.

Following a determination of the assessed uses, an evaluation of the potential “footprint” of glyphosate use patterns (i.e., the area where pesticide application occurs) is determined. This “footprint” represents the initial area of concern, based on an analysis of available land cover data for the state of California. The initial area of concern is defined as all land cover types and the stream reaches within the land cover areas that represent the labeled uses described above. Based on glyphosate use patterns, the entire state of California is considered to be the initial area of concern.

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

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

Due to the lack of a defined no effect concentration in a subchronic freshwater fish study from the open literature (Jiraungkoorskul et. al., 2003), the spatial extent of the action area (i.e., the boundary where exposures and potential effects are less than the Agency's LOC) for glyphosate cannot be determined. Therefore, it is assumed that the action area encompasses the entire state of California, regardless of the spatial extent (i.e., initial area of concern or footprint) of the pesticide use(s).

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."[4] 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 glyphosate (*e.g.,* runoff, spray drift, etc.), and the routes by which ecological receptors are exposed to glyphosate *(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 to 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.

A complete discussion of all the toxicity data available for this risk assessment, including resulting measures of ecological effect selected for each taxonomic group of concern, is included in Section 4 of this document. A summary of the assessment endpoints and

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

measures of ecological effect selected to characterize potential assessed direct and indirect CRLF risks associated with exposure to glyphosate is provided in **Table 2.11**.

Table 2.11 Assessment Endpoints and Measures of Ecological Effects	
Assessment Endpoint	**Measures of Ecological Effects**[5]
Aquatic-Phase CRLF *(Eggs, larvae, juveniles, and adults)*[a]	
Direct Effects	
1. Survival, growth, and reproduction of CRLF	1a. Amphibian acute LC_{50} (ECOTOX) or most sensitive fish acute LC_{50} (guideline or ECOTOX) if no suitable amphibian data are available: bluegill sunfish 96-hr LC_{50}: 43 mg a.e./L. Formulations: fish acute LC_{50} terrestrial uses: 3.17 mg/L and aquatic uses: 824 mg/L 1b. Amphibian chronic NOAEC (ECOTOX) or most sensitive fish chronic NOAEC (guideline or ECOTOX): chronic study with leopard frog NOAEC/LOAEC: 1.8/>1.8 mg a.e./L. Formulations: chronic study with leopard frog LOAEC: 1.9 mg formulation/L terrestrial uses; Study not available for aquatic uses. 1c. Amphibian early-life stage data (ECOTOX) or most sensitive fish early-life stage NOAEC (guideline or ECOTOX): study not available
Indirect Effects and Critical Habitat Effects	
2. Survival, growth, and reproduction of CRLF individuals via indirect effects on aquatic prey food supply (*i.e.*, fish, freshwater invertebrates, non-vascular plants)	2a. Most sensitive fish, aquatic invertebrate, and aquatic plant EC_{50} or LC_{50} (guideline or ECOTOX): bluegill sunfish 96-hr LC_{50}: 43 mg a.e./L; water flea 48-hr EC_{50}: 53.2 mg a.e./L; green algae 96-hr EC_{50}: 12.1 mg a.e./L. For formulations: freshwater fish terrestrial uses 3.17 mg/L and aquatic uses: 824 mg/L; freshwater invertebrates terrestrial uses 3 mg/L and aquatic uses 164.3 mg/L; non-vascular plants: EC_{50}: 0.39 mg/L (terrestrial and aquatic uses) 2b. Most sensitive aquatic invertebrate and fish chronic NOAEC (guideline or ECOTOX): Life cycle study with fathead minnow NOAEC/LOAEC: 25.7/>25.7 mg a.e./L; water flea chronic NOAEC: 49.9 mg a.e./L. For formulations: studies not available.
3. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat, cover, food supply, and/or primary productivity (*i.e.*, aquatic plant community)	3a. Vascular plant acute EC_{50} (duckweed guideline test or ECOTOX vascular plant): duckweed growth inhibition EC_{50}: 11.9 mg a.e./L. For formulations, terrestrial uses 2 mg/L and aquatic uses 25 mg/L. 3b. Non-vascular plant acute EC_{50} (freshwater algae or diatom, or ECOTOX non-vascular): green algae 96-hr EC_{50}: 12.1 mg a.e./L. For formulations, 0.39 mg/L for terrestrial and aquatic uses.
4. Survival, growth, and reproduction of CRLF	4a. Distribution of EC_{25} values for monocots

[5] All registrant-submitted and open literature toxicity data reviewed for this assessment are included in Appendix J.

Table 2.11 Assessment Endpoints and Measures of Ecological Effects	
Assessment Endpoint	**Measures of Ecological Effects**[5]
individuals via effects to riparian vegetation	(seedling emergence, vegetative vigor, or ECOTOX): EC_{25} seedling emergence: >4 to >5 lbs a.e/A; EC_{25} vegetative vigor: 0.16 – 0.98 lbs a.e./A 4b. Distribution of EC_{25} values for dicots (seedling emergence, vegetative vigor, or ECOTOX): EC_{25} seedling emergence: > 4 to > 5 lbs a.e/A; EC_{25} vegetative vigor: 0.074 – 0.89 lbs a.e./A
Terrestrial-Phase CRLF *(Juveniles and adults)*	
Direct Effects	
5. Survival, growth, and reproduction of CRLF individuals via direct effects on terrestrial phase adults and juveniles	5a. Most sensitive bird[b] or terrestrial-phase amphibian acute LC_{50} or LD_{50} (guideline or ECOTOX): bobwhite acute LD_{50}: > 3196.3 mg a.e./kg bw; bobwhite subacute dietary LC_{50}: > 4971.2 ppm a.e. 5b. Most sensitive bird[b] or terrestrial-phase amphibian chronic NOAEC (guideline or ECOTOX): bobwhite quail reproduction NOAEC 830 ppm a.e.
Indirect Effects and Critical Habitat Effects	
6. Survival, growth, and reproduction of CRLF individuals via effects on terrestrial prey (*i.e.,*terrestrial invertebrates, small mammals , and frogs)	6a. Most sensitive terrestrial invertebrate and vertebrate acute EC_{50} or LC_{50} (guideline or ECOTOX)[c]: honey bee acute contact LD_{50} > 100 µg a.i./bee; rat LD_{50}: >4800 mg a.e./kg; bobwhite acute LD_{50}: > 3196.3 mg a.e./kg bw; bobwhite subacute dietary LC_{50}: > 4971.2 ppm a.e. 6b. Most sensitive terrestrial invertebrate and vertebrate chronic NOAEC (guideline or ECOTOX): No chronic terrestrial invertebrate study available; bobwhite quail reproduction NOAEC 830 ppm a.e.; rat reproduction study NOAEL: 500 mg a.e./kg bw/day, NOAEC: 10000 ppm
7. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat (*i.e.*, riparian and upland vegetation)	7a. Distribution of EC_{25} values for monocots (seedling emergence, vegetative vigor, or ECOTOX): EC_{25} seedling emergence: >4 - >5 lbs a.e/A; EC_{25} vegetative vigor: 0.16 – 0.98 lbs a.e./A 4b. Distribution of EC_{25} values for dicots (seedling emergence, vegetative vigor, or ECOTOX): EC_{25} seedling emergence: >4 - >5 lbs a.e/A; EC_{25} vegetative vigor: 0.074 – 0.89 lbs a.e./A

[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 glyphosate 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 glyphosate effects data are available.

Adverse modification to the critical habitat of the CRLF includes, but is not limited to, the following, as specified by USFWS (2006):

1. Alteration of water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs.
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.
4. Significant alteration of channel/pond morphology or geometry.
5. Elimination of upland foraging and/or aestivating habitat, as well as 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.

Measures of such possible effects by labeled use of glyphosate on critical habitat of the CRLF are described in **Table 2.12**. 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 2.12 Summary of Assessment Endpoints and Measures of Ecological Effect for Primary Constituent Elements of Designated Critical Habitat	
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.	a. Most sensitive aquatic plant EC_{50} (guideline or ECOTOX): duckweed growth inhibition EC_{50}: 11.9 mg a.e./L. For formulations, 0.39 mg/L (freshwater diatom) for both terrestrial and aquatic uses. b. Distribution of EC_{25} values for terrestrial monocots (seedling emergence, vegetative vigor, or ECOTOX): EC_{25} seedling emergence: >4 - >5 lbs a.e/A; EC_{25} vegetative vigor: 0.16 – 0.98 lbs a.e./A c. Distribution of EC_{25} values for terrestrial dicots (seedling emergence, vegetative vigor, or ECOTOX): EC_{25} seedling emergence: >4 - >5 lbs a.e/A; EC_{25} vegetative vigor: 0.074 – 0.89 lbs a.e./A
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.	a. Most sensitive EC_{50} values for aquatic plants (guideline or ECOTOX): duckweed growth inhibition EC_{50}: 11.9 mg a.e./L. For formulations: freshwater diatom 96-hr EC_{50}: 0.39 mg/L b. Distribution of EC_{25} values for terrestrial monocots (seedling emergence or vegetative vigor, or ECOTOX): EC_{25} seedling emergence: >4 - >5 lbs a.e/A; EC_{25} vegetative vigor: 0.16 – 0.98 lbs a.e./A c. Distribution of EC_{25} values for terrestrial dicots (seedling emergence, vegetative vigor, or ECOTOX): EC_{25} seedling emergence: >4 - >5 lbs a.e/A; EC_{25} vegetative vigor: 0.074 – 0.89 lbs a.e./A
Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.	a. Most sensitive EC_{50} or LC_{50} values for fish or aquatic-phase amphibians and aquatic invertebrates (guideline or ECOTOX): bluegill sunfish 96-hr LC_{50}: 43 mg a.e./L; water flea 48-hr EC_{50}: 53.2 mg a.e./L. For formulations: freshwater fish: terrestrial uses 3.17 mg/L and aquatic uses: 824 mg/L; freshwater invertebrates terrestrial uses 3 mg/L and aquatic uses 164.3 mg/L b. Most sensitive NOAEC values for fish or aquatic-phase amphibians and aquatic invertebrates (guideline or ECOTOX): chronic study with leopard frog NOAEC/LOAEC: 1.8/>1.8 mg a.e./L.; water flea chronic NOAEC: 49.9 mg a.e./L. For formulations: chronic study with leopard frog LOAEC: 1.9 mg formulation/L terrestrial uses; studies not available for aquatic uses or for aquatic invertebrates.
Reduction and/or modification of aquatic-based food sources for pre-metamorphs (*e.g.,* algae)	a. Most sensitive aquatic plant EC_{50} (guideline or ECOTOX): green algae EC_{50}: 12.1 mg a.e./L. For formulations: freshwater diatom 96-hr EC_{50}: 0.39 mg/L

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	a. Distribution of EC_{25} values for monocots (seedling emergence, vegetative vigor, or ECOTOX): EC_{25} seedling emergence: >4 - > 5 lbs a.e/A; EC_{25} vegetative vigor: 0.16 – 0.98 lbs a.e./A b. Distribution of EC_{25} values for dicots (seedling emergence, vegetative vigor, or ECOTOX): EC_{25} seedling emergence: >4 - >5 lbs a.e/A; EC_{25} vegetative vigor: 0.074 – 0.89 lbs a.e./A c. Most sensitive food source acute EC_{50}/LC_{50} and NOAEC values for terrestrial vertebrates (mammals) and invertebrates, birds or terrestrial-phase amphibians, and freshwater fish: rat LD_{50}: >4800 mg a.e./kg; honey bee acute contact LD_{50} > 100 µg a.i./bee; bobwhite acute LD_{50}: > 3196.3 mg a.e./kg bw; bobwhite subacute dietary LC_{50}: > 4971.2 ppm a.e. and bluegill sunfish 96-hr LC_{50}: 43 mg a.e./L. Chronic NOAEC: rat reproduction study NOAEL: 500 mg a.e./kg bw/day, NOAEC: 10000 ppm; no chronic terrestrial invertebrate study available; bobwhite quail reproduction NOAEC 830 ppm a.e.and life cycle study with fathead minnow NOAEC/LOAEC: 25.7/>25.7 mg a.e./L.
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 glyphosate to the environment. The following risk hypotheses are presumed for this endangered species assessment:

The labeled use of glyphosate 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 glyphosate release mechanisms, biological receptor types, and effects endpoints of potential concern. The conceptual models for aquatic and terrestrial phases of the CRLF are shown in **Figures 2.4 and 2.5**, respectively, and the conceptual models for the aquatic and terrestrial PCE components of critical habitat are shown in **Figures 2.6 and 2.7**, respectively. Exposure routes shown in dashed lines are not quantitatively considered because the contribution of those potential exposure routes to potential risks to the CRLF and modification to designated critical habitat is expected to be negligible.

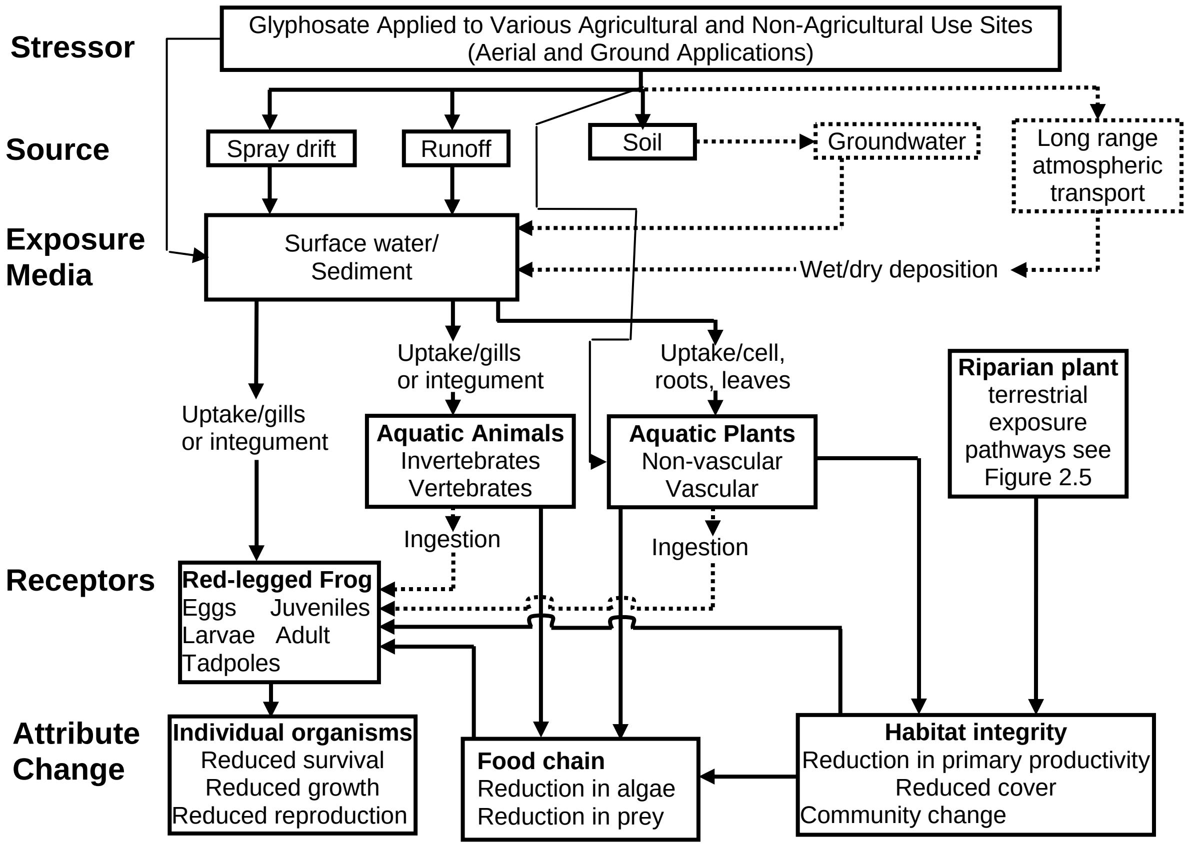


Figure 2.4 Conceptual Model for Aquatic-Phase of the CRLF

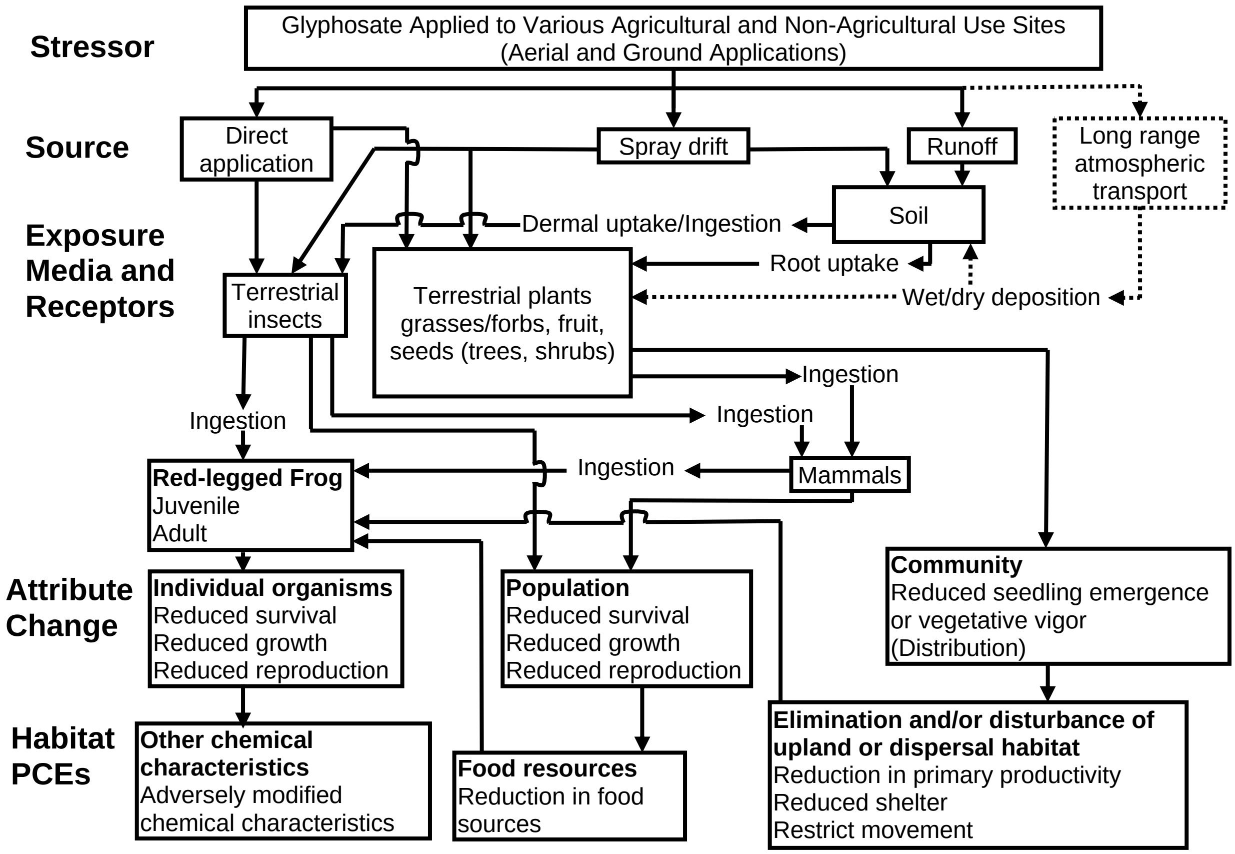


Figure 2.5 Conceptual Model for Terrestrial-Phase of the CRLF

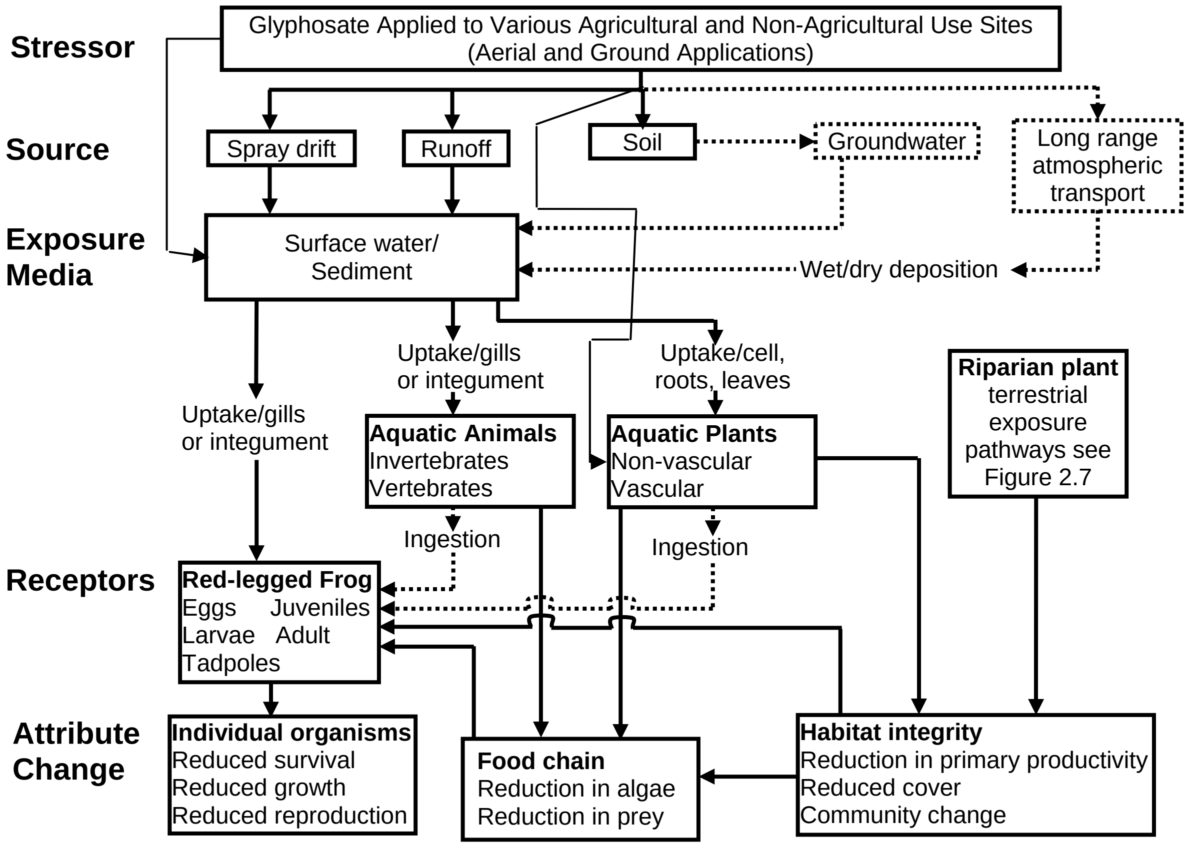


Figure 2.6 Conceptual Model for Pesticide Effects on Aquatic Component of CRLF Critical Habitat

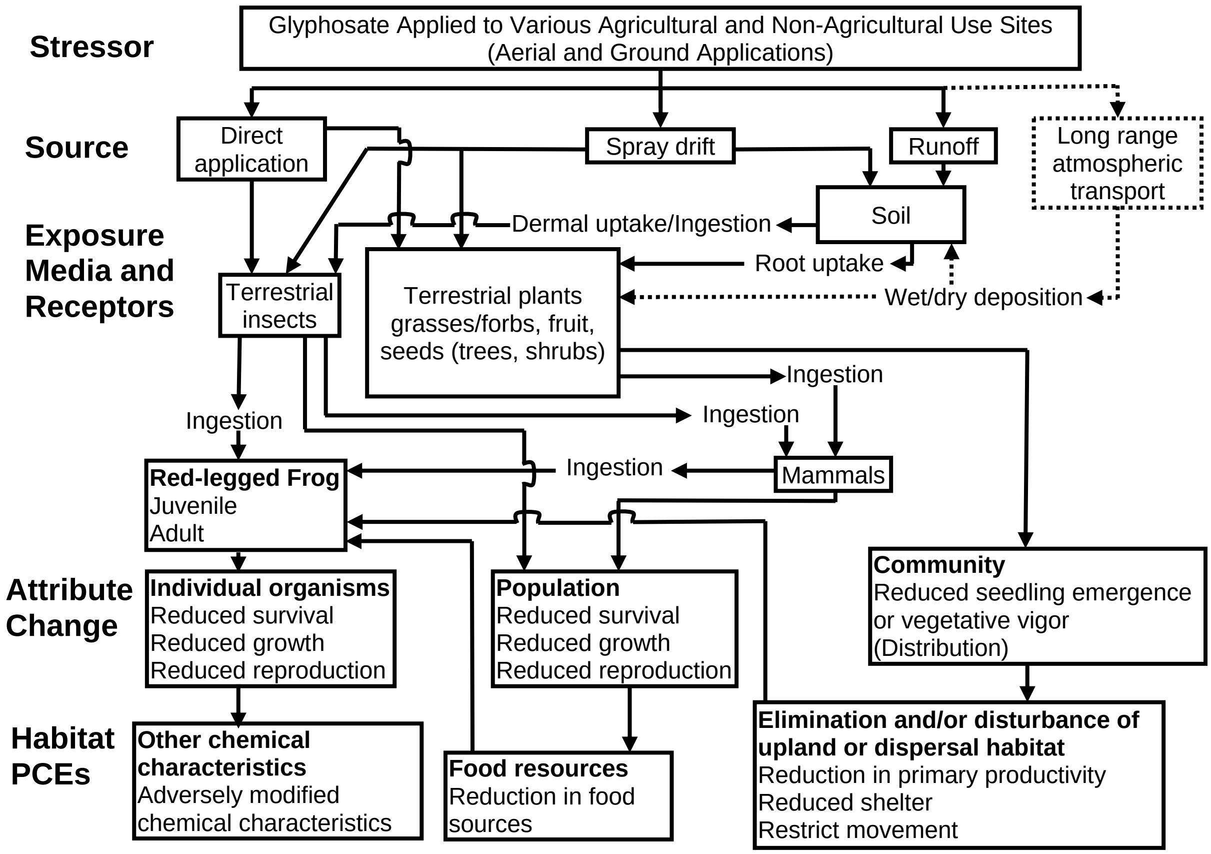


Figure 2.7 Conceptual Model for Pesticide Effects on Terrestrial Component of CRLF Critical Habitat

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 glyphosate 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 RQ-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 glyphosate is estimated using the probit dose-response slope and either the LOC (discussed below) or actual calculated RQ value.

2.10.1 Measures to Evaluate the Risk Hypothesis and Conceptual Model

2.10.1.1 Measures of Exposure

The physical/chemical properties and environmental fate data for glyphosate, along with available monitoring data, indicate that runoff and spray drift are the principal potential transport mechanisms of glyphosate to the aquatic and terrestrial habitats of the CRLF. Based on its low vapor pressure and Henry's Law constant, long range atmospheric transport is not expected to be an important transport mechanism. In this assessment, transport of glyphosate through runoff and spray drift is considered in deriving quantitative estimates of glyphosate 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 glyphosate using maximum labeled application rates and methods of application. For aquatic exposure, a Tier I approach is used unless there are LOC exceedances. The model used to predict aquatic exposure from terrestrial applications of glyphosate is the screening model GENEEC2. The model used to predict aquatic exposure from terrestrial applications of glyphosate formulations and from aquatic applications of both glyphosate and glyphosate formulations, is a simple dilution calculation based on the standard pond scenario. The model used to predict terrestrial EECs on food items is T-REX. The model used to derive EECs relevant to terrestrial and wetland plants is TerrPlant. These models are parameterized using relevant reviewed registrant-submitted environmental fate data in support of glyphosate registration.

Exposure estimates for the aquatic-phase CRLF and for fish and aquatic invertebrates (serving as potential prey) in water bodies exposed to spray drift or runoff from terrestrial applications of glyphosate are derived using the Tier I simulation model GENEEC2 (Version 2.0; August 1, 2001). GENEEC2 uses a standard pond scenario, which assumes application of the active ingredient 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. GENEEC2 considers adsorption of the pesticide to soil or sediment, incorporation of the pesticide at application, direct deposition of spray drift into the water body, and degradation of the pesticide in soil before runoff and within the water body. It is a single event model, meaning that it assumes one single large rainfall/runoff event occurs and removes a large quantity of pesticide at one time from the field to a pond.

Aquatic exposure resulting from terrestrial applications of glyphosate formulations or from aquatic applications of either glyphosate or glyphosate formulations is estimated using a simple dilution calculation based on the standard pond scenario and assuming that the entire applied mass is dispersed evenly in the standard water body. For terrestrial applications of glyphosate formulations, the calculation uses default spray drift parameters to estimate applied mass, and for aquatic applications, the application rate defines the mass applied.

Exposure estimates for the terrestrial-phase CRLF and terrestrial invertebrates and mammals (serving as potential prey) assumed to be in the target area or in an area exposed to spray drift are derived using the T-REX model (version 1.3.1, 12/07/2006). This model incorporates the Kenega nomograph, as modified by Fletcher *et al.* (1994), which is based on a large set of actual field residue data. The upper limit values from the nomograph represented the 95th percentile of residue values from actual field measurements (Hoerger and Kenega, 1972). For modeling purposes, direct exposures of the CRLF to glyphosate 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 glyphosate are bound by using the dietary based EECs for small insects and large insects.

EECs for terrestrial plants inhabiting dry and wetland areas are derived using TerrPlant (version 1.2.2, 12/26/2006). This model uses estimates of pesticides in runoff and in spray drift to calculate EECs. EECs are based upon solubility, application rate and minimum incorporation depth.

The spray drift model, AgDRIFT is used to assess exposures of terrestrial phase CRLF and its prey to glyphosate deposited on terrestrial habitats by spray drift. In addition to the buffered area from the spray drift analysis, the downstream extent of glyphosate 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 the ECOTOXicology database (ECOTOX), a source for locating single chemical toxicity data for aquatic life, terrestrial plants, and wildlife. The database was searched in order to provide more ecological effects data and in an attempt to bridge existing data gaps. 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 glyphosate 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 glyphosate 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 glyphosate risks, the 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 LOCs (USEPA, 2004) (see **Appendix C**).

For this endangered species assessment, listed species LOCs are used for comparing RQ values for acute and chronic exposures of glyphosate directly to the CRLF. If estimated glyphosate exposure to the CRLF resulting from a particular use is 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 glyphosate exposure to CRLF prey resulting from a particular use is sufficient to exceed the listed species LOC, then the effects determination for that use is a “may affect.” If the RQ being considered also exceeds the non-listed species acute risk LOC, then the effects determination is a LAA. If the acute RQ is between the listed species LOC and the non-listed acute risk species LOC, then further lines of evidence (*i.e.*

probability of individual effects, species sensitivity distributions) are considered in distinguishing between a determination of NLAA and a LAA. When considering indirect effects to the CRLF due to effects to algae as dietary items or plants as habitat, the non-listed species LOC for plants is used because the CRLF does not have an obligate relationship with any particular aquatic and/or terrestrial plant. If the RQ being considered for a particular use exceeds the non-listed species LOC for plants, the effects determination is "may affect". Further information on LOCs is provided in **Appendix C**.

2.10.2 Data Gaps

The environmental fate and ecological effects databases for glyphosate are complete for the CRLF assessment. All fate and ecological effects study requirements have been satisified and there are no data gaps.

3. Exposure Assessment

Glyphosate is formulated as a liquid concentrate that can be applied through ground or aerial application. Risks from ground boom and aerial applications are considered in this assessment because they are expected to result in the highest off-target levels of glyphosate due to generally higher spray drift levels. Ground boom and aerial modes of application tend to use lower volumes of application applied in finer sprays than applications coincident with sprayers and spreaders and thus have a higher potential for off-target movement via spray drift.

3.1 Label Application Rates and Intervals

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

Currently registered agricultural uses of glyphosate relevant to CRLF critical habitat in California include, among others, use on row crops, cotton, nuts, melons, citrus, grapes, berries and other fruit, corn, wheat, and potatoes as well as use on turf, ornamentals, and forest trees. There are many non-agricultural uses of glyphosate as well, including application to rights of way and around buildings, structures, and paved areas. Additionally, for some uses, glyphosate is labeled for direct aquatic application. The uses being assessed, both for glyphosate and its formulations, were summarized previously in **Table 2.6**.

3.2 Aquatic Exposure Assessment

3.2.1 Modeling Approach

Aquatic EECs of glyphosate and glyphosate formulations are derived using a Tier I screening level approach. There are a variety of types of uses, including terrestrial and aquatic applications, either as glyphosate or as glyphosate formulations. Each type of use has different fate and exposure issues and so requires different methods to determine EECs, as described below. For all types of uses, only the highest labeled application rates are considered. If estimates using Tier I modeling and high application rates do not exceed LOCs, then further refinement is not required.

For all uses, exposure estimates are generated using the standard pond scenario and 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 Modeling Calculations

3.2.2.1 Direct Aquatic Applications

The highest potential aquatic exposure for glyphosate results from uses which allow application directly to a water body. For both glyphosate and its formulations, peak aquatic exposure from these direct aquatic applications was estimated by calculating simple dilution in the standard pond, which has a volume of 20,000 m^3 and a surface area of 1 ha. In this calculation, an aquatic EEC is determined by dividing the mass of glyphosate applied to the pond by the volume of the pond, representing the peak exposure in a well-mixed water body.

$$\text{EEC } (kg/L) = \frac{[\text{Seasonal application rate } (lb/A) * 1.12\ (kg\ ha^{-1}/lb\ A^{-1}) * 1\ ha/pond]}{[20{,}000{,}000\ L/pond]}$$

Chronic EECs are not estimated because the simple dilution calculation does not account for chemical and environmental fate processes that affect longer term exposure, such as abiotic and biotic degradation, volatilization, and partitioning to sediment. For the same reason, this calculation cannot account for multiple applications and so, in order to be conservative, it is assumed that the maximum seasonal application rate has been applied in a single application. Further refinement of estimates of chronic exposure or of exposure from single applications is not required unless there are LOC exceedances.

As listed in Table 2.6, the maximum seasonal application rates for aquatic uses are 3.75 lb ae/A for glyphosate and 32.9 lb formulation/A for formulations.

3.2.2.2 Terrestrial Applications

Although direct aquatic applications are expected to lead to the highest exposure concentrations, surface water exposures for terrestrial uses of glyphosate and its formulations have also been calculated, for characterization purposes.

For terrestrial appliations of glyphosate, EECs are quantitatively estimated using the Tier I simulation model GENEEC2 (Version 2.0; August 1, 2001), based on the standard pond scenario. The modeled application site is not crop-specific and represents a generic vulnerable site where high concentration levels are expected due to the occurrence of environmental conditions, including weather and soils, known to favor transport to and persistence in surface water. A summary of the GENEEC2 model inputs used in assessing aquatic exposure from terrestrial applications of glyphosate are provided in **Table 3.1**.

