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Ecotoxicological Risk Assessment for Roundup® Herbicide

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I. Introduction

Glyphosate-based weed control products are among the most widely used broad-spectrum herbicides in the world. The herbicidal properties of glyphosate were discovered in 1970, and commercial formulations for nonselective weed control were first introduced in 1974 (Franz et al. 1997). Formulations of glyphosate,

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including Roundup® Herbicide (RU)[1] (Monsanto Company, St. Louis, MO), have been extensively investigated for their potential to produce adverse effects in nontarget organisms. Governmental regulatory agencies, international organizations, and others have reviewed and assessed the available scientific data for glyphosate formulations and independently judged their safety. Conclusions from three major organizations are publicly available and indicate RU can be used with minimal risk to the environment (Agriculture Canada 1991; USEPA 1993a; WHO 1994). Several review publications are available on the fate and effects of RU or glyphosate in the environment (Carlisle and Trevors 1988; Smith and Oehme 1992; Malik et al. 1989; Rueppel et al. 1977; Sullivan and Sullivan 1997; Forestry Canada, 1989). In addition, several books have been published about the environmental and human health considerations of glyphosate and its formulations (Grossbard and Atkinson 1985; Franz et al. 1997). In addition, RU and other glyphosate formulations have been selected for use in a number of weed control programs for state and local jurisdictions in the United States. Many of these uses require that ecological risk assessments be conducted in the form of Environmental Impact Statements or Environmental Assessments. These documents are comprehensive and specific to local use situations. Documents are available for risk assessments in Texas, Washington, Oregon, Pennsylvania, New York, Virginia, and other states (USDA 1989, 1992, 1996, 1997; USDI 1989; Washington State DOT 1993).

The purpose of this assessment was to expand the available ecotoxicology data and apply current ecological risk assessment methodologies to the evaluation of potential acute and chronic effects of RU (technical glyphosate acid and a surfactant) on nontarget species in the environment. The sources of information used in this assessment include both published and proprietary research reports on RU, glyphosate, and the major metabolite of glyphosate (aminomethylphosphonic acid, AMPA), as well as the surfactant used in formulations. In this effort, the authors have had the cooperation of the Monsanto Company, St. Louis, MO, which has provided complete access to its database of studies and other documentation. In general, the scientific studies carried out by Monsanto were conducted for regulatory purposes and comply with accepted protocols. Studies on glyphosate products commercially available from other manufacturers have been conducted, but this information was not available for use in this assessment.

II. Problem Formulation

A. Risk Assessment Methodology

The framework used for this ecological risk assessment includes three major phases: (1) a problem formulation phase; (2) a data analysis phase (exposure

[1] Abbreviations: a.e., acid equivalents; AMPA, aminomethylphosphonic acid; HQ, hazard quotient; IPA, isopropylamine; LOEL, lowest-observed effect level; NML, no-mortality level; NOAEC, no-observed-adverse-effect concentration; NOAEL, no-observed-adverse-effect level; NOEC, no-observed-effect concentration; NOEL, no-observed-effect level; NTP, National Toxicology Program; POEA, polyethoxylated tallowamine; RU, Roundup®; TRV, toxicity reference value; USEPA, United States Environmental Protection Agency; WHO, World Health Organization.

assessment and toxicological assessment); and (3) a risk characterization phase (CCME 1996; Environment Canada 1997; USEPA 1998). The problem formulation phase was an information gathering and interpretation stage, in which the assessment was focused and the approach for critical areas of interest planned. During this phase, the bounds of the assessment were established by delineating the assessment and measurement endpoints. During the data analysis phase, estimates of exposure and dose–response relationships were compiled and evaluated. During the risk characterization phase, the results of the exposure and effect analyses were combined to determine the potential for adverse effects. Specific details of how the current risk assessment was adapted to these various risk assessment phases are described in detail next.

General Approach.

The focus of this assessment was on ecotoxicological risk associated with the use of RU, specifically considering the direct effects of RU, glyphosate, and the associated surfactant on nontarget organisms. When terrestrial ecosystems are treated with herbicides, vegetation that is one of the main determinants of animal habitats is removed. Consequently, habitat change associated with herbicide use can be expected. When herbicides are applied to aquatic ecosystems, it is expected that there will be direct effects on plants and potentially secondary effects at the community level caused by release of nutrients and organic carbon that can affect dissolved oxygen. The assessment does not address ecological disturbances associated with vegetation changes in the treated areas. The broader question of the relative risks and benefits of programs to control weeds was beyond the scope of this assessment. Direct effects of herbicides on plants are an expected consequence of these programs, and it must be recognized that these management practices will cause alterations of the terrestrial ecosystem and may affect nontarget species.

An initial assessment was made (Tier I) by use of "worst-case" assumptions to calculate a very conservative hazard quotient. The approach employed was similar to the hyperconservative quotient method (Environment Canada 1997). Tier I hazard quotients are designed to be protective, and where an extreme exposure level does not affect the most sensitive species identified in laboratory tests, there is a high degree of confidence that risk will be minimal. If effects are predicted using the extreme exposure scenario, a more realistic exposure scenario is examined and compared to potentially affected species (Tier II). On completion of the refined risk assessment, if significant residual risk potential still exists, recommendations are made to acquire more scientific information or to prepare steps toward mitigation.

Literature Review. The data used in this assessment were assembled from the peer-reviewed, open literature and from proprietary studies (Monsanto Company, St. Louis, MO) conducted to support the registration of glyphosate and RU herbicide. In recent years, two reviews have been completed regarding the toxicity of glyphosate, glyphosate formulations, and surfactants to various types of aquatic and terrestrial wildlife. In 1993, the U.S. Environmental Protection Agency released a document on glyphosate entitled "Re-registration Eligibility

Decision" (USEPA 1993a). In 1994, the World Health Organization released its review on glyphosate entitled "Glyphosate: Environmental Health Criteria 159" (WHO 1994). Both these documents have been extensively peer reviewed, and the information and discussions in these reviews served as the foundation for the current assessment.

Regardless of its origin, all data used in the development of the risk assessment were carefully scrutinized. The literature database contained a high degree of variability in both focus and quality. Critical assessment criteria were established and applied to select the data used in the assessment. The criteria utilized to evaluate data quality included the following:

1. Standard endpoints reported (survival, growth, or reproduction)
2. Fundamental aspects of experimental design reported such as
   Source and characteristics of test material
   Number of animals used, control mortality
   Measurement and reporting of dissolved oxygen for aquatic testing
3. Key elements of data evaluation reported, such as data evaluated and type of statistical analysis

Endpoints. The U.S. EPA Ecological Risk Assessment Guidelines (USEPA 1998) established a problem formulation phase where the objective of the risk assessment is clearly established. An important component of problem formulation includes a clear delineation of assessment endpoint(s) and associated measurement endpoints (also called measures of effect). Assessment endpoints are explicit expressions of the environmental value to be protected (USEPA 1998). The risk assessment presented here is generic in scope and, to achieve the comprehensive assessment goals for all uses of RU, a general integrative assessment endpoint has been selected. The assessment endpoint was defined as "no reduction in populations of non-target organisms as a result of direct toxic effects associated with the use of Roundup® Herbicide." Measurement endpoints are measurable qualities related to the valued characteristic chosen as the assessment endpoint (Suter 1993). The quantitative value that represents a measurement endpoint should be linked to the assessment endpoint in such a way that the goals of the risk assessment can be attained. In this assessment, survival during "acute" exposure scenarios, and survival, growth, and/or reproduction during "chronic" exposure scenarios were chosen as measurement endpoints. In the risk assessment, measurement endpoints have been linked with the assessment endpoint by evaluating individual performance in a toxicity assay and inferring population performance. Specifically, it was assumed that at concentrations where no effects were observed on survival, growth, or reproduction of individuals, then the populations would not be impacted. The no mortality level for the most sensitive species for which toxicity information was available was used as a surrogate for the most sensitive species in the community. Thus, protection of the most sensitive species should be protective of community structure and function.

Potentially Exposed Groups and Representative Taxa. Although primarily employed to control unwanted vegetation within an agricultural setting, glyphosate-containing herbicides are also used in industrial, ornamental garden, aquatic weed control, and residential weed management. Based on these broad uses, organisms in both aquatic and terrestrial environments are potentially exposed. Representative plants and animals are listed along with the major route of environmental exposure (Table 1).

Fish, amphibians, and aquatic invertebrates were selected as surrogate organisms for potentially exposed aquatic animals. Fish occupy a number of trophic levels. They are susceptible to contaminant exposure through their diet, but direct uptake of waterborne chemicals via the gill is often the primary route of exposure. Amphibians were included because they often have life history strategies quite different from fish. Just as important, however, are those organisms that occupy lower trophic levels. Aquatic microorganisms, invertebrates, and plants are extremely important to the functioning of ecosystems. These organisms make up the base of the food chain and are responsible for degrading detritus and for the primary production of organic raw materials that subsequently fulfill the nutrient requirements of other organisms.

Soil microorganisms, terrestrial invertebrates, and nontarget plants would mainly be exposed through direct contact with the herbicide during application and through interaction with the surface soil. Ingestion of contaminated foodstuffs would be expected to be the primary route of herbicide exposure for birds and mammals. To capture the diversity of species potentially exposed to RU, a range of ingestion rates for birds and mammals were considered. In general,

Table 1. Potentially exposed organisms based on exposure pathway evaluation of Roundup® Herbicide.

Environmental compartment	Potentially exposed organism groups	Major route of environmental exposure
Aquatic	Microorganisms (e.g., algae)	Water
	Macrophytes (e.g., duckweed)	Water
	Invertebrates (e.g., *Daphnia*)	Water
	Amphibians (e.g., *Xenopus* sp.)	Water
	Fish (e.g., trout, bluegill)	Water
Soil	Soil microorganisms (e.g., bacteria, fungi)	Soil
	Soil invertebrates (e.g., earthworm)	Soil
Terrestrial	Beneficial arthropods (e.g., honey bees, lacewing)	Direct contact, spray drift
	Nontarget plants (e.g., fencerows)	Spray drift
	Birds (e.g., quail)	Diet (e.g., seeds/fruits, insects, animal tissue)
	Mammals (e.g., mouse)	Diet (e.g., seeds/fruits, insects, foliage, animal tissue)

smaller birds and mammals have greater rates of metabolism and higher food ingestion rates relative to body weight. Small mammals are thus a more conservative model than larger ones because the dose to the smaller animals for any given environmental concentration in food is greater. Large animals were included to keep a perspective on the potential exposure of certain relevant large herbivores, such as deer.

Exposure Assessment Approach.

In the Tier I assessment, both the maximum acute and chronic levels of exposure were estimated for each potentially exposed taxonomic group. Because RU components, glyphosate and surfactant, could co-occur during acute exposure, acute exposure levels were based on estimates of the intact RU formulation. As the levels of exposure under chronic conditions would be strongly influenced by even minor differences in fate of the components, chronic exposure levels were determined separately for glyphosate and surfactant. Actual measures in the environment were given priority for designation of maximum exposure levels. In some cases, insufficient information on measured field concentrations was available to define an exposure level (e.g., depth of soil collected, depth of water collected, application rates, application method). In those cases, a model based on conservative input parameters was used to calculate an exposure level.

The maximum acute exposure range for aquatic and soil organisms considered that either zero or 50% of RU was intercepted by target vegetation. Bare ground application would be equivalent to the 0% interception scenario. The maximum chronic exposure range for aquatic and soil organisms considered interception (0% or 50%), but also a range of dissipation rates for glyphosate and the surfactant. Exposures of soil organisms, such as microbes and earthworms, or aquatic organisms, such as fish, amphibians, and invertebrates, were based on the concentration of RU or its components in soil or water, respectively.

The major route of exposure for birds and mammals is likely to be via the diet, so that estimation of exposures for birds and mammals require consideration of body size and food ingestion rates. A range of ingestion rates was considered. Generic food ingestion equations derived for all birds or for all mammals were used to estimate food consumption for small and large animals (Nagy 1987). The resultant ingestion rate (g food/d) was divided by the body weight to give a daily proportion of food ingestion (g food/g bw/d). These values were used to calculate RU exposure for several different types of dietary items, such as foliage, berries, and seeds. The exposure estimates were based on conservative assumptions, including (1) that the organisms spend the entire time in the treated area, and (2) that all dietary items contain the maximum possible concentration of RU.

Toxicity Assessment Approach.