Table 3.1. GENEEC2 Inputs for Aquatic EECs from Terrestrial Applications of Glyphosate

Input Parameter	Value	Comment	Source
Application Rate and Method	7.95 lb ae/A; Aerial spray	For forestry, the use with the maximum labeled applicatiom rate.	Product labels
Application Details	Fine to medium droplet size Not wetted in No buffer		EFED Defaults
K_{oc}	3100 mL/g_{oc}	Lowest non-sand value from five soils	MRID 44320646
Solubility in Water	12,000 mg/L		Product Chemistry
Aerobic Soil Metabolism Half-life	5.4 days	90% upper confidence bound on the mean	MRIDs 42372501, 44320645
Hydrolysis at pH 7	0 days	Stable to hydrolysis	MRID 00108192, 44320642
Aquatic Photolyis	0 days	Stable to photolysis	MRID 41689101; 44320643
Aerobic Aquatic Metabolism	21 days	Single value x 3	MRID 41723601

For terrestrial application of formulations, partitioning and degradation properties for each formulation component in runoff suggest that the final proportion of the residues of these components in the receiving surface waters would not represent what was introduced and what was tested in an aquatic organism toxicity study using the formulated product. For this reason, spray drift is assumed to be the only route of aquatic exposure to the formulation as introduced. The mass of mesotrione from terrestrial applications expected to reach the water body through drift was estimated based on the default assumption of 5% drift for aerial spray. The simple dilution method, described in Section 3.2.2.1, is then applied to determine a peak aquatic EEC. Chronic EECs cannot be estimated because there are no fate data available for formulated products to allow for simulation of dissipation processes. For terrestrially applied formulation, the maximum seasonal application rate is 34.0 lb formulation/A, shared by a variety of non-crop uses, including rights-of-way, rangeland, ornamental, non-agricultural, and residential uses.

3.2.3 Results

EECs for terrestrial and aquatic applications of both glyphosate and its formulations are presented in **Table 3.2**. These EECs are based on the maximum labeled use from each category. Glyphosate EECs represent ug ae/L and formulation EECs represent ug formulation/L. GENEEC2 model outputs and simple dilution calculations are included in **Appendix D**.

Table 3.2. Aquatic EECs for Glyphosate and its Formulations

Use Type	Exposure Routes	Model	Peak	21-Day Avg EEC	60-Day Avg EEC
GLYPHOSATE (ug ae/L)					
Terrestrial	Runoff, spray drift	GENEEC2	87.2	69.0	45.8
Aquatic	Direct application	Simple Dilution	210	NA	NA
FORMULATIONS (ug formulation/L)					
Terrestrial	Spray drift	Simple Dilution	95.2	NA	NA
Aquatic	Direct Application	Simple Dilution	1840	NA	NA

3.2.4 Existing Monitoring Data

A critical step in the process of characterizing EECs is comparing the modeled estimates with available surface water monitoring data. Monitoring of glyphosate and/or AMPA (major biotransformation product) is not extensive, mostly because of the lack of appropriate analytical chemistry methods to identify/quantify glyphosate and AMPA prior to 2001, when a method was developed by the USGS with a method reporting limit of 0.1 μg/L for both species.

Included in this assessment are California-specific glyphosate and AMPA monitoring data for both surface and groundwater from the USGS NAWQA program (http://water.usgs.gov/nawqa). Several open literature studies monitoring glyphosate at sites outside of California are discussed here as well because they are targeted to specific use sites and so provide insight into potential off-site transport of glyphosate. The California Department of Pesticide Regulation (CDPR) surface water monitoring database (http://www.cdpr.ca.gov/docs/emon/surfwtr/surfdes.htm) does not include glyphosate or AMPA as analytes and so will not be discussed further.

3.2.4.1 USGS NAWQA Surface Water Data

In California, the NAWQA database includes monitoring for glyphosate and AMPA in surface water at three locations, although this monitoring does not target specific chemicals or uses. At each location, 16 to 19 samples were collected between October 2002 and September 2003, generally every two to four weeks. Results are reported in **Table 3.3**. At a mixed use site in Merced County, glyphosate and AMPA were detected above the reporting limit of 0.1 µg/L at one sampling event (8/07/2003), at levels of 0.18 µg/L and 0.22 µg/L, respectively. At a mixed use site in San Joaquin county, glyphosate was detected four times over the sampling period (0.13 to 0.24 µg/L) but AMPA was detected in every sample (0.12 µg/L to 0.56 µg/L). The glyphosate detections showed no temporal pattern. AMPA showed peaks on 3/11/2003 (0.36 µg/L) and on 8/06/2003 (0.56 µg/L). At the only agricultural site, in Stanislaus county, glyphosate was detected in all but one of the samples and AMPA was detected in all samples. At this site, glyphosate detections were low (≤ 0.2 µg/L) until a peak concentration of 7.5 µg/L was reached on 3/12/03. Concentrations steadily decreased for 6 weeks and then remained ≤1.2 µg/L throughout the rest of the sampling period. AMPA levels were lower, with a maximum detected value of 1.1 µg/L reached on 7/24/03.

Table 3.3. NAWQA Surface Water Sampling Results in California

Site Location	Use Type	# of samples	Glyphosate		AMPA	
			# Detects	**Range** (µg/L)	**# Detects**	**Range** (µg/L)
Merced	Mixed	19	1	0.18	1	0.22
San Joaquin	Mixed	16	4	0.13 – 0.24	16	0.12 – 0.56
Stanislaus	Agriculture	16	15	0.10 – 7.46	16	0.23 – 1.07

3.2.4.2 USGS NAWQA Groundwater Data

In California, the NAWQA program monitored for glyphosate and AMPA in groundwater at 48 wells in 7 counties, although this monitoring does not target specific chemicals or uses. Neither compound was detected, although some sampling had reporting limits higher than 0.1 µg/L (0.15 µg/L for glyphosate and 0.31 µg/L for AMPA). This sampling included 30 sites in primarily agricultural areas in Fresno, Kings, Madera, Merced, San Joaquin, Stanislaus, and Tulare Counties and 18 urban sites in Sacramento County.

3.2.4.3 Additional Studies

A USGS study sampled for glyphosate and AMPA in overland flow and in surface water in the Leary Weber Ditch Basin, Hancock County, Indiana (Baker et al., 2006). The 2.5

mi^2 study basin is primarily agricultural (87%), farmed with corn and soybeans, and flow in the ditch is dominated by tile-drain contributions. Overland flow and surface water samples were collected during two storm events occurring one to two weeks following pesticide application. Glyphosate and AMPA were detected in all overland flow samples (n=12). In the first storm event, glyphosate concentrations in overland flow were approximately 300 to 500 ppb and in the second event, concentrations were approximately 30 to 60 ppb. The median concentration of AMPA in all runoff samples was ~30 ppb. In surface water in the Leary Weber Ditch, glyphosate and AMPA were detected in 13 and 15 of 19 samples, respectively. The maximum glyphosate concentration was ~7 ppb and the median concentration was ~0.2 ppb. The maximum AMPA concentration was ~1 ppb and the median was slightly above the detection limit of 0.1 ppb. (Concentrations were only reported in charts, not numerically, so exact values are not available.)

3.2.4.4 Atmospheric Monitoring Data

Available studies monitoring atmospheric transport in the Central Valley and Sierra Nevada do not include glyphosate as an analyte (http://www.cdpr.ca.gov/docs/empm/pubs/tac/tacstdys.htm; http://www.nature.nps.gov/air/Studies/air_toxics/wacap.cfm). Some monitoring of glyphosate in rainwater has been conducted, but has found only local effects attributed to spray drift, as discussed in Section 2.4.2.

3.2.5 Spray Drift Buffer Analysis

In order to determine terrestrial and aquatic habitats of concern due to glyphosate exposures through spray drift, it is necessary to estimate the distance that spray applications can drift from the treated area and still be present at concentrations that exceed levels of concern. An analysis of spray drift distances was completed using AgDrift Tiers 1 and 3.

Based on glyphosate use patterns, the entire state of California is considered to be the initial area of concern. As stated previously, due to the lack of a defined no effect concentration in a subchronic freshwater fish study from the open literature (Jiraungkoorskul et. al., 2003), the spatial extent of the action area for glyphosate cannot be determined. Therefore, it is assumed that the action area also encompasses the entire state of California. Therefore, buffers can be estimated for a specific use; however, for aggregate uses, the widest buffer for both terrestrial and aquatic uses would be applied and would effectively be the entire state.

The spray drift buffer analysis is presented in the Risk Description, Section 5.2.3.2 under Terrestrial Plants.

3.2.6 Downstream Dilution Analysis

As stated above, for glyphosate, both the initial area of concern and the action area are considered to be the entire state of California. Due to the fact that the glyphosate labels allow for aquatic uses in multiple types of water bodies, multiple applications within a specific watershed may occur within the same time frame. As a result, there is potentially no input of “glyphosate clean" water to dilute existing concentrations of glyphosate downstream because it could be applied in the downstream waterbodies as well. Therefore, no credible watershed dilution can be done. For that reason, a downstream dilution analysis was not conducted.

3.3 Terrestrial Animal Exposure Assessment

T-REX (Version 1.3.1) is used to calculate dietary and dose-based EECs of glyphosate for the CRLF and its potential prey (*e.g.* small mammals and terrestrial insects) inhabiting terrestrial areas. EECs used to represent the CRLF are also used to represent exposure values for frogs serving as potential prey of CRLF adults. T-REX simulates a 1-year time period. For this assessment, spray applications of glyphosate are considered as discussed below.

Terrestrial EECs for foliar formulations of glyphosate were derived for the uses summarized in Table 3.7. A magnitude of residue study for alfalfa (MRID 45646001) provided sufficient data to generate a foliar dissipation half-life for glyphosate. Two half-lifes were generated, 4 and 7 days. The 7 day value was selected as a conservative estimate for use in T-REX. Use-specific input values, including number of applications, application rate and application interval are provided in **Table 3.4**. An example output from T-REX is available in **Appendix E**.

Table 3.4. Input Parameters for Foliar Applications Used to Derive Terrestrial EECs for Glyphosate with T-REX

Use Scenario (Application method)	Application rate (lbs ae/A)	Number of Applications
Forestry and areas with impervious surfaces (aerial)	7.95	1
Alfalfa, avocado, forestry, nursery, rangeland, residential and turf (ground)	3.75	2
Almond, fruit, grape and olive (ground)	3.84	2
Citrus, cole crop, lettuce, melon, onion, potato and wine grape (ground)	3.85 1st application 2.3 2nd application	2
Corn, cotton, garlic, impervious surfaces, row crop, strawberry and wheat (ground)	3.75 1st application 2.25 2nd application	2
Corn (aerial) and wheat (ground)	0.75	8
Rangeland (ground)	1.54	5
Rangeland (aerial)	0.387	20
Right of way (aerial)	7.5	1
Right of way (ground)	3.69	2

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

For modeling purposes, exposures of the CRLF to glyphosate through contaminated food are estimated using the EECs for the small bird (20 g) which consumes small insects. Dietary-based and dose-based exposures of potential prey are assessed using the small mammal (15 g) which consumes short grass. Upper-bound Kenega nomogram values reported by T-REX for these two organism types are used for derivation of EECs for the CRLF and its potential prey (**Table 3.5**). Only the values for chronic exposure are provided because the acute avian oral and dietary and mammalian oral studies showed no mortalities at the highest dose/concentration tested. Dietary-based EECs for small and large insects reported by T-REX as well as the resulting adjusted EECs are available in **Table 3.6**. An example output from T-REX v. 1.3.1 is available in **Appendix E**.

Table 3.5 Upper-bound Kenega Nomogram EECs for Dietary- and Dose-based Exposures of the CRLF and its Prey to Glyphosate

Use	EECs for CRLF		EECs for Prey (small mammals)	
	Dietary-based EEC (ppm)	Dose-based EEC (mg/kg-bw)	Dietary-based EEC (ppm)	Dose-based EEC (mg/kg-bw)
Forestry (aerial) and areas with impervious surfaces 7.95 lbs a.e./A	1073.25	Not applicable	1908.00	1819.13
Alfalfa, avocado, forestry, nursery, rangeland, residential and turf 3.75 lbs/A	632.81	Not applicable	1125.00	1072.6
Almond, fruit, grape and olive 3.84 lb/A	648.00	Not applicable	1152.00	1098.34
Citrus, cole crop, lettuce, melon, onion, potato and wine grape 3.85 first application, 2.3 second application lb/A	388.13 - 649.69	Not applicable	690.00 - 1155.00	657.86 - 1101.2
Corn, cotton, garlic, impervious surfaces, row crop, strawberry and wheat 3.75 first application, 2.25 second application lb/A	379.69 – 889.92	Not applicable	675.00 – 1582.07	643.56 - 1508.38
Corn and wheat 0.75 lb/A	135.00	Not applicable	240.00	228.82
Rangeland 1.54 lb/A	276.93	Not applicable	492.32	469.39
Rangeland 0.387 lb/A	104.49	Not applicable	185.76	177.11
Right of way 7.5 lb/A	1012.50	Not applicable	1800.00	1716.16
Right of way 3.69 lb/A	622.69	Not applicable	1107.00	1055.44

Table 3.6. EECs (ppm) for Indirect Effects to the Terrestrial-Phase CRLF via Effects to Terrestrial Invertebrate Prey Items		
Use	**Small Insect**	**Large Insect**
Forestry (aerial) and areas with impervious surfaces 7.95 lbs a.e./A	1073.25	119.25
Alfalfa, avocado, forestry, nursery, rangeland, residential and turf 3.75 lbs/A	632.81	70.31
Almond, fruit, grape and olive 3.84 lb/A	648.00	72.00
Citrus, cole crop, lettuce, melon, onion, potato and wine grape 3.85 first application, 2.3 second application lb/A	388.13 - 649.69	43.13 – 72.19
Corn, cotton, garlic, impervious surfaces, row crop, strawberry and wheat 3.75 first application, 2.25 second application lb/A	379.69 – 889.92	42.19 – 98.88
Corn and wheat 0.75 lb/A	135.00	15.00
Rangeland 1.54 lb/A	276.93	30.77
Rangeland 0.387 lb/A	104.49	11.61
Right of way 7.5 lb/A	1012.50	112.5
Right of way 3.69 lb/A	622.69	69.19

3.4 Terrestrial Plant Exposure Assessment

TerrPlant (Version 1.1.2) is used to calculate EECs for non-target plant species inhabiting dry and semi-aquatic areas. Parameter values for application rate, drift assumption and incorporation depth are based upon the use and related application method (**Table 3.7**). A runoff value of 0.05 is utilized based on glyphosate's solubility, which is classified by TerrPlant as >100 mg/L. For aerial and ground application methods, drift is assumed to be 5% and 1%, respectively. EECs relevant to terrestrial plants consider pesticide concentrations in drift and in runoff. These EECs are listed by use in **Table 3.7**. An example output from TerrPlant v.1.2.2 is available in **Appendix F.**

Table 3.7 TerrPlant Inputs and Resulting EECs for Plants Inhabiting Dry and Semi-aquatic Areas Exposed to Glyphosate via Runoff and Drift						
Use	**Application rate (lbs a.i./A)**	**Application method**	**Drift Value (%)**	**Spray drift EEC (lbs a.i./A)**	**Dry area EEC (lbs a.i./A)**	**Semi-aquatic area EEC (lbs a.i./A)**
Alfalfa, avocado, corn, cotton, forestry, garlic, impervious, residential, row crop, strawberry, wheat	3.75	Foliar - Ground	1	0.0375	0.225	1.913
Almond, fruit, grape, olive	3.84	Foliar -Ground	1	0.0384	0.230	1.96
Citrus	3.85	Foliar -Ground	1	0.0385	0.231	1.964
Cole crop, lettuce, melon, onion, potato, wine grape	3.85	Foliar -Aerial	5	0.1925	0.385	2.118

Table 3.7 TerrPlant Inputs and Resulting EECs for Plants Inhabiting Dry and Semi-aquatic Areas Exposed to Glyphosate via Runoff and Drift						
Use	Application rate (lbs a.i./A)	Application method	Drift Value (%)	Spray drift EEC (lbs a.i./A)	Dry area EEC (lbs a.i./A)	Semi-aquatic area EEC (lbs a.i./A)
Corn	0.75	Foliar -Aerial	5	0.0375	0.075	0.4125
Forestry	7.95	Foliar -Aerial	5	0.3975	0.795	4.3725
Impervious	7.95	Foliar -Ground	1	0.0795	0.477	4.0545
Nursery, rangeland, sugar beet, tomato, turf	3.75	Foliar -Aerial	5	0.1875	0.375	2.0625
Rangeland	1.54	Foliar -Ground	1	0.0154	0.0924	0.7854
Rangeland	0.387	Foliar -Aerial	5	0.01935	0.0387	0.21285
Rights of way	7.5	Foliar -Aerial	5	0.375	0.75	4.125
Rights of way	3.69	Foliar -Ground	1	0.0369	0.2214	1.8819
Wheat	0.75	Foliar -Ground	1	0.0075	0.045	0.3825

4. Effects Assessment

This assessment evaluates the potential for glyphosate 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 glyphosate and its salts.

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 an ECOTOX search on 12/21/2007. 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 glyphosate.

Citations of all the open literature studies are attached in **Appendix G**. This includes all studies that were 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). **Appendix G** 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 H**.

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 glyphosate. A summary of the available aquatic and terrestrial ecotoxicity information, use of the probit dose response relationship, and the incident information for glyphosate are provided in Sections 4.1 through 4.4, respectively.

A large number of toxicity studies on glyphosate and/or its formulated products, especially acute toxicity studies have either been submitted to the Agency or are available in the open literature. The vast majority of these studies are on glyphosate formulations with mammals and aquatic species. Due to the proprietary nature of the surfactants and other inerts in the formulated products, the submitted studies with the associated data evaluation records (DERs) and the studies from the open literature did not usually report any details on the formulations tested other than a generic trade name, such as Roundup or Rodeo and the percent active ingredient. Often, the active ingredient was not identified in the submitted study report or the DER as to whether or not it was glyphosate or one of its salts that was tested. This was also true of the open literature. Most results were not expressed in terms of glyphosate acid equivalents. Therefore, the ecotoxicity data on formulations are presented in terms of the trade name, active ingredient tested (if available) and the percent active ingredient. Where available, the name of the surfactant present in the formulated product is noted. If the active ingredient and percent active ingredient are reported, then the results from the studies are expressed in terms of acid equivalents. In some cases, a best guess was made as to the active ingredient tested based on what is known to be in trade name products. Toxicity endpoint values for the isopropylamine salt of glyphosate (IPA) were converted to acid equivalents by multiplying by 0.74, the ratio of the molecular weight of glyphosate to the IPA salt. The trisodium diglyphosate (sesquisodium salt) toxicity endpoints values were converted to acid equivalents by multiplying by 0.42 and the glyphosate ammonium salt values were converted to acid equivalents by multiplying by 0.77.

Appendix I includes a summary of the mammalian data utilized for the most current assessment of human health risk for glyphosate. These data are used for determination of the action area and potential sublethal effects.

Acute toxicity data are available for fish, aquatic invertebrates and birds with the degradate, aminomethyl phosphonic acid (AMPA). AMPA appears to be less acutely toxic than the parent to freshwater fish and invertebrates and birds. Tables of these studies are provided after the data on the technical material and formulations with the appropriate taxonomic group.

Summary tables of all the available ecotoxicity information for the glyphosate formulated products and degradate are presented in **Appendix J**, incorporated along with the ecotoxicity studies conducted with the technical material glyphosate and/or its salts.

Toxicity data on mixtures were obtained from both the studies submitted to the Agency and from those found in the open literature from ECOTOX. The glyphosate team was unable to obtain copies of all the open literature studies on mixtures. Therefore, the bibliographic references for these studies are included in **Appendix A.** One submitted study was available for a mixture of glyphosate and oxyfluorfen tested on green algae (MRID 45906008). This study is summarized in **Table 4.23**. Many acute mammalian studies were conducted with mixtures of glyphosate and other active ingredients. These are also discussed in **Appendix A**.

4.1 Toxicity of Glyphosate to Aquatic Organisms

Tables 4.1 and **4.2** summarize 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 tabular summary of submitted and any open literature data considered relevant to this ecological risk assessment for the CRLF is presented below.

The available toxicity data on technical glyphosate and/or its isopropylamine salt (IPA) with aquatic-phase amphibians indicate that glyphosate is less toxic to the selected amphibian species tested than to the selected freshwater fish species tested. In order to protect the wider range of aquatic-phase amphibians (including the CRLF) which may be more sensitive than those amphibians that were tested, the more conservative endpoints from freshwater fish were selected for assessment of risk. Endpoints from the amphibian studies, presented along with the uncertainties associated with these studies, were used as conservative estimates if the endpoints could conceivably be lower than those selected from the fish studies. These endpoints are summarized in the following tables.

Table 4.1 Freshwater Aquatic Toxicity Profile for Glyphosate and/or Its Salts

Assessment Endpoint	Species	Toxicity Value Used in Risk Assessment	Citation MRID # /Date	Comment
Acute Direct Toxicity to Aquatic-Phase CRLF	Bluegill sunfish (*Lepomis macrochirus*)	96-hr. LC_{50}: 43 mg a.e./L*	44320630/1995	Acceptable
Chronic Direct Toxicity to Aquatic-Phase CRLF	Fathead minnow (*Pimephales promelas*) Leopard Frog (*Rana pipiens*)	NOAEC: 25.7 mg a.e./L (highest concentration tested) NOAEC: 1.8 mg a.e./L (highest concentration tested)	00108171/1975 46650501/2004	Acceptable. Frog study endpoint was used in assessment as a conservative estimate. Supplemental
Indirect Toxicity to Aquatic-Phase CRLF via Acute Toxicity to Freshwater Invertebrates (i.e. prey items)	Midge (*Chironomus plumosus*)	48-hr LC_{50}: 53.2 mg a.e./L	00162296/1979	Acceptable
Indirect Toxicity to Aquatic-Phase CRLF via Chronic Toxicity to Freshwater Invertebrates (i.e. prey items)	Water flea (*Daphnia magna*)	NOAEC: 49.9 mg a.e./L	00124763/1982	Acceptable. LOAEC: 95.7 mg a.e./L based on reduced reproductive capacity.
Indirect Toxicity to Aquatic-Phase CRLF via Toxicity to Non-vascular Aquatic Plants	Green algae (*Selenastrum capricornutum*)	4-day EC50: 12.1 mg a.e./L	40236901/1987	Acceptable
Indirect Toxicity to Aquatic-Phase CRLF via Toxicity to Vascular Aquatic Plants	Duckweed (*Lemna gibba*)	14-day EC50: 11.9 mg a.e./L	44320638/1996	Acceptable

*a.e. = expressed in terms of acid equivalents for glyphosate

Some glyphosate formulations have been found to be more toxic to aquatic organisms than technical glyphosate. Therefore, endpoints for assessment of risk to glyphosate formulations were selected. In California, one of the more toxic surfactants is not allowed to be applied directly to aquatic sites (polyoxy ethylene fatty amine or POEA). Therefore, for aquatic organisms, separate endpoints were selected for terrestrial uses where the POEA surfactant is allowed and for aquatic uses where this surfactant is not allowed. For aquatic animals, significant differences in toxicities between the formulations containing POEA and those that do not contain the surfactant are observed. For assessment of risk, exposure to the formulations is expressed in terms of EEC of the formulation rather than to the glyphosate acid equivalent. For consistency of units, the toxicity endpoints are also expressed in terms of concentration of formulation rather than the glyphosate acid equivalent.

For terrestrial uses, the most conservative endpoints from all the active formulations were selected. For aquatic uses, endpoints needed to be selected from studies on formulations that do not contain the POEA surfactant. Since it was not always possible to tell which formulations tested did not have the POEA surfactant, whenever possible, endpoints were selected from studies conducted with formulations that are currently labeled for aquatic use. This was not possible for the aquatic plant studies. The studies on aquatic plants were conducted with a product with the same basic name that has two separate labels, one for terrestrial uses and one for aquatic uses. It could not be determined from the aquatic plant studies whether or not they were conducted with the formulation for terrestrial uses or with the formulation for aquatic uses. The two formulations are different in terms of the inerts; however, the formulation for terrestrial uses does not have the POEA surfactant in it. Therefore, as a conservative estimate, the studies on this formulation were utilized for the assessment of risk to aquatic plants following exposure to formulations.

Table 4.2 Freshwater Aquatic Toxicity Profile for Glyphosate Formulations

Assessment Endpoint	Species	Toxicity Value Used in Risk Assessment	Citation MRID # /Date	Comment
Acute Direct Toxicity to Aquatic-Phase CRLF				Both studies supplemental
Terrestrial Applications	Rainbow trout (*Oncorhynchus mykiss*)	96-hr LC_{50}: 3.17 ppm formulation	40098001/1986	Roundup: 30% a.i.
Aquatic Applications	Rainbow trout (*Oncorhynchus mykiss*)	96-hr LC_{50}: 824 ppm formulation	45374001/1999	Glyphosate (360 g/L SL) 27% a.i.
Chronic Direct Toxicity to Aquatic-Phase CRLF				Supplemental
Terrestrial Applications	Leopard Frog (*Rana pipiens*)	LOAEC: 1.9 mg formulation/L	46650501/2004	No NOAEC
Indirect Toxicity to	Water flea			Both studies

Table 4.2 Freshwater Aquatic Toxicity Profile for Glyphosate Formulations				
Assessment Endpoint	**Species**	**Toxicity Value Used in Risk Assessment**	**Citation MRID # /Date**	**Comment**
Aquatic-Phase CRLF via Acute Toxicity to Freshwater Invertebrates (i.e. prey items)	(*Daphnia magna*) for both application types			acceptable
Terrestrial Applications		48-hr EC_{50}: 3 ppm formulation	00162296/1979	30.3% Glyphosate IPA
Aquatic Applications		48-hr EC_{50}: 164.3 ppm formulation	45374003/1999	27.25% Glyphosate (360 g/L SL formulation
Indirect Toxicity to Aquatic-Phase CRLF via Toxicity to Non-vascular Aquatic Plants Terrestrial and Aquatic Applications	Freshwater diatom (*Navicula pelliculosa*)	96-hr EC_{50}: 0.39 ppm formulation	45666701/2001	Acceptable Glyphosate (glyphos) 31.0%
Indirect Toxicity to Aquatic-Phase CRLF via Toxicity to Vascular Aquatic Plants	Duckweed (*Lemna gibba*) for both application types			
Terrestrial Applications		14-day EC_{50}: 4.9 ppm formulation	44125714/1984	Supplemental Glyphosate IPA salt (Roundup 41%)
Aquatic Applications		7-day EC_{50}: 25 ppm formulation	45666704/2001	Glyphosate (glyphos) 31.0% Acceptable

Toxicity to aquatic fish and invertebrates is categorized using the system shown in **Table 4.3** (U.S. EPA, 2004). Toxicity categories for aquatic plants have not been defined.

Table 4.3 Categories of Acute Toxicity for Aquatic Organisms	
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 and Aquatic-Phase Amphibians

Glyphosate toxicity data are available for both freshwater fish and aquatic-phase amphibians. The freshwater fish data were used as a surrogate to estimate direct acute risks to the CRLF because the endpoints from the fish data are more conservative. For chronic risk, the amphibian endpoint is utilized; however, it is noted that both the fish and frog NOAECs are non-definitive (i.e., no effects were observed at the highest concentration tested and there was no LOAEC). In addition, the frog study is classified as supplemental. This study had some significant uncertainties associated with water quality and high mortality rates in the controls.

Freshwater fish toxicity data were also used to assess potential indirect effects of glyphosate to the CRLF. Effects to freshwater fish resulting from exposure to glyphosate have the potential to 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 tabular summary of acute and chronic freshwater fish data, including data from the open literature, is provided below in Sections 4.1.1.1 through 4.1.1.3. Many acute toxicity studies are available for glyphosate formulations, with LC_{50}'s ranging from 1 to > 1000 mg/L. Because the number of fish studies on formulations is so extensive, only those studies which are referenced in the document are provided here. The remainder of the studies are summarized in tables in **Appendix J**. Acute toxicity data on the degradate, AMPA and two surfactants are also summarized.

4.1.1.1 Freshwater Fish: Acute Exposure (Mortality) Studies

Glyphosate and Its Salts Technical Material

Table 4.4 summarizes acute toxicity studies with freshwater fish on technical glyphosate and its salts. Study data are available for bluegill sunfish, rainbow trout, fathead minnow and channel catfish and are expressed in terms of glyphosate acid equivalents for comparison purposes. The data from these studies are so variable within each species that it is not possible to determine a range of sensitivities.

Table 4.4. Freshwater Fish Acute Toxicity for Technical Glyphosate and Its Salts					
Species	**% Active Ingredient***	**96-hour LC_{50} NOAEC (mg a.e./L)*/ Slope**	**Toxicity Category[2]**	**MRID #/Year**	**Study Classification**
Bluegill sunfish (*Lepomis macrochirus*)	95.6	LC_{50}: **43 (30.6 - 53.5)**[3] NOAEC: 30.6 Slope: Not available	Slightly toxic	44320630/1995	Acceptable
Bluegill sunfish (*Lepomis macrochirus*)	83	LC_{50}: 99.6 (92.1 - 107.9)[1] NOAEC: 83 Slope: Not available	Slightly toxic	00108205/1978	Acceptable
Bluegill sunfish (*Lepomis macrochirus*)	96.7	LC_{50}: 100.2 (78.7 - 114.5)[4] NOAEC not reported Slope: Not available	Practically nontoxic	00162296/1979	Acceptable
Rainbow trout (*Oncorhynchus mykiss*)	83	LC_{50}: 71.4 (58.1-84.8) NOAEC: 34.9 Slope: Not available	Slightly toxic	00136339/1978	Acceptable
Rainbow trout (*Oncorhynchus mykiss*)	96.7	LC_{50}: 100.2 (85.9 - 121.6)[4] NOAEC not reported Slope: Not available	Practically nontoxic	00162296/1979	Acceptable
Rainbow trout (*Oncorhynchus mykiss*)	95.6	LC_{50}: 128.1 (95.6 - 172.1) NOAEC: 30.6 Slope: Not available	Practically nontoxic	44320629/1995	Acceptable
Fathead minnow (*Pimephales promelas*)	96.7	LC_{50}: 69.4 (56.5 - 85.9)[4] NOAEC not reported Slope: Not available	Slightly toxic	00162296/1979	Acceptable
Channel catfish (*Ictalurus punctatus*)	96.7	LC_{50}: 93 (78.7 - 114.5)[4] NOAEC not reported Slope: Not available	Slightly toxic	00162296/1979	Acceptable

* a.i. = active ingredient; a.e. = acid equivalent
[1] Range is 95% confidence interval for endpoint
[2]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[3] **Bold** and shaded value will be used to calculate risk quotients
[4] Study conducted with the isopropylamine salt

Glyphosate and Its Salts Formulations

Table 4.5 summarizes selected acute toxicity studies on freshwater fish with several glyphosate and glyphosate salt formulations. Submitted data on glyphosate formulations indicate that some of the formulations are more toxic to freshwater fish than technical glyphosate itself. Studies have indicated that one surfactant, polyethoxylated tallowamine (referred to as polyoxy ethylene fatty amine or POEA) is probably the reason for the increased toxicity of some of the glyphosate formulations (Giesy, 2000; USDA, 2003; MRID 00162296). For example, in one study (MRID 00162296), fathead minnows were exposed to either technical isopropylamine salt of glyphosate (IPA), a glyphosate IPA formulation or the POEA surfactant. The resultant acute LC_{50}s were 69.4, 1.7 and 2.0 mg/L, respectively.

For the studies selected for the quantitative assessment of risk, the units for the formulations are expressed in both acid equivalents and in mg/L formulation. As stated previously, for terrestrial uses, the most conservative endpoint from all the active formulations was selected. For aquatic uses, the endpoint was selected from a study conducted with a formulation that is currently labeled for aquatic use.

The acute toxicity values between freshwater fish species are not sufficiently consistent to determine a range of sensitivities for freshwater fish. For example, one review indicates that the salmonids are more sensitive to glyphosate than other species of fish (USDA, 2003); however, the available data here do not necessarily support this statement. Data from the open literature (ECOTOX) provide some information on sublethal effects (see Section 4.1.1.3).

Also stated previously, the form of glyphosate (acid or salt) and the surfactants present in each of the formulations tested are either ambiguously reported or not reported at all. However, the Roundup® formulations generally have the IPA salt, a surfactant and water (Geisy, 2000). The formulations of Roundup® that have been tested often contain the POEA surfactant.

Note that when the acute LC_{50}s for the formulations are expressed in terms of glyphosate acid equivalents, they are not identical to the LC_{50} values for the same studies considered in previous risk assessments or reviews. The LC_{50} values are normally lower when expressed in terms of acid equivalents.