Although measures of both hazard (toxicity) and exposure must be considered in a risk assessment, it is sometimes useful to classify chemicals based on ranges of the relative potencies. Hazard classification used in this review is based on guidance from the U.S. EPA (USEPA 1985a, b, c) (Table 2). Survival, growth, or reproduction were used to characterize the toxicity of RU or its constituents. Measurement endpoints in this evaluation were LC_{50}, EC_{50}, no-observed-effect concentration (NOEC), or no-observed-adverse-effect concentration (NOAEC). LC_{50} refers to the estimated concentration that will cause mortality in 50% of a test population, and EC_{50} refers to the concentration that will cause a specified effect, such as a decrease in growth, in 50% of a test population. The NOEC is the greatest concentration tested that caused no observed effects in test organisms in acute or chronic studies. The NOAEC may be considered when tested concentrations produce effects that are not obviously adverse to the organism, such as stimulatory effects on growth. The toxicity values established through a review of the literature were used to derive toxicity reference values (TRVs), and were defined as the maximum exposure concentration that would not cause deleterious impacts on populations of plants, animals, and other biota. Both acute and chronic TRVs were derived for potentially exposed groups. Acute TRVs were established using the following process. (1) For each taxonomic group, the most sensitive species was identified based on the least EC_{50} or LC_{50} values. (2) If an experimental NOEC had been identified for that species, then that NOEC was selected as the acute TRV. (3) If an experimental NOEC was not determined, then a no-mortality level (NML) (actually a 1 in 10,000 mortality level) was derived using a 5-fold safety factor as described by Urban and Cook (1986). The NML is equivalent to a probability of mortality of 0.0001. Chronic TRVs were estimated based on the NOEC from the most sensitive species in chronic tests with glyphosate. If chronic studies were available for RU, and the RU NOEC was less than the glyphosate chronic NOEC, then the RU NOEC (expressed as glyphosate acid equivalents, a.e.) was used to estimate the glyphosate chronic TRV. This method provides additional conservatism to the risk assessment. If a NOEC for only one species was available to estimate the chronic TRV, then an additional 2-fold application factor was applied. For certain aquatic organisms, it was necessary to establish a TRV where no chronic data were available. In those cases, the chronic TRV was estimated by applying a 20-fold application factor to the LC_{50} or EC_{50} (International Joint Commission 1975).

Table 2. Toxicity classifications for aquatic and avian species.

U.S.EPA toxicity classification[a]	European Toxicity Classification[b] (Aquatic)	Acute aquatic LC_{50} or EC_{50} (mg/L)	Avian dietary LC_{50} (mg/kg)
Practically nontoxic	—	>100	>5000
Slightly toxic	Harmful	>10, ≤100	>1000, ≤5000
Moderately toxic	Toxic	>1, ≤10	>500, ≤1000
Highly toxic	Very toxic	≥0.1, ≤1	>50, ≤500
Very highly toxic	Very toxic	<0.1	≤50

[a]USEPA 1985a,b,c.
[b]European Council 1993.

Risk Characterization Approach.

Risk was characterized by calculation of a hazard quotient (HQ), derived by comparing the acute or chronic TRVs with the maximum acute or chronic exposure levels, respectively. The HQ in this assessment is similar to the risk quotient used for pesticide risk assessment by the U.S. EPA (Urban and Cook 1986). A HQ less than or equal to 1.0 in the Tier I analysis suggests minimal risk. A HQ greater than 1.0 does not suggest that effects would be expected to occur, but rather that a more critical assessment of the assumptions used in Tier I would be warranted. In Tier II analyses, more realistic assumptions and mitigating factors are considered to generate a more environmentally relevant exposure scenario. If the HQ still exceeds 1.0, additional steps to refine the assessment, such as collection of actual environmental concentrations under a range of exposure and mitigation scenarios, may be appropriate.

B. Characteristics of Roundup® Formulations and Components

RU Formulations of Glyphosate.

There are a number of formulations of glyphosate-based herbicides, all of which have the same basic ingredients: the isopropylamine (IPA) salt of glyphosate; a surfactant; and water. RU is the trade name used in North America. Language considerations and differing business needs have resulted in the marketing of this formulation in some countries using a variety of other brand names (such as Sting, Alphee, Azural, and Faena). RU or these closely related products are qualitatively the same, varying in the amount of glyphosate and the amount and type of surfactant; however, the IPA salt of glyphosate is the active ingredient in all the formulations. Most often, the concentration of glyphosate in these formulations is 360 g acid equivalents (a.e.) per liter. This, however, is not always the case, and in certain formulations the base formulation is diluted with water to create more dilute products that contain 240, 160, 120, or 9 g a.e./L.

A polyethoxylated tallowamine surfactant (CAS number 61791-26-2; abbreviated POEA), which is a mixture of polyethoxylated long-chain alkylamines synthesized from animal-derived fatty acids, is the predominant surfactant used in glyphosate-based products. This surfactant, MON 0818 (a code designation for the preparation of POEA used in Monsanto formulations), is added to facilitate the penetration of glyphosate through cuticular waxes on target plants. The surfactant is typically 15% or less of the formulation, and this concentration was used for the purpose of this risk assessment. Circumstances in which the use of different formulations could lead to substantially different scientific conclusions, such as the role of surfactants in aquatic toxicity, were considered in the risk assessment.

Most formulations of glyphosate have a surfactant added. However, Rodeo® Herbicide is a formulation of glyphosate registered in the U.S. for aquatic uses that does not contain a surfactant. Because no surfactant is included in the product as sold, a surfactant must be added to effectively control weeds. The user can select a surfactant that meets the specific needs of the weed control program.

The POEA surfactant can be mixed with Rodeo so that the tank mix is qualitatively the same as RU. The two factors that most often guide surfactant selection are efficacy and potential toxicity to nontarget aquatic organisms. As is shown later, POEA is more toxic to aquatic animals than is the active herbicidal ingredient glyphosate. Most surfactants have LC_{50} values for aquatic animals in the range of 1 to >100 mg/L (USEPA 1986), which would be classified as highly to practically nontoxic (see Table 2). The least POEA LC_{50} for aquatic animals is 0.65 mg/L (Folmar et al. 1979), which is at the upper end of the toxicity range for surfactants. Therefore, to be conservative, the RU formulation, containing POEA, was used in the risk assessment for aquatic uses because this should be protective of other surfactants as well.

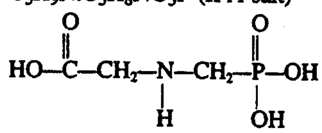
Wherever possible, this document has converted measures to metric units of weight, volume, and area. Some reports have expressed glyphosate concentrations in pounds or gallons per unit area, using acid equivalents (a.e.) or isopropylamine salt active ingredient (a.i.). Conversions to metric units have been made to simplify direct comparison of exposure or fate data. The standard RU formulation of 360 g glyphosate a.e./L is equivalent to 0.75 lb glyphosate a.e./qt, or 1.0 lb glyphosate a.i./qt. An application rate of 1 qt RU/A is equivalent to 0.75 lb glyphosate a.e./A (acre), or 0.84 kg glyphosate a.e./ha (hectare). When converting between glyphosate a.e. and RU concentrations, it was assumed that 1 mg of RU contained 0.31 mg glyphosate acid equivalents. When converting between acid equivalents (a.e.) and active ingredient (a.i.), 1 mg a.i. was assumed to contain 0.75 mg a.e.