Table 4.5. Freshwater Fish Acute Toxicity for Glyphosate Formulations						
Chemical (Active Ingredient)	**Species**	**% a.i.***	**96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope**	**Toxicity Category[1]**	**MRID #/Year**	**Study Classification**
Glyphosate IPA (Roundup)*	Rainbow trout (*Oncorhynchus mykiss*)	30	LC_{50}: 1 (0.8 - 1.2)[2] (**3.17** mg formulation/L) NOAEC: N.R.* Slope:N.R.	Highly toxic	40098001/1986	Supplemental
Glyphosate (360 g/L SL)	Rainbow trout (*Oncorhynchus mykiss*)	27	LC_{50}: 224.5 (160.1 - 280.0) (**824** mg formulation/L) NOAEC: 160 Slope:N.R.	Practically non-toxic	45374001/1999	Supplemental
Glyphosate IPA (Roundup with POEA surfactant)	Fathead minnow (*Pimephales promelas*)	30	LC_{50}: 1.7 (1.4 - 2.1) NOAEC: N.R. Slope:N.R.	Moderately toxic	00162296/1979	Supplemental
Glyphosate IPA (Roundup)	Bluegill sunfish (*Lepomis macrochirus*)	31	LC_{50}: 1.8 (1.4 - 2.6) NOAEC: 0.7 Slope:N.R.	Moderately toxic	00124760/1982	Acceptable
Glyphosate IPA (Roundup)	Rainbow trout (*Oncorhynchus mykiss*)	31	LC_{50}: 2.5 (2.0 - 3.1) NOAEC: 1.8 Slope:N.R.	Moderately toxic	00124761/1982	Supplemental
Glyphosate IPA (Roundup)	Fathead minnow (*Pimephales promelas*)	41	LC_{50}: 2.9 (1.7 - 4.9) NOAEC: 1.7 Slope:N.R.	Moderately toxic	00070896/1980	Acceptable
Glyphosate IPA	Bluegill sunfish (*Lepomis macrochirus*)	30	LC_{50}: 3 (2.4 - 3.7) NOAEC: N.R. Slope:N.R.	Moderately toxic	40098001/1986	Supplemental
Glyphosate IPA (Roundup)	Bluegill sunfish (*Lepomis macrochirus*)	41	LC_{50}: 4.3 (2.7 - 7.3) NOAEC: 2.7 Slope:N.R.	Moderately toxic	00070897/1980	Acceptable
Glyphosate IPA (Roundup)	Channel catfish (*Ictalurus punctatus*)	41	LC_{50}: 4.9 (2.9 - 8.0) NOAEC: 2.9 Slope:N.R.	Moderately toxic	00070894/1980	Supplemental

Table 4.5. Freshwater Fish Acute Toxicity for Glyphosate Formulations						
Chemical (Active Ingredient)	**Species**	**% a.i.***	**96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope**	**Toxicity Category[1]**	**MRID #/Year**	**Study Classification**
Glyphosate IPA (Roundup)	Rainbow trout ((*Salmo gairdneri*)	36	LC_{50}: 5.5 - 9.2 (4.2 - 13) NOAEC: 4.2 Slope:N.R.	Moderately toxic	40579203/1986	Supplemental
Glyphosate IPA (Roundup)	Chinook Salmon (*Oncorhynchus tschawytscha*)	36	LC_{50}: 7.1 (5.9 - 9.7) NOAEC: <1.3 Slope:N.R.	Moderately toxic	40579201/1986	Not classified 10% mortality at 1.3 (loss of equilibrium and mobility)
Glyphosate IPA (Roundup)	Coho Salmon (*Oncorhynchus kisutch*)	36	LC_{50}: 8.2 (4.2 – 13.4) NOAEC: 3.42 Slope:N.R.	Moderately toxic	40579202/1986	Supplemental
Glyphosate IPA with X-77 surfactant	Rainbow trout (*Oncorhynchus mykiss*)	5	LC_{50}: 9.4 (7.0 - 12.4) NOAEC: 7 Slope:N.R.	Moderately toxic	00078664/1980	Acceptable
Glyphosate IPA with Geronol CF/AR surfactant	Rainbow trout (*Oncorhynchus mykiss*)	45	LC_{50}: > 450 (N.A.) mg a.e./L or > 1000 mg formulation/L NOAEC: 1000 mg formulation/L Slope:N.A.	Practically non-toxic	44738201/1996	Not classified

* a.i. = active ingredient; a.e. = acid equivalent; IPA = isopropylamine salt; NR = not reported; NA = not available
[1]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[2] Range is 95% confidence interval for endpoint
[3] **Bolded** and shaded values will be used to calculate risk quotients

Table 4.6 summarizes submitted acute toxicity studies on freshwater fish with two surfactants, POEA and geronol, an alkyl polyoxy ethylene phosphoric acid ester. The studies with POEA indicate that it is slightly to highly toxic with similar toxicity values in rainbow trout, fathead minnows and channel catfish and slightly less toxic to bluegill sunfish. Geronol does not appear to be toxic to zebra fish.

Table 4.6. Freshwater Fish Acute Toxicity for Surfactants Used with Glyphosate Formulations

Chemical	Species	% a.i.[1]	96-hour LC_{50}/NOAEC (mg/L)/Slope	Toxicity Category[2]	MRID #/Year	Study Classification
Polyoxy ethylene fatty amine (POEA)	Rainbow trout (*Oncorhynchus mykiss*)	100	LC_{50}: 1 (1.2 - 1.7)[3] NOAEC and slope not reported	Highly toxic	00162296/1979	Acceptable
Polyoxy ethylene fatty amine (POEA)	Fathead minnow (*Pimephales promelas*)	100	LC_{50}: 2 (1.5 - 2.7) NOAEC and slope not reported	Moderately toxic	00162296/1979	Acceptable
Polyoxy ethylene fatty amine (POEA)	Channel catfish (*Ictalurus punctatus*)	100	LC_{50}: 3 (2.5 - 3.7) NOAEC and slope not reported	Moderately toxic	00162296/1979	Acceptable
Polyoxy ethylene fatty amine (POEA)	Bluegill sunfish (*Lepomis macrochirus*)	100	LC_{50}: 13 (10.0 - 17.0) NOAEC and slope not reported	Slightly toxic	00162296/1979	Acceptable
Surfactant Geronol CF/AR (alkyl polyoxy ethylene phosphoric acid ester)	Zebra fish (*Brachydanio rerio*)	100	LC_{50}: >100 (N.A.) NOAEC and slope not reported	Practically non-toxic	44738201/ Summary from another study	Not classified

[1] a.i. = active ingredient, assumed 100% for technical material
[2]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[3] Range is 95% confidence interval for endpoint.

The acute toxicity study with rainbow trout (**Table 4.7**) indicates that the degradate, aminomethyl phosphonic acid (AMPA) is less toxic to freshwater fish than the parent glyphosate.

Table 4.7. Freshwater Fish Acute Toxicity for Aminomethyl Phosphonic Acid (AMPA) Degradate of Glyphosate						
Chemical	**Species**	**% a.i.[1]**	**96-hour LC_{50}/NOAEC (mg/L)/Slope**	**Toxicity Category[2]**	**MRID #/Year**	**Study Classification**
AMPA	Rainbow trout (Oncorhynchus mykiss)	94.38	LC50: 499 (391 - 647) NOAEC: 174 Slope: 6.42	Practically nontoxic	43334713/1991	Acceptable

[1] a.i. = active ingredient, assumed 100% for technical material
[2]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[3] Range is 95% confidence interval for endpoint.

4.1.1.2 Freshwater Fish: Chronic Exposure (Growth/Reproduction) Studies

No effects were observed at the highest level tested, 25.7 mg a.e./L in a life cycle study with technical glyphosate in fathead minnows. No other chronic studies were found with freshwater fish, including in the open literature; however, subchronic studies were found in the open literature. Sublethal effects from these studies are summarized in Section 4.1.1.3. No appropriate chronic toxicity data for either the surfactants or the degradate have been located.

Table 4.8. Freshwater Fish Chronic Toxicity for Technical Glyphosate and Its Salts				
Species	**% Active Ingredient**	**NOAEC/LOAEC (mg acid equivalent/L)**	**MRID #/Year**	**Study Classification**
Fathead minnow (*Pimephales promelas*)	87.3	25.7/>25.7[1]	00108171/1975	Acceptable

4.1.1.3 Freshwater Fish: Sublethal Effects and Additional Open Literature Information

None of the open literature data provided more conservative endpoints that may be used in a quantitative estimate of risk. Several studies were published that concentrated on potential sublethal effects following glyphosate exposure, particularly on a microscopic and biochemical level. In addition, at least one study examined potential behavioral effects. Any sublethal effects observed in the submitted acute toxicity studies on the technical material are also summarized in **Table 4.9**. Observed sublethal effects in the chronic studies are already summarized in other sections of the ecological effects characterization section. For freshwater fish, sublethal data from the open literature and submitted studies are available for tilapia, topmouth gudgeon, rainbow trout, north African catfish and Lee Koh. The formulations, Roundup®, Vision® and glyphosate with several different surfactants and glyphosate were tested. The NOAECs for sublethal effects range from 8 ppb to 30.6 ppm. The lowest NOAEC is 8 ppb, based on an increase in wigwag behavior in rainbow trout at the LOAEC of 46 ppb following exposure to Vision®, a formulation containing the toxic surfactant, POEA. The highest NOAEC is 30.6 ppm, based on dark coloration in rainbow trout at the LOAEC of 53.6 ppm following exposure to 95.6% glyphosate. Other studies show sublethal effects on several organs (gills, liver and kidneys) and various systemic enzymes, plus some behavioral and neurophysiological changes. In addition, in a fish mutagenicity study, Roundup induced erythrocyte micronuclei at 42, 85 and 170 mg/kg. Unless they can be quantitatively associated with mortality, growth or reproduction, sublethal effects are not included in the quantitative assessment of risk; however, they are discussed in the risk description.

Table 4.9. Freshwater Fish Sublethal Effects From Submitted and Open Literature Studies

Species	Chemical	NOAEC	LOAEC:Effects	MRID/ECOTOX Reference No.
Nile tilapia (*O. niloticus*)	Roundup (48% a.e.)	Not determined	5 ppm: gills: filament cell proliferation, lamellar cell hyperplasia, lamellar fusion, epithelial lifting, and aneurysm. Liver: vacuolation of hepatocytes and nuclear pyknosis. Kidneys: dilation of Bowman's space and accumulation of hyaline droplets in the tubular epithelial cells. Significant increase in aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase activities. Decreased activity.	E096917 – **This study used to determine Action Area**
Nile tilapia (*O. niloticus*)	Roundup (48% a.e.)	5 ppm	15 ppm: gills: mucosal cells of laminar epithelium - loss of microridges and appearance of intercellular spaces; thickening of primary epithelium, edema, lifting and fusion of secondary lamellae – may impair respiratory function. Liver: progressive reduction and fragmentation of RER; swollen mitochondria; increases in number and sizes of lysosomes and lipid droplets; infiltration of leukocytes; increased hepatocyte size with pyknotic nuclei and presence of vacuoles. Kidney: degeneration of nuclear membrane; mitochondrial contraction and/or swelling; accumulation of large electron dense particles; increase in number and size	E096937

Table 4.9. Freshwater Fish Sublethal Effects From Submitted and Open Literature Studies				
Species	**Chemical**	**NOAEC**	**LOAEC:Effects**	**MRID/ECOTOX Reference No.**
			of lysosomes and apical vacuoles; some cellular necrosis. Increased plasma aspartate and alanine aminotransferase and alkaline phosphatase activities at 15 ppm.	
Topmouth gudgeon (*pseudorasobora parva*)	Glyphosate IPA salt (41%)	Not determined	1 ppm: Initial possible inhibition of liver esterase activity and then possible induction of enzyme activity. Not dose dependent.	E097111
Rainbow trout (*O. mykiss*)	Vision (356 g/L glyphosate acid with surfactant)	8 ppb	45.75 ppb: increase in wigwag behavior (one of agonistic behaviors). No effects on growth, foraging variables or antagonistic activity; no evidence of neoplasia or melanomacrophages and no increase in gill lesions at 45.75 ppb (highest concentration tested).	E097714
Rainbow trout (*O. mykiss*)	Glyphosate (assumed technical) and combinations with surfactants R-11 and Target Prospeador Acitvator	1.25 ppm (glyphosate alone)	Rainbow trout vitellogenin assay. Estrogenic effects. No effects with glyphosate alone. When combined with surfactants at 1.25 ppm, trends indicated elevated vitellogenin.	E080643
North African catfish (*Clarius gariepinus*)	Roundup (no other identification)	Not determined	3.9 ppm: Increased plasma AST, ALP, ALT levels.	E097133
Rainbow trout (*O. mykiss*)	Technical glyphosate 95.6%	30.6 ppm	53.6 ppm: dark coloration	MRID 44320629
T. rendalli	Roundup® (480g/l) and surfactant	No NOAEL	42 mg/kg. Fish erythrocyte micronucleus assay. Pesticide applied by injection. Roundup induced micronuclei at 42, 85 and 170 mg/kg	E074478
Rainbow trout *(O. mykiss)*	Roundup® 143 g/L	0.01 ppm	0.1 ppm. Olfactory-mediated behavioral and neurophysiological response. Over a concentration range that does not result in acute toxicity, trout detect Roundup but do not avoid it. Above that concentration, they avoid it (≥ 10 ppm). Study found that behavioral responses may be more sensitive tox. endpoints than neurophysiological responses.	E089625 Tierney 2007
Rainbow trout *(O. mykiss)*	Roundup® 356 g/L glyphosate IPA MON 02139	30 ppm	40 ppm. Fish tend to avoid concentrations that are lethal (40 ppm and above). 96-hr LC_{50} 54.8 in the lab and 52 in the field. No mortality at 2.2 kg a.e./ha, 10x and 100x field dose.	E010471
Rainbow trout *(O. mykiss)*	Vision® 356 g a.e./L with either 10% or	Avoidance: 27 ppm	96 hr LC_{50}: 100 ppm (7.5%); 75 ppm (10%); 27 ppm (15%).	E05182

Table 4.9. Freshwater Fish Sublethal Effects From Submitted and Open Literature Studies				
Species	**Chemical**	**NOAEC**	**LOAEC:Effects**	**MRID/ECOTOX Reference No.**
	15% surfactant (POEA). 7.5% surfactant tested in acute study	(15%) & 75 ppm (10%) Other behavior 6.75 ppm (15%) & 18.75 ppm (10%)	Avoidance behavior LOAEC: 150 ppm (10%); 54 ppm (15%) Other behavior LOAEC: Erratic swimming & rapid respiration 13.5 ppm (15%); erratic swimming & labored respiration 37.5 ppm (10%)	
Tilapia (*Oreochromis niloticus*) Lee Koh (*Cyprinus carpio*)	Roundup® 30.5% w/w glyphosate	0.31 ppm for tilapia 1.7 ppm for Lee Koh	Tilapia: 0.55 ppm: erratic swimming. 96-hr LC_{50}: 2.3 ppm. Lee Koh: LC_{50}: 3.1 ppm. LOAEC not provided.	E03296

4.1.1.4 Aquatic-phase Amphibian: Acute and Chronic Studies

Acute and chronic studies have been conducted on glyphosate, both technical and formulations with various frog species. These studies indicate that the frog is generally either equally or less susceptible to glyphosate toxicity than fish. **Tables 4.10 – 4.14** summarize the submitted frog studies for technical glyphosate, its salts, and formulations. Data are also available on the surfactant, POEA. MRID 46650501 tested the green frog (*Rana clamitans*, Gosner stage 25) with technical glyphosate (isopropylamine salt (IPA)), an IPA formulation with 15% POEA, and POEA. The acute LC_{50}'s were >17.9, 2.0 and 2.2 mg/L, respectively, with technical IPA and the IPA formulation expressed in terms of glyphosate acid equivalents. This study indicates that aquatic amphibians are also susceptible to POEA toxicity.

Forty-two day studies with leopard frog (*Rana pipiens*) larvae indicate that a formulation containing 15% POEA and the POEA surfactant itself are more toxic to the frogs than the technical IPA salt (MRID 46650501).

Table 4.10 Aquatic-Phase Amphibian Acute Toxicity for Technical Glyphosate and Its Salts					
Species	**% Active Ingredient***	**96-hour LC_{50} NOAEC (mg a.e./L)*/ Slope**	**Toxicity Category[2]**	**MRID #/Year**	**Study Classification**
Australian tree frog (*Litoria moorei*) Tadpole	96	LC50: 103.2 (43.2 - 172.8)[1] NOAEL: N.R.* Slope: N.R.	Practically nontoxic	43839601/1995	Supplemental
Australian frog (*Crinia insignifera*) Adult	96	LC50: 75 (60.4-92.7) NOAEL: N.R. Slope: N.R.	Slightly toxic	43839601/1995	Supplemental
Green Frog (*Rana clamitans*) Gosner Stg 25	Tech[4]	LC50: >17.9 (NR) NOAEL: NR Slope: NR	Slightly toxic	46650501/2001	Supplemental

* a.i. = active ingredient; a.e. = acid equivalent; N.R. = not reported
[1] Range is 95% confidence interval for endpoint
[2]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[3] Study conducted with the isopropylamine salt

Table 4.11 Aquatic-Phase Amphibian Acute Toxicity for Glyphosate Formulations						
Chemical	**Species**	**% a.i.***	**96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope**	**Toxicity Category[1]**	**MRID #/Year**	**Study Classification**
Glyphosate- IPA (Cosmo Flux Coca mix)	Africian clawed frog (*Xenopus laevis*) Larvae	18	LC50: 1.1 (0.56 - 2.3) or 10 mg/L formulation NOAEL: 0.14 Slope: 4.92	Moderately toxic	46873601/2006	Supplemental
Glyphosate IPA (Cosmo Flux Poppy mix)	Africian clawed frog (*Xenopus laevis*) Larvae	0.0205	LC50: 1.3 (0.92 - 1.8) or 16 mg/L formulation NOAEL: 0.43 Slope: NA*	Moderately toxic	46873602/2006	Supplemental
Glyphosate IPA (Roundup Original with 15% POEA)	Green Frog (*Rana clamitans*) Gosner Stg 25	NR	LC50: 2 (1.9-2.2) or 6.5 mg/L formulation NOAEL: NR* Slope: NR	Moderately toxic	46650501/2001	Supplemental

Table 4.11 Aquatic-Phase Amphibian Acute Toxicity for Glyphosate Formulations						
Chemical	**Species**	**% a.i.***	**96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope**	**Toxicity Category[1]**	**MRID #/Year**	**Study Classification**
Glyphosate IPA (Roundup Transorb with 15% POEA)	Green Frog (*Rana clamitans*) Gosner Stg 25	NR	LC50: 2.2 (2.1-2.4) or 7.2 mg/L formulation NOAEL: NR Slope: NA	Moderately toxic	46650501/2001	Supplemental
Glyphosate IPA (Roundup Original with 15% POEA)	Leopard Frog (*Rana pipiens*) Gosner Stg 25	NR	LC50: 2.9 (NR) or 9.2 mg/L formulation NOAEL: NR Slope: NR	Moderately toxic	46650501/2000	Supplemental
Glyphosate IPA (Roundup Original with 15% POEA)	American toad (*Bufo americanus*) Gosner Stg 25	NR	LC50: <4.0 (NR) or < 12.9 mg/L formulation NOAEL: NR Slope: NR	Moderately toxic	46650501/1994	Supplemental
Glyphosate IPA (Roundup with 15% POEA)	Wood Frog (*Rana sylvatica*) Gosner Stg 25	NR	LC50: 5.1 (4.9-5.4) or 16.5 mg/L formulation NOAEL: NR Slope: NR	Moderately toxic	46650501/1994	Supplemental
Glyphosate IPA (Roundup 360)	Australian tree frog (*Litoria moorei*) Tadpole	30.3	LC50: 5.6 (4.4 - 7.1) or 18.5 mg/L formulation NOAEL: N.R. Slope: N.R.	Moderately toxic	43839601/1995	Supplemental
Glyphosate IPA (Roundup Original with 15% POEA)	Leopard Frog (*Rana pipiens*) Gosner Stg 20	NR	LC50: 6.5 (6.1-6.8) or 20.9 mg/L formulation NOAEL: NR Slope: NA	Moderately toxic	46650501/1994	Supplemental
Glyphosate IPA (Roundup Original with 15% POEA)	Green frog (*Rana clamitans*) Gosner Stg 20	NR	LC50: 7.1 (6.6-7.6) or 22.8 mg/L formulation NOAEL: NR Slope: NR	Moderately toxic	46650501/1994	Supplemental
Glyphosate IPA (Roundup Original with 15% POEA)	American toad (*Bufo americanus*) Gosner Stg 20	NR	LC50: 8 (NR) or 25.8 mg/L formulation NOAEL: NR Slope: NR	Moderately toxic	46650501/1994	Supplemental

Table 4.11 Aquatic-Phase Amphibian Acute Toxicity for Glyphosate Formulations						
Chemical	**Species**	**% a.i.***	**96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope**	**Toxicity Category[1]**	**MRID #/Year**	**Study Classification**
Glyphosate IPA (Roundup Original with 15% POEA)	Wood Frog (*Rana sylvatica*) Gosner Stg 20	NR	LC50: > 8 (NR) or > 25.8 mg/L formulation NOAEL: NR Slope: NR	Moderately toxic	46650501/1994	Supplemental
Glyphosate IPA (Glyphos AU with 3-7% POEA)	Green Frog (*Rana clamitans*) Gosner Stg 25	NR	LC50: 8.9 (8.6-9.2) or 28.6 mg/L formulation NOAEL: NR Slope: NR	Moderately toxic	46650501/2001	Supplemental
Glyphosate IPA (Roundup Biactive with 10-20% unspecified surfactant)	Green frog (*Rana clamitans*) Gosner Stg 25	NR	LC50: >17.9 (NR) or > 57.7 mg/L formulation NOAEL: NR Slope: NR	Slightly toxic	46650501/2001	Supplemental
Glyphosate IPA (Glyphos BIO with 3-7% POEA)	Green Frog (*Rana clamitans*) Gosner Stg 25	NR	LC50: >17.9 (NR) or >57.7 mg/L formulation NOAEL: NR Slope: NR	Slightly toxic	46650501/2001	Supplemental
Glyphosate IPA (Roundup 360)	Australian frog (*Crinia insignifera*) Adult	30.3	LC50: 30.4 (0-infinity) or 100.2 mg/L formulation NOAEL: N.R. Slope: N.R.	Slightly toxic	43839601/1995	Supplemental
Glyphosate IPA (Roundup 360)	Australian frog (*Crinia insignifera*) Tadpole	30.3	48 hr LC50: 38.2 (30.2 - 48.8) or 125.9 mg/L formulation NOAEL: N.R. Slope: N.R.	Slightly toxic	43839601/1995	Supplemental
Glyphosate IPA (with surfactant Geronol CF/AR	Common froglet (*Ranidella signifera*) Tadpole	45	LC50: >450 (N.A.) or >1000 mg/L formulation NOAEL: 1000 Slope: N.A.	Practically nontoxic	44738201/1996	Supplemental
Glyphosate IPA (Roundup Biactive))	Common froglet (*Ranidella signifera*) Tadpole	36	LC50: >360 (N.A) or >1000 mg/L formulation NOAEL: <800 Slope: N.A.	Practically nontoxic	44738201/1996	Supplemental

Table 4.11 Aquatic-Phase Amphibian Acute Toxicity for Glyphosate Formulations						
Chemical	**Species**	**% a.i.***	**96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope**	**Toxicity Category[1]**	**MRID #/Year**	**Study Classification**
Glyphosate IPA (with surfactant Geronol CF/AR	Common froglet (*Ranidella signifera*) Tadpole	36	LC50: >360 (N.A) or >1000 mg/L formulation NOAEL: 1000 Slope: N.A.	Practically nontoxic	44738201/1996	Supplemental
Glyphosate IPA (with surfactant Geronol CF/AR	Common froglet (*Ranidella signifera*) Tadpole	10	LC50: >100 (N.A.) or >1000 mg/L formulation NOAEL: 1000 Slope: N.A.	Practically nontoxic	44738201/1996	Supplemental

* a.i. = active ingredient; a.e. = acid equivalent; IPA = isopropylamine salt, N.A. = not available, N.R. = not reported
[1]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[2] Range is 95% confidence interval for endpoint

Table 4.12. Aquatic-Phase Amphibian Acute Toxicity for POEA Surfactant Used with Glyphosate Formulations						
Chemical	**Species**	**% a.i.[1]**	**96-hour LC_{50}/NOAEC (mg/L)/Slope**	**Toxicity Category[2]**	**MRID #/Year**	**Study Classification**
Polyoxy ethylene fatty amine (POEA or MON 0818)	Green Frog (*Rana clamitans*) Gosner Stg 25	69-73	LC50: 2.2 (2.1-2.4) NOAEC: NR* Slope: NR	Moderately toxic	46650501/2001	Supplemental

* NR = not reported
[1] a.i. = active ingredient, assumed 100% for technical material
[2]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[3] Range is 95% confidence interval for endpoint.

Table 4.13. Aquatic Phase Amphibian Chronic Toxicity for Technical Glyphosate IPA Salt and IPA Salt Formulations				
Species	**% Active Ingredient**	**NOAEC/LOAEC (mg acid equivalent/L)**	**MRID #/Year**	**Study Classification**
Leopard Frog (*Rana pipiens*)	Tech IPA (assumed 100%)	NOAEC/LOAEC: **1.8/>1.8**[1]	46650501/2004	Supplemental
Leopard Frog (*Rana pipiens*)	Roundup Original & Transorb 15% POEA	NOAEC/LOAEC: **0.6/1.8**[1] decr. percentage larvae surviving to reach Stage 42 and length at metamorphosis. Incr. time to metamorphosis, mixed-sex gonads and tail damage. Gosner stage 25, larvae treated with Roundup® Original at 1.8 mg a.e/L or with Roundup® Transorb at 0.6 and 1.8 mg a.e./L exhibited significantly higher thyroid hormone mRNA expression than controls.	46650501/2004	Supplemental
[1] **Bold** and shaded value will be used to calculate risk quotients				

Table 4.14 Aquatic-Phase Amphibian Chronic Toxicity for POEA Surfactant Used with Glyphosate Formulations					
Chemical	**Species**	**% a.i.**[1]	**NOAEC/ LOAEC (mg a.i./L)**	**MRID #/Year**	**Study Classification**
Polyoxy ethylene fatty amine (POEA or MON 0818)	Leopard Frog (*Rana pipiens*) Larvae	Tech	NOAEC/ LOAEC: 0.6/1.8	46650501/2004	Supplemental
[1] a.i. = active ingredient, assumed 100% for technical material					

4.1.1.5 Aquatic Amphibian Sublethal Effects and Additional Open Literature Information

Some of the open literature studies on amphibians provide additional information that may be of use in the risk characterization for glyphosate. These studies are summarized in **Table 4.15**.

Table 4.15. Aquatic Amphibian Sublethal Effects From Submitted and Open Literature Studies				
Species	**Chemical**	**NOAEC**	**LC_{50} or LOAEC:Effects**	**MRID/ ECOTOX Ref. No.**
Green frog *(Rana pipiens)*	Vision® (contains POEA surfactant)	Not determined for mortality	LOAEC for mortality: 0.75 ppm a.e. at pH 7.5. Note: higher pH (7.5) versus 5.5 increases acute toxicity	E072794
African clawed frog (*Xenopus laevis*)	Rodeo® (480 g a.e./L no surfactant) Roundup® (356 g ae/L with POEA surfactant)	5 ppm a.e. (Roundup®) and 2000 ppm a.e. (Rodeo®)	Frog embryo teratogenesis assay. LC_{50}'s: POEA (6.8 ppm), Roundup® (9.3 ppm a.e.), Rodeo® (7297 ppm a.e.). No significant increases in embryo malformations for either formulation.	E053090
Crinia insignifera, *Heleioporus eyrei*, *Limnodynastes dorsalis,and Litoria moorei*	Glyphosate, glyphosate IPA, Roundup®, Touchdown® and Roundup® Biactive	N/A	48-hr acute LC_{50}'s (formulations) for tadpoles, metamorphs and adults between 2.9 and >360 mg a.e./L with Roundup® (MON 2139) as the most toxic formulation to Roundup® Biactive as the least toxic formulation. Glyphosate IPA salt alone (LC_{50}: 466 mg a.e./L) less toxic than glyphosate acid (LC_{50}: 81.2 – 121 mg a.e./L), probably due to acid intolerance. Slight differences in species sensitivity *L moorei* tadpoles more sensitive than other tadpoles; adult and new metamorphs less sensitive than tadpoles.	E071857
Leopard frog (*Rana pipiens*), Green frog, (*Rana clamitans*) American toad, (*Bufo americanus*), Africian clawed frog (*Xenopus laevis*)	Vision® (contains POEA surfactant)	N/A	96-hr acute studies. Toxicity enhanced by elevated pH with Surfactant POEA (15%) hypothesized as major source of pH interaction. LC_{50}'s (mga.e./L) pH 6.0 pH 7.5 Leopard frog embryo* 15.1 7.5 Leopard frog larvae* 1.8 1.1 Green frog embryo 5.3 4.1 Green frog larvae 3.5 1.4 American toad embryo 4.8 6.4 American toad larvae 2.9 1.7 Africian clawed frog embryo 15.6 7.9 African clawed frog larvae 2.1 0.88 *Gosner 8-25 = embryo, Gosner 25 = larvae Growth inhibition in surviving frogs observed with clawed frog, green frog and leopard frog	E072795
Scinax nasicus tadpoles Gosner stages 25-26 (prometamorphic)	Glyfos (48% IPA + 15% POEA)	N/A	96-hr acute LC_{50}: 2.64 mg glyphos/L (1.95 mg a.e./L). Malformations (craniofacial and mouth deformities, eye abnormalities and bent curved tails) increase with increased time and mortality.	E071969

Table 4.15. Aquatic Amphibian Sublethal Effects From Submitted and Open Literature Studies				
Species	**Chemical**	**NOAEC**	**LC_{50} or LOAEC:Effects**	**MRID/ ECOTOX Ref. No.**
Western chorus frog (*Pseudacris triseriata*) and Plains leopard frog (*Rana blairi*) tadpoles Gosner stage 25	Kleeraway Grass and Weed Killer RTU (IPA 0.75%, surfactant – ethoxylated tallowamine).		Concentration levels 750, 75, 7.5 or 0.75 ppm IPA. 24-hr exposure period. No frogs survived 7.5 – 750 ppm. Western chorus frogs slightly more sensitive. No effect on growth or final Gosner stage.	E61464
Rana cascadae larvae	Roundup® 50.2%	Not determined for time to metamorph-osis	LOAEL 1 ppm. Concentration levels 0.96 and 1.94 ppm for 43 days. None survived to metamorphosis at 1.94 ppm (mean time 7.5 days). Bent tails and slow swimming ability before death. Metamorphosis occurred more rapidly in treated frogs with decreased size and mass. Unclear from this study as to whether or not LOAEL is in terms of a.e..	E096423

4.1.2 Toxicity to Freshwater Invertebrates

Freshwater aquatic invertebrate toxicity data were used to assess potential indirect effects of glyphosate to the CRLF. Effects to freshwater invertebrates resulting from exposure to glyphosate have the potential to 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 in Sections 4.1.2.1 through 4.1.2.3.

4.1.2.1 Freshwater Invertebrates: Acute Exposure Studies

The acute toxicity endpoint for aquatic invertebrates is taken from the study on early fourth instar midge larvae, maintained in laboratory cultures. As with freshwater fish, many studies are available on formulations. Because the number of studies on formulations is so extensive, only a few of the studies are summarized here. The remainder of the studies are summarized in tables in **Appendix J**. One study (MRID 00162296) tested glyphosate technical, a glyphosate IPA formulation and the surfactant, POEA on the midge. The EC_{50}'s were: 53.2, 13.3 and 13 mg/L. The EC_{50}'s for the technical material and the formulation are expressed in terms of glyphosate acid equivalents. As with freshwater fish and amphibians, this study indicates that the increased toxicity of the formulations with the surfactant, POEA are probably due to the surfactant.

For formulations, as with freshwater fish, for terrestrial uses, the most conservative endpoint from all the active formulations was selected. For aquatic uses, the endpoint was selected from a study that was conducted with a formulation that is currently labeled for aquatic use.

Table 4.16. Freshwater Invertebrates Acute Toxicity for Technical Glyphosate*					
Species	**% a.i.***	**48-hour EC_{50} - LC_{50}/ NOAEC (mg a.e./L)*/ Slope**	**Toxicity Category[1]**	**MRID #/Year**	**Study Classification**
Midge (*Chironomus plumosus*)	96.7	LC_{50}: **53.2 (30.0 - 93.8)**[3] NOAEC: N.R. Slope: N.R.	Slightly toxic	00162296/1979	Acceptable
Water flea (*Daphnia magna*)	95.6	EC_{50}: 128.1 (95.6 - 172.1) NOAEC: 95.6 Slope: N.R.	Practically nontoxic	44320631/1995	Acceptable
Water flea (*Daphnia magna*)	83	EC_{50}: 647.4 (577.7 - 725.4) NOAEC: 464.8 Slope: N.R.	Practically nontoxic	00108172/1978	Acceptable

* No technical glyphosate salts were tested; a.i. = active ingredient; a.e. = acid equivalent, N.R. = not reported
[1]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[2] **Bolded** and shaded value will be used to calculate risk quotients
[3] Range is 95% confidence interval for endpoint

Table 4.17. Freshwater Invertebrates Acute Toxicity for Glyphosate Formulations						
Chemical	**Species**	**% a.i.***	**48-hour EC_{50} - LC_{50}/ NOAEC (mg a.e./L)*/ Slope**	**Toxicity Category[1]**	**MRID #/Year**	**Study Classification**
Glyphosate IPA (Roundup with POEA surfactant)	Water flea (*Daphnia magna*)	30.3	EC50: 2.2 (1.9 - 2.5); **formulation: 3** NOAEC: N.R. Slope: N.R.	Moderately toxic	00162296/1979	Acceptable
Glyphosate (360 g/L SL formulation)	Water flea (*Daphnia magna*)	27.25	EC50: 44.8 (38.0 - 52.0); **formulation: 164.3** NOAEC: 26 Slope: 7.6	Slightly toxic	45374003/1999	Acceptable

Table 4.17. Freshwater Invertebrates Acute Toxicity for Glyphosate Formulations						
Chemical	**Species**	**% a.i.***	**48-hour EC_{50} - LC_{50}/ NOAEC (mg a.e./L)*/ Slope**	**Toxicity Category[1]**	**MRID #/Year**	**Study Classification**
Glyphosate IPA (Roundup)	Water flea (*Daphnia magna*)	41.36	EC50: 1.6 (1.4 - 1.9)[2] NOAEC: 0.6 Slope: 5.4	Moderately toxic	00070893/1980	Acceptable
Glyphosate IPA (Roundup)	Crayfish (*Orconectes nais*)	30.3	LC50: 5.2 (4.1 - 6.4) NOAEC: N.R. Slope: N.R.	Moderately toxic	40098001/1986	Supplemental
Glyphosate IPA (Roundup)	Scud (*Gammarus pseudolimnaeus*)	31	LC50: 13 (9.6 - 19.2) NOAEC: 1.4 Slope: 2.33	Slightly toxic	00124762/1982	Supplemental
Glyphosate IPA (Roundup with POEA surfactant)	Midge (*Chironomus plumosus*)	30.3	LC50: 13.3 (7.0 - 23.7) NOAEC: N.R. Slope: N.R.	Slightly toxic	00162296/1979	Acceptable
Glyphosate IPA (no surfactant)	Water flea (*Daphnia magna*)	62.4	EC50: 401.3 (347.7 - 470.5) NOAEC: 147.8 Slope: 7.6	Practically nontoxic	00078663/1981	Acceptable
Glyphosate IPA with surfactant Geronol CF/AR	Water flea (*Daphnia carinata*)	36	EC50: 220 (194 – 252) (610 (540 - 700) mg formulation/L) NOAEC: 49 or 135 mg formulation/L Slope: N.R.	Practically nontoxic	44738201/1996	Not classified

* a.i. = active ingredient; a.e. = acid equivalent; IPA = isopropylamine salt
[1]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[2] Range is 95% confidence interval for endpoint
[3]**Bolded** and shaded value will be used to calculate risk quotients

Table 4.18. Freshwater Invertebrates Acute Toxicity for Surfactants Used with Glyphosate Formulations						
Chemical	**Species**	**% a.i.***	**48-hour EC_{50} - LC_{50}/ NOAEC (mg/L)/ Slope**	**Toxicity Category[1]**	**MRID #/Year**	**Study Classification**
Surfactant Geronol CF/AR (alkyl polyoxy ethylene phosphoric acid)	Daphnia (*Daphnia magna*)	Tech.	EC50: 48 NOAEC: Slope: N.A.	Slightly toxic	44738201/1996	Not classified
MON 0818 (POEA)	Midge (*Chironomus plumosus*)	100	LC50: 13 (7.1-24.0)[2] NOAEC: N.R. Slope: N.R.	Slightly toxic	00162296/1979	Acceptable

* a.i. = active ingredient, assumed 100% for technical.
[1]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[2] Range is 95% confidence interval for endpoint

The acute toxicity study with the water flea (Table 4.19) indicates that the degradate, aminomethyl phosphonic acid (AMPA) is less toxic to freshwater invertebrates than the parent glyphosate.

Table 4.19. Freshwater Invertebrates Acute Toxicity for Aminomethyl Phosphonic Acid (AMPA) Degradate of Glyphosate						
Chemical	**Species**	**% a.i.[1]**	**48-hour LC_{50}/NOAEC (mg/L)/Slope**	**Toxicity Category[2]**	**MRID #/Year**	**Study Classification**
AMPA	Water flea (*Daphnia magna*)	94.38	EC50: 683 (553 - 1010) NOAEC: 320 Slope: N.A.	Practically nontoxic	43334715/1994	Acceptable

[1] a.i. = active ingredient, assumed 100% for technical material
[2]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[3] Range is 95% confidence interval for endpoint, N.A. = not available

4.1.2.2 Freshwater Invertebrates: Chronic Exposure Studies

Table 4.20. Freshwater Invertebrates Chronic Toxicity for Technical Glyphosate IPA Salt				
Species	**% Active Ingredient**	**NOAEC/LOAEC (mg acid equivalent/L)**	**MRID #/Year**	**Study Classification**
Water flea (*Daphnia magna*)	99.7	**49.9**/95.7[1]	00124763/1982	Acceptable
[1]**Bold** value will be used to calculate risk quotients				

4.1.2.3 Freshwater Invertebrates: Open Literature Data

There are additional freshwater invertebrate toxicity data, including sublethal effects information, available in the open literature (for references and other details see **Appendices G and H**). None of the toxicological endpoints identified in the open literature studies are more sensitive than the most sensitive acute and chronic endpoints available in the submitted studies (see Sections 4.1.2.1 – 4.1.2.2).

4.1.3 Toxicity to Aquatic Plants

Aquatic plant toxicity studies were used as one of the measures of effect to evaluate whether or not glyphosate has the potential to affect primary production and the availability of aquatic plants as food for CRLF tadpoles. Primary productivity is essential for indirectly supporting the growth and abundance of the CRLF.

Two types of studies were used to evaluate the potential of glyphosate to affect aquatic plants. Laboratory and field studies were used to determine whether or not glyphosate has the potential to cause direct effects to aquatic plants. A tabular summary of the laboratory data and freshwater field studies for aquatic plants is provided in Sections 4.1.3.1 and 4.1.4.

4.1.3.1 Aquatic Plants: Laboratory Data

For aquatic vascular plants, the endpoint is selected from a duckweed study (MRID 44320638). This study does not fulfill guideline requirements because it needs phytotoxicity data; however, this is a 14-day study and it has a lower EC_{50} value than any of the other studies. Therefore, this study is selected for the vascular plant endpoint. For aquatic non-vascular plants, the endpoint is selected from a toxicity study on green algae (MRID 40236901). This study appears to have fewer uncertainties than MRID 40236904. Therefore, the endpoint is selected from this study. Again, as with other aquatic species, some of the formulations appear to be more toxic than the technical material.

Table 4.21. Aquatic Vascular and Nonvascular Freshwater Plant Toxicity Studies for Technical Glyphosate				
Species	**% Active Ingredient***	**EC_{50} NOAEC (mg a.e./L)*/ Slope**	**MRID #/Year**	**Study Classification**
Vascular Plants				
Duckweed (*Lemna gibba*)	95.6	14-day EC50: **11.9 (9.4-14.9)** NOAEC: 1.3 Slope: N.R.	44320638/1996	Supplemental
Duckweed (*Lemna gibba*)	96.8	7-day EC50: 23.2 (20.3 - 27.1) NOAEC: 7.3 Slope: 2.91	45773101/2002	Acceptable
Duckweed (*Lemna gibba*)	96.6	14-day EC50: 20.8 (N.R.) NOAEC: <1.8 Slope: N.R.	40236905/1987	Acceptable
Non-vascular Plants				
Green algae (*Selenastrum capricornutum*)	96.6	4-day EC50: **12.1 (11.5 - 12.9)** NOAEC: N.R. Slope: 12	40236901/1987	Acceptable
Bluegreen algae (*Anabaena flos-aquae*)	96.6	4-day EC50: 11.4 (10.5 - 12.1) NOAEC: N.R. Slope: 3.53	40236904/1987	Acceptable
Green algae (*Selenastrum capricornutum*)	95.6	5-day EC50: 13.4 (9.6 - 19.1) NOAEC: 9.6 Slope: N.R.	44320637/1995	Acceptable
Bluegreen algae (*Anabaena flos-aquae*)	95.6	5-day EC50: 14.3 (9.3 - 25.8) NOAEC: 11.5 Slope: N.R.	44320639/1996	Acceptable
Freshwater diatom (*Navicula pelliculosa*)	95.6	5-day EC50: 16.3 (11.5 - 22.9) NOAEC: 1.7 Slope: N.R.	44320641/1996	Acceptable
Freshwater diatom (*Navicula pelliculosa*)	96.6	7-day EC50: 37.3 (34.8 - 41.5) NOAEC: 18.5 Slope: 5.87	40236902/1987	Acceptable

* a.i. = active ingredient; a.e. = acid equivalent; N.R. = Not reported
[1] Range is 95% confidence interval for endpoint
[2] **Bold** value will be used to calculate risk quotients

Table 4.22. Aquatic Vascular and Nonvascular Freshwater Plant Toxicity Studies for Glyphosate Formulations					
Chemical	**Species**	**% a.i.***	**EC_{50}/ NOAEC (mg a.e.*/L)/ Slope**	**MRID #/Year**	**Study Classification**
Vascular Plants					
Glyphosate IPA salt* (glyphos (glyphosate product))	Duckweed (*Lemna gibba*)	31.0	7-Day EC50: 7.7 (7.1 - 8.3) [1] Formulation: **25** NOAEC: 0.29 Slope: 4.76	45666704/2001	Acceptable
Glyphosate IPA salt (Roundup 41%)	Duckweed (*Lemna minor*)	30.3	14-day EC50: 1.5 (N.R.) ; for formulation: **4.9** NOAEC: N.R. Slope: N.R.	44125714/1984	Supplemental
Glyphosate IPA salt (TEP Roundup)	Duckweed (*Lemna minor*)	NR	48 hr. EC50: >16.91 (N.A.) NOAEC: 16.91 Slope: N.A.	44125713/1989	Supplemental
Glyphosate IPA salt (Roundup, % not reported)	Duckweed (*Lemna minor*)	N.R.	14-day EC50: 2.0 (N.R.) NOAEC: N.R. Slope: N.R.	44125714/1984	Supplemental
Nonvascular Plants					
Glyphosate monoammonium salt	Green algae (*Selenastrum capricornutum*)	68.5	72-hr EC50: 1.85 (1.3 - 2.3) NOAEC: 0.61 Slope: N.R.	45777403/1999	Supplemental
Glyphosate monoammonium salt	Green algae (*Selenastrum capricornutum*)	64.9	72-hr EC50: 11.2 (10 - 12.6) NOAEC: 1.58 Slope: N.R.	45767102/2002	Supplemental
Glyphosate IPA salt with surfactant Geronol CF/AR	Green algae (*Selenastrum capricornutum*)	36	72-hr EC50: 97 (85 - 111) NOAEC: 73 Slope: N.A.	44738201/1996	Supplemental
Glyphosate IPA salt with surfactant Geronol CF/AR	Green algae (*Selenastrum capricornutum*)	36	72-hr EC50: 39 (33 - 45) NOAEC: 16 Slope: N.A.	44738201/1996	Supplemental
Glyphosate (glyphos)	Freshwater diatom (*Navicula pelliculosa*)	31.0	96-hr EC50**: 0.12 (0.11 – 0.13)** [2]; for formulation: **0.39** NOAEC: 0.082 Slope: 8.78	45666701/2001	Acceptable
Glyphosate IPA salt (glyphos (glyphosate product))	Green algae (*Selenastrum capricornutum*)	31.0	96-hr EC50: 0.68 (0.57 - 0.81) NOAEC: 0.43 Slope: 4.47	45666702/2001	Acceptable

* a.i. = active ingredient; a.e. = acid equivalent; IPA = isopropylamine salt; NR = not reported; NA = not available
[1] Range is 95% confidence interval for endpoint
[2] **Bolded** and shaded value will be used to calculate risk quotients

Table 4.23. Aquatic Nonvascular Freshwater Plant Toxicity Studies on Glyphosate Mixtures					
Chemical	**Species**	**% a.i.***	**EC_{50}/ NOAEC (mg a.e.*/L)/ Slope**	**MRID #/Year**	**Study Classification**
Nonvascular Plants					
Glyphosate acid-equivalent (IPA)/Oxyfluorfen mix	Green algae (*Selenastrum capricornutum*)	32	96-hr EC50: 0.0026 (0.0021 – 0.0033)[1] NOAEC: 0.00045 Slope: 3.96	45906008/2001	Acceptable
* a.i. = active ingredient; a.e. = acid equivalent; IPA = isopropylamine salt; [1]Range is 95% confidence interval for endpoint					

4.1.3.2 Aquatic Plants: Open Literature Data

Three studies on 3 different species of green algae were conducted which provide lower 96-hr EC_{50}'s based on cell counts (growth) correlated with absorbance over time for 96 hours on a Shimadzu UV-2401 PC Spectrophotometer. All of these studies were performed by the same group of scientists and published in different papers. In the first study, conducted with 95% technical material (not stated if glyphosate or the IPA of glyphosate), the 96-hr EC_{50} was 3.530 mg/L for *Chlorella pyrenoidosa* (Ma et.al 2001, ECOTOX reference 61983). In the second study (Ma et al., 2002, ECOTOX reference 65938), the 96 hr. EC_{50} for *Chlorella vulgaris* was 4.70 mg/L. This was again conducted with a 95% technical product. The study authors used the CAS number for glyphosate, not IPA, so it is assumed that this is the acid. The third study, conducted with *Raphidocelis subcapitata (Selenastrum capricornutum)* (Ma et al., 2006, ECOTOX ref. 83543), the 96 hr. acute toxicity value is 5.56 mg/L. Again, the study was conducted with 95% technical product, which is presumed to be the glyphosate acid. The results from these studies are discussed and compared to the aquatic exposure values in the risk characterization section (Section 5.2.2.1).

4.1.4 Freshwater Field/Mesocosm Studies

A study was conducted to examine the effects of glyphosate on the biomass of predators, tadpoles/small herbivores, zooplankton and periphyton, the survival of predators, the abundance of zooplankton, and survival of tadpole species in mesocosm study units (1200L tanks (Relyea, ECOTOX ref. 89112)). A simulated application rate of 6.4 mL/m^2 with a 25.2% formulation was used, providing a nominal concentration of 3.8 mg a.i.L. Species used in the mesocosms were reported to be naturally co-occurring and at loading rates similar to what are found in the field. The study was conducted for 13 days under static conditions following a single spray application. Under the conditions tested, species richness was reduced by 22% with Roundup®. Roundup® completely eliminated two species of tadpoles (leopard frogs and gray tree frogs) and nearly eliminated wood frogs (98% mortality), resulting in a 70% decline in the species richness of tadpoles. It is not clear from the methods section which specific formulation of the pesticide was used; however, the study authors state that the formulation of glyphosate (Roundup) contains polyethoxylated tallowamine (POEA). Although Roundup appeared to be associated with a high mortality rate in amphibian larvae, amphibian mortality in controls ranged from approximately 30 to 80%. The relatively high mortality rate with control tadpole species was likely due to predation from spotted salamanders and predacious beetles; however, it is difficult to interpret glyphosate-related mortality given the extent of mortality in controls for some tadpole species. It is noteworthy that while increased mortality of amphibian larvae appeared to be associated with glyphosate treatment, red-spotted salmanders were not affected.

A study was conducted with glyphosate to determine whether or not glyphosate plus the surfactant, polyethoxylated tallow amine (POEA) affects survival of anurans, either in aquatic environments (mesocosms) and/or terrestrial environments (semi-dry tanks; Relyea, ECOTOX Ref. 86885). The pesticide was applied by a direct overspray. In an aquatic larvae study, a factorial combination of glyphosate present or absent with three different soil treatments (no soil, sand, and loam) was tested. The concentration of glyphosate was reportedly based on the label recommended application rate (i.e., a nominal concentration of 3.8 mg a.i./L (simulated application rate of 1.6 mL a.i./m^2)). Roundup® Weed and Grass Killer was tested (25.2% active ingredient plus POEA surfactant). For the terrestrial juvenile study, glyphosate with POEA surfactant was tested in comparison to a control. The nominal amount tested was 6.5 mL at a rate of 1.6 mg a.i./m^2. There were three replicates, each time with a different amphibian species.

The results of the study suggested that exposure to nominal concentrations of Roundup® Weed and Grass Killer at a rate equivalent to 1.6 mg a.i./m^2 (3.8 mg a.i./L) for 20 days, decreased survival of leopard frogs, American toads and gray tree frogs [aquatic phase] larvae by over 73%. American toad larvae were the most sensitive with only 20% survival followed by gray tree frog (50% survival) and leopard frog (75%) survival compared to controls with >80% survival). It is not clear whether the toxicity can be attributed to glyphosate alone, the surfactant polyethoxylated tallowamine (POEA) alone, or to the combination of glyphosate and POEA. Although the study suggests that presence of soil did not decrease the toxicity of Roundup®, it is also not clear whether the amount of soil added to each of the study units was adequate to test this hypothesis. Exposure of juvenile [terrestrial phase] wood frogs, tree frogs and American toads to Roundup at a rate of 1.6 mg a.i./m^2 resulted in over 64% decrease in survival across species after 24 hours. It is not clear how the terrestrial exposure of Roundup® to terrestrial-phase juvenile frogs relates to conditions that may exist in the field. The moist paper towel would likely prolong exposure beyond what may typically be encountered in the field.

A mesocosm study was conducted with a glyphosate formulation (13% a.i.) applied to 1,200L outdoor cattle troughs containing three aquatic-phase amphibian species (leopard frog, gray tree frog and the American toad) with and without predators (red-spotted newt or *Dytiscus* beetles). Exposure was static for 23 days (Relyea et. al, ECOTOX Ref. 86886). Although there was uncertainty associated with the application rates and the specific formulation used, study units were apparently treated at a nominal concentration of 1.3 mg glyphosate/L. Glyphosate treatment reduced overall tadpole survival and biomass. American toad larvae were the most sensitive with only 20% survival followed by gray tree frog (50% survival) and leopard frog (75%) survival compared to controls with >80% survival). Glyphosate had no effect on the survival of red-spotted newts. The study design is not sufficient to determine whether the decreased survival/biomass associated with exposure to Roundup is due to glyphosate or to some other component of the formulated product. While the study authors speculate on the potential role of the surfactant, polyethoxylated tallowamine (POEA), in causing the observed effects on anuran larvae, the study does not test this potential relationship.

Chemical and biological monitoring studies were conducted in 51 different wetlands to quantify the magnitude of contamination by glyphosate formulation Vision® (Thompson et. al, ECOTOX Ref. 72797). Wetlands were classified as over-sprayed, adjacent, or buffered in relation to the operational target spray blocks. Aqueous concentrations of glyphosate in buffered wetlands were below the level of detection (<0.02 mg a.i./L) in 14 of the 16 buffered wetlands. Mean glyphosate concentrations in the buffered wetlands (0.03 mg a.i./L) were significantly ($p<0.05$) less than that of either adjacent (0.18 mg a.i./L) or over-sprayed wetlands (0.33 mg a.i./L). Biomonitoring of caged amphibians larvae showed no significant effect on mean 48-hr mortality of either green leopard frogs (*Rana pipiens*) or green frogs (*R. clamitans*) exposed *in situ*. Percent mortality was not significantly correlated with exposure concentrations. The authors conclude that there were no statistically significant differences in mortality between treatment sites; however, leopard frog and green frog larvae had 14.2% and 35.6% mortality in over-sprayed areas. Buffered areas with the lowest mean concentrations (0.03 mg a.i./L) of glyphosate had larval mortality for leopard frog larvae (15%) and green frog larvae (25.7%) roughly similar to oversprayed areas. The authors conclude that glyphosate exposures typically occurring in forest wetlands are insufficient to induce significant acute mortality in native amphibian larvae. No raw data were included in the study; however, the results suggest that there was a large amount of variability that could have obscured detecting treatment effects especially given that these were naturally occurring wetlands that represented a range of environmental conditions. Additionally, since concentrations of the surfactant (MON0818) were not measured, it is uncertain as to the extent that this co-formulant was present in any of the aquatic habitats studied.

Open Literature Studies

Aquatic vascular plants

For most of the studies on vascular plants, there are insufficient details in the articles to accurately determine concentration levels tested. For other studies, the endpoints were higher than those found in the submitted studies.

Aquatic nonvascular plants

Of the available open literature studies from which data may be extracted for comparing the results with the submitted studies, 3 studies, on 3 different species of green algae provide lower 96-hr EC_{50}'s based on cell counts (growth) correlated with absorbance over time for 96 hours on a Shimadzu UV-2401 PC Spectrophotometer. All of these studies were performed by the same group of scientists and published in different papers.

The papers were not thoroughly reviewed for acceptability according to Agency guidelines; however, they are discussed in this section and compared to the highest aquatic EEC. In the first study, conducted with 95% technical material (not stated if glyphosate or the IPA of glyphosate), the 96-hr EC_{50} was 3.530 mg/L for *Chlorella pyrenoidosa* (Ma et.al 2001, ECOTOX reference 61983). Comparing that value to the highest EEC of 222.9 ppb, the RQ would be 0.06, significantly lower than the LOC for aquatic plants. In the second study (Ma et al., 2002, ECOTOX reference 65938), the 96 hr. EC_{50} for *Chlorella vulgaris* was 4.70 mg/L. This was again conducted with a 95% technical product. The study authors used the CAS number for glyphosate, not IPA, so it is assumed that this is the acid. The resulting highest RQ from this study would be 0.05. The third study, conducted with *Raphidocelis subcapitata (Selenastrum capricornutum)* (Ma et al., 2006, ECOTOX ref. 83543), the 96 hr. acute toxicity value is 5.56 mg/L with a resulting RQ of 0.04. Again, the study was conducted with 95% technical product, which is presumed to be the glyphosate acid. Even with these lower endpoints, the LOC for aquatic plants would not be exceeded.

4.2 Toxicity of Glyphosate to Terrestrial Organisms

Tables 4.24 and 4.25 summarize 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.

Table 4.24 Terrestrial Toxicity Profile for Glyphosate and/or Its Salts				
Endpoint	**Species**	**Toxicity Value Used in Risk Assessment**	**Citation MRID#/Date**	**Comment**
Acute Direct Toxicity to Terrestrial-Phase CRLF (LD_{50})	Bobwhite quail (*Colinus virginianus*)	LD_{50}: >1912 mg/kg bw	44320626/1997	Acceptable
Acute Direct Toxicity to Terrestrial-Phase CRLF (LC_{50})	Bobwhite quail (*Colinus virginianus*)	LC_{50}: >4971.2 PPM	44320628/1997	Acceptable
Chronic Direct Toxicity to Terrestrial-Phase CRLF	Bobwhite quail (*Colinus virginianus*)	Reproduction study NOAEC: 830 PPM	108207/1978	Acceptable LOAEC: >830 PPM (highest concentration tested).
Indirect Toxicity to Terrestrial-Phase CRLF (via acute toxicity to mammalian prey items)	Rat (*rattus norvegicus*)	LD_{50} >4800 mg/kg bw	43728003/1989	Acceptable
Indirect Toxicity to Terrestrial-Phase CRLF (via	Rat (*rattus norvegicus*)	NOAEL: 500 mg/kg bw/day; NOAEC: 10000 ppm	41621501/1990	Acceptable Reproduction study parental/pup LOAEL: 1500 mg/kg

Table 4.24 Terrestrial Toxicity Profile for Glyphosate and/or Its Salts				
Endpoint	**Species**	**Toxicity Value Used in Risk Assessment**	**Citation MRID#/Date**	**Comment**
chronic toxicity to mammalian prey items)				bw/day; LOAEC: 30000 ppm (soft stools, decreased body weight gain and food consumption in parents and decreased body weight gain during lactation in pups).
Indirect Toxicity to Terrestrial-Phase CRLF (via acute toxicity to terrestrial invertebrate prey items)	Honey bee (*Apis mellifera*)	48 hr LD_{50} (O): >100 µg/bee	00026489/1972	Acceptable
Indirect Toxicity to Terrestrial- and Aquatic-Phase CRLF (via toxicity to terrestrial plants)	Seedling Emergence Monocots	EC25: >5 LB/A	40159301/1987	Acceptable
	Seedling Emergence Dicots	EC25: > 5 LB/A	40159301/1987	Acceptable
	Vegetative Vigor Monocots	EC25**:** 0.16 LB/A	44125715/45045101/ 1995	Acceptable
	Vegetative Vigor Dicots	EC25: 0.074 LB/A	44320636/1996	Acceptable

For birds and mammals, the endpoints following acute exposure are not discrete and a quantitative estimate of risk could not be done. However, for registered formulation products, there is one avian study and 4 mammalian studies with discrete values. For estimation of risk, these studies were matched with the specific labeled rates and uses. Endpoints for these studies are summarized in **Table 4.25**.

Table 4.25 Terrestrial Toxicity Profile for Glyphosate Formulations				
Endpoint	**Species**	**Toxicity Value Used in Risk Assessment**	**Citation MRID#/Date**	**Comment**
Acute Direct Toxicity to Terrestrial-Phase CRLF (LD_{50})	Bobwhite quail (*Colinus virginianus*)	LD_{50}: 1651mg/kg bw	45777402/1999	Acceptable Glyphosate monoammonium salt (MON 14420)
Indirect Toxicity to Terrestrial-Phase CRLF (via acute toxicity to	Rat (*rattus norvegicus*)	LD_{50}: 3750 mg/kg bw	41305404/1989	Acceptable

Table 4.25 Terrestrial Toxicity Profile for Glyphosate Formulations				
Endpoint	**Species**	**Toxicity Value Used in Risk Assessment**	**Citation MRID#/Date**	**Comment**
mammalian prey items)	Rat (*rattus norvegicus*)	LD_{50}: 5000 mg/kg bw	41142304/1989	Acceptable
	Rat (*rattus norvegicus*)	LD_{50}: 5827 mg/kg bw	44615502/1998	Acceptable
	Rat (*rattus norvegicus*)	LD_{50}: 3803 mg/kg bw	44918601/1999	Acceptable

Acute toxicity to terrestrial animals is categorized using the classification system shown in **Table 4.4** (U.S. EPA, 2004). Toxicity categories for terrestrial plants have not been defined.

Table 4.26 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 glyphosate; therefore, acute and chronic avian toxicity data are used to assess the potential direct effects of glyphosate to terrestrial-phase CRLFs.

4.2.1.1 Birds: Acute Exposure (Mortality) Studies

Acute toxicity data on selected avian species are available for technical glyphosate, several formulations and the AMPA degradate. Based on the available studies, glyphosate is at the most, only slightly toxic. It does not appear that the formulations are any more toxic than the technical material. The AMPA degradate is not more toxic than the parent either. **Tables 4.27 – 4.29** summarize these studies.

Table 4.27. Avian Acute Toxicity for Technical Glyphosate						
Chemical	**Species**	**% a.i.[1]**	**LD_{50}/ LC_{50} NOAEL/ NOAEC (mg a.e./kg bw or ppm a.e.)[1]**	**Toxicity Category[2]**	**MRID #/Year**	**Study Classification**
Glyphosate	Bobwhite quail (*Colinus virginianus*)	83	LD50: **>3196** mg a.e./kg bw	Slightly toxic	00108204	Acceptable No treatment-related mortalities.
Glyphosate	Mallard duck (*Anas platyrhynchos*)	98.5	LC50: >4570 (N.A.) PPM NOAEC: 4570.4	Slightly toxic	108107/37765/1973	Acceptable No mortalities at any concentration
Glyphosate	Bobwhite quail (*Colinus virginianus*)	98.5	LC50: >4570 (N.R.) PPM NOAEC: 4570	Slightly toxic	00076492/1973	Acceptable No mortalities at any concentration
Glyphosate	Bobwhite quail (*Colinus virginianus*)	95.6	LD50: >1912 (N.A.) mg/kg bw NOAEL: 1912	Slightly toxic	44320626/1997	Acceptable No mortalities at any dose
Glyphosate	Mallard duck (*Anas platyrhynchos*)	95.6	LC50: >4971 (N.A.) PPM NOAEC: 4971.2	Slightly toxic	44320627/1998	Acceptable No mortalities at any concentration
Glyphosate	Bobwhite quail (*Colinus virginianus*)	95.6	LC50: **>4971** (N.A.) PPM NOAEC: 4971.2	Slightly toxic	44320628/1997	Acceptable No mortalities at any concentration

[1] a.i. = active ingredient; a.e. = acid equivalent
[2]Based on LC_{50} (ppm): < 50 very highly toxic; 50 - 500 highly toxic; 501 - 1000 moderately toxic; 1001-5000 slightly toxic; >5000 practically non-toxic; based on LD_{50} (mg/kg bw): < 10 very highly toxic; 10 - 50 highly toxic; 51 - 500 moderately toxic; 501-2000 slightly toxic; >2000 practically non-toxic
[3] Range is 95% confidence interval for endpoint, N.A. = not available, N.R. = not reported
[4] **Bolded** and shaded value will be used to calculate risk quotients.

Table 4.28 Avian Acute Toxicity for Glyphosate Formulations						
Chemical	**Species**	**% a.i.[1]**	**LD_{50}/ LC_{50} NOAEL/ NOAEC (mg a.e./kg bw or ppm a.e.)[1]**	**Toxicity Category[2]**	**MRID #/Year**	**Study Classification**
Trisodium diglyphosate/Urea (Polado formula; MON 8000)	Bobwhite quail (*Colinus virginianus*)	75	LD50: >780 (N.R.) PPM NOAEC: 780	Slightly toxic	00085638/1980	Supplemental
Trisodium diglyphosate/Urea (Polado formula; MON 8000)	Bobwhite quail (*Colinus virginianus*)	75	LC50: >1770 (N.R.) PPM NOAEC: 1770	Slightly toxic	00085639/1981	Supplemental
Trisodium diglyphosate/Urea (Polado formula; MON 8000)	Mallard duck (*Anas platyrhynchos*)	75	LC50: >1770 (N.R.) PPM NOAEC: 315	Slightly toxic	00085640/1980	Supplemental
Glyphosate monoammonium salt (MON 14420)	Bobwhite quail (*Colinus virginianus*)	68.5	LD50: 1131 (925 - 1541) mg/kg bw (**1651** mg formulation/ kg bw)[4] NOAEL: 333	Slightly toxic	45777402/1999	Acceptable
Glyphosate isopropylamine salt (MON65005)	Mallard duck (*Anas platyrhynchos*)	31.32	LC50> 1760 (N.A.) PPM NOAEC: 1760	Slightly toxic	44465701/1997	Acceptable
Glyphosate isopropylamine salt (MON65005)	Bobwhite quail (*Colinus virginianus*)	31.32	LC50> 1760 (N.A.) PPM NOAEC: 1760	Slightly toxic	44465702/1997	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent
[2]Based on LC_{50} (ppm): < 50 very highly toxic; 50 - 500 highly toxic; 501 - 1000 moderately toxic; 1001-5000 slightly toxic; >5000 practically non-toxic; based on LD_{50} (mg/kg bw): < 10 very highly toxic; 10 - 50 highly toxic; 51 - 500 moderately toxic; 501-2000 slightly toxic; >2000 practically non-toxic
[3] Range is 95% confidence interval for endpoint, N.A. = not available, N.R. = not reported
[4] **Bolded** and shaded value will be used to calculate risk quotients.

Table 4.29. Avian Acute Toxicity for Aminomethyl Phosphonic Acid (AMPA) Degradate of Glyphosate						
Chemical	**Species**	**% a.i.[1]**	**LD_{50}/ LC_{50} NOAEL/ NOAEC (mg a.e./kg bw or ppm a.e.)/ Slope[1]**	**Toxicity Category[2]**	**MRID #/Year**	**Study Classification**
AMPA	Bobwhite quail (*Colinus virginianus*)	87.8	LD50: >1976 (N.A.) mg/kg NOAEL: 1185 Slope: N.A.	Slightly toxic	43334709/1991	Acceptable
AMPA	Bobwhite quail (*Colinus virginianus*)	87.8	LC50: >4934 (N.A.) PPM NOAEC: 4934 Slope: N.A.	Slightly toxic	43334710/1994	Acceptable
AMPA	Mallard duck (*Anas platyrhynchos*)	87.8	LC50: >4934 (N.A.) PPM NOAEC: 4934 Slope: N.A.	Slightly toxic	43334711/1994	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent
[2]Based on LC_{50} (ppm): < 50 very highly toxic; 50 - 500 highly toxic; 501 - 1000 moderately toxic; 1001-5000 slightly toxic; >5000 practically non-toxic; based on LD_{50} (mg/kg bw): < 10 very highly toxic; 10 - 50 highly toxic; 51 - 500 moderately toxic; 501-2000 slightly toxic; >2000 practically non-toxic
[4] Range is 95% confidence interval for endpoint, N.A. = not available

4.2.1.2 Birds: Chronic Exposure (Growth, Reproduction) Studies

Neither reproductive effects nor effects on growth were observed following chronic exposure to either mallards or bobwhite quail.

Table 4.30. Avian Chronic Toxicity for Technical Glyphosate						
Chemical	**Species**	**% a.i.[1]**	**LD_{50}/ LC_{50} NOAEL/ NOAEC (mg a.e./kg bw or ppm a.e.)[1]**	**Toxicity Category[2]**	**MRID #/Year**	**Study Classification**
Glyphosate	Mallard duck (*Anas platyrhynchos*)	90.4	LOAEC: >27 (N.A.) PPM NOAEC: 27	N.A	00036328/113457/1975	Supplemental
Glyphosate	Mallard duck (*Anas platyrhynchos*)	83	LOAEC: >830 (N.A.) PPM NOAEC: 830	N.A.	111953/1978	Acceptable
Glyphosate	Bobwhite quail (*Colinus virginianus*)	83	LOAEC: >830 (N.A.) PPM NOAEC: **830**	N.A.	108207/1978	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent
[2] Range is 95% confidence interval for endpoint, N.A. = not applicable
[3] **Bolded** shaded value will be used to calculate risk quotients.

4.2.1.3 Birds: Open Literature Data

There are additional avian toxicity data, including sublethal effects information, available in the open literature (for details see **Appendix H**). None of the toxicological endpoints identified in the open literature studies are more sensitive than the most sensitive acute and chronic endpoints available in the submitted avian toxicity studies (see Sections 4.2.1.1 – 4.2.1.2).

There was one subchronic study on the effects of the formulation, Roundup "(360 g/l of glyphosate, 480 g/l of IPA salt and 684 g/l of other inert ingredients)" on the epididymal region of drakes (*Anas platyrhynchos*) (Oliveira et. al. 2007, ECOTOX Reference No. 97136). The formulation was administered by gavage to three groups of 6 adult drakes for 15 days at 0 (distilled water), 5 and 100 mg/kg bw. There was a significant reduction (90%, $p \leq 0.05$) in plasma testosterone levels after treatment at both dose levels when compared to the control group. The report stated that "alterations in the structure of the testis and epididymal region…with changes in the expression of androgen receptors restricted to the testis" were observed. The authors also stated that "the effects were mostly dose dependent, indicating that this herbicide may cause disorder in the morphophysiology of the male genital system of animals". Further studies would be needed to determine whether or not these observed effects would affect avian (or, in this case, terrestrial-phase amphibian) reproduction.

4.2.1.4 Terrestrial-phase Amphibian Acute and Chronic Studies

No toxicity studies on glyphosate are available for terrestrial-phase amphibians.

4.2.2 Toxicity to Mammals

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

4.2.2.1 Mammals: Acute Exposure (Mortality) Studies

The acute toxicity studies on the technical material indicate that glyphosate is practically non-toxic to mammals. Hundreds of studies are available on formulations. Most of the LD_{50}'s are greater than the highest dose tested. Only a small sample of the studies on the formulations is presented here. The rest of the studies are presented in **Appendix J**.

Table 4.31. Mammalian Acute Toxicity for Technical Glyphosate						
Chemical	**Species**	**% a.i.[1]**	**LD_{50} (mg a.e./kg bw)[1]**	**Toxicity Category[2]**	**MRID No.**	**Study Classification**
Glyphosate	Rat (*Rattus norvegicus*)	96	**>4800**	Practically non-toxic	43728003	Acceptable
Glyphosate	Rat (*Rattus norvegicus*)	95	>4750	Practically non-toxic	45058306	Acceptable
Glyphosate	Rat (*Rattus norvegicus*)	97.2	>4860 up and down	Practically non-toxic	46760505	Acceptable
Glyphosate	Rat (*Rattus norvegicus*)	88	>4400	Practically non-toxic	44320604	Acceptable
Glyphosate	Rat (*Rattus norvegicus*)	95	>4750 up and down	Practically non-toxic	46998805	Acceptable
Glyphosate	Rat (*Rattus norvegicus*)	76	>3800	Practically non-toxic	41400601	Acceptable
Glyphosate	Rat (*Rattus norvegicus*)	96	>1920	Slightly toxic or less	44142104	Acceptable
Glyphosate	Rat (*Rattus norvegicus*)	95.4	>4770 up and down	Practically non-toxic	46816107	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent
[2]Based on LD_{50} (mg/kg bw): < 10 very highly toxic; 10 - 50 highly toxic; 51 - 500 moderately toxic; 501-2000 slightly toxic; >2000 practically non-toxic.
[3] **Bolded** shaded value will be used to calculate risk quotients.