Physical and Environmental Characteristics of RU Components.

Environmental Chemistry and Metabolism of Glyphosate. The available data on the physical and chemical properties of glyphosate have been reviewed extensively (Mackay et al. 1997); representative values are given (Table 3). Glyphosate is an amphoteric compound with several pK_a values. The polarity of glyphosate makes it practically insoluble in organic solvents. The amphoteric nature of glyphosate accounts for its relatively great K_d for binding to soil particles. Because of this characteristic, glyphosate herbicides are only effective when applied directly to the plant surface. Once glyphosate enters the soil, it is essentially unavailable to plants due to its very high affinity for soil. This quality explains why glyphosate-treated areas can be planted with crops soon after application.

Technical grade glyphosate acid manufactured by Monsanto averages 96% purity on a dry weight basis. By-products of synthesis constitute the remainder, in which individual component concentrations are less than 1% (w/w). By-product contents of pesticides are regulated by government authorities. A data package containing this information has been developed, submitted, and approved for Monsanto-produced glyphosate. Technical grade glyphosate has been used as the test material in the toxicological testing discussed in this assessment, and the by-products in technical grade glyphosate have not changed significantly

Table 3. Physical and chemical properties of glyphosate.

Common name	Glyphosate
Synonyms	*N*-(Phosphonomethyl)glycine (acid) Glyphosate isopropylamine salt (IPA salt)
Chemical formula	$C_3H_8NO_5P$ (acid) $C_3H_9N.C_3H_8NO_5P$ (IPA salt)
Chemical structure	 (acid)
CAS No.	1071-83-6 (acid) 38641-94-0 (IPA salt)
Molecular weight	169.09 (acid) 227.2 (IPA salt)
Physical state and color	Crystalline powder, white
Melting point[a]	200 °–230 °C
Boiling point	No data available
Water solubility[a]	10,000–15,700 mg/L at 25 °C
Octanol/water partition Coefficient: log K_{ow}[a]	−4.59 to −1.70
Vapor pressure[a]	2.59×10^{-5} Pa at 25 °C
Henry's law constant[a]	1.41×10^{-5} Pa-m^3/mol
Sorption partition coefficient: K_d[b]	3–1,188; geometric mean ($n = 28$), 64
Sorption partition coefficient: K_{oc} (L/kg)[b]	9–60,000; geometric mean ($n = 28$), 2,072

[a]Mackay et al. (1997).
[b]Range for agricultural and forest soils: Gerritse et al. (1996); Glass (1987); Cheah et al. (1996); Nomura and Hilton (1977); Hance (1976); Brightwell and Malik (1978); Livingston et al. (1986); Piccolo et al. (1994).

over the course of toxicological testing. Because the reported findings of toxicological studies using technical grade glyphosate include any effects that could result from by-products, such effects are embodied in the resulting risk characterization and assessment.

Glyphosate Uptake into Plants. Application of RU to target vegetation is achieved through direct spray onto foliage. Glyphosate is assimilated by leaves and rapidly translocated within the phloem. This property accounts for its activity as a systemic herbicide (Franz et al. 1997). Adsorption of RU into plants depends largely on diffusion. Factors influencing diffusion include surface area coverage and concentration (Kirkwood 1987), which are affected by spray volume and droplet size. A key factor influencing RU absorption is the nature of the plant cuticle that acts as a barrier to the penetration of chemicals. As discussed earlier, a surfactant is used to facilitate the uptake of glyphosate. Surfactants serve a number of functions including acting as a wetting agent, or spreader, to reduce the surface tension between the leaf surface and the spray droplet.

Glyphosate Mechanism of Action. Glyphosate-based herbicides have a broad spectrum of activity toward plants. Activity toward animals is small because the mode of action for glyphosate is a biochemical pathway apparently unique to plants and some microorganisms. The mechanism by which glyphosate is toxic to plants has been reported in detail (Franz et al. 1997; Cole 1985). Glyphosate inhibits plant growth by inhibiting the production of essential aromatic amino acids through competitive inhibition of the enzyme enolpyruvylshikimate phosphate (EPSP) synthase. This is a key enzyme in the shikimic acid pathway for the synthesis of chorismate (Fig. 1), which is a precursor for the essential amino acids phenylalanine, tyrosine, and tryptophan. Other factors, including species of plant and growth conditions, may affect uptake and thus the effectiveness of and the response to glyphosate.

III. Exposure Assessment

A. Use Patterns

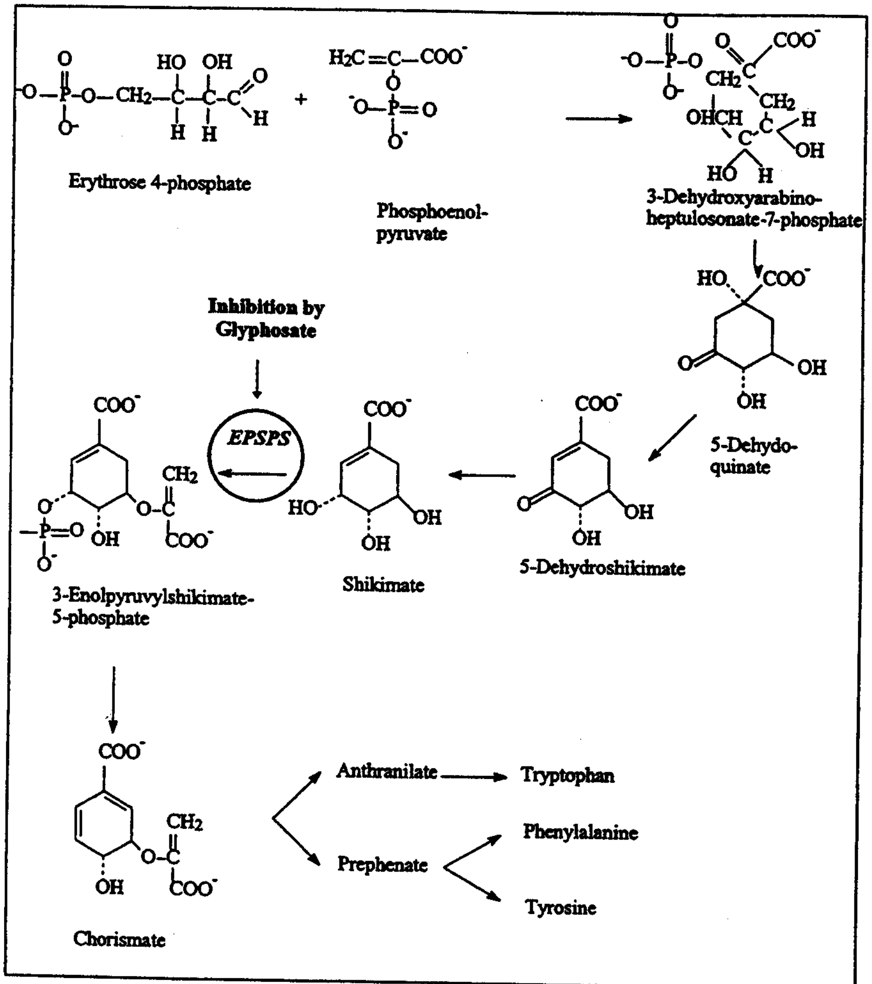
Types of Uses.