Table 4.32 Mammalian Acute Toxicity for Glyphosate Formulations						
Chemical	**Species**	**% a.i.[1]**	**LD_{50} (mg a.e./kg bw a.e.)[1]**	**Toxicity Category[2]**	**MRID No.**	**Study Classification**
HM-2028 (Glyphosate: 11.4%)	Rat (*Rattus attus norvegicus*)	11.4	357	Moderately toxic when reported as a.e. due to low percentage of a.i.	46714802	Acceptable – **not registered in California**
MON 20033	Rat (*rattus norvegicus*)	63	3150 (**5000** mg formulation/ kg)	Practically nontoxic	41142304	Acceptable
MON 77063	Rat (*rattus norvegicus*)	65.4	2599 (**5827** mg formulation/ kg)	Practically nontoxic	44615502	Acceptable

Table 4.32 Mammalian Acute Toxicity for Glyphosate Formulations						
Chemical	**Species**	**% a.i.[1]**	**LD_{50} (mg a.e./kg bw a.e.)[1]**	**Toxicity Category[2]**	**MRID No.**	**Study Classification**
Glyphosate IPA	Rat (*Rattus norvegicus*)	22.9	724 (**3803** mg formulation/ kg)	Slightly toxic	44918601	Acceptable
MON 20047	Rat (*rattus norvegicus*)	18.4	460 – 690 (**3750** mg formulation/ kg)	Moderately toxic when reported as a.e. due to low percentage of a.i.	41305404	Acceptable
ClearOut 41 (41% glyphosate IPA)	Rat (*Rattus norvegicus*)	30.3	>606	Slightly toxic	44883104	Acceptable
Clearout 62 (62% glyphosate IPA)	Rat (*Rattus norvegicus*)	62	>1240	Slightly toxic	45657801	Acceptable
GF-1667 (62.1% glyphosate dimethylammonium salt)	Rat (*Rattus norvegicus*)	49	>2450	Practically nontoxic	46730705	Acceptable
HM-0548 5905-LTE Mixture of ammonium salt (19.68%) and IPA (13.36%)	Rat (*Rattus norvegicus*)	25	>1250	Slightly toxic	47236803	Acceptable
MON 60696 (70.1% monoammonium salt)	Rat (*Rattus norvegicus*)	54	>2700	Practically nontoxic	43049302	Acceptable
MON 78634 (71.8% ammonium salt)	Rat (*Rattus norvegicus*)	65.2	>1304	Slightly toxic	46087001	Acceptable
Nufarm RUP0532 (41% Glyphosate as IPA and ammonium salts)	Rat (*Rattus norvegicus*)	30.3	>1515	Slightly toxic	45386802	Acceptable
56077-LL - Phoss-8	Rat (*Rattus norvegicus*)	80	>4000	Practically nontoxic	45044402	Acceptable
Roundup L&G Ready to Use (glyphosate IPA)	Rat (*Rattus norvegicus*)	0.85	>40	Highly toxic when reported as a.e. due to low percentage of a.i.	41395601	Acceptable
Spray–Charlie (44% GLY-41 (524-475 with 41% IPA)	Rat (*Rattus norvegicus*)	15.2	>760	Slightly toxic	45929403	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent
[2]Based on LD_{50} (mg/kg bw): < 10 very highly toxic; 10 - 50 highly toxic; 51 - 500 moderately toxic; 501-2000 slightly toxic; >2000 practically non-toxic.
[3] **Bolded** shaded value will be used to calculate risk quotients.

4.2.2.2 Mammals: Chronic Exposure (Growth, Reproduction) Studies

The chronic mammalian endpoint is selected from a 2-generation reproduction study in the rat. In this dietary study, the parental/systemic NOAEL is 500 mg/kg/day in both sexes and the LOAEL is 1500 mg/kg/day based on soft stools, decreased body weight gain and food consumption. The reproductive NOAEL is ≥ 1500 mg/kg/day (HDT) in both sexes. The offspring NOAEL is 500 mg/kg/day in both sexes with a LOAEL of 1500 mg/kg/day based on decreased body weight gain during lactation.

There is a lower endpoint based on maternal mortality in the rabbit developmental toxicity study. The maternal NOAEL is 175 mg/kg bw/day and the maternal LOAEL is 350 mg/kg/day based on mortality, diarrhea, soft stools, and nasal discharge. The chronic mammalian endpoint was not selected from this study because it is believed that the effects may be acid effects from glyphosate acid, administered as a bolus dose by gavage. It may not occur through the diet with mammals. Several of the deaths were due to gastroenteritis and/or caecal ulcerations. Similar effects (stomach hemorrhages) were observed in the rat developmental toxicity study at higher dose levels.

Table 4.33. Mammalian Chronic Toxicity for Technical Glyphosate					
Chemical	**Species**	**% a.i.[1]**	**NOAEL/ NOAEC (mg a.e./kg bw or ppm a.e.)[1]**	**MRID #/Year**	**Study Classification**
Glyphosate	Rat (*rattus norvegicus*)	97.67	2-generation reproduction study **Parental/Systemic NOAEL: 500 mg/kg/day (10,000 ppm) LOAEL: 1500 mg/kg/day (30,000 ppm)** Reproductive NOAEL: 1500 mg/kg/day (HDT) **Offspring NOAEL: 500 mg/kg/day (10,000 ppm) LOAEL: 1500 mg/kg/day**	41621501/1990	Acceptable
Glyphosate	Rat (*rattus norvegicus*)	100%	3-generation reproduction study Parental/Systemic, Offspring and Reproductive NOAELs: 30 mg/kg/day (highest dose tested).	00081674; 00105995 1981; 1982	Acceptable
Glyphosate	Rabbit (*Oryctolagus cuniculus*)	98.7	Developmental toxicity study Maternal NOAEL = 175 mg/kg/day LOAEL = 350 mg/kg/day based on mortality, diarrhea, soft stools, and nasal discharge. Developmental NOAEL = 350 mg/kg/day (HDT) LOAEL = not established.	00046363/1980	Acceptable
Glyphosate	Rat (*rattus norvegicus*)	98.7	Maternal NOAEL = 1000 mg/kg/day LOAEL = 3500 mg/kg/day based on inactivity, mortality, stomach hemorrhages and reduced body weight gain. Developmental NOAEL = 1000 mg/kg/day LOAEL = 3500 mg/kg/day based on increased incidence in the number of fetuses and litters with unossified sternebrae and decreased fetal body weight.	00046362/1980	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent
[2] Range is 95% confidence interval for endpoint, N.A. = not applicable
[3] **Bolded** shaded value will be used to calculate risk quotients.

4.2.3 Toxicity to Terrestrial Invertebrates

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

4.2.3.1 Terrestrial Invertebrates: Acute Exposure (Mortality) Studies

Studies on terrestrial invertebrates are available on both the technical material and on formulations. The studies indicate that glyphosate does not appear to be very toxic to terrestrial invertebrates. The formulations do not appear to be more toxic than the technical material.

Table 4.34. Acute Toxicity Studies on Terrestrial Invertebrates for Technical Glyphosate

Chemical	Species	% a.i.[1]	LD_{50}/ LC_{50} NOAEL/ NOAEC	MRID #/Year	Study Classification
Glyphosate	Honey bee (*Apis mellifera*)	98.5	48 hr LD50 (O): **>100** (N.R.)[2] **µg/bee**[3] NOAEL: N.R. Slope: N.R.	00026489/1972	Acceptable
Glyphosate	Honey bee (*Apis mellifera*)	98.5	48 hr LD50 (C): >100 (N.R.) µg/bee NOAEL: N.R. Slope: N.R.	00026489/1972	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent
[2] Range is 95% confidence interval for endpoint, N.R. = not reported; O = oral study; C = contact study
[3] **Bolded** shaded value will be used to calculate risk quotients.

Table 4.35. Acute Toxicity Studies on Terrestrial Invertebrates for Glyphosate Formulations

Chemical	Species	% a.i.[1]	LD_{50}/ LC_{50} NOAEL/ NOAEC	MRID #/Year	Study Classification
Glyphosate monoammonium salt (MON78568)	Honey bee (*Apis mellifera*)	65.6	48 hr LD50 (C): >100 (N.A.)[2] µg/bee NOAEL: 100 Slope: N.R.	45767104/2001	Not classified
Glyphosate monoammonium salt (MON78568)	Honey bee (*Apis mellifera*)	65.6	48 hr LD50 (O): >76.23 (N.A.) µg a.e./bee NOAEL: <76.23 µg a.e./bee Slope: N.R.	45767104/2001	Not classified

Table 4.35. Acute Toxicity Studies on Terrestrial Invertebrates for Glyphosate Formulations					
Chemical	**Species**	**% a.i.[1]**	**LD_{50}/ LC_{50} NOAEL/ NOAEC**	**MRID #/Year**	**Study Classification**
Glyphosate monoammonium salt (MON78568)	Predatory mite (*Typhlodromus pyri*)	64.9	7 D LD50 (C): 1200 (839-1786) g a.e./ha (1.1 lb/A) NOAEL: 216 Slope: N.R.	45767105/2002	Not classified
Glyphosate monoammonium salt (MON78568)	Predatory mite (*Typhlodromus pyri*)	64.9	7 D LD50 (C): >4320 (N.R.) g/ha (>3.85 lb/A) NOAEL: 216 Slope: N.R.	45767106/2002	Not classified
Glyphosate monoammonium salt (MON78568)	Predatory mite (*Typhlodromus pyri*)	64.9	14 - 21 D LD50 (C): N.A. (N.A.) g/ha NOAEL: 216 or <119 (no dose-response) (<0.11 lb/A) Slope: N.A.	45767106/2002	Not classified
Glyphosate monoammonium salt (MON78568)	Earthworm (*Eisenia fetida*)	64.9	14 D LD50 (C): >6560 (N.A.) mg/kg soil NOAEL: 6560 Slope: N.R.	45767109/2001	Not classified
Glyphosate monoammonium salt (MON78568)	Parasitic wasp (*Aphidius rhopalosiphi*)	64.9	48 hr - 13 days LD50 (C): >108 (N.R.) g a.e./ha (>0.096 lb/A) NOAEL: Not established Slope: N.R.	45767107/2002	Not classified
Glyphosate monoammonium salt (MON78568)	Parasitic wasp (*Aphidius rhopalosiphi*)	64.9	48 hr - 13 days LD50 (C): >4320 (N.R.) g/ha (>3.86 lb/A) NOAEL: 4320 Slope: N.R.	45767107/2002	Not classified
Glyphosate monoammonium salt (MON78568)	Parasitic wasp (*Aphidius rhopalosiphi*)	64.9	48 hr - 13 days LD50 (C): >4320 (N.R.) g a.e./ha (>3.86 lb/A) NOAEL: 4320 Slope: N.R.	45767108/2002	Not classified
Glyphosate monoammonium salt (MON78568)	Lacewing (*Chrysoperla carnia*)	64.9	Up to 10 days LD50 (C): >4320 (N.R.) g/ha (>3.86 lb/A) NOAEC: 4320 Slope: N.R.	45767110/2002	Not classified
Glyphosate IPA salt (MON 2139)	Honey bee (*Apis mellifera*)	36	48 hr LD50 (O): >100 (N.R.) µg/bee NOAEL: N.R. Slope: N.R.	00026489/1972	Acceptable
Glyphosate IPA salt (MON 2139)	Honey bee (*Apis mellifera*)	36	48 hr LD50 (C): >100 (N.R.) µg/bee NOAEL: N.R. Slope: N.R.	00026489/1972	Acceptable

Table 4.35. Acute Toxicity Studies on Terrestrial Invertebrates for Glyphosate Formulations					
Chemical	**Species**	**% a.i.**[1]	**LD_{50}/ LC_{50} NOAEL/ NOAEC**	**MRID #/Year**	**Study Classification**
Glyphosate IPA salt (MON65005)	Honey bee (*Apis mellifera*)	31.32	48 hr LD50 (C): >31.3 (N.A.) µg a.e./bee NOAEL: 319 Slope: N.A.	44465703/1997	Acceptable
Glyphosate IPA salt (MON 77360)	Honey bee (*Apis mellifera*)	30.0	48 hr LD50 (C): >30 (NA) µg/bee NOAEL: 30 Slope: NA	45370301/2001	Acceptable
Glyphosate IPA salt (MON 77360)	Honey bee (*Apis mellifera*)	30.0	48 hr LD50 (O): >30 (NA) µg/bee NOAEL: 15 Slope: NA	45370302/2001	Supplemental

[1] a.i. = active ingredient; a.e. = acid equivalent/ IPA = isopropylamine; N.R. = not reported; O = oral study; C = contact study
[2] Range is 95% confidence interval for endpoint,

4.2.3.2 Terrestrial Invertebrates: Open Literature Studies

Open literature data on glyphosate, its salts and/or formulations included a large number of efficacy studies which were not useful for a quantitative assessment of risk. Those studies which could possibly be used were either tested at lower concentrations than the submitted studies with no effects or insufficient information was provided on the test material to determine the concentration levels tested for either the active ingredient and/or the glyphosate acid equivalent.

4.2.4 Toxicity to Terrestrial Plants

Terrestrial plant toxicity data are used to evaluate the potential for glyphosate to affect riparian zone and upland vegetation within the action area for the CRLF. Impacts to riparian and upland (i.e., grassland, woodland) vegetation have the potential to 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.

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

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

The results of the Tier II seedling emergence and vegetative vigor toxicity tests on non-target plants are summarized below in **Tables 4.36 and 4.37.**

Table 4.36 Vegetative Vigor Study on Terrestrial Plants with Technical Glyphosate

Chemical	Species	% a.i.[1]	EC_{25}/ NOAEC (EC_{05}) (lbs a.e./Acre[1]	MRID #/Year	Study Classification
Monocots					
Glyphosate	Oat (*Avena sativa*)	96.6	21 D EC25: 0.4 (N.R.) LB/A NOAEC/EC05: 0.14 Slope: 2.3	43088701/1994	Acceptable
Glyphosate	Corn (*Zea mays*)	96.6	21 D EC25: 0.43 (N.R.) LB/A NOAEC/EC05: 0.07 Slope: 3.7	43088701/1994	Acceptable
Glyphosate	Onion (*Allium cepa*)	96.6	21 D EC25: 0.83 (N.R.) LB/A NOAEC/EC05: 0.56 Slope: 2.4	43088701/1994	Acceptable
Glyphosate	Ryegrass (*Lolium perenne*)	96.6	21 D EC25: 0.98 (N.R.) LB/A NOAEC/EC05: 0.56 Slope: 4.9	43088701/1994	Acceptable
Dicots					
Glyphosate	Tomato (*Lycopersicon esculentum*)	96.6	21 D EC25: 0.11 (N.R.) LB/A NOAEC/EC05: 0.035 Slope: 3.4	43088701/1994	Acceptable
Glyphosate	Cucumber (*Cucumis sativus*)	96.6	21 D EC25: 0.46 (N.R.) LB/A NOAEC/EC05: 0.14 Slope: 2.6	43088701/1994	Acceptable
Glyphosate	Lettuce (*Lactuca sativa*)	96.6	21 D EC25: 0.4 (N.R.) LB/A NOAEC/EC05: 0.28 Slope: N.R.	43088701/1994	Acceptable
Glyphosate	Cabbage (*Brassica oleracea*)	96.6	21 D EC25: 0.3 (N.R.) LB/A NOAEC/EC05: 0.14 Slope: N.R.	43088701/1994	Acceptable
Glyphosate	Soybean (*Glycine max*)	96.6	21 D EC25: 0.42 (N.R.) LB/A NOAEC/EC05: 0.28 Slope: N.R.	43088701/1994	Acceptable

Table 4.36 Vegetative Vigor Study on Terrestrial Plants with Technical Glyphosate					
Chemical	**Species**	**% a.i.**[1]	**EC_{25}/ NOAEC (EC_{05}) (lbs a.e./Acre**[1]	**MRID #/Year**	**Study Classification**
Glyphosate	Radish (*Rhaphanus sativus*)	96.6	21 D EC25: 0.14 (N.R.) LB/A NOAEC/EC05: 0.035 Slope: N.R.	43088701/1994	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent; N.R. = Not reported
[2] **Bold** value will be used to calculate risk quotients.

Studies on Formulations

Table 4.37 Terrestrial Plant Studies with Glyphosate Formulations					
Chemical	**Species**	**% a.i.**[1]	**EC_{25}/ NOAEC (EC_{05}) (lbs a.e./Acre**[1]	**MRID #/Year**	**Study Classification**
Seedling Emergence Studies					
Monocots					
Glyphosate(80WDG formulation)	Veg.Crops(10 Sp.) (Monocots & Dicots)	75	29 D EC25: >4.5 (N.R.) NOAEC/EC05: 3.6 Slope: N.R.	44125712/1996	Acceptable
Glyphosate IPA salt CP-70139	Oat (*Avena sativa*), Rice (*Oryza sativa*), Sorghum (*Sorghum bicolor*), Barnyard grass (*Echinochloa crusgalli*)	50	14 D EC25: >5 (N.R.) NOAEC/EC05: N.R. Slope: N.R.	40159301/1987	Acceptable
Glyphosate(80WDG formulation)	Veg.Crops(10 Sp.) (Monocots & Dicots)	48.3	4WKS EC25: >4 (N.A.) NOAEC/EC05: 4 Slope: N.A.	44320635/1996	Acceptable
Dicots					
Glyphosate(80WDG formulation)	Veg.Crops(10 Sp.) (Monocots & Dicots)	75	29 D EC25: >4.5 (N.R.) NOAEC/EC05: 3.6 Slope: N.R.	44125712/1996	Acceptable
Glyphosate IPAsalt CP-70139	Soybean, Sugarbeet, Buckwheat, Cocklebur, Crabgrass, Panicum grass, Downy brome, Velvetleaf, Smartweed, Morning glory, Lambsquarter, Hemp	50	14 D EC25: >5 (N.R.) NOAEC/EC05: N.R. Slope: N.R.	40159301/1987	Acceptable
Glyphosate(80WDG formulation)	Veg.Crops(10 Sp.) (Monocots & Dicots)	48.3	4WKS EC25: >4 (N.A.) NOAEC/EC05: 4 Slope: N.A.	44320635/1996	Acceptable
Vegetative Vigor Studies					
Monocots					

Table 4.37 Terrestrial Plant Studies with Glyphosate Formulations					
Chemical	**Species**	**% a.i.[1]**	**EC_{25}/ NOAEC (EC_{05}) (lbs a.e./Acre[1]**	**MRID #/Year**	**Study Classification**
Glyphosate(80WDG formulation)	Onion (*Allium cepa*)	75	27 D EC25: 0.28 (N.R.) NOAEC/EC05: 0.14 Slope: N.R.	44125715/45045 101/1995	Acceptable
Glyphosate(80WDG formulation)	Sorghum (*Sorghum bicolor*)	75	27 D EC25**: 0.16** (N.R.) NOAEC/EC05: 0.07 Slope: N.R.	44125715/45045 101/1995	Acceptable
Glyphosate(80WDG formulation)	Wheat (*Triticum aestivum*)	75	27 D EC25: 0.22 (N.R.) NOAEC/EC05: 0.1 Slope: N.R.	44125715/45045 101/1995	Acceptable
Glyphosate(80WDG formulation)	Corn (*Zea mays*)	75	27 D EC25: 0.35 (N.R.) NOAEC/EC05: 0.18 Slope: N.R.	44125715/45045 101/1996	Acceptable
Glyphosate(80WDG formulation)	Corn (*Zea mays*)	48.3	48WKS EC25: 0.227 (N.R.) NOAEC/EC05: 0.148 Slope: N.R.	44320636/1996	Acceptable
Glyphosate(80WDG formulation)	Purple nutsedge (*Cyperus rotundus*)	48.3	4WKS EC25: 0.805 (N.R.) NOAEC/EC05: 0.445 Slope: N.R.	44320636/1996	Acceptable
Glyphosate(80WDG formulation)	Wheat (*Triticum aestivum*)	48.3	4WKS EC25: 0.176 (0.138-0.183 a.e.) NOAEC/EC05: 0.049 Slope: N.R.	44320636/1996	Acceptable
Glyphosate(80WDG formulation)	Oat (*Avena sativa*)	48.3	4WKS EC25: 0.201 (N.R.) NOAEC/EC05: 0.148 Slope: N.R.	44320636/1996	Acceptable
Dicots					
Glyphosate(80WDG formulation)	Garden pea (*Pisum sativum*)	75	27 D EC25: 0.89 (N.R.) NOAEC/EC05: 0.45 Slope: N.R.	44125715/45045 101/1995	Acceptable
Glyphosate(80WDG formulation)	Sugarbeet (*Beta vulgaris*)	75	27 D EC25: 0.21 (B.R.) NOAEC/EC05: 0.12 Slope: N.R.	44125715/45045 101/1995	Acceptable
Glyphosate(80WDG formulation)	Sunflower (*Helianthus annus*)	75	27 D EC25: 0.16 (N.R.) NOAEC/EC05: 0.08 Slope: N.R.	44125715/45045 101/1995	Acceptable
Glyphosate(80WDG formulation)	Radish (*Rhaphanus sativus*)	75	27 D EC25: 0.09 (N.R.) NOAEC/EC05: 0.02 Slope: N.R.	44125715/45045 101/1995	Acceptable

Table 4.37 Terrestrial Plant Studies with Glyphosate Formulations					
Chemical	**Species**	**% a.i.[1]**	**EC_{25}/ NOAEC (EC_{05}) (lbs a.e./Acre[1]**	**MRID #/Year**	**Study Classification**
Glyphosate(80WDG formulation)	Soybean (*Glycine max*)	75	27 D EC25: 0.32 (N.R.) NOAEC/EC05: 0.12 Slope: N.R.	44125715/45045 101/1995	Acceptable
Glyphosate(80WDG formulation)	Cucumber (*Cucumis sativus*)	75	27 D EC25: 0.45 (N.R.) NOAEC/EC05: 0.16 Slope: N.R.	44125715/45045 101/1995	Acceptable
Glyphosate(80WDG formulation)	Sugarbeet (*Beta vulgaris*)	48.3	4WKS EC25: 0.277 (N.R.) NOAEC/EC05: 0.148 Slope: N.R.	44320636/1996	Acceptable
Glyphosate(80WDG formulation)	Radish (*Rhaphanus sativus*)	48.3	4WKS EC25: 0.235 (N.R.) NOAEC/EC05: 0.148 Slope: N.R.	44320636/1996	Acceptable
Glyphosate(80WDG formulation)	Soybean (*Glycine max*)	48.3	4WKS EC25: 0.126 (N.R.) NOAEC/EC05: 0.049 Slope: N.R.	44320636/1996	Acceptable
Glyphosate(80WDG formulation)	Lettuce (*Lactuca sativa*)	48.3	4WKS EC25: 0.217 (N.R.) NOAEC/EC05: 0.148 Slope: N.R.	44320636/1996	Acceptable
Glyphosate(80WDG formulation)	Cucumber (*Cucumis sativus*)	48.3	4WKS EC25: **0.074** (N.R.) NOAEC/EC05: 0.049 Slope: N.R.	44320636/1996	Acceptable
Glyphosate(80WDG formulation)	Rape (*Brassica compestris*)	48.3	4WKS EC25: 0.098 (0.065-0.084) NOAEC/EC05: 0.049 Slope: N.A.	44320636/1996	Acceptable
Glyphosate(80WDG formulation)	Okra (*Hibiscus esculentus*)	48.3	4WKS EC25: 0.172 (N.R.) NOAEC/EC05: 0.049 Slope: N.R.	44320636/1996	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent; N.R. = Not reported; IPA = isopropylamine
[2] **Bolded** shaded value will be used to calculate risk quotients.

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

The Agency uses the probit dose response relationship as a tool for providing additional information on the potential for acute direct effects to individual listed species and aquatic animals that may indirectly affect the listed species of concern (U.S. EPA, 2004). As part of the risk characterization, an interpretation of acute RQ for listed species is discussed. This interpretation is presented in terms of the chance of an individual event (i.e., mortality or immobilization) should exposure at the EEC actually occur for a species with sensitivity to glyphosate 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.

4.4 Incident Database Review

A review of the EIIS database for ecological incidents involving glyphosate and its salts (PC Codes 417300, 103601, 103603, 103604 and 103607) was completed on 08/11/2008. The results of this review for terrestrial, plant, and aquatic incidents are discussed below in Sections 4.4.1 through 4.4.3, respectively. A complete list of the incidents involving glyphosate and its salts, including associated uncertainties is included as **Appendix K**.

4.4.1 Terrestrial Incidents

One incident report for technical glyphosate was filed on 6/13/2006. The certainty code was classified as **unlikely**. This incident was for a registered use on sunflowers, broadcast spray. It was reported that 1 american kestrel, 1 robin, 5 grackels, 597 horned larks, an unknown number of kangaroo rats, a few lark buntings, 1633 mourning doves, 5 red-winged blackbirds, 12 sparrows, 150 unknown birds and 5 western meadowlarks were killed upon ingestion of the herbicide.

Five incident reports for glyphosate isopropylamine salt were filed, 2 in 1993, 1 in 1994, 1 in 1996 and 1 in 2004 for uses on corn, field, home/lawn and a tree farm. One report did not file a specific use. The certainty indices were from unlikely to probable. The unlikely report was for incapacitation of a duck and mortality in 2 geese following inhalation. The possible reports were for mortality in an unknown quantity of birds from drift, mortality in 3 birds from drift and mortality in several dogs from runoff. The probable report was for incapacitation of two iguanas following ingestion of glyphosate.

4.4.2 Plant Incidents

For glyphosate, 63 incidents were reported for mostly plant damage to a wide variety of plants from either direct treatment or spray drift. The reports were filed from 1992 – 2008 with the certainty code ranging from possible to highly probable. The majority of the reports were either probable or highly probable.

For the isopropylamine salt of glyphosate, 443 incident reports were filed for a wide variety of terrestrial plants, particularly agricultural crops and grass. There were only a few incidents of trees being damaged or killed. The majority of the reports were rated as probable but there were some highly probable incidents and a number of possible incidents. The reports were filed from 1990 – 2006 with a large number of accidental misuses and of unknown legality. Plant damage and mortality were the main issues with drift as the main exposure route.

4.4.3 Aquatic Incidents

For glyphosate, two incident reports were filed in which 1 carp and 1 catfish were incapacitated and 20 goldfish were killed upon ingestion of glyphosate. The certainty index was possible for both incidents. The reports were filed in 2003.

For the isopropylamine salt of glyphosate, 16 incident reports were filed from 1990 – 2003. The certainty indices ranged from unlikely to highly probable. There was one accidental misuse in which thousands of shad were killed upon ingestion. It was not stated what the application method was, but this was the one report that was rated highly probable. Three other misuses were reported and the remainder were either registered uses (majority) or unknown. Eight of the reports were from runoff, 2 ingestion, 1 pond treatment and 1 skin contact. The others were either unknown or not reported. Fifteen reported mortality and 2 reported incapacitation. All of the reports were on fish. The numbers of fish killed ranged from 9 to thousands.

5. Risk Characterization

Risk characterization is the integration of the exposure and effects characterizations. Risk characterization is used to determine the potential for direct and/or indirect effects to the CRLF or for modification to its designated critical habitat from the use of glyphosate in California. The risk characterization provides an estimation (Section 5.1) and a description (Section 5.2) of the likelihood of adverse effects. In addition, it includes risk assessment assumptions, limitations, and uncertainties as well as a comprehensive conclusion regarding the likelihood of adverse effects to the CRLF or its designated critical habitat (i.e., "no effect," "likely to adversely affect," or "may affect, but not likely to adversely affect").

5.1 Risk Estimation

Risk is estimated by calculating the ratio of exposure to toxicity. This ratio is the risk quotient (RQ), which is then compared to pre-established acute and chronic levels of concern (LOCs) for each category evaluated (**Appendix C**). For acute exposures to the CRLF and its animal prey in aquatic habitats, as well as terrestrial invertebrates, the LOC for listed species is 0.05. For acute exposures to the CRLF and mammals in terrestrial habitats, the LOC for listed species 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 (**Tables 3.3 – 3.5**) based on the label-recommended glyphosate usage scenarios summarized in **Table 2.5** and the appropriate aquatic toxicity endpoint from **Tables 4.1 and 4.2**. 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 glyphosate (**Tables 3.8 and 3.9**) and the appropriate toxicity endpoint from **Tables 4.24 and 4.25**. Exposures are also derived for terrestrial plants, as discussed in Section 3.3 and summarized in **Table 3.10**, based on the highest application rates of glyphosate use within the action area.

5.1.1 Exposures in the Aquatic Habitat

As stated in the Ecological Effects Characterization Section (**Section 4.1**), although glyphosate appears to be less toxic to amphibians than to freshwater fish, an endpoint from the amphibian studies would be used as a conservative estimate if the amphibian endpoint could conceivably be lower than the one selected from the fish studies. This is the case with the chronic endpoint for direct effects to the CRLF. Both the fish and amphibian chronic studies show no toxicity, with the NOAEC at the highest concentration tested. The NOAEC from the amphibian study is lower than the NOAEC from the fish study. Therefore, as a conservative estimate of risk, the chronic endpoint for direct effects to the CRLF was selected from the amphibian study.

Also noted in **Section 4.1**, some formulations have been found to be more toxic to aquatic organisms than glyphosate on an acid equivalent basis. For assessment of risk following exposure to formulations, the most conservative endpoints from all available formulation data were selected for terrestrial uses where the POEA surfactant is allowed and separate endpoints were selected from studies on formulations without POEA for aquatic uses where this surfactant is not allowed. Wherever possible, endpoints for aquatic uses were selected from studies conducted with formulations that are currently labeled for aquatic use. For aquatic plants, due to a similarity in the product label name, it could not be determined from the aquatic plant studies whether or not they were conducted with a formulation labeled for terrestrial uses or with a formulation labeled for aquatic uses. The two formulations are different in terms of the inerts; nevertheless, the formulation for terrestrial uses does not have the POEA surfactant in it. Therefore, as a conservative estimate, these studies were utilized for the assessment of risk to aquatic plants following exposure to formulations labeled for aquatic use. Exposure to the formulations is expressed in terms of EEC of the formulation rather than to the glyphosate acid equivalent. For consistency of units, the toxicity endpoints are also expressed in terms of concentration of formulation rather than the glyphosate acid equivalent.

Data from several studies indicate that the toxicity of glyphosate in aquatic environments, particularly for some of the formulations, is pH and temperature dependent. This may be enhanced by the presence of surfactants. These two potential factors are not accounted for in this assessment.

5.1.1.1 Direct Effects to Aquatic-Phase CRLF

Glyphosate

Direct effects to the aquatic-phase CRLF are based on the highest peak aquatic EEC and the lowest acute and chronic toxicity values for freshwater fish and/or amphibians. There are no acute or chronic LOC exceedances. The highest aquatic EEC (210 ppb) was generated from the registered use for management of aquatic plants at 3.75 lbs a.e./A and was calculated by assuming direct application to water by a simple dilution. As a conservative estimate, the RQs following chronic exposure were calculated from the peak EEC. EECs for chronic exposure would only have been estimated if the chronic RQs exceeded the chronic LOC for aquatic animals using the conservative peak value. The highest acute RQ is < 0.01, using the lowest EC_{50} value of 43000 ppb a.e. from the acute toxicity study with Bluegill sunfish (*Lepomis macrochirus*; MRID 44320630). This value is less than the acute LOC of 0.05 for listed aquatic animals. For mortality following acute exposure, the probability of an individual effect at the acute RQ is 1 in 5.0E+24 (1 in 4.8E+05 to 7.0E+94) using a default slope assumption of 4.5 with lower and upper 95% confidence intervals of 2 and 9, respectively (Urban and Cook, 1986).

The highest chronic RQ is 0.12, using the lowest NOAEC of 1800 ppb a.e. (highest concentration tested, no LOAEC) from the chronic toxicity study in the leopard frog (*Rana pipiens*; MRID 46650501). It is noted that there is considerable uncertainty associated with this study due to the relatively high rate of mortality in the control groups (38%) and insufficient analysis of the water quality; however, the study does provide the most conservative estimate of risk. The RQ is less than the chronic aquatic LOC of 1 for aquatic animals.

Based on the highest acute RQ of less than 0.01 and the highest chronic RQ of 0.12, glyphosate is not expected to directly affect the aquatic-phase of the CRLF when the risks are estimated from the toxicity endpoints with the technical material. The preliminary effect determination is “no effect.”

Formulations

Risk from Terrestrial Uses

Aquatic EECs for formulations were estimated from spray drift only for each potential scenario (see **section 3.2.1.3**). The most conservative acute toxicity LC_{50} value for a formulation is 3170 ppb formulation from a study on rainbow trout (MRID 40098001). Using the highest peak aquatic EEC for formulations (95.2 ppb for forestry (aerial, 34 lbs formulation/A)), the highest acute RQ for freshwater fish is 0.03, which is less than the acute aquatic listed species LOC of 0.05. There are no exceedances for any of the other uses. There are no acceptable chronic toxicity studies on formulations with freshwater fish; however, there was one report of a 42-day chronic study conducted with several formulations on leopard frog larvae (MRID 46650501). These formulations contain the toxic surfactant, POEA. The LOAEC is 1900 ppb formulation based on decreased length at metamorphosis and percentage of larvae surviving to reach Stage 42 and increased time to metamorphosis, mixed-sex gonads and tail damage. Again, it is noted that there is considerable uncertainty associated with this study. Nevertheless, as a conservative estimate, a comparison of the LOAEC of 1900 ppb with the peak aquatic EEC value of 95.2 ppb (the chronic 60-day EEC for formulations cannot be estimated), the chronic RQ would be a value that is greater than 0.05. For the chronic LOC of 1 to be exceeded, the NOAEC for the study would have to be less than 95.2 ppb or 20 times less than the LOAEC. Due to the fact that some of the results are highly variable with lack of statistical significance in some key parameters, the study data are only being used as a bounding value for potential risk to the aquatic-phase CRLF following exposure to formulations used in terrestrial scenarios. Therefore, based on the weight of the evidence, including the use of the highest peak EEC for a chronic EEC value, the preliminary effect determination for formulations (direct effect: terrestrial uses) is "no effect."