The use of glyphosate-based herbicides includes agriculture, industrial, ornamental garden, and residential weed management. In agriculture, the use of glyphosate is increasing, particularly in applications involving genetically modified plant varieties selected for the ability to tolerate glyphosate treatment (Roundup Ready®). Other agricultural uses for glyphosate-based products include its use by farmers as a routine step in field preparation. Nonagricultural users include public utilities, municipalities, or state transportation departments where RU is used for the control of weeds or noxious plants. A variety of formulations of RU are available under different brand names registered for use worldwide for control of vegetation in viticulture, orchards, agriculture, aquaculture, and forestry, as well as for residential use.

Application Techniques and Use Rates.

For commercial uses, the typical methods for applying RU involve spraying aqueous solutions either from ground mechanical equipment, with hand-held sprayers, or by licensed aerial applicators. Ground sprayers are the primary method of application in agricultural uses. The application rate is dependent on the weeds that are to be controlled. Rates for specific weeds are listed in the product labels (available directly from Monsanto or from the Monsanto internet website: *www.monsanto.com*).

The timing and frequency of RU application depends on the target weed species and must be within certain maximum allowed levels specified on the label. In the U.S., annual maximum use rates, defined as the sum of all glyphosate applications made to a given site during 1 year, are limited to no more than 6.73 kg a.e./ha for crops (equivalent to 21.7 L RU/ha) and no more than 8.92 kg a.e./ha (28.7 L RU/ha) for noncrop uses. The same annual limits, based



Adapted from Franz et al. 1997.

Fig. 1. Mechanism of action for glyphosate in plants.

on glyphosate acid content, apply to other RU brands that contain different concentrations. For single applications, rates between 0.84 to 2.52 kg a.e./ha are most common, with maximum single application rates up to 4.2 kg a.e./ha, depending on the species and growth stage of plants to be controlled. Mixtures for application are made by diluting the concentrate in 30–375 L water/ha. For aerial application, a lesser volume is used, such as 30–140 L of water. Typical water volumes for ground applications are about 180 L/ha (Monsanto, personal communication, 1998).

A single application is designed to effectively kill plants present at the time of treatment. Complete season-long control may require several applications of glyphosate products to plants emerging at different times. The frequency of application in agricultural situations with glyphosate-tolerant crops may allow several over-the-top treatments at lesser rates to achieve season-long in-crop weed control (Monsanto, personal communication, 1998). Regardless of whether a conventional crop or a glyphosate-tolerant one is grown, the annual maximum total is 6.73 kg a.e./ha in any cropping situation. Annual maximum treatment rates are set based on efficacy studies, are later specified in the registration, and are not linked to risk assessment issues.

B. Environmental Fate and Transport

Movement off Treated Areas: Drift.

Glyphosate and POEA. Glyphosate has no significant vapor pressure; therefore, loss of glyphosate to the atmosphere via vaporization from treated surfaces is negligible (Brønstad and Friestad 1985; Malik et al. 1989; Agriculture Canada 1991; Franz et al. 1997). However, offsite movement of glyphosate is possible through spray drift (Payne 1992; Atkinson 1985; Marrs et al. 1989, 1993). Although the spray drift of pesticides is not compound specific, this is relevant when nontarget effects of RU are considered, and several studies have specifically addressed the issue. The potential for spray drift during applications is dependent on local weather conditions, particularly wind speed and the type of spray equipment used. Spray application of herbicides in agricultural areas is typically not conducted under adverse weather conditions, such as wind speeds greater than 5 m/s or temperature inversions. When drift does occur, there is a rapid decline in surface deposition with increasing distance from the target site for both ground and aerial applications. Spray drift studies conducted in Europe indicate that drift from low boom ground applications typical of the majority of row crop agriculture is approximately 4% at a distance of 1 m from the edge of the field (Ganzelmeier et al. 1995). At 5 m, the drift value is 0.6% of the application rate.

For aerial application, drift will be somewhat greater than for ground applications because the spray boom is a greater distance from the target crop or soil. In a forestry application with a glyphosate-based product, it has been reported that at 25 m or greater from the application site, deposition was typically less than 10% of the application rate and less than 1% at 75 m or greater (Payne et al. 1990). Another study reported that spray deposition decreased to less than 10% of the application rate in the first 30 m downwind and to less than 5% at a distance of 200 m (Riley et al. 1991). Variation in canopy height and aerial application techniques (spray boom and nozzle design and alignment) have been noted to have significant impact on drift (Teske et al. 1997).

Other studies suggest that drift rates would be greater. For instance, residues have been measured 400 m downwind from ground applications (Yates et al. 1978). This research was conducted nearly 20 yr ago, and the technology for application and restrictions on time of application have changed significantly. Some of the nozzles and pressure settings in the original research were intentionally designed to increase drift. Similarly, greater levels of drift have been reported (Payne and Thompson 1992), but these authors intentionally selected conditions that would yield greater levels of drift than would be expected under normal use patterns. In the latter studies, the same authors conclude that, with proper precautions, herbicides could be applied in a wider range of wind speeds than that currently used without causing increased environmental impact in sensitive areas. During the 1990s, significant advances in the understanding of factors causing spray drift have led to inclusion on product labels of specific directions to reduce herbicide drift. These recommendations primarily focus on use of the maximum droplet size that will provide adequate coverage and on reducing air turbulence caused by the aircraft. Recommendations also call for use of smoke generators, to determine the drift distance, and the movement of the edge of the application area away from the edge of the field, to contain the majority of the drift within the confines of the field. The AgDrift™ software model developed by the Spray Drift Task Force for the U.S. EPA predicts that drift will be about 40% of that reported by Payne et al. (Teske et al. 1997).