Risk from Aquatic Uses

For accessing acute risk to formulations labeled for aquatic use, the endpoint was selected from a fish study for which there was a matching label which has aquatic uses (MRID 45374001, rainbow trout study with a glyphosate SL formulation, Reg. No. 100-1135) with an LC_{50} value of 824 ppm or 824000 ppb formulation). There was also a bluegill sunfish study with a non-discrete LC_{50} that was greater than 183700 ppb formulation; however, the rainbow trout study was selected because the LC_{50} is a discrete value.
The peak aquatic EEC estimated on a formulation basis for direct application to water (use on aquatic plants at 32.9 lbs formulation/A) is 1840 ppb. Comparing the peak aquatic EEC with the toxicity endpoint of 824000 ppb, the RQ for direct application to water is < 0.01. This is less than the aquatic listed species LOC of 0.05. Therefore, for freshwater fish, surrogate for the aquatic phase CRLF, the preliminary effect determination (direct effect: aquatic uses) is "no effect."

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

Glyphosate

Indirect effects of glyphosate 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 acute toxicity value (EC_{50}) for aquatic non-vascular plants. With the highest peak EEC of 210 ppb and the most conservative 96-hr EC_{50} of 12100 ppb from a study on green algae (MRID 40236901), the highest RQ for non-vascular plants would be 0.02. This is less than the LOC of 1 for aquatic plants. Therefore, glyphosate is not expected to indirectly affect the aquatic-phase of the CRLF through the diet (tadpoles) or habitat from aquatic non-vascular plants. The preliminary effect determination for glyphosate is “no effect.”

Formulations

Risk from Terrestrial Uses

As with fish, it is noted that some formulations can be considerably more toxic to non-vascular aquatic plants. The study with the lowest 96-hr EC_{50} on a formulation basis (390 µg glyphos/L) was conducted with freshwater diatom (*Navicula pelliculosa*; MRID 45666701). Using the highest peak EEC for formulations registered for terrestrial uses (95.2 ppb), the highest RQ for non-vascular plants is 0.24, which is less than the LOC of 1 for aquatic plants. Therefore, the preliminary effect determination for formulations (indirect effect: diet or habitat terrestrial uses) is “no effect.”

Risk from Aquatic Uses

As with freshwater fish, formulations containing the toxic surfactant, POEA are not allowed to be used in aquatic applications. Therefore, for accessing risk to nonvascular aquatic plants, a study was selected in which the formulation was known not to contain POEA. This study is the same one as selected above with an EC_{50} of 390 µg glyphos/L. As stated previously, a glyphos product is available for aquatic uses. It is unclear as to whether or not this study was conducted with the exact formulation because there are glyphos products for terrestrial uses and glyphos products for aquatic uses. This study was selected as the most conservative endpoint, assuming that the product tested was for aquatic uses.

The peak aquatic EEC estimated on a formulation basis for direct application to water (use on aquatic plants) is 1840 ppb. The RQ is direct application to water is 4.7. This is higher than the LOC for aquatic plants of 1. Therefore, with the formulations, the preliminary effect determination (indirect effect: diet or habitat aquatic uses) is “may affect.”

Aquatic Invertebrates

Glyphosate

Indirect acute effects to the aquatic-phase CRLF via effects to prey (invertebrates) in aquatic habitats are based on the highest peak EECs from the registered uses (aquatic plant management) and the lowest acute toxicity value for freshwater invertebrates. For chronic risks, as with freshwater fish, the peak EEC and the lowest chronic toxicity value for invertebrates are used to derive RQs. There are no LOC exceedances with risk estimations based on the highest peak (210 ppb) EECs generated from the registered uses (management of aquatic plants). The highest acute RQ is < 0.01, using the lowest EC_{50} value of 53200 ppb a.e. from the acute toxicity study with the midge (*Chironomus plumosus*; MRID 00162296). This value is less than the acute LOC of 0.05 for listed aquatic animals. The highest chronic RQ is also <0.01, using the most conservative NOAEC of 49900 ppb a.e. from the chronic toxicity study in daphnia (*Daphnia magna*; MRID 00124763). This value is less than the chronic LOC of 1 for aquatic animals. Therefore, glyphosate is not expected to indirectly affect the aquatic-phase of the CRLF via direct effects on aquatic invertebrates and the preliminary effect determination is “no effect.”

Formulations

Risk from Terrestrial Uses

As with fish and aquatic non-vascular plants, it is noted that some formulations can be considerably more toxic to freshwater invertebrates. The most conservative EC_{50} on a formulation basis is 3 mg/L formulation (3000 ppb) (*daphnia magna* with a 41% glyphosate IPA formulation, MRID 00162296). Using the highest peak EEC for formulations registered for terrestrial use (95.2 ppb), the highest RQ for aquatic invertebrates is 0.03, which is less than the listed species LOC of 0.05 for aquatic invertebrates. None of the other uses exceed any of the acute aquatic invertebrate LOCs. There are no acceptable chronic toxicity studies on formulations with freshwater invertebrates. Therefore, no RQs were estimated. The preliminary effect determination for formulations (indirect effect: reduction in prey - terrestrial uses) is “no effect.”

Risk from Aquatic Uses

An acute toxicity study was found on a freshwater invertebrate for which there was a matching label with aquatic uses (MRID 45374003; daphnia study with a glyphosate SL formulation; 360g/L; Reg. No. 100-1135) with an EC_{50} value of 164.3 ppm formulation (164300 ppb). The peak formulation EEC for aquatic uses is 1840 ppb. This provides an RQ of 0.01 following acute exposure, which does not exceed the acute aquatic LOC for listed species. Therefore, for formulations (indirect effect: reduction in prey - aquatic uses) is “no effect.”

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 are used to assess potential indirect effects to the CRLF based on a reduction in freshwater fish and frogs as food items. Based on an acute RQ of <0.01 and a chronic RQ of 0.12 for the aqueous-phase CRLF, glyphosate is not expected to indirectly affect the adult aquatic-phase CRLFs when the risks are estimated from the toxicity endpoints with the technical material. The preliminary effect determination is "no effect." For acute risk from formulations, the highest acute RQ from terrestrial applications is 0.03, which is less than the acute LOC of 0.05 for listed aquatic animals. The highest chronic RQ is a value that would be greater than 0.05 based on the conservative peak aquatic EEC value of 95.2 ppb and a chronic LOAEC of 1900 ppb. As stated previously, for the chronic LOC of 1 to be exceeded, the NOAEC for the study would have to be 20 times less than the LOAEC. In addition, some of the results are highly variable with lack of statistical significance in some key parameters. Therefore, for formulations (terrestrial uses), the preliminary effect determination is "no effect". For formulations labeled for aquatic use, the highest acute RQ is < 0.01. Therefore, the preliminary effect determination for formulations (aquatic uses) is also "no effect".

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

Glyphosate

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. For both non-vascular and vascular plants, the LOC for aquatic plants is not exceeded with the highest peak EEC generated from the registered uses (management of aquatic plants). The risk to non-vascular plants is summarized in **Section 5.1.2.2.** The highest RQ for non-vascular plants is 0.02. For vascular plants, the highest RQ is also 0.02, based on the peak EEC of 210 ppb and an EC_{50} of 11900 ppb a.e. (MRID 44320638) for duckweed. Glyphosate is not expected to indirectly affect the aquatic-phase CRLF through habitat from aquatic vascular and non-vascular plants. The preliminary effect determination is "no effect."

Formulations

Risk from Terrestrial Uses

As stated previously, the EC_{50} of 390 µg glyphos/L with freshwater diatom, an aquatic non-vascular plant, provides an RQ of 0.24 with the highest peak EEC of 95.2 ppb for a formulation. This is less than the LOC of 1 for aquatic plants. The EC_{50} for aquatic vascular plants was selected from a duckweed study (MRID 44125714): 4.9 ppm (4900 ppb) on a formulation basis. The resulting RQ is 0.02, which is less than the LOC of 1 for aquatic plants.

Therefore, the preliminary effect determination for formulations (indirect effect: habitat and/or primary productivity - terrestrial uses) is "no effect."

<u>Risk from Aquatic Uses</u>

As stated previously, the same endpoint for non-vascular plants (390 ppb) is used for aquatic uses. For vascular plants, only a 7-day study is available on a formulation which does not contain POEA. As with the non-vascular plants, this study was conducted with glyphos, which has a formulation for aquatic uses. The endpoint for the duckweed study is 25 ppm or 25000 ppb (MRID 45666704). Again, the peak aquatic EEC estimated on a formulation basis for direct application to water (use on aquatic plants) is 1840 ppb. The RQ for vascular plants, direct application to water is 1840/25000 or 0.07. This is lower than the LOC for aquatic plants of 1. However, the RQ for non-vascular plants is 4.7. Therefore, with some of the formulations, the preliminary effect determination (indirect effect: habitat and/or primary productivity - aquatic uses) is "may affect."

5.1.2 Exposures in the Terrestrial Habitat

5.1.2.1 Direct Effects to Terrestrial-phase CRLF

Glyphosate

As previously discussed in **Section 3.3**, potential direct effects to terrestrial-phase CRLFs are based on foliar applications of glyphosate. Potential direct acute effects to the terrestrial-phase CRLF are derived by considering dose- and dietary-based EECs modeled in T-REX for a small bird (20 g) consuming small invertebrates (**Table 3.8**) and acute oral and subacute dietary toxicity endpoints for avian species. There were no mortalities in any of the available acute avian studies. Therefore, no RQs were calculated. The highest dose/concentration tested in the acute avian studies were >3196.3 mg a.e./kg bodyweight (83% technical) and >4971.2 mg a.e./kg diet (95.6% technical), both with bobwhite quail.

Potential direct chronic effects of glyphosate 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.

Table 5.1 shows that the chronic avian LOC is exceeded for birds consuming small invertebrates for the following uses: forestry (7.95 lbs a.e./A, aerial); uses on areas with impervious surfaces (ground: i.e., agricultural/farm structures/buildings and equipment, commercial storage/warehouse premises, household/domestic dwellings outdoor premises, industrial areas, non-agricultural outdoor buildings/structures, path/patios, paved areas (private roads/sidewalks) and urban areas (7.95 lbs a.e./A, ground) and for rights of way uses (7.5 lbs a.e./A, aerial). None of the other uses exceed the chronic avian LOC. It is noted that there were no effects in the chronic avian study at the highest concentration tested; however, the preliminary effect determination is "may affect."

Table 5.1 Summary of Chronic RQs* Used to Estimate Direct Effects to the Terrestrial-phase CRLF (non-granular application)	
Use (Application Rate)	**Dietary-based Chronic RQ[1]**
Forestry and Areas with Impervious Surfaces 7.95 lbs a.e./A (aerial), 1 application/year	**1.29**
California Rights of Way 7.5 lbs a.e./A (aerial), 1 application/year	**1.22**
Almond, fruit, grape and olive 3.84 lbs a.e./A, 2 applications/year, 14 day application interval	0.78
* = LOC exceedances (chronic RQ $\geq$ 1) are bolded and shaded. [1] Based on avian NOAEC of 830 ppm a.e. (MRID 00108207)	

Formulations

Acute oral and acute dietary avian studies have been conducted on some formulations. As with the technical material, most of the LD_{50}/LC_{50}'s are higher than the highest dose/concentrations tested. There is one study in which there is a definitive LD_{50}. This study was conducted with bobwhite quail on the glyphosate monoammonium salt (MON 14420: 68.5% w/w glyphosate, MRID 45777402). The LD_{50} is 1651 mg formulation/kg bodyweight on a formulation basis. For this formulation, there is a specific label with application rates ranging from 5.5 to 1.1 lbs formulation/A. These uses were modeled with this particular LD_{50} for this particular formulation, assuming one application per year and a half-life of 7 days. The RQs (diet of small invertebrates) for all use scenarios exceed the acute avian LOC of 0.1 for listed species (see **Table 5.2** below). With this particular formulation, any application rate of 0.8 lbs formulation/A and above will exceed the acute avian LOC of 0.1 for listed species.

For other formulations, RQs were not calculated because the LD_{50}'s were higher than the highest dose/concentration tested. The highest dose/concentration tested in these studies were: >2510 mg formulation/kg bodyweight (MRID 00085638) and >5620 mg formulation/kg diet (MRID 00085639) on bobwhite quail with trisodium diglyphosate/Urea (Polado formula (MON 8000)).

Table 5.2. Summary of Acute RQs* on Formulations Used to Estimate Direct Effects to the Terrestrial-phase CRLF (non-granular application)	
Use (Application Rate)	**Dose-based acute RQ[1]**
MON 14420[2]	
Industrial areas outdoor (5.5 lbs formulation/A)	**0.71**
Ornamental lawns and turf (2.2 lbs formulation/A)	**0.28**
Ornamental lawns and turf (1.1 lbs formulation/A)	**0.14**
* = LOC exceedances (acute RQ $\geq$ 0.1) are bolded and shaded. [1] Based on avian LD_{50} of 1651 mg formulation/kg bw [2] Registration Number 524-424	

Therefore, the preliminary effect determination for formulations (direct effect on terrestrial-CRLF) is "may affect."

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

5.1.2.2.1 Terrestrial Invertebrates

In order to assess the risks of glyphosate to terrestrial invertebrates, which are considered prey of CRLF in terrestrial habitats, the honey bee is used as a surrogate for terrestrial invertebrates. The toxicity value for terrestrial invertebrates is calculated by multiplying the lowest available acute contact LD_{50} of >100 µg a.i./bee (MRID 00026489) by 1 bee/0.128g, which is based on the weight of an adult honey bee. EECs (µg a.i./g of bee) calculated by T-REX for small and large insects are then divided by the calculated toxicity value for terrestrial invertebrates (>781.25 µg a.i./g of bee) to estimate the RQ. Although the acute LD_{50} value is not a discrete value, it is noted that there is 27% mortality at this dose level. Since mortality was observed, the T-REX model was used to estimate upper bound RQs for terrestrial invertebrates. The results show that for small insects, all of the RQs for all uses could exceed the LOC of 0.05 for listed terrestrial invertebrates. Uses on forestry, areas with impervious surfaces at 7.95 lbs a.e./A and rights of way could exceed the acute LOC of 0.5 for non-listed species. For large insects, uses on forestry, areas with impervious surfaces and rights of way could exceed the acute LOC of 0.05 for listed species. None of the other uses exceed the acute LOC for listed species. Due to the fact that the acute LD_{50} value for the honey bee is not a discrete value and that there is mortality at the single limit dose tested, the RQs could exceed the acute LOC for listed species at all application rates for small insects and at the higher application rates for large insects. In addition, the RQs could exceed the acute LOC for non-listed species at the higher application rates for small insects. Therefore, there is an uncertainty for terrestrial invertebrate species. **Table 5.3** summarizes the results.

Table 5.3. Summary of Upper-Bound RQs Used to Estimate Indirect Effects to the Terrestrial-phase CRLF via Direct Effects on Terrestrial Invertebrates as Dietary Food Items		
Use	**Small Insect RQ***	**Large Insect RQ***
Forestry and Areas with Impervious Surfaces 7.95 lbs a.e./A	**<1.4**	**<0.15**
California rights of way 7.5 lbs a.e./A	**<1.3**	**<0.14**
California corn and wheat 0.75 lbs a.e./A	**<0.17**	<0.02
California rangeland 1.54 lbs a.e./A	**<0.35**	<0.04
California rangeland 0.387 lbs a.e./A	**<0.1**	<0.01
* = LOC exceedances (RQ ≥ 0.05) are bolded and shaded. Because a definitive endpoint was not established for terrestrial invertebrates (i.e., the value is greater than the highest test concentration), the RQ represents an upper bound value.		

Potential risk to terrestrial invertebrates is further discussed in the risk description section. The preliminary effect determination is "may affect."

5.1.2.2.2 Mammals

Glyphosate

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. EECs are divided by the toxicity value to estimate acute and chronic dose-based RQs as well as chronic dietary-based RQs. There were no mortalities in any of the available acute mammalian studies. Therefore, no RQs were calculated.

Table 5.4 summarizes the risk quotients for small mammals eating short grass with chronic exposure to glyphosate. The chronic RQs exceed the chronic LOC for small mammals on a dose-basis for use rates of 3.84 lb/A and above.

Table 5.4. Summary of Chronic RQs* Used to Estimate Indirect Effects to the Terrestrial-phase CRLF via Direct Effects on Small Mammals as Dietary Food Items (non-granular application)		
Use Scenario **Application Rate (# Applications per year/Interval (days))**	**Chronic RQ**	
	Dose-based Chronic RQ[1]	**Dietary-based Chronic RQ[2]**
Forestry (aerial) and areas with impervious surfaces 7.95 lbs a.e./A (1)	**1.66**	0.19
Right of way 7.5 lbs a.e./A (1)	**1.56**	0.18
Citrus, cole crop, lettuce, melon, onion, potato and wine grape 3.85 first application, 2.3 second application lbs a.e./A (2/14)	0.6 – **1.00**	0.07 – 0.12
Almond, fruit, grape and olive 3.84 lbs a.e./A (2/14)	**1.00**	0.12
Corn, cotton, garlic, impervious surfaces, row crop, strawberry and wheat 3.75 first application, 2.25 second application lbs a.e./A (2/14)	0.59 – 0.98	0.07 - 0.11
Alfalfa, avocado, forestry, nursery, rangeland, residential and turf 3.75 lbs a.e./A (2/14)	0.98	0.11
Right of way 3.69 lbs a.e./A (2/14)	0.96	0.11

* = LOC exceedances (chronic RQ $\geq$ 1) are bolded and shaded.
[1] Based on dose-based EEC and glyphosate rat NOAEL of 500 mg/kg-bw (MRID 41621501).
[2] Based on dietary-based EEC and glyphosate rat NOAEC = 10000 mg/kg-diet.

Formulations

Many acute oral toxicity studies have been conducted on formulations with the rat. As with the technical material, most of the LD_{50}'s are higher than the highest dose/concentrations tested. There are six submitted studies in which there are definitive LD_{50}'s. Label matches were conducted for each of these products and estimates were made as to how much of the formulated product could be applied in pounds per acre before exceeding the acute LOC for listed mammals. A label match-up with one of these products (MRID 46714802) determined that it is not registered in California (Registration number 5905-560). Other labels state that "not all products recommended on this label are registered for use in California". The specific formulation uses that are not allowed in California are not detailed here. For the five formulations in which there are definitive acute mammalian LD_{50}'s available and in which at least some of the uses may be allowed in California, **Table 5.5** provides that application rates in terms of pounds formulated product per acre that would exceed the acute mammalian listed species LOC for that product. An assumption is made that the product is applied only once per season. It is noted that for many labels, there are other products that are submitted to the Agency that use the same acute toxicity studies for their labels.

Table 5.5. Application Rates with Formulations Exceeding the Acute Mammalian LOC for Listed Species for Specific Formulations with Definitive Acute Mammalian LD_{50} Values – Small Mammals Eating Short Grass		
Registration Number	**Acute Mammalian LD_{50} mg/kg bw (MRID No.)**	**Application Rate Exceeding Dose-Based Acute RQ (lb formulation/A)[1,2]**
524-440	3750 (41305404)	3.5
62719-323	3803 (44918601)	3.5
524-504	5827 (44615502)	5.5
524-435	5000 (41142304)	5
524-424	2686 (40853903)	2.5
[1]LOC exceedances (acute RQ $\geq$ 0.1) [2] Assuming only 1 application per season		

The preliminary effect determination for both glyphosate and formulations (indirect effect on terrestrial-CRLF: diet) is “may affect.”

5.1.2.2.3 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. See **Section 5.1.2.1** and associated table (**Table 5.1**) for results. No acute RQs were calculated for birds because there were no mortalities in any of the available acute avian studies.

Since the chronic avian LOC was exceeded for birds consuming small invertebrates for forestry and rights of way uses (aerial application) and for uses with areas with impervious surfaces, the preliminary effect determination is “may affect.”

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 (the most sensitive EC_{25}’s were used rather than the NOAEC or EC_{05} because there are no obligate relationships between the CRLF and any terrestrial plant species). The most sensitive toxicity thresholds are 0.16 (monocot – dry weight) and 0.074 (dicot - phytotoxicity) lb ae/acre from the vegetative vigor studies. No effects were observed in the seedling emergence studies. RQs were estimated using the Terrplant (Version 1.2.2) model for the various uses of glyphosate in California.

Tables 5.6 and 5.7 summarize the risks to monocots and dicots from glyphosate uses with both ground and aerial spray applications. None of the RQs for terrestrial plants living in either dry or semi-aquatic areas exposed to the combined deposition estimates from runoff and spray drift exceed the terrestrial plant LOC of 1. The terrestrial plant LOC is exceeded for both monocots and dicots when they are exposed to glyphosate via spray drift for aerial uses at 3.75 lbs a.e./A and above and for ground uses with impervious surfaces at 7.95 lbs a.e./A. The preliminary effect determination is "may affect". An example output from TerrPlant v.1.2.2 is provided in **Appendix F.**

Table 5.6 RQs* for Monocots Inhabiting Dry and Semi-Aquatic Areas Exposed to Glyphosate via Runoff and Drift

Use Scenario	Application rate (lbs a.i./A)	Application method	Drift Value (%)	Spray drift RQ	Dry area RQ	Semi-aquatic area RQ
Alfalfa, avocado, corn, cotton, forestry, garlic, impervious, residential, row crop, strawberry, wheat	3.75	Ground	1	0.23	<0.1	<0.38
Almond, fruit, grape, olive	3.84	Ground	1	0.24	<0.1	<0.39
Citrus	3.85	Ground	1	0.24	<0.1	<0.39
Cole crop, lettuce, melon, onion, potato, wine grape	3.85	Aerial	5	**1.20**	<0.1	<0.42
Corn	0.75	Aerial	5	0.23	<0.1	<0.1
Forestry	7.95	Aerial	5	**2.48**	<0.16	<0.87
Impervious	7.95	Ground	1	0.5	<0.1	<0.81
Nursery, rangeland, sugar beet, tomato, turf	3.75	Aerial	5	**1.17**	<0.1	<0.41
Rangeland	1.54	Ground	1	<0.1	<0.1	<0.16
Rangeland	0.387	Aerial	5	0.12	<0.1	<0.1
Rights of way	7.5	Aerial	5	**2.34**	<0.15	<0.83
Rights of way	3.69	Ground	1	0.23	<0.1	<0.38
Wheat	0.75	Ground	1	<0.1	<0.1	<0.1

* = LOC exceedances (RQ $\geq$ 1) are bolded and shaded.

Table 5.7 RQs* for Dicots Inhabiting Dry and Semi-Aquatic Areas Exposed to Glyphosate via Runoff and Drift						
Use Scenario	**Application rate (lbs a.i./A)**	**Application method**	**Drift Value (%)**	**Spray drift RQ**	**Dry area RQ**	**Semi-aquatic area RQ**
Alfalfa, avocado, corn, cotton, forestry, garlic, impervious, residential, row crop, strawberry, wheat	3.75	Ground	1	0.51	<0.1	<0.38
Almond, fruit, grape, olive	3.84	Ground	1	0.52	<0.1	<0.39
Citrus	3.85	Ground	1	0.52	<0.1	<0.39
Cole crop, lettuce, melon, onion, potato, wine grape	3.85	Aerial	5	**2.60**	<0.1	<0.42
Corn	0.75	Aerial	5	0.51	<0.1	<0.1
Forestry	7.95	Aerial	5	**5.37**	<0.16	<0.87
Impervious	7.95	Ground	1	**1.07**	<0.1	<0.81
Nursery, rangeland, sugar beet, tomato, turf	3.75	Aerial	5	**2.53**	<0.1	<0.41
Rangeland	1.54	Ground	1	0.21	<0.1	<0.16
Rangeland	0.387	Aerial	5	0.26	<0.1	<0.1
Rights of way	7.5	Aerial	5	**5.07**	<0.15	<0.83
Rights of way	3.69	Ground	1	0.50	<0.1	<0.38
Wheat	0.75	Ground	1	0.10	<0.1	<0.1
* = LOC exceedances (RQ ≥ 1) are bolded and shaded.						

5.1.3 Primary Constituent Elements of 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).

The preliminary effects determination for aquatic-phase PCEs of designated habitat related to potential effects on aquatic and/or terrestrial plants is “may affect”, based on the risk estimation provided in **Sections 5.1.1.2, 5.1.1.3**, and **5.1.2.3**.

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 glyphosate on this PCE, acute and chronic freshwater fish and invertebrate toxicity endpoints, as well as 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**. For freshwater fish and invertebrates, there are no acute or chronic aquatic LOC exceedances for glyphosate or for formulations with the highest peak EECs generated from the registered uses (aquatic weed management). The LOC for aquatic plants is not exceeded with the highest peak EEC generated from the uses involving direct application to water (aquatic weed management) for glyphosate a.e. but is exceeded for direct application to water for formulations. Based on an acute RQ of 4.7 for formulations for aquatic non-vascular plants, the preliminary effect determination for the PCE, “alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source” is “no effect” for glyphosate and “may affect” for formulations.

5.1.3.2 Terrestrial-Phase (Upland Habitat and Dispersal Habitat)

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 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 preliminary effects determination for terrestrial-phase PCEs of designated habitat related to potential effects on terrestrial plants is “may affect”, based on the risk estimation provided in **Section 5.1.2.3**.

The third terrestrial-phase PCE is “reduction and/or modification of food sources for terrestrial phase juveniles and adults.” To assess the impact of glyphosate 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**. There were no mortalities for glyphosate a.e. in either the acute avian or the acute mammalian studies. Therefore, no RQs were calculated. The chronic avian LOC is exceeded for birds consuming small invertebrates for forestry uses with aerial application, for uses with areas with impervious surfaces and for rights of way (aerial application). The chronic RQs exceed the chronic LOC for small mammals on a dose-basis for every use rate of 3.84 lbs a.e./A and above. The LOC for listed terrestrial invertebrates is exceeded for all uses for small insects. The LOC for listed terrestrial invertebrates for large insects is exceeded for all uses at 7.5 lbs a.e./A and above (see **Table 5.4** for application rates and scenarios). Therefore, the preliminary effect determination is "may affect."

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 5.2.1.2**. There were no mortalities in the acute avian studies. Therefore, no RQs were calculated. The chronic avian LOC is exceeded for birds consuming small invertebrates for forestry uses with aerial application, for uses with areas with impervious surfaces and for rights of way (aerial application). Therefore, the preliminary effect determination is "may affect."

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.

If the RQs presented in the Risk Estimation (**Section 5.1**) show no direct or indirect effects for the CRLF, and no modification to PCEs of the CRLF's designated critical habitat, a "no effect" determination is made, based on glyphosate's use within the action area. However, if 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 glyphosate. A summary of the results of the risk estimation (*i.e.*, "no effect" or "may affect" finding) is provided in **Table 5.8** for direct and indirect effects to the CRLF and in **Table 5.9** for the PCEs of designated critical habitat for the CRLF.

Table 5.8 Preliminary Effects Determination Summary for Glyphosate - Direct and Indirect Effects to CRLF		
Assessment Endpoint	**Preliminary Effects Determination**	**Basis For Preliminary Determination**
Aquatic Phase (eggs, larvae, tadpoles, juveniles, and adults)		
Survival, growth, and reproduction of CRLF individuals via direct effects on aquatic phases	No effect	No LOC exceedances for freshwater fish and/or aquatic-phase amphibians following either acute or chronic exposures to either glyphosate (a.e.) or to its formulations.
Survival, growth, and reproduction of CRLF individuals via effects to food supply (*i.e.*, freshwater invertebrates, non-vascular plants)	May affect	No LOC exceedances for freshwater invertebrates following either acute or chronic exposures and no LOC exceedances for aquatic non-vascular plants following acute exposure with glyphosate (a.e.). With formulations, no LOC exceedances for freshwater invertebrates following acute exposures from either terrestrial or aquatic uses and for non-vascular plants from terrestrial uses; however, there are LOC exceedances for aquatic non-vascular plants following acute exposures from aquatic uses (32.9 lbs formulation/A).
Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat, cover, and/or primary productivity (*i.e.*, aquatic plant community)	May affect	No LOC exceedances for aquatic non-vascular and vascular plants with glyphosate a.e.. With formulations, no LOC exceedances for aquatic vascular and non-vascular plants following acute exposures from terrestrial uses; however, there are LOC exceedances with non-vascular plants following acute exposures from aquatic uses (32.9 lbs formulation/A).
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.	May affect	The terrestrial plant LOC is exceeded for spray drift for both monocots and dicots for aerial uses at 3.75 lbs a.e./A and above and for ground uses on areas with impervious surfaces at 7.95 lbs a.e./A.
Terrestrial Phase (Juveniles and adults)		
Survival, growth, and reproduction of CRLF individuals via direct effects on terrestrial phase adults and juveniles	May affect	Chronic avian LOC exceeded for forestry, uses on areas with impervious surfaces and rights of way (7.5 lbs a.e./A and above). Acute avian LOC for listed species exceeded for one formulation for application rates of 0.8 lbs formulation/A and above: Industrial areas outdoors and non-agricultural rights of way (5.5 lbs formulation/A), ornamental lawns and turf (1.1 – 2.2 lbs formulation/A
Survival, growth, and reproduction of CRLF individuals via effects on prey (*i.e.*, terrestrial invertebrates, small terrestrial mammals and terrestrial phase amphibians)	May affect	See box above for terrestrial phase amphibians. For small insects, LOC for listed species potentially exceeded for all uses (0.387 lbs a.e./A and above) and LOC for non-listed species potentially exceeded for forestry, areas with impervious surfaces and rights of way (7.5 lbs a.e./A and above). For large

Table 5.8 Preliminary Effects Determination Summary for Glyphosate - Direct and Indirect Effects to CRLF		
Assessment Endpoint	**Preliminary Effects Determination**	**Basis For Preliminary Determination**
		insects, LOC for listed species potentially exceeded forestry, areas with impervious surfaces and rights of way. Acute RQs for small mammals exceed acute mammal LOC for listed species with 4 formulations at 3.5 lbs formulation/A and above. Chronic RQs for small mammals exceed chronic mammalian LOC on a dose-basis for every use scenario at 3.84 lbs a.e./A and above.
Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat (*i.e.*, riparian vegetation)	May affect	Terrestrial plant LOC exceeded for spray drift for both monocots and dicots for aerial uses at 3.75 lbs a.e./A and above and for ground uses with impervious surfaces at 7.95 lbs a.e./A.

Table 5.9 Preliminary Effects Determination Summary for Glyphosate PCEs of Designated Critical Habitat for the CRLF		
Assessment Endpoint	**Preliminary Effects Determination**	**Basis For Preliminary Determination**
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.	May affect	The terrestrial plant LOC is exceeded for spray drift for both monocots and dicots for aerial uses at 3.75 lbs a.e./A and above and for ground uses with impervious surfaces at 7.95 lbs a.e./A. No LOC exceedances for aquatic non-vascular and vascular plants with glyphosate a.e.. With formulations, no LOC exceedances for aquatic vascular and non-vascular plants following acute exposures from terrestrial uses; however, there are LOC exceedances for non-vascular plants following exposures from aquatic uses (management of aquatic plants at 32.9 lbs formulation/A).
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.	May affect	The terrestrial plant LOC is exceeded for spray drift for both monocots and dicots for aerial uses at 3.75 lbs a.e./A and above and for ground uses with impervious surfaces at 7.95 lbs a.e./A. No LOC exceedances for aquatic non-vascular and vascular plants with glyphosate a.e.. With formulations, no LOC exceedances for aquatic vascular and non-vascular plants following acute exposures from terrestrial uses; however, there are LOC exceedances with non-vascular plants following exposures from aquatic uses (management of

Table 5.9 Preliminary Effects Determination Summary for Glyphosate PCEs of Designated Critical Habitat for the CRLF		
Assessment Endpoint	**Preliminary Effects Determination**	**Basis For Preliminary Determination**
		aquatic plants at 32.9 lbs formulation/A).
Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.	May affect	For glyphosate a.e.: no aquatic LOC exceedances for freshwater fish and invertebrates and aquatic plants with the highest peak EEC (management of aquatic plants). For formulations: no LOC exceedances for freshwater invertebrates and fish following acute exposure from either terrestrial or aquatic uses. No LOC exceedances for non-vascular plants following acute exposures from terrestrial uses; however, there are LOC exceedances following acute exposures from aquatic uses (management of aquatic plants at 32.9 lbs formulation/A).
Reduction and/or modification of aquatic-based food sources for pre-metamorphs (e.g., algae)	May affect	Glyphosate a.e.: no exceedance of aquatic plant LOC with the highest peak EEC (management of aquatic plants at 3.75 lbs a.e./A). Formulations: no LOC exceedances for aquatic non-vascular plants following acute exposures from terrestrial uses; however, there are LOC exceedances following exposures from aquatic uses (management of aquatic plants at 32.9 lbs formulation/A).
Terrestrial Phase PCEs (Upland Habitat and Dispersal Habitat)		
Elimination and/or disturbance of upland habitat; ability of habitat to support food source of CRLFs: Upland areas within 200 ft of the edge of the riparian vegetation or dripline surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance	May affect	The terrestrial plant LOC is exceeded for spray drift for both monocots and dicots for aerial uses at 3.75 lbs a.e./A and above and for ground uses with impervious surfaces at 7.95 lbs a.e./A.
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	May affect	The terrestrial plant LOC is exceeded for spray drift for both monocots and dicots for aerial uses at 3.75 lbs a.e./A and above and for ground uses with impervious surfaces at 7.95 lbs a.e./A.
Reduction and/or modification of food sources for terrestrial phase juveniles and adults	May affect	Chronic avian LOC exceeded for forestry, uses on areas with impervious surfaces and rights of way (7.5 lbs a.e./A and above). Acute avian LOC for listed species exceeded for one formulation for application rates of 0.8 lbs formulation/A and above. Acute RQs for small mammals exceed acute mammal LOC for listed species with 4 formulations at 3.5 lbs formulation/A and above. Chronic RQs for small

Table 5.9 Preliminary Effects Determination Summary for Glyphosate PCEs of Designated Critical Habitat for the CRLF		
Assessment Endpoint	**Preliminary Effects Determination**	**Basis For Preliminary Determination**
		mammals exceed chronic mammalian LOC on a dose-basis for every use scenario at 3.84 lbs a.e./A and above. For small insects, LOC for listed species potentially exceeded for all uses (0.387 lbs a.e./A and above) and LOC for non-listed species potentially exceeded for forestry, areas with impervious surfaces and rights of way (7.5 lbs a.e./A and above). For large insects, LOC for listed species potentially exceeded forestry, areas with impervious surfaces and rights of way.
Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	May affect	Chronic avian LOC exceeded for forestry, uses on areas with impervious surfaces and rights of way (7.5 lbs a.e./A and above). Acute avian LOC for listed species exceeded for one formulation for application rates of 0.8 lbs formulation/A and above.

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 glyphosate.

Glyphosate

The acute and chronic RQs for direct effects to the aquatic-phase CRLF are presented in **Section 5.1.1.1**. As stated previously, none of the RQs exceed either the acute or chronic LOCs for freshwater fish (surrogate for the CRLF).

The highest peak aquatic EEC for glyphosate is 210 ppb for aquatic plant management. Monitoring data (NAQWA) indicate surface water concentrations ranging from 0.1 – 7.46 ppb. These concentrations are more than an order of magnitude lower than the estimated concentration utilized in the risk estimations.

The probability of an individual effect at the RQ (<0.01) is <1 in 8.9E+18 (1 in 3.2E+04 to 1.0E+72). The acute fish studies provided no slope. Therefore, a default slope of 4.5 (confidence limits 2 - 9) was used to estimate the probability.

For freshwater fish, the data on the technical material from the acute toxicity studies are so variable within each species that it is not possible to provide a sensitivity analysis. For amphibians, acute toxicity data are available on 3 species: the Australian tree frog, Australian frog and the green frog. The data are not sufficient to indicate a range of sensitivities for frogs. The acute toxicity values range from > 17.9 to 103.2 ppm. The study with the lowest potential endpoint (green frog: >17.9 ppm) does not provide a definitive endpoint. For the other two species, the confidence interval for the Australian tree frog (LC_{50}: 103.2 (43.2 - 172.8)) overlaps with the confidence interval for the Australian frog (LC_{50}: 75 (60.4-92.7)). Therefore, for the technical material, a species sensitivity analysis could not be conducted.

Formulations

Terrestrial Uses

Acute risk from formulated products (terrestrial uses) were estimated using the most conservative LC_{50} and peak aquatic EEC. The acute aquatic LOCs were not exceeded. Therefore, the acute aquatic LOCs would not be exceeded for any of the uses. Risks from chronic exposure were estimated from a chronic toxicity study on a formulated product with leopard frog larvae. As stated previously, this study has no NOAEC; however, the NOAEC would have to be 20 times less than the LOAEC for the chronic LOC for aquatic animals to be exceeded. Due to the fact that there was high variability in the results and either minimal or a lack of statistical significance for some of the key parameters, the NOAEC is probably closer to the LOAEC than a value that is 20 times less than the LOAEC.

Aquatic Uses

Acute risk from formulated products (aquatic uses) were estimated using a fish study that had a matching label which has aquatic uses and the peak aquatic EEC. The acute aquatic LOCs were not exceeded.

As stated in the Ecological Effects Characterization section (Section 4), submitted data on formulations indicate that some of the formulations are more toxic to freshwater fish than glyphosate itself, particularly those studies which tested formulations with one type of surfactant, polyethoxylated tallowamines (polyoxy ethylene fatty amine). Other surfactants do not appear to increase the toxicity of glyphosate except the X-77 surfactant with an acute LC_{50} value of 9.4 in rainbow trout when mixed with the isopropylamine salt (MRID 00078664). The data from the formulation studies do not provide a pattern consistent enough to determine a range of sensitivities for freshwater fish. One review indicates that the salmonids are more sensitive to glyphosate than other species of fish (USDA, 2003); however, the available data here are not sufficient to indicate that that is the case. The acute LC_{50}'s from studies conducted with formulations and freshwater fish range from 1 ppm (rainbow trout, MRID 40098001) for Roundup™ (most likely with the polyethoxylated tallowamine surfactant) to >1000 ppm (rainbow trout, geronol surfactant, MRID 44738201). The range of acute toxicity values are likely more related to the various formulations tested rather than to the sensitivities of the various freshwater fish species tested. The Roundup formulations have been tested the most and the acute LC_{50} values for the various fish species are all between 1 and 10 ppm. For example, submitted acute toxicity study values (LC_{50} in ppm) for freshwater fish species with Roundup formulations are: rainbow trout (1 - 9.2 (MRID 40098001, 00124761, 00162296 and 40579203)); bluegill sunfish (1.8 - 4.3 (MRID 00124760, 40098001 and 00070897)); fathead minnow (2.9 (MRID 00070896)); channel catfish (4.9 (MRID 00070894)); Chinook salmon (7.1 (MRID 40579201)) and Coho salmon (8 (MRID 40579202)). Formulations with other surfactants ("W", "X-77", "AA" and geronol provided a wide range of LC_{50} values, starting from 9.4 ppm to >1000 ppm.

For amphibians, a few studies were conducted on multiple formulations with several species of frogs. As with freshwater fish, the acute LC_{50}'s range from 1.1 ppm to 1000 ppm with the differences more likely related to the various formulations tested rather than to the sensitivities of the various frog species. For example, data on Roundup®, containing the polyethoxylated tallowamine surfactant provide LC_{50} values in a tight range from 2 ppm in the green frog (Gosner Stage 25) to 8 ppm with the wood frog (Gosner Stage 20). Again, as with the freshwater fish, these values are all between 1 and

10 ppm. In a series of studies reported by Howe et al. (MRID 46650501) which examined the acute and chronic effects of glyphosate alone, the surfactant polyethoxylated tallowamine (POEA) and glyphosate formulated products on 4 aquatic phase amphibian (anuran) species, the data indicated that younger amphibian larvae (Gosner stage 20) were less sensitive to acute lethality from the toxic surfactant-containing formulations than older larvae (Gosner stage 25). At stage 25, *R. clamitans* (green frog) was the most sensitive (96-hr LC_{50} = 6.5 mg/L or 2.0 mg a.e./L) and *R sylvatica* (wood frog) was the most tolerant (96-hr LC_{50} = 16.5 mg/L or 5.1 mg a.e./L).

Table 5.10. Aquatic-Phase Amphibian Acute Toxicity for Roundup Formulation

Chemical	Species	96-hour LC_{50} (mg a.e.*/L)	MRID #/Year
Glyphosate IPA (Roundup)	Green Frog (*Rana clamitans*) Gosner Stg 25	LC_{50}: 2 (1.9-2.2)[1]	46650501/2001
Glyphosate IPA (Roundup)	Leopard Frog (*Rana pipiens*) Gosner Stg 25	LC_{50}: 2.9 (NR)	46650501/2000
Glyphosate IPA (Roundup)	American toad (*Bufo americanus*) Gosner Stg 25	LC_{50}: 4.2 (NR)	46650501/1994
Glyphosate IPA (Roundup)	Wood Frog (*Rana sylvatica*) Gosner Stg 25	LC_{50}: 5.1 (4.9-5.4)	46650501/1994
Glyphosate IPA (Roundup)	Leopard Frog (*Rana pipiens*) Gosner Stg 20	LC_{50}: 6.5 (6.1-6.8)	46650501/1994
Glyphosate IPA (Roundup)	Green frog (*Rana clamitans*) Gosner Stg 20	LC_{50}: 7.1 (6.6-7.6)	46650501/1994
Glyphosate IPA (Roundup)	American toad (*Bufo americanus*) Gosner Stg 20	LC_{50}: 8 (NR)	46650501/1994
Glyphosate IPA (Roundup)	Wood Frog (*Rana sylvatica*) Gosner Stg 20	LC_{50}: 8 (NR)	46650501/1994

*a.e. = acid equivalent; IPA = isopropylamine salt, N.R. = not reported
[1] Range is 95% confidence interval for endpoint

Incident Data

Two incident reports were filed for glyphosate and 16 incidents were filed for the isopropylamine salt of glyphosate. For glyphosate, with a certainty index of possible, 2 fish were incapacitated and 20 fish were killed following exposure. For the isopropylamine salt of glyphosate, the certainty indices of the reports ranged from unlikely to highly probable. There was one accidental misuse in which thousands of shad were killed upon ingestion. Drums of Roundup were found floating in the water with the dead fish. This was the one report that was rated highly probable. The fish kill was more likely due to the surfactant in the Roundup formulation rather than from glyphosate itself. Eight of the incidents were from runoff, 2 from ingestion, 1 from pond treatment and 1 from skin contact. Fifteen reported mortality and 2 reported incapacitation. The numbers of fish killed ranged from 9 to thousands.

Open Literature Data and Sublethal Effects

For freshwater fish, as stated previously, none of the data from the open literature provided more conservative endpoints that could be used in a quantitative estimate of risk. Sublethal data are available for muliple fish species with technical glyphosate, the formulations, Roundup® and Vision® and with glyphosate with several different surfactants. The NOAECs for sublethal effects range from 8 ppb to 30.6 ppm. The lowest NOAEC/LOAEC is 8/46 ppb, based on an increase in wigwag behavior in rainbow trout following a 2 month exposure to Vision®, a formulation containing the toxic surfactant, POEA (E097714). In order to do a comparison of the NOAEC with the modeled chronic EEC for a formulation, only the EECs from terrestrial uses may be used because POEA is not allowed in formulations with aquatic uses. In addition, the results are expressed in terms of the acid equivalent, glyphosate. Therefore, based on the information provided in the paper, the lowest NOAEC/LOAEC may be converted to 25.8/148.3 ppb formulation. As stated previously, the chronic EEC for terrestrial formulations could not be estimated. If the peak EEC of 95.2 ppb for terrestrial formulations is used as a very conservative estimate, the comparison shows that the peak EEC is in between the NOAEC and the LOAEC for this behavioral effect. Therefore, there is an uncertainty as to whether or not this sublethal effect may occur in freshwater fish near areas where glyphosate is applied.

In amphibians, sublethal data from the open literature are available for green, African clawed, leopard, moaning, bull, motorbike, cascades and Western chorus frog; sign-bearing froglet; lesser snouted treefrog and the American toad. The lowest NOAEC is < 1 ppm and the highest is 4000 ppm. At a LOAEC of 1 ppm, metamorphosis occurred more rapidly in treated frogs with decreased size and mass when Cascade frogs were tested with Roundup®. In that study, no NOAEC was determined. At 6000 ppm with the Rodeo® formulation, there was a decrease in African clawed frog embryo growth. Other studies show malformations (craniofacial and mouth deformities, eye abnormalities and bent curved tails) and slow swimming abilities at lethal concentrations. Again, using the peak EEC of 95.2 ppb for terrestrial uses, a comparison of the LOAEC of 1000 ppb with the peak EEC shows that the concentration at which the LOAEC is observed is an order of magnitude higher than the peak EEC. The NOAEC could easily be at a concentration that is higher than the peak EEC.

For tables detailing these studies, see section 4.1.1.5 in the Ecological Effects Characterization section.

Effect Determination

Based on the weight of the evidence, the final effect determination is “no effect” for direct effects to the aquatic-phase CRLF. This determination is based on the lack of LOC exceedances following either acute or chronic exposures for both glyphosate and formulations, the low monitoring data in surface water when compared to the modeled concentrations and the low probability of an individual effect. The accidental misuse where thousands of fish were killed involved Roundup, a formulation which often contains the toxic surfactant, POEA, which is not allowed for aquatic uses in California.

5.2.1.2 Terrestrial-Phase CRLF

Glyphosate

Acute RQs were not calculated because there were no mortalities up to and including the highest dose/concentration tested. There are also no sublethal effects in any of the avian studies on the technical material. The chronic avian studies also do not have any effects at the highest concentrations tested. If comparisons are made between the terrestrial EECs estimated from T-REX and the highest dose tested in the acute oral studies, the results show that all of the EEC values are lower, but at application rates above 0.75 lbs a.e./A, the EECs are greater than $1/10^{th}$ of the highest dose tested in the studies. For that reason, there is an uncertainty for listed avian species (the LOC for listed avian species is 0.1). For any of the uses at application rates of 7.5 lbs a.e./A and above, the EEC values are greater than half the highest dose/concentration tested in the acute avian studies. Therefore, for those applications, there is uncertainty for non-listed species (the LOC for non-listed species is 0.5). A similar situation holds true for the subacute dietary-based EECs. The acute dietary-based EEC's are greater than 10% of the highest concentration tested in the avian subacute feeding studies for application rates higher than 2.35 lbs a.e./A.

The probability of an individual effect at the LOC (0.1: no RQs) is <1 in 2.94E+05 (<1 in 4.40E+01 to <1 in 8.86E+18). The acute bird studies provided no slope. Therefore, a default slope of 4.5 (confidence limits 2 - 9) was used to estimate the probability.

The chronic avian study showed no effects at the highest concentration tested. As stated in the risk estimation section, uses with application rates of 7.5 lbs a.e./A and higher have EECs that are higher than the highest concentration tested in the chronic avian study. Following chronic exposure, the RQs exceed the chronic LOC of 1 for consumption of broadleaf plants and small insectivorous mammals at rates of 7.5 lbs a.e./A and above. The RQs drop below the chronic LOC at lower application rates. These RQ values are conservative because there is no LOAEL from the chronic avian study.

Formulations

As stated in the risk estimation section, most of the available avian studies on formulations indicate LD_{50}/LC_{50} values greater than the highest dose/concentration tested. For the one study which has a definitive acute toxicity value, the application rates from the specific label for which this study was submitted indicate an exceedance of the acute avian LOC for all use rates, including the highest rate (5.5 lbs formulation/A (industrial outdoors)) to the lowest rate (1.1 lbs formulation/A (ornamental lawns and turf)). The dose-based acute RQs are 0.71 and 0.14, respectively, for a diet of small invertebrates.

The probability of an individual effect for the formulation at the LOC would be the same as the probability for the technical material (see above). The probability of an individual effect at the RQ of 0.53 is 1 in 9.32 (1 in 3.44 to 1 in 1.53E+02) and at the RQ of 0.11 is 1 in 1.25E+05 (1 in 3.62E+01 to 1 in 3.19E+17). The acute bird study provided no slope. Therefore, a default slope of 4.5 (confidence limits 2 - 9) was used to estimate the probability. This model assumes a dose-response. The mortality in the acute oral study on the formulation did not provide a dose-response. There was one mortality at the second highest dose level and complete mortality at the highest dose level. Therefore, a significant uncertainty is associated with using this probit model for estimating the probability of an individual effect.

T-HERPS

As stated above, the acute avian LOC of 0.1 was exceeded on a dose-basis for all use rates from the specific label for the formulated product. Therefore, for direct effects to the terrestrial-phase CRLF following exposure to a formulation, the model, T-HERPS (v. 1.0) was used to further define the risk to herpetofauna following acute exposure to this formulated product on a dose-basis. Modeling the avian data in T-HERPS to estimate direct effects to the terrestrial-phase CRLF indicates that medium and large-sized herps eating small herbivorous mammals on a dose-basis may be at risk following acute exposure at the labelled application rate of 5.5 lb/A. At 2.2 lb/A, the risk for large-sized herps drops below the LOC; however, the risk to medium-sized herps remains above the LOC. At 1.1 lbs formulation/A, the risk to medium-sized herps continues to remain above the LOC.

The following tables provide the results from T-HERPS for industrial outdoor uses at 5.5 lbs formulation/A and for ornamental lawns and turf at 2.2 and 1.1 lbs formulation/A.

Table 5.11. Upper Bound Kenaga, Terrestrial Herpetofauna Dose-Based Risk Quotients Used to Estimate Direct Effects to the Terrestrial-Phase CRLF Following Acute Exposure to a Formulation on Industrial Outdoor Areas at 5.5 lb Formulation/A[1]

Size Class (grams)	Adjusted LD50	EECs and RQs									
		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects		Small Herbivore Mammals		Small Insectivore Mammal		Small Amphibians	
		EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
1.4	1651.00	28.85	0.02	3.21	<0.01	N/A	N/A	N/A	N/A	N/A	N/A
37	1651.00	28.35	0.02	3.15	<0.01	822.79	**0.50**[2]	51.42	0.03	0.98	<0.01
238	1651.00	18.58	0.01	2.06	<0.01	127.91	**0.08**	7.99	<0.01	0.64	<0.01

[1]Registration Number 524-424
[2] **Bolded** values exceed the acute terrestrial LOC of 0.1 for listed species

Table 5.12. Upper Bound Kenaga, Terrestrial Herpetofauna Dose-Based Risk Quotients Used to Estimate Direct Effects to the Terrestrial-Phase CRLF Following Acute Exposure to a Formulation on Ornamental Lawns and Turf at 2.2 lb Formulation/A[1]											
Size Class (grams)	**Adjusted LD50**	**EECs and RQs**									
		Broadleaf Plants/ Small Insects		**Fruits/Pods/ Seeds/ Large Insects**		**Small Herbivore Mammals**		**Small Insectivore Mammal**		**Small Amphibians**	
		EEC	**RQ**	**EEC**	**RQ**	**EEC**	**RQ**	**EEC**	**RQ**	**EEC**	**RQ**
1.4	1651.00	11.54	0.01	1.28	<0.01	N/A	N/A	N/A	N/A	N/A	N/A
37	1651.00	11.34	0.01	1.26	<0.01	329.12	**0.20**[2]	20.57	0.01	0.39	<0.01
238	1651.00	7.43	<0.01	0.83	<0.01	51.16	0.03	3.20	<0.01	0.26	<0.01

[1]Registration Number 524-424
[2] **Bolded** values exceed the acute terrestrial LOC of 0.1for listed species

Table 5.13. Upper Bound Kenaga, Terrestrial Herpetofauna Dose-Based Risk Quotients Used to Estimate Direct Effects to the Terrestrial-Phase CRLF Following Acute Exposure to a Formulation on Ornamental Lawns and Turf at 1.1 lb Formulation/A[1]											
Size Class (grams)	**Adjusted LD50**	**EECs and RQs**									
		Broadleaf Plants/ Small Insects		**Fruits/Pods/ Seeds/ Large Insects**		**Small Herbivore Mammals**		**Small Insectivore Mammal**		**Small Amphibians**	
		EEC	**RQ**	**EEC**	**RQ**	**EEC**	**RQ**	**EEC**	**RQ**	**EEC**	**RQ**
1.4	1651.00	5.77	<0.01	0.64	<0.01	N/A	N/A	N/A	N/A	N/A	N/A
37	1651.00	5.67	<0.01	0.63	<0.01	164.56	**0.10**[2]	10.28	0.01	0.20	<0.01
238	1651.00	3.72	<0.01	0.41	<0.01	25.58	0.02	1.60	<0.01	0.13	<0.01

[1]Registration Number 524-424
[2] **Bolded** values either exceed or equal the acute terrestrial LOC of 0.1 for listed species

Open Literature Studies

No additional acute avian studies were found in the open literature to further inform this risk assessment on a quantitative basis. However, as stated previously, there was one 15-day gavage study in which the formulation, Roundup was observed to reduce plasma testosterone levels after treatment of 5 mg/kg bw and above. In addition, "alterations in the structure of the testis and epididymal region…with changes in the expression of androgen receptors restricted to the testis" were observed.

Incident Data

One incident report on glyphosate was categorized as unlikely. This report is not summarized here. For glyphosate isopropylamine salt, one incident connected with use on corn was reported where an unknown quantity of birds were killed following exposure through drift after a broadcast spray. This was an accidental misuse. This report was classified as possible. In a second incident, also categorized as possible, 3 birds (species unknown) were killed following exposure through drift after an unknown application. The legality of the application is unknown.

Effect Determination

Based on the weight-of-evidence, the final effect determination is LAA. This is based on the following points:

- Although no effects were observed in the avian reproduction studies, the concentration levels tested were sufficiently low that at application rates of 7.5 lbs a.e./A and above, the terrestrial dietary EECs are greater than the highest concentration tested in the avian reproduction studies. This creates an uncertainty for direct effects following chronic exposure.
- An open literature study on the effects of the formulation, Roundup on the epididymal region of drakes indicates that there may be some potential effects on the morphophysiology of the male duck reproductive system at dose levels as low as 5 mg/kg bw. This study supports the uncertainty associated with the potential risk following chronic exposure at higher application rates.
- One formulation has a discreet LD_{50} value. Comparing that value to the terrestrial EECs generated from the specific label for that formulation indicates that the acute avian dose-based LOC is exceeded for all application rates (1.1 to 5.5 lb formulation/A) listed on the label.
- The T-HERPS model for the formulation mentioned above indicates that medium and large-sized herps eating small herbivorous mammals on a dose-basis may be at risk following acute exposure at the highest application rate. At the lower application rates on the label, the potential risk to medium-sized herps still remains.
- The incident data, although categorized as possible, indicates that if the acute exposure is sufficiently high, there may be some avian (and thus, CRLF) mortality following acute exposure through drift.
- As stated previously, between 1999 and 2006, glyphosate was reportedly used in all 58 counties in California with the total amount approximately 7.8 million pounds (a.i.) in 2006 (CDPR PUR). In addition, glyphosate has a number of residential and industrial uses that are not represented in these data. Landscape maintenance and rights of way are among the highest usages in the counties which may have some currently CRLF occupied areas (**Tables 2.7 – 2.9** and **Figure 2.2**).

5.2.2 Indirect Effects (via Reductions in Prey Base)

5.2.2.1 Algae (non-vascular plants)

Glyphosate

As stated in the risk estimation section, the highest RQ for non-vascular plants is 0.02. This was based on the lowest available EC_{50} of 12100 ppb for green algae and the highest peak EEC of 210 ppb for management of aquatic plants. The preliminary effect determination was "no effect." Also stated previously, the monitoring data indicated the highest EEC of 7.46 ppb, at least an order of magnitude lower than the modeled concentrations utilized in the risk estimations.

Formulations

Terrestrial Uses

Again, as stated in the risk estimation section, the highest RQ for non-vascular plants following terrestrial application of a formulation is 0.243 using the lowest 96-hr EC_{50} of 0.39 mg/L (390 µg/L) with the freshwater diatom and the highest peak EEC for formulations of 95.2 ppb. This is less than the LOC of 1 for aquatic plants.

Aquatic Uses

The highest RQ for non-vascular plants following aquatic application of a formulation is 4.7 using the lowest EC_{50} of 0.39 mg/L and the peak EEC for formulations following aquatic uses of 1.84 ppm. This is greater than the LOC of 1 for aquatic plants.

Open Literature Data

Of the available open literature studies from which data may be extracted for comparing the results with the submitted studies, 3 studies, on 3 different species of green algae provide lower 96-hr EC_{50}'s based on cell counts (growth) correlated with absorbance over time for 96 hours on a Shimadzu UV-2401 PC Spectrophotometer. All of these studies were performed by the same group of scientists and published in different papers. The papers were not thoroughly reviewed for acceptability according to Agency guidelines; however, they are discussed in this section and compared to the highest aquatic EEC. In the first study, conducted with 95% technical material (not stated if glyphosate or the IPA of glyphosate), the 96-hr EC_{50} was 3.530 mg/L for *Chlorella pyrenoidosa* (Ma et.al 2001, ECOTOX reference 61983). Comparing that value to the highest EEC of 222.9 ppb, the RQ would be 0.06, significantly lower than the LOC for aquatic plants. In the second study (Ma et al., 2002, ECOTOX reference 65938), the 96 hr. EC_{50} for *Chlorella vulgaris* was 4.70 mg/L. This was again conducted with a 95% technical product. The study authors used the CAS number for glyphosate, not IPA, so it is assumed that this is the acid. The resulting highest RQ from this study would be 0.05. The third study, conducted with *Raphidocelis subcapitata (Selenastrum capricornutum)* (Ma et al., 2006, ECOTOX ref. 83543), the 96 hr. acute toxicity value is 5.56 mg/L with a resulting RQ of 0.04. Again, the study was conducted with 95% technical product, which is presumed to be the glyphosate acid. Even with these lower endpoints, the LOC for aquatic plants would not be exceeded.

Based on the weight-of-evidence, final effects determination is LAA for indirect effects, reduction in prey base (aquatic non-vascular plants) following application of a formulation registered for aquatic uses. The effects determination is based on an exceedance of the LOC for aquatic non-vascular plants. As with avian species, glyphosate is used in all 58 counties in California. Landscape maintenance and rights of way are among the highest usages in the counties which may have some currently CRLF occupied areas.

5.2.2.2 Aquatic Invertebrates

Glyphosate

The potential for glyphosate 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.

The acute and chronic RQs for indirect effects to the aquatic-phase CRLF (reduction in aquatic invertebrate prey) are presented in **Section 5.1.1.2**. As stated previously, none of the RQs exceed either the acute or chronic LOC for freshwater invertebrates. Monitoring data (NAQWA) indicate surface concentrations ranging from 0.1 – 7.46 ppb. These concentrations are at least an order of magnitude lower than the modeled concentrations utilized in the risk estimations.

The probability of an individual effect at the RQ (<0.01) is <1 in 8.9E+18 (1 in 3.2E+04 to 1.0E+72). The acute aquatic invertebrate studies provided no slope. Therefore, a default slope of 4.5 (confidence limits 2 - 9) was used to estimate the probability. The percentage effect to the freshwater invertebrate population prey base is < 1.1E-17 percent.

Formulations

Terrestrial uses

As stated previously, comparing the most conservative EC_{50} of 3 mg formulation/L with the highest peak EEC for formulations of 95.2 ppb formulation, the highest RQ for aquatic invertebrates is 0.03, which is less than the LOC of 0.05 for aquatic invertebrates. Therefore, none of the other uses will exceed the acute aquatic invertebrate LOC.

Aquatic uses

Again, the LOC for aquatic animals is not exceeded with the acute toxicity study endpoint of 164.3 ppm formulation and the peak EEC of 1.84 ppm formulation.

Open Literature

There were no open literature studies on aquatic invertebrates that would further inform this assessment of risk. All of the studies provide endpoints that are greater than the most sensitive endpoints used in this assessment.

The acute and chronic RQs for glyphosate are below the acute and chronic LOC's for aquatic animals (highest acute and chronic RQ's are both < 0.01). For formulations, the acute LOC for listed aquatic invertebrates is not exceeded for either terrestrial or aquatic uses (acute RQs are 0.03 and 0.01, respectively). Again, as stated previously, monitoring data (NAQWA) indicate surface concentrations ranging from 0.1 – 7.46 ppb. These concentrations are at least an order of magnitude lower than the modeled concentrations utilized in the risk estimations.

Based on the weight of the evidence, the final effect determination is "no effect" for indirect effects, reduction in prey base for the aquatic-phase CRLF. This determination is based on the lack of LOC exceedances following either acute or chronic exposures for both glyphosate and formulations, the low monitoring data in surface water when compared to the modeled concentrations, the low probability of an individual effect and the low percentage effect to the freshwater invertebrate population prey base.

5.2.2.3 Fish and Aquatic-phase Frogs

As stated previously, for both glyphosate and formulations, none of the RQs exceed the acute and/or chronic LOCs for freshwater fish (surrogate for the CRLF) and aquatic-phase amphibians. Risks from chronic exposure to formulations (terrestrial uses with the conservative peak EEC and the LOAEC from a leopard frog study) were not considered to be of a concern. The probability of an individual effect at the RQ (<0.01) is <1 in 8.9E+18 (1 in 3.2E+04 to 1.0E+72) and percentage effect to the freshwater fish/aquatic-phase amphibian population prey base is 1 in 1.1E-19.

Based on the weight-of-evidence, the final effects determination is "no effect" for this endpoint (indirect effects: fish and frogs - reduction in prey base). This determination is based on the lack of LOC exceedances following either acute or chronic exposures for both glyphosate and formulations, the low monitoring data in surface water when compared to the modeled concentrations, the low probability of an individual effect and the low percentage effect to the freshwater fish and amphibian population prey base.

5.2.2.4 Terrestrial Invertebrates

Glyphosate

When the terrestrial-phase CRLF reaches juvenile and adult stages, its diet is mainly composed of terrestrial invertebrates. As stated in the risk estimation section, the LD_{50} value for terrestrial invertebrates is not a discrete value (>781.25 µg a.i./g of bee). Using the terrestrial EECs estimated from the model, T-REX, this is equivalent to a rate of >

5.79 lb a.e./A. It is noted that there is 27% mortality at this dose level. Therefore, the terrestrial model, T-REX was used to estimate upper bound RQs for terrestrial invertebrates.

As stated previously, the results show that the RQs could exceed the acute LOC for listed species at all application rates for small insects and at the higher application rates for large insects. In addition, the RQs could exceed the acute LOC for non-listed species at the higher application rates for small insects. At the highest upper bound RQ (<1.4 with forestry uses), the chance of an individual effect to small insects is <1 in 1.34 with a <75% percentage effect to the terrestrial invertebrate prey base. At the lowest upper bound RQ (<0.01 with rangeland), the chance of an individual effect to large insects is <8.86E+18 with a <1.13E-17 percentage effect to the terrestrial invertebrate prey base.

Formulations

On an acid equivalent basis, the formulations were tested at lower concentrations than the technical material, ranging from 0.096 lbs a.e./A to 3.86 lbs a.e./A and/or 30 to 100 µg a.e./bee (technical material was tested at 100 µg/bee or as stated above, 5.79 lbs a.e./A.). With the following exception, all of the LD_{50} or LC_{50} values for formulations exceeded the highest dose/concentration tested. There was one formulation which had a discrete 7-day LD_{50} value for 1 – 2 day old predatory mites (*Typhlodromus pyri*) of 1.1 lb a.e./A. This is 7 times less than the highest application rate for glyphosate products on an acid equivalent basis. It is for glyphosate monoammonium salt (MRID 45767105; MON78568). There is no specific registered product in the United States with this name. Therefore, it remains an uncertainty whether or not currently registered glyphosate products may affect terrestrial invertebrates.

Open Literature Data

Open literature data on glyphosate, its salts and/or formulations included a large number of efficacy studies which were not useful for a quantitative assessment of risk. Those studies which could possibly be used were either tested at lower concentrations than the submitted studies with no effects or insufficient information was provided on the test material to determine the concentration levels tested for either the active ingredient and/or the glyphosate acid equivalent.

Based on the weight-of-evidence, the final effects determination is LAA for indirect effects on terrestrial invertebrates as dietary food items. The effects determination is based on a potential exceedance of the LOC for listed terrestrial invertebrates at all application rates (small invertebrates), for non-listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above (small invertebrates) and for listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above (large invertebrates). The probability of an individual effect and the percentage population effects are expected to be high at the higher application rates. As with avian species, glyphosate is used in all 58 counties in California. Landscape maintenance and rights of way are among the highest usages in the counties which may have some currently CRLF occupied areas.

5.2.2.5 Mammals

Glyphosate

Life history data for terrestrial-phase CRLFs indicate that large adult frogs consume terrestrial vertebrates, including mice. For glyphosate, acute RQs were not calculated for mammals because there were no mortalities up to and including the highest doses tested. In addition, no sublethal effects were reported in any of the acute mammalian studies on the technical material. If comparisons are made between the terrestrial EECs estimated from T-REX and the highest dose tested in the acute study, the results show that all of the EEC values are lower, but at application rates of 3.75 lbs a.e./A and above, the EECs are greater than $1/10^{th}$ of the highest dose tested in the acute mammalian studies. None of the EECs are higher than 20% of the highest dose tested. For that reason, there is an uncertainty for listed mammalian species but the uncertainty is considerably less for non-listed mammalian species. For example, for forestry uses at 7.95 lb a.e./A, the highest use rate, the dose-based EEC for small mammals eating short grass is 1819.13 mg a.e./kg bw. The adjusted acute LD_{50} for a 15 g herbivore mammal is 10549.59. One thousand eight hundred nineteen divided by 10549.59 is greater than 0.1, the LOC for listed species but less than 0.5, the LOC for non-listed species. The probability of an individual effect at the LOC (0.1: no RQs) is <1 in 2.94E+05 (<1 in 8.86E+18 to <1 in 4.40E+01). The acute mammal studies provided no slope. Therefore, a default slope of 4.5 (confidence limits 2 - 9) was used to estimate the probability. The percentage effect to the mammalian prey base would be <1%.

The chronic RQs exceed the chronic LOC for small mammals on a dose-basis for use rates of 3.84 lbs a.e./A and above. This includes many crops, forestry, areas with impervious surfaces and rights of way. The RQs range from 0.16 to 1.66.

Formulations

As stated in the risk estimation section, most of the available mammalian studies on formulations indicate LD_{50} values greater than the highest dose tested. For the five studies which have definitive acute toxicity values, the application rates from the specific labels for which these studies were submitted indicate an exceedance of the acute mammalian LOC for use rates of 2.5 lbs formulation/A for one label, 3.5 lbs formulation/A and above for two labels, 5 lbs formulation/A for the third label and 5.5 lbs formulation/A for the fourth label.

The probability of an individual effect for the formulation at the LOC would be the same as the probability for the technical material (see above). Since the use rates from the labels for these products were not individually modeled, the probability at the RQ was not estimated. For these formulations, at the LOC (i.e., the application rates below which listed species would not expected to be affected), the percentage effect to the mammalian prey base would be <0.01%.

Note: There is a reproduction/developmental screening study on POEA, the toxic surfactant. This has a lower endpoint than the reproduction study on glyphosate (NOAEL: 300 ppm ((14.9 - 16.6 mg/kg bw/day (M) and 18.9 - 19.5 mg/kg bw/day (F)) and LOAEL 1000 ppm (52.8 – 56.1 mg/kg bw/day (M) and 64.9 – 66.6 mg/kg bw/day (F) based on increased mean number of unaccounted-for sites, litter loss, decreased mean number of pups born live, litter size and postnatal survival from birth to PND 4. The effects are not reproducible in second generation. This may impact risk to mammals following chronic exposure to one of the formulations containing the POEA surfactant.

Open Literature Data

No additional mammalian studies were found in the open literature to further inform this risk assessment on a quantitative basis. Most of the studies were field studies to observe indirect effects to various small mammal populations in forests following terrestrial plant reduction from glyphosate applications. These studies would be supportive of indirect effects related to changes in the riparian habitat of the CRLF.