Because the possibility of drift during herbicide application cannot be excluded, drift was considered in exposure calculations for certain nontarget organisms. For this assessment, the drift rate was assumed to be 10%. Although surfactant-specific drift studies were not available, it was assumed that drift rates would be similar to those for glyphosate.

Movement off Treated Areas: Leaching or Runoff.

Glyphosate. Although glyphosate is very soluble in water, its strong sorption to soils limits mobility. Consequently, glyphosate is unlikely to leach into groundwater or runoff significantly into surface water following application (Brønstad and Friestad 1985; Hance 1976; Roy et al. 1989a; Malik et al. 1989; Feng and Thompson 1990; Horner 1990; WHO 1994; Miller et al. 1995). The immobility of glyphosate and AMPA is supported by numerous laboratory studies (Sprankle et al. 1975; Rueppel et al. 1977; Sanchez-Martin et al. 1994; Crisanto et al. 1994), and forestry and agricultural field studies (Roy et al. 1989a; Feng and Thompson 1990; Horner 1990; Newton et al. 1994; WHO 1994). In field studies, the immobility of glyphosate in soils has been demonstrated by the lack of detectable concentrations of glyphosate in runoff waters from forest ecosystems receiving glyphosate treatment (Newton et al. 1984; Roy et al. 1989a). In laboratory studies and field studies using no-tillage agricultural soils, the maximum concentration of glyphosate in runoff waters was less than 1.9% of the applied dose (Rueppel et al. 1977; Edwards et al. 1980). This maximum runoff concentration reported in the field study was for 1 d after a heavy rainfall on soils receiving glyphosate at an application rate of 8.96 kg a.e./ha. Cumulative data compiled from a 3-yr monitoring period indicated that less than 1% of the applied glyphosate was found in runoff and of that minimal amount, 99% was found following the first rainfall event. The model used in this assessment assumed a runoff potential of 2%, which exceeds the potential runoff demonstrated in all the studies surveyed.

Some authors have reported that glyphosate can be readily desorbed from soil and has the potential to be extensively mobile in the soil environment (Piccolo et al. 1994). However, these conclusions are not considered to be representative of most conditions, because the experiment did not reflect field conditions. The starting concentration of glyphosate in solution was extremely great (50–300 mg a.e./L), and the ratio of water to soil was 25:1. These factors resulted in unrealistic concentrations, ranging from 213 to 2115 mg a.e./kg in the soil following adsorption. Maximum concentrations in soil following field use are typically 1–5 mg a.e./kg. The equilibration time for the adsorption and desorption steps was only 2 hr, which does not allow all adsorption processes to occur. Thus, the results from this study should not be extrapolated to field conditions, where glyphosate concentrations and water:soil ratios are much less and adsorption processes can fully proceed.

Some reports of leaching have been published in the literature. Follow-up investigations have shown that many of these reported detectable values were analytical artifacts. The results of monitoring of pesticides in groundwater by local authorities in Germany between 1989 and 1994 have been reported (Greenpeace 1995). Detectable concentrations of glyphosate were observed at 7 of 424 localities, but only two of these concentrations were greater than 0.1 μg a.e./L (maximum, 0.35 μg a.e./L). The 0.1 μg/L concentration is a regulatory level for drinking water quality in parts of Europe (European Council 1980). Examination of the report revealed that the reported positive results from four of the sites were the result of misinterpretation of the data, and one site used an analytical method inappropriate for the detection of glyphosate (Institut Fresenius 1998). Of the two remaining sites, one had poor quality analytical data, and subsequent samples taken at both sites showed no detectable glyphosate, indicating that, if it had been present, it did not persist in the wells.

The U.S. EPA has also reported the results of groundwater monitoring studies in which glyphosate was reported to be detected in 7 of 247 wells (USEPA 1992). Further investigation of the reports concluded that these detections could be attributed to errors in analysis, point-source contamination, or inappropriate analytical methodology; none were determined to be valid detections. Although glyphosate has been reported in well water at an electrical substation in Newfoundland (Smith et al. 1996), this is not indicative of the leaching potential of glyphosate under agricultural conditions. Electrical substations in Newfoundland are typically built on gravel platforms above excavated ground, after removal of the top soil horizon, and are subjected to relatively great rates of precipitation. The site was treated with 4.6 kg a.e./ha, followed by 4.3 kg a.e./ha 1 mon later. A maximum concentration of 45 μg a.e./L was detected 7 wk after initial application, and the level declined to 13 μg a.e./L at 37 wk. The site where glyphosate was detected was situated on a limestone bed without a soil-restricting layer and thus highly permeable.

Polyethoxylated Tallowamine (POEA). Based on adsorption and degradation data for POEA surfactant used in RU, leaching and runoff potential is expected to be small, based on adsorption and degradation data for the surfactant. POEA strongly adsorbs to soil. The K_{oc}s in three different soil types were estimated to range from 2500 to 9600 (Marvel et al. 1974). POEA that was adsorbed to soil was not readily desorbed; even using ammonium hydroxide as the extracting solvent removed less than 20% of the POEA adsorbed to soil. Thus, the mobility of POEA in soil is expected to be less than 2%.

Dissipation in Soil.

Glyphosate. Dissipation of a substance from soils, in this review, is defined as loss by chemical breakdown or irreversible movement to other environmental compartments. The dissipation of glyphosate from soils in the environment is predominantly due to biodegradation, which is mediated primarily by bacteria and fungi. The main metabolic pathway in soil is degradation of glyphosate to AMPA, which is further metabolized to carbon dioxide (Sprankle et al. 1975; Rueppel et al. 1977; Malik et al. 1989) (Fig. 2). A second pathway, involving