Based on the weight-of-evidence, the final effects determination is LAA for indirect effects, reductions in prey base based on the potential risk to mammals following chronic exposure. The uncertainties associated with acute exposure to glyphosate and its formulations are considered to be insignificant because the CRLF does not have an obligate relationship with mammals, none of the EECs for acute exposure to glyphosate are higher than 20% of the highest dose tested (at which there was no effect) and the percentage effect to the mammalian prey base and the probability of an individual effect for both glyphosate and its formulations are considered to be low. Again, glyphosate is used in all 58 counties in California with landscape maintenance and rights of way among the highest usages in the counties which may have some currently CRLF occupied areas.

5.2.2.6 Terrestrial-phase Amphibians

Terrestrial-phase adult CRLFs also consume frogs. RQ values representing direct exposures of glyphosate to terrestrial-phase CRLFs are used to represent exposures of glyphosate to frogs in terrestrial habitats. Acute RQs for avian species (surrogate to CRLF) were not calculated because there were no mortalities up to and including the highest dose/concentrations tested; however, there is an uncertainty in the potential risk. Although all of the terrestrial EEC values are lower, many are greater than $1/10^{th}$ of the highest dose/concentration tested in the acute avian studies. The chronic avian study showed no effects at the highest concentration tested; however, again, there is an uncertainty in the potential risk with uses with application rates of 7.5 lbs a.e./A and higher because the terrestrial EECs that are higher than the highest concentration tested in the chronic avian study. T-HERPS indicated that herps eating broadleaf plants, small insects and small herbivorous mammals on a dietary-basis may be at risk following chronic exposure at application rates of 7.5 lb/A and above.

Based on the weight-of-evidence, the final effects determination is likely to adversely affect (LAA) (see section 5.2.1.2 for supporting statements).

5.2.3 Indirect Effects (via Habitat Effects)

5.2.3.1 Aquatic Plants (Vascular and Non-vascular)

Glyphosate

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

Potential indirect effects to the CRLF based on impacts to habitat and/or primary production were assessed using RQs from freshwater aquatic vascular and non-vascular plant data. For aquatic plants, the LOC is not exceeded for glyphosate in acid equivalents with the highest peak EEC generated from the registered uses (direct application to water). Based on an RQ of 0.02 for both vascular and non-vascular plants, glyphosate is not expected to indirectly affect the aquatic-phase of the CRLF through the diet (tadpoles) or habitat from aquatic non-vascular plants.

Risk to Emergent Aquatic Vegetation - Risk from Spray Drift Adjacent to Habitat Area

Sections 5.1.2.3 and 5.2.3.2 describe the risk to the terrestrial plant community. Risks to emergent plants following spray drift may be assessed using the same parameters. Using the most sensitive EC_{25} values for both dicots and monocots, the RQs range from <0.1 – 5.37 with application rates ranging from 0.387 (aerial) to 7.95 (aerial) lbs ae/A. Based on the EC_{25} ranges (see **Section 5.2.3.2**), those monocots and dicots with EC_{25} values of 0.4 lb a.e./A or greater will not exceed the terrestrial plant LOC with the highest terrestrial EECs from forestry uses at 7.95 lbs. a.e./A. Since some of the EC_{25}'s for both monocots and dicots are greater than 0.4 lb a.e./A, it is possible that not all emergent aquatic plants will be affected following spray drift alone. Spray drift buffers are estimated in Section 5.2.2.4.

Formulations

For formulations, again, the LOC for aquatic plants is not exceeded with the highest peak EEC generated from terrestrial applications for both non-vascular and vascular plants (highest RQs are 0.24 and 0.02, respectively). Following aquatic applications, the LOC is exceeded for non-vascular plants (RQ = 4.7) but is not exceeded for non-vascular plants (RQ = 0.07).

Open Literature Data

Open literature data for aquatic non-vascular plants are described in section 5.2.2.1. For most of the studies on vascular plants, there are insufficient details in the articles to accurately determine concentration levels tested. For other studies, the endpoints were higher than those found in the submitted studies.

As stated previously, monitoring data are at least an order of magnitude lower than the modeled concentrations utilized in the risk estimations.

Based on the weight-of-evidence, the final effects determination is LAA for aquatic plants (indirect effects: habitat) (see Section 5.2.2.1. for supporting evidence).

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.

As stated in the risk estimation section, none of the RQs for terrestrial plants living in either dry or semi-aquatic areas exposed to the combined deposition estimates from runoff and spray drift exceed the terrestrial plant LOC. The RQ values for monocots and dicots inhabiting dry and semi-aquatic areas are derived by comparing the combined deposition estimates from runoff and spray drift to adverse effect levels measured in seedling emergence studies. For glyphosate, there were no effects in the seedling emergence studies. Therefore, it follows that RQs estimated from seedling emergence values would be low. For estimation of risk from spray drift alone, the exposure from spray drift is compared to the more sensitive measure of effect, either seedling emergence or vegetative vigor. The results of these calculations are RQ values for monocots and dicots inhabiting adjacent and semi-aquatic areas and exposed to drift only. For aerial uses at application rates of 3.75 lbs a.e./A and above and for ground uses with impervious surfaces at 7.95 lbs a.e./A the RQs from spray drift for both monocots and dicots exceed the terrestrial plant LOC of 1.

The seedling emergence EC_{25} values for monocots and dicots are all greater than 4 lbs a.e./A. The RQs with the terrestrial uses of glyphosate for monocots and dicots inhabiting dry and semi-aquatic areas (runoff and spray drift), utilizing the seedling emergence EC_{25} values of > 4 lbs a.e./A range from < 0.1 to < 0.87.

For spray drift only, the RQs range from <0.1 – 5.37 with application rates ranging from 0.387 to 7.95 lbs ae/A. These values were derived from the most sensitive EC_{25} value of 0.074 lb ae/A (dicots). The EC_{25} values range from 0.074 to 0.89 lbs a.e./A for dicots and from 0.16 – 0.98 lbs a.e./A for monocots from the vegetative vigor studies. Based on these ranges, those monocots and dicots with EC_{25} values of 0.4 lb a.e./A or greater will not exceed the terrestrial plant LOC with the highest terrestrial EECs from forestry uses at 7.95 lbs. a.e./A. From the most sensitive vegetative vigor study this would include cucumber and garden pea for dicots and purple nutsedge for monocots. These risk estimates are based on terrestrial plant toxicity data for a limited set of agricultural plants. Therefore, there are uncertainties associated with potential toxicity to the wide variety of non-agricultural plants inhabiting the CRLF habitat. Even if glyphosate only kills the most sensitive terrestrial plants, the habitat may still be sufficiently modified to the point such that it is no longer viable CRLF habitat.

The glyphosate labels state that it is a postemergent, systemic herbicide. It is generally non-selective and gives broad-spectrum control of many annual weeds, woody brush and trees. For tree, vine and shrub crops, the general precautions state that extreme care must be exercised to avoid contact of herbicide solution, spray, drift or mist with foliage or green bark of trunk, branches, suckers, fruit or other parts of trees, canes and vines. Therefore, it is expected that glyphosate applications can affect both herbaceous and woody vegetation, especially when the exposure is via drift. This is supported by the incident data. For glyphosate, 63 incidents were reported for mostly plant damage to a wide variety of plants from either direct treatment or spray drift. For the isopropylamine salt of glyphosate, 443 incident reports were filed for a wide variety of terrestrial plants, particularly agricultural crops and grass. There were a few incidents of trees being damaged or killed. Plant damage and mortality were the main issues with drift as the main exposure route. Studies in the open literature were mainly efficacy studies or studies on fungi and were not useful as support for this risk assessment.

Based on the weight-of-evidence, the final effect determination is “LAA” for indirect effects: reduction in terrestrial plant community - riparian and upland habitat. This determination is based on LOC exceedances for terrestrial plants (both monocots and dicots) following spray drift at aerial application rates of 3.85 lbs a.e./A and above and at a ground application rates of 7.5 lbs a.e./A and above. Because the RQs for terrestrial plants are relatively low, sufficient buffers may mitigate the concern for the terrestrial habitat associated with the CRLF and reduce the determination to NLAA. Again, glyphosate is used in all 58 counties in California with landscape maintenance and rights of way among the highest usages in the counties which may have some currently CRLF occupied areas.

Spray Drift Buffer Analysis

As stated previously, the entire state of California is considered to be both the initial area of concern and the action area. Therefore, spray drift buffers can be estimated for a specific use; however, for aggregate uses, the widest buffer for both terrestrial and aquatic uses would be applied and would effectively be the entire state.

For a specific use, in order to determine terrestrial and aquatic habitats of concern due to glyphosate exposures through spray drift, it is necessary to estimate the distance that spray applications can drift from the treated area and still be present at concentrations that exceed levels of concern. The quantitative estimations of risk indicate that terrestrial plants generate the highest RQ risk values. Therefore, the spray drift analysis was conducted with the most sensitive endpoint for terrestrial plants. Using the most sensitive terrestrial plant endpoint with the AgDrift model in the Tier I aerial mode with the default droplet size distribution ASAE very fine to fine, the spray drift buffers for use rates of 7.5 lbs a.e./A and above exceed the 1,000 foot range. Therefore, the AgDrift Tier 3 model for aerial applications was used with a maximum downwind distance of 3000. This distance goes slightly beyond the maximum limit of the model and is thus an uncertainty.

In order to characterize the portion of the action area for a specific use that is relevant to the CRLF and specific to the area where the effects determination (*e.g.*, NLAA versus LAA) could be made, an analysis was conducted using the most sensitive non-listed plant EC_{25} of 0.074 lbs ai/acre. Typically the NOAEC is used when there is an obligate relationship between the species being assessed and listed plants (or other taxa). However, there is no obligate relationship between the CRLF and any listed plant; therefore, the LAA/NLAA determination would be based on the area defined by the non-listed species LOC (*e.g.*, EEC/EC_{25}).

For glyphosate uses, the maximum estimated distance is 2785 feet for aerial application on forestry at 7.95 lbs a.e./A. All of the estimations are based on a default droplet size distribution ASAE of very fine to fine. The next largest buffer is 2631 feet for forestry and rights of way aerial application at 7.5 lbs a.e./A. The remainder of the uses have reduced buffer distances for lower application rates. A summary of the modeled distances by application rate is presented in **Table 5.14.**

Table 5.14. Predicted Terrestrial Spray Drift Dissipation Distances for Glyphosate From AgDrift

Application Rate (lbs. a.e./A)/ Method	Uses Represented	Buffer Distance for Non-listed Plants Distance (ft)[1]
3.75 Ground	Alfafa, avocado, corn, cotton, forestry, garlic, impervious, residential, row crop, strawberry, wheat	125[2]
3.84 Ground	Almond, fruit, grape, olive	125[2]
3.85 Ground	Citrus	125[2]
3.85 Aerial	Cole crop, lettuce, melon, onion, potato, wine grape	1768[2]
0.75 Aerial	Corn	312[2]
7.5 Aerial	Forestry	2631[2]
7.95 Aerial	Forestry	2785[2,3]
7.95 Ground	Impervious	259

Table 5.14. Predicted Terrestrial Spray Drift Dissipation Distances for Glyphosate From AgDrift		
Application Rate (lbs. a.e./A)/ Method	**Uses Represented**	**Buffer Distance for Non-listed Plants Distance (ft)[1]**
3.75 Aerial	Nursery, rangeland, sugar beet, tomato, turf	1768[2]
1.54 Ground	Rangeland	53[2]
0.387 Aerial	Rangeland	135[2]
7.5 Aerial	Rights of way	2631[2]
3.69 Ground	Rights of way	125[2]
0.75 Ground	Wheat	25[2]

[1] The EC_{25} value is used to define the buffer associated with the relevant portion of the action area.
[2] AgDrift with droplet size distribution ASAE very fine to fine with high boom for ground applications and Tier 3 for aerial with maximum downwind distance of 3000 if needed (this is an uncertainty)
[3] Some of the forestry labels state: "do not use nozzles or nozzle configurations that dispense spray as fine spray droplets". In those cases, using the AgDrift Aerial Tier 3 model with a fine to medium droplet size would provide a reduced buffer distance of 1122 feet.

Open Literature

The open literature studies do not generally have endpoints that can be compared to those in the submitted studies. The studies are mostly on fungus and/or are efficacy studies.

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).

The effect determinations for indirect effects to the CRLF via direct effects to aquatic and terrestrial plants are used to determine whether modification to critical habitat may occur.

For aquatic plants, the aquatic plant LOC is not exceeded for glyphosate a.e. for both vascular and non-vascular plants; however, for formulations, the LOC is exceeded for non-vascular plants (aquatic applications). The LOC is not exceeded for either terrestrial applications (both vascular and non-vascular plants) or for aquatic applications (vascular plants).

For terrestrial plants, the terrestrial plant LOC for both monocots and dicots is exceeded following spray drift from aerial uses at application rates of 3.75 lbs a.e./A and above and from ground uses at a rate of 7.5 lbs a.e./A and above. Risks to emergent aquatic plants following spray drift were also assessed using the same model as the terrestrial plant community.

The effect determinations for both aquatic and terrestrial plants are "LAA" based on LOC exceedances for non-vascular aquatic plants following application of a formulation directly to water and for aquatic emergent plants and terrestrial plants following aerial application at rates of 3.75 lbs a.e./A and above and following ground applications at rates of 7.5 lbs a.e./A and above. As stated previously, glyphosate is used in every county in the state of California.

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 (discussed above), 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.

For glyphosate, the acute and chronic RQs for direct effects to the aquatic-phase CRLF do not exceed either the acute or chronic LOC for freshwater fish and amphibians. Acute RQs from formulated products, both terrestrial and aquatic uses also do not exceed the acute LOC for freshwater fish and amphibians. The final effect determination is "no effect" for direct effects to the aquatic-phase CRLF.

For freshwater invertebrates, none of the acute or chronic RQs exceed either the acute or chronic aquatic LOC for either glyphosate a.e. or for formulations. In addition, the probit analysis indicates that the probability of an individual effect and the percentage effect to the freshwater invertebrate population prey base would be very low and the monitoring data are considerably lower than the modeled concentrations utilized in the risk assessment. Based on the weight-of-evidence, the final effect determination is "no effect" for aquatic invertebrates.

For freshwater fish as food items, as stated above, the final effect determination is "no effect".

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.

For terrestrial plants, the risk from spray drift from aerial uses at application rates of 3.75 lbs a.e./A and above and ground uses at rates of 7.5 lbs a.e./A and above exceed the LOC of 1 for both monocots and dicots. The final effect determination for terrestrial plants is "LAA".

The third terrestrial-phase PCE is "reduction and/or modification of food sources for terrestrial phase juveniles and adults." To assess the impact of glyphosate on this PCE, acute and chronic toxicity endpoints for terrestrial invertebrates, mammals, and terrestrial-phase frogs are used as measures of effects.

For mammals, based on the weight-of-evidence, the final effects determination is LAA for indirect effects, reductions in prey base based on the potential risk following chronic exposure. The chronic RQs exceed the chronic LOC for small mammals on a dose-basis for use scenarios at application rates of 3.84 lbs a.e./A and above. This includes many crops, forestry, areas with impervious surfaces and rights of way. The RQs range from 0.16 to 1.66.

For terrestrial-phase amphibians, based on the weight-of-evidence, the final effect determination is LAA. This is based on the following statements. The concentration levels tested in the chronic avian studies were sufficiently low that at application rates of 7.5 lbs a.e./A and above, the terrestrial dietary EECs are greater than the highest concentration tested in the avian reproduction studies. This creates an uncertainty for direct effects following chronic exposure. This is supported by an open literature study on the effects of the formulation, Roundup on the epididymal region of drakes indicates that there may be some potential effects on the morphophysiology of the male duck reproductive system at dose levels as low as 5 mg/kg bw. The acute avian dose-based LOC is exceeded for one formulation at all application rates (1.1 to 5.5 lb formulation/A) listed on the label. When modeled using the T-HERPS model, the potential risk remains. The incident data, although categorized as possible, indicates that if the acute exposure is sufficiently high, there may be some avian (and thus, CRLF) mortality following acute exposure through drift.

For terrestrial invertebrates, based on the weight-of-evidence, the final effects determination is LAA. The effects determination is based on a potential exceedance of the LOC for listed terrestrial invertebrates at all application rates (small invertebrates), for non-listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A (small invertebrates) and for listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above (large invertebrates). The probability of an individual effect and the percentage population effects are expected to be high at the higher application rates.

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.

For direct effects, as stated in the amphibian paragraph provided above, based on the weight-of-evidence, the final effect determination is “LAA” based on the uncertainty associated with chronic exposure and supporting evidence from an open literature study, exceedances of the acute avian LOC for a formulation with a discreet LD_{50} value and supporting incident data that there may be some avian (and thus, CRLF) mortality following acute exposure through drift.

For indirect effects, the final effect determination is “LAA” for mammals (potential risk following chronic exposure), terrestrial invertebrates (potential exceedance of the LOC for listed small invertebrates at all application rates, for non-listed small invertebrates at the higher application rates and for listed large invertebrates at the higher application rates. For amphibians, again as stated above, the final effect determination is “LAA”.

6. Uncertainties

6.1 Exposure Assessment Uncertainties

6.1.1 Environmental Fate Data

Factors controlling the persistence, transformation, and transport of pesticides depend on the characteristics of the soil and microbial population. Studies that satisfy Agency environmental fate data requirements are conducted in limited systems (soil; water-sediment) and may not represent all of the potential use environments. Glyphosate is widely used in the United States and for multiple uses at the same site. The behavior of glyphosate based on data in a limited number of test systems extrapolated to multiple sites may overestimate or underestimate the exposure to glyphosate in specific sites and season. Environmental fate data used in estimating exposure concentrations do not specifically take into account the pH dependent dissociation and speciation of glyphosate in aquatic systems.

There are no environmental fate data available to describe the behavior of end use products in which glyphosate is formulated with a surfactant, leading to some uncertainty in the estimated exposures for these products.

6.1.2 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 dependent on pest resistance, timing of applications, cultural practices, and market forces.

6.1.3 Aquatic Exposure Modeling of Glyphosate

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

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

Aquatic exposure to glyphosate was assessed using a Tier I approach, which is designed as a coarse screen and estimates conservative pesticide concentrations in surface water from a few basic chemical parameters and pesticide label use and application information. Tier 1 is used to screen chemicals to determine which ones potentially pose sufficient risk to warrant higher level modeling. Most aquatic EECs were generated using simple dilution calculations based on the mass of pesticide and the volume of the water body. These calculations do not account for any dissipation or degradation processes and so are likely to overestimate exposure. Exposure to glyphosate from terrestrial applications was estimated using the Tier I model GENEEC2.

For terrestrial uses, assumptions made about transport of the pesticide to the water body lead to some uncertainty in these estimates. Both the simple dilution calculation and the GENEEC2 modeling are based on the default assumption that 5% of applied pesticide is transported to the water body through spray drift. For formulations, quantitative exposure modeling for formulations is limited based on the expectation that the varying physical-chemical properties of individual components of pesticide formulations will result in progressively different formulation constituents in environmental media over time. As the proportions of formulation components in environmental media differ from the proportions in the tested formulation, the assumption that environmental residues are toxicologically equivalent to tested formulations cannot be supported beyond the time period immediately following product application. For this reason, spray drift of formulation directly to the water body is the only transport route considered for estimating formulation EECs.

To account for uncertainties associated with modeling, available monitoring data were compared to calculated estimates of peak EECs for the different uses. As discussed above, the NAWQA database includes data for glyphosate concentrations measured in surface waters at 3 sites in California, one receiving runoff from agricultural areas and two from mixed use areas. The specific use patterns (e.g. application rates and timing, crops) associated with the use areas are unknown, however, they are assumed to be representative of potential glyphosate terrestrial use areas. Glyphosate was detected most frequently at the agricultural site, where the highest measured concentration was 7.5 ug/L, an order of magnitude lower than the peak EEC for terrestrial applications of glyphosate estimated using Tier I modeling. Monitoring is not expected to capture peak concentrations due to limited sampling frequency. Monitoring only considers individual compounds, the active ingredient and its metabolites, and does not reflect exposure to formulated products. Additionally, there are no monitoring data available for direct aquatic applications of glyphosate, which are likely to have higher exposures than terrestrial applications.

6.1.4 Potential Groundwater Contributions to Surface Water Chemical Concentrations

Although the potential impact of discharging groundwater on CRLF populations is not explicitly delineated, it should be noted that groundwater could provide a source of pesticide to surface water bodies – especially low-order streams, headwaters, and groundwater-fed pools. This is particularly likely if the chemical is persistent and mobile. Soluble chemicals that are primarily subject to photolytic degradation will be very likely to persist in groundwater, and can be transportable over long distances. Similarly, many chemicals degrade slowly under anaerobic conditions (common in aquifers) and are thus more persistent in groundwater. Much of this groundwater will eventually be discharged to the surface – often supporting stream flow in the absence of rainfall. Continuously flowing low-order streams in particular are sustained by groundwater discharge, which can constitute 100% of stream flow during baseflow (no runoff) conditions. Thus, it is important to keep in mind that pesticides in groundwater may have a major (detrimental) impact on surface water quality, and on CRLF habitats.

SciGrow may be used to determine likely 'high-end' groundwater vulnerability, with the assumption (based upon persistence in sub- and anoxic conditions, and mobility) that much of the compound entering the groundwater will be transported some distance and eventually discharged into surface water. Although concentrations in a receiving water body resulting from groundwater discharge cannot be explicitly quantified, it should be assumed that significant attenuation and retardation of the chemical will have occurred prior to discharge. Nevertheless, groundwater could still be a significant consistent source of chronic background concentrations in surface water, and may also add to surface runoff during storm events (as a result of enhanced groundwater discharge typically characterized by the 'tailing limb' of a storm hydrograph).

6.1.5 Usage Uncertainties

County-level usage data were obtained from California's Department of Pesticide Regulation Pesticide Use Reporting (CDPR PUR) database. Four years of data (2002 – 2005) were included in this analysis because statistical methodology for identifying outliers, in terms of area treated and pounds applied, was provided by CDPR for these years only. No methodology for removing outliers was provided by CDPR for 2001 and earlier pesticide data; therefore, this information was not included in the analysis because it may misrepresent actual usage patterns. CDPR PUR documentation indicates that errors in the data may include the following: a misplaced decimal; incorrect measures, area treated, or units; and reports of diluted pesticide concentrations. In addition, it is possible that the data may contain reports for pesticide uses that have been cancelled. The CPDR PUR data does not include home owner applied pesticides; therefore, residential uses are not likely to be reported. As with all pesticide usage data, there may be instances of misuse and misreporting. The Agency made use of the most current, verifiable information; in cases where there were discrepancies, the most conservative information was used.

6.1.6 Terrestrial Exposure Modeling of Glyphosate

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 glyphosate 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 glyphosate from multiple applications, each application of glyphosate 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/AGDISP 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/AGDISP 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 (*e*.g., aerial), release heights and wind speeds. Alterations in any of these inputs would change the area of potential effect.

6.2 Effects Assessment Uncertainties

6.2.1 Age Class and Sensitivity of Effects Thresholds

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

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

6.2.2 Use of Surrogate Species Effects Data

Limited toxicity tests and open literature data on glyphosate are available for frogs or any other aquatic-phase amphibian; therefore, freshwater fish are used as surrogate species for aquatic-phase amphibians. Although limited data are available for glyphosate, the available open literature information on glyphosate toxicity to aquatic-phase amphibians shows that acute and chronic ecotoxicity endpoints for aquatic-phase amphibians are generally less sensitive than freshwater fish. Therefore, endpoints based on freshwater fish ecotoxicity data are assumed to be protective of potential direct effects to aquatic-phase amphibians including the CRLF, and extrapolation of the risk conclusions from the most sensitive tested species to the aquatic-phase CRLF is likely to overestimate the potential risks to those species. Efforts are made to select the organisms most likely to be affected by the type of compound and usage pattern; however, there is an inherent uncertainty in extrapolating across phyla. In addition, the Agency's LOCs are intentionally set very low, and conservative estimates are made in the screening level risk assessment to account for these uncertainties.

6.2.3 Sublethal Effects

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

Sublethal effects from exposure to glyphosate are presented throughout the Ecological Effects Section (Section 4.0). To the extent to which sublethal effects are not considered in this assessment, the potential direct and indirect effects of glyphosate 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 Assessment of Risk to Terrestrial Species

Many of the ecological effects studies on terrestrial species did not show any effects at the highest dose/concentration tested. This included the acute toxicity studies on birds, mammals and invertebrates as well as the chronic avian study. For the acute toxicity studies, the dose/concentration levels were relatively high. With the exception of one scenario with terrestrial invertebrates, the terrestrial EECs were all lower than the highest dose/concentration tested in the acute studies; however, they are sufficiently high that there is an uncertainty in the acute risk to these taxonomic groups. The terrestrial EEC for chronic exposure is higher than the highest concentration tested in the chronic avian study. Due to the uncertainty of risk to avian species at concentration levels higher than those tested in the chronic study, this was considered to be a potential risk to this taxonomic group. These uncertainties may lead to an overestimation of risk to these taxomonic groups.

7. Risk Conclusions

In fulfilling its obligations under Section 7(a)(2) of the Endangered Species Act, the information presented in this endangered species risk assessment represents the best data currently available to assess the potential risks of glyphosate 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 glyphosate. Additionally, the Agency has determined that there is the potential for modification of CRLF designated critical habitat from the use of the chemical.

This assessment indicates that direct effects to the terrestrial-phase CRLF eating broadleaf plants, small insects and small herbivorous mammals on a dietary-basis may be at risk following chronic exposure to glyphosate at application rates of 7.5 lb a.e./A and above (forestry, areas with impervious surfaces and rights of way). In addition, for one particular formulation (Registration No. 524-424), medium and large-sized CRLF's eating small herbivorous mammals on a dose-basis may be at risk following acute exposure at an application rate of 5.5 lb formulation/A (industrial outdoor uses). At the lowest application rate of 1.1 lb formulation/A, there is potential risk to medium-sized CRLF's eating small herbivorous mammals on a dose-basis (ornamental lawns and turf).

Indirect effects to both the aquatic- and terrestrial-phase CRLF, based on reduction in prey base may occur with the following taxonomic groups: aquatic nonvascular plants with products specifically labelled for aquatic use; small insects with any use and large insects at application rates of 7.5 lb a.e./A and above; terrestrial phase amphibians following chronic exposure at application rates of 7.5 lb a.e./A and above; terrestrial phase amphibians following acute exposure to one particular formulation (Registration No. 524-424), at application rates of 1.1 lbs formulation/A and above (ornamental lawns and turf and industrial outdoor uses) and mammals following chronic exposure at application rates of 3.84 lbs a.e./A and above (i.e., many crops, forestry, rights of way and areas with impervious surfaces).

Indirect effects to both the aquatic- and terrestrial-phase CRLF, based on habitat effects may occur with aquatic non-vascular plants with products specifically labelled for aquatic use and with aquatic emergent plants and terrestrial plants (both monocots and dicots) following spray drift with aerial application at rates of 3.75 lbs/A and above (most crops, forestry, rangeland, residential, rights of way and turf) and with ground applications on areas with impervious surfaces at a rate of 7.95 lbs/A.

Buffers were estimated for specific uses associated with the risk to terrestrial plants. As stated previously, because the initial footprint and the action area encompass the entire state of California, for aggregate uses, the widest buffer for both terrestrial and aquatic uses would be applied and would effectively be the entire state. For similar reasons, the downstream analysis was not conducted. There is potentially no input of "glyphosate-clean" water to dilute existing concentrations of glyphosate downstream because it could be applied in the downstream waterbodies as well. In addition, no reference maps have been generated because the glyphosate uses overlap all of the frog habitat.

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 Tables 7.1 and 7.2. 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 2**.

Table 7.1 Effects Determination Summary for Glyphosate Use and the CRLF

Assessment Endpoint	Effects Determination [1]	Basis for Determination
Survival, growth, and/or reproduction of CRLF individuals	LAA[1]	**Potential for Direct Effects**
		***Aquatic-phase (Eggs, Larvae, and Adults)*:** The acute and chronic LOCs for freshwater fish and aquatic-phase amphibians are not exceeded for either glyphosate, its salts or its formulations.
		***Terrestrial-phase (Juveniles and Adults)*:** The chronic LOC for avian species (surrogate for CRLF) is exceeded at application rates of 7.5 lb a.e./A and above (forestry, areas with impervious surfaces and rights of way). The acute LOC for one particular formulation is exceeded for medium and large- and for medium-sized CRLF's eating small herbivorous mammals on a dose-basis at application rates of 5.5 (highest rate: industrial outdoor uses) and 1.1 (lowest rate: ornamental lawns and turf) lb formulation/A, respectively. For the formulation, the probability of an individual effect at the RQs for the highest and lowest application rates are 1 in 9.32 and 1 in 1.25E+05, respectively. Initial area of concern and action area are the entire state of California. Glyphosate is used in all 58 counties in California with landscape maintenance and rights of way among the highest usages in the counties which may have some currently CRLF occupied areas.
		Potential for Indirect Effects
		Aquatic prey items, aquatic habitat, cover and/or primary productivity The acute and chronic LOCs for freshwater invertebrates are not exceeded for glyphosate, its salts or formulations. In addition, the probit analysis indicates that the probability of an individual effect and the percentage effect to the freshwater invertebrate population prey base would be very low, and the monitoring data are considerably lower than the modeled concentrations utilized in the risk assessment**.** For non-vascular plants, the LOC for aquatic plants is exceeded for formulations specified for aquatic uses. For vascular plants, the LOC for aquatic plants is not exceeded for either glyphosate, its salts or its formulations; however, for aquatic emergent plants, the terrestrial plant LOC is exceeded following spray drift with aerial applications at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A. The acute and chronic LOC for freshwater fish and aquatic-phase amphibians are not exceeded for either glyphosate, its salts or its formulations.
		Terrestrial prey items, riparian habitat For terrestrial invertebrates, the upper bound RQs for small insects exceed the LOC for listed terrestrial invertebrates for all uses and for non-listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above. The upper bound

Table 7.1 Effects Determination Summary for Glyphosate Use and the CRLF		
Assessment Endpoint	**Effects Determination [1]**	**Basis for Determination**
		RQs for large insects exceed the LOC for listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above. At the highest upper bound RQ (<1.4 at 7.95 lbs a.e./A with uses on forestry and areas with impervious surfaces), the chance of an individual effect is <1 in 1.34 with a <75% percentage effect to the terrestrial invertebrate prey base. At the lowest upper bound RQ (<0.01 with 0.387 lbs a.e./A on rangeland), the chance of an individual effect is <8.86E+18 with a <1.13E-17 percentage effect to the terrestrial invertebrate prey base. The chronic RQs exceed the chronic LOC for small mammals on a dose-basis for application rates of 3.84 lbs/A and above (i.e., most crops, forestry, areas with impervious surfaces and rights of way). The chronic LOC for avian species (surrogate for CRLF) is exceeded at application rates of 7.5 lb a.e./A and above (forestry, areas with impervious surfaces and rights of way). The acute LOC for one particular formulation is exceeded for medium and large- and for medium-sized CRLF's eating small herbivorous mammals on a dose-basis at application rates of 5.5 (highest rate: industrial outdoor uses) and 1.1 (lowest rate: ornamental lawns and turf) lb formulation/A, respectively. For the formulation, the probability of an individual effect at the RQs for the highest and lowest application rates are 1 in 9.32 and 1 in 1.25E+05, respectively. For terrestrial plants, the LOC is exceeded following spray drift with aerial applications at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A. Initial area of concern and action area are the entire state of California.

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

Table 7.2 Effects Determination Summary for Glyphosate Use and CRLF Critical Habitat Impact Analysis		
Assessment Endpoint	**Effects Determination [1]**	**Basis for Determination**
Modification of aquatic-phase PCE	Habitat modification[1]	For terrestrial plants, the LOC is exceeded following spray drift with aerial applications at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A. For non-vascular plants, the LOC for aquatic plants is exceeded, only for formulations specified for aquatic uses. For vascular plants, the LOC for aquatic plants is not exceeded for either glyphosate, its salts or its formulations; however, for aquatic emergent plants, the terrestrial plant LOC is exceeded following spray drift with aerial applications at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A. The acute and chronic LOCs for freshwater fish and aquatic-phase amphibians are not exceeded for either glyphosate, its salts or its formulations. The acute and chronic LOCs for freshwater invertebrates are not exceeded for glyphosate, its salts or formulations. In addition, the probit analysis indicates that the probability of an individual effect and the percentage effect to the freshwater invertebrate population prey base would be very low.

Table 7.2 Effects Determination Summary for Glyphosate Use and CRLF Critical Habitat Impact Analysis		
Assessment Endpoint	**Effects Determination [1]**	**Basis for Determination**
Modification of terrestrial-phase PCE	Habitat modification[1]	For terrestrial plants, the LOC is exceeded following spray drift with aerial applications at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A. The chronic LOC for avian species (surrogate for CRLF) is exceeded at application rates of 7.5 lb a.e./A and above (forestry, areas with impervious surfaces and rights of way). The acute LOC for one particular formulation is exceeded for medium and large- and for medium-sized CRLF's eating small herbivorous mammals on a dose-basis at application rates of 5.5 (highest rate: industrial outdoor uses) and 1.1 (lowest rate: ornamental lawns and turf) lb formulation/A, respectively. For the formulation, the probability of an individual effect at the RQs for the highest and lowest application rates are 1 in 9.32 and 1 in 1.25E+05, respectively. For terrestrial invertebrates, the upper bound RQs for small insects exceed the LOC for listed terrestrial invertebrates for all uses and for non-listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above. The upper bound RQs for large insects exceed the LOC for listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above. At the highest upper bound RQ (<1.4 at 7.95 lbs a.e./A with uses on forestry and areas with impervious surfaces), the chance of an individual effect is <1 in 1.34 with a <75% percentage effect to the terrestrial invertebrate prey base. At the lowest upper bound RQ (<0.01 with 0.387 lbs a.e./A on rangeland), the chance of an individual effect is <8.86E+18 with a <1.13E-17 percentage effect to the terrestrial invertebrate prey base. The chronic RQs exceed the chronic LOC for small mammals on a dose-basis for application rates of 3.84 lbs/A and above (i.e., most crops, forestry, areas with impervious surfaces and rights of way).

[1] Habitat Modification or No effect (NE)

Based on the conclusions of this assessment, a formal consultation with the U. S. Fish and Wildlife Service under Section 7 of the Endangered Species Act should be initiated to determine whether there are reasonable and prudent alternatives and/or measures to reduce and/or eliminate potential incidental take.

When evaluating the significance of this risk assessment's direct/indirect and 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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