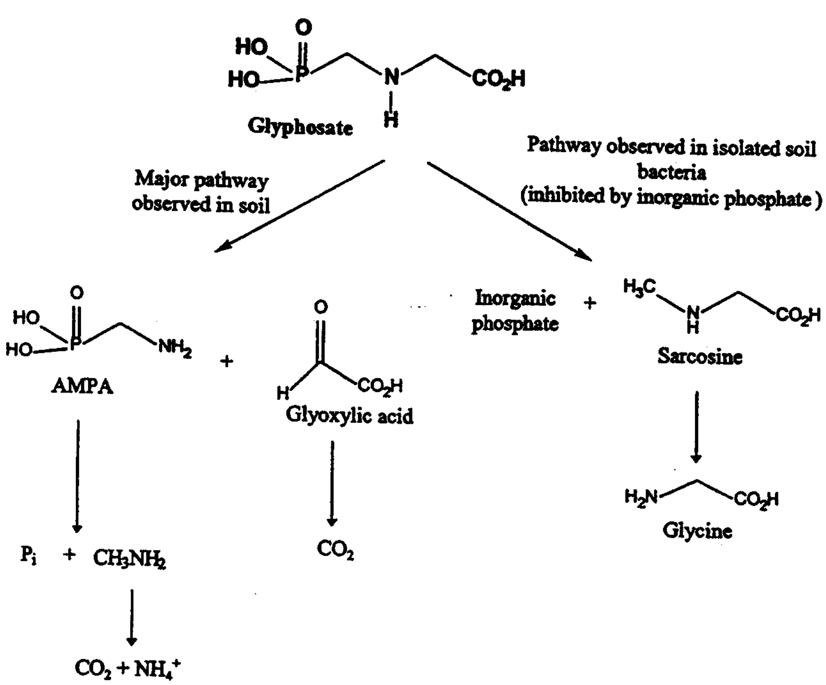


Fig. 2. Degradation pathway of glyphosate in soil. Adapted from Franz et al. 1997.

cleavage of the C–P bond to give inorganic phosphate and sarcosine, has also been observed with isolated soil bacteria in the absence of phosphate (Shinabarger and Braymer 1986; Kishore and Jacob 1987; Pipke et al. 1987). Abiotic degradation processes such as photolysis and hydrolysis contribute little to the dissipation of glyphosate in the environment (Sprankle et al. 1975; Nomura and Hilton 1977; Rueppel et al. 1977; Torstensson 1985; Brønstad and Friestad 1985; Tooby 1985; Malik et al. 1989; WHO 1994).

The rate of decrease of glyphosate concentrations in soil matrices depends on the overall microbial activity of the treated soil (Carlisle and Trevors 1988; Moshier and Penner 1978). Field studies indicate that glyphosate typically dissipates rapidly from both simple ecosystems, such as agricultural, and more complex ecosystems, such as forests, regardless of the diverse edaphic and climatic conditions (Newton et al. 1984; Ragab et al. 1985; Torstensson et al. 1989; Roy et al. 1989a; Feng and Thompson 1990). The characteristic differences between forestry and agricultural soils, such as pH, organic matter content, temperature, and moisture, result in different dissipation rates among ecosystems (Stark 1983; Torstensson 1985). Following application in forest ecosystems, the time for 50% dissipation (DT_{50}) for glyphosate in soils ranges from 1.4 to 60 d (Allan and Klein 1983; Edwards 1981; Newton et al. 1984; Roy et al. 1989a; Torstensson et al. 1989; Feng and Thompson 1990). In agricultural soils, half-lives range from 1.7 to 197.3 d but are typically less than 60 d (Ragab et al. 1985; Oppenhuizen and Goure 1993; Heinonen-Tanski et al. 1985; Mestdagh 1979; Danhaus 1984; Oppenhuizen 1993). The most comprehensive study was an 18-mon field study of eight different sites across the U.S. representing a range of climatological conditions and soil types. The DT_{50} ranged from 1.7 to 141.9 d, with a median of 14.9 d (Oppenhuizen 1993). When RU was applied to exposed soils at these test sites at annual use rates of 8.9–9.9 kg a.e./ha, the geometric and arithmetic means for DT_{50} were 17.5 and 41 d, respectively. These results are consistent with the results of laboratory studies where the DT_{50} values in a variety of different soil types have been reported to be less than 60 d (Sprankle et al. 1975; Rueppel et al. 1977; Nomura and Hilton 1977; Smith and Aubin 1993). A summary of the DT_{50} values of glyphosate in several soil types following either forest or row-crop applications is presented in Table 4 and Fig. 3. The percent rank was derived from the method of Parkhurst et al. (1995) (percent rank = rank/$(n + 1) \times 100$). The soil DT_{50} values used in the prediction of chronic glyphosate concentrations in soil were 32 and 95 d, which represent the arithmetic mean and 90th centile of the measured field DT_{50} values, respectively.

Aminomethylphosphonic Acid (AMPA). Microbial degradation of AMPA, the major glyphosate metabolite, has been reported in laboratory studies to proceed at a slower rate than for glyphosate (Rueppel et al. 1977). Transient increases in soil concentrations of AMPA in the field have been reported in both forestry and agricultural ecosystems as glyphosate is converted to AMPA and then subsequently degraded (Müller et al, 1981; Ragab et al. 1985; Roy et al. 1989a;

Table 4. Measured dissipation times of glyphosate following field application of formulated herbicide.

Soil type	Location	DT_{50} (days)	Reference
Agricultural	Canada	6–21	Oppenhuizen and Goure (1993)
Agricultural	Canada	<10	Ragab et al. (1985)
Agricultural	Finland	<58	Heinonen-Tanski et al. (1985)
Agricultural	France	5–197.3	Mestdagh (1979)
Agricultural	Sweden	1.2–24.3	Mestdagh (1979)
Agricultural	USA	27.3–55.5	Danhaus (1984)
Agricultural	USA	1.7–141.9	Oppenhuizen (1993)
Forestry	Canada	45–60	Feng and Thompson (1990)
Forestry	Canada	1.4–17.8	Allan and Klein (1983)
Forestry	Canada	24	Roy et al. (1989a)
Forestry	Sweden	<60	Torstensson et al. (1989)
Forestry	USA	40.2	Newton et al. (1984)
Forestry	USA	30.9	Edwards (1981)
Statistical summary ($n = 47$):			
Range		1.2–197.3	
10th centile		5	
Geometric mean		17	
Arithmetic mean		32	
90th centile		95	

Feng and Thompson 1990; Newton et al. 1994). The slower degradation rate for the AMPA may result from its greater adsorption to soils and decreased availability to be acted on by microbial degradation processes (Rueppel et al 1977; Nomura and Hilton 1977; Torstensson and Stark 1979). The median field degradation half-life of AMPA, estimated from glyphosate field dissipation studies that were conducted at eight sites in the U.S. and three sites in Canada, was reported to be approximately 145 d, with a range of 76 to 240 d (Oppenhuizen 1993; Oppenhuizen and Goure 1993; Gustafson and Bleeke 2000).

Polyethoxylated Tallowamine (POEA). When degradation of POEA was investigated in three soils (silt loam, silty clay loam, and sandy loam), microbial degradation was the primary process, with minimal degradation occurring under sterile conditions (Marvel et al. 1974). The estimated degradation half-life for parent POEA was less than 1 wk and possibly as short as 1–2 d. Approximately 25%–30% of applied ^{14}C-POEA was mineralized to CO_2 within 7 wk. Because few measured data were available for POEA dissipation, conservative estimates

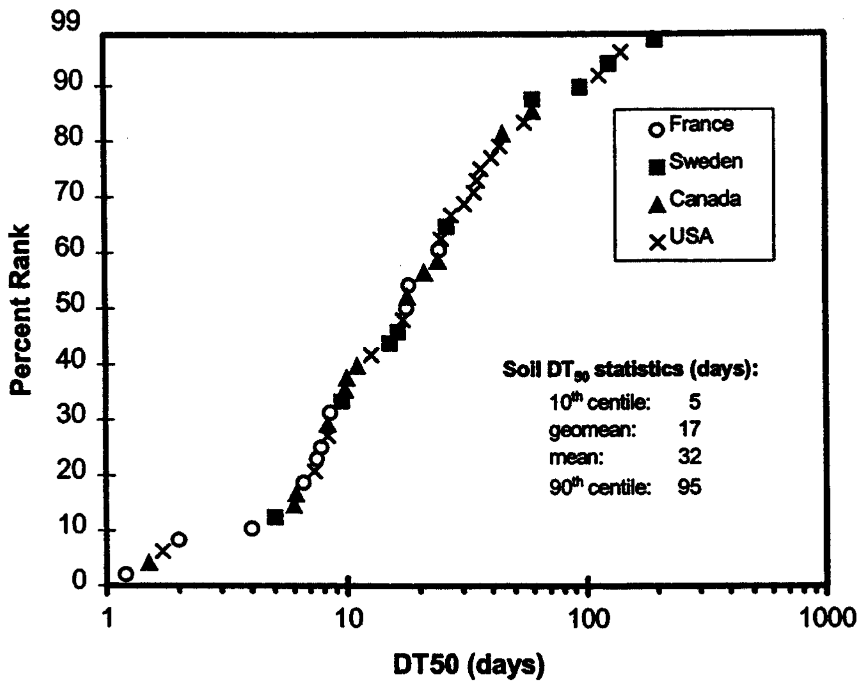


Fig. 3. Distribution of glyphosate 50% dissipation times (DT_{50}s) in soil from field dissipation studies following terrestrial use.

were used in this assessment. Thus, the range of half-life values for POEA in soil assumed in this assessment was 7–14 d.

Dissipation in Water.

Glyphosate. Both field and laboratory studies have reported microbial degradation of glyphosate to AMPA and CO_2 in aquatic environments (Brightwell and Malik 1978), and rapid dissipation from both flowing and standing surface waters (Brønstad and Friestad 1985). The results of field studies indicate that 50% of the concentration of glyphosate initially found in water dissipates within time periods ranging from a few days to 2 wk (Newton et al. 1984, 1994; Edwards 1981; Horner 1990; Goldsborough and Brown 1993). The principal factors contributing to the dissipation of glyphosate in flowing waters, such as streams, include tributary dilution, dispersion, and loss through processes such as adsorption to suspended particulate matter or sediments and microbial degradation (Bowmer 1982; Edwards 1981; Feng et al. 1990). The rate of glyphosate dissipation from nonflowing waters, such as ponds, is mainly a function of the local conditions and therefore is considered site specific. Chemical, physical, and biological factors determine the dissipation of available glyphosate from the environment (Goldsborough and Beck 1989). Based on the data summarized above, a conservative range of aquatic half-life values was estimated to be from 7 to 14 d.

Aminomethylphosphonic Acid (AMPA). Dissipation of AMPA in aquatic environments has been demonstrated in studies with glyphosate, in which AMPA is formed by degradation of glyphosate and then dissipates (Brightwell and Malik 1978; Goldsborough and Brown 1993). The rate of dissipation can be estimated from field studies of glyphosate, and is in the range of 7–14 d (Horner and Kunstman 1988). Thus, the half-life of AMPA in aquatic environments is comparable to that of glyphosate.

Polyethoxylated Tallowamine (POEA). In natural waters containing suspended sediment, such as lakes, ponds, and rivers, POEA is degraded through microbial processes (Banduhn and Frazier 1974). The half-life of POEA (DT_{50}) was estimated to be less than 3–4 wk. Mineralization of 40%–50% of the applied ^{14}C-POEA to $^{14}CO_2$ has been demonstrated to occur in 14 wk. Because a limited number of measurements were available, a conservative range of aquatic half-life values was estimated. The half-life range for POEA assumed in this assessment was 21–42 d.

C. Environmental Concentrations from Terrestrial Uses

Acute Scenario.

Soil. A number of studies have been conducted to evaluate the concentrations of glyphosate in soil that result from the direct application of RU to bare ground (Table 5). Nearly all reported concentrations of glyphosate in soil have been less than 5 mg a.e./kg. There are two reports of concentrations significantly greater than 5 mg a.e./kg, but for the purposes of determining a maximum level of glyphosate in soil they were considered outliers, and not used. The greatest reported concentration was 39.8 mg a.e./kg following an aerial application of 2.0 kg a.e./ha in a Canadian forest (Feng and Thompson 1990). However, an artificial deposit collector at the same site also showed unusually great residues, and the authors of the study noted that the large deposition rate could be attributed to application error. The other large value that was not included was a reported concentration of 17 mg a.e./kg in loam soil samples in Finland following an application of 2.6 kg a.e./ha (Müller et al. 1981). The same article also reported a maximum concentration of 3.8 mg a.e./kg in a fine silt soil. The differences between the two soils may be due to the organic carbon content of the Finnish loam soil (44%, versus 1.5% for the fine silt). Moisture contents for

Table 5. Concentrations of glyphosate in soil shortly after direct application of formulated herbicide.

Type	Location	Year	Application rate (kg/ha)	Sampling depth (cm)	Soil concentration (mg a.e./kg dw)	Reference
Agricultural soils:						
Clay loam	Arizona, USA	1991	9.1	15	2.23	Oppenhuizen (1993)
Fine sand	Laukaa, Finland	1978–1980	1.4	5	1.6	Heinonen-Tanski et al. (1985)
Loam	Minnesota, USA	1991	9.0	15	1.82	Oppenhuizen (1993)
Loam	Ohio, USA	1991	9.1	15	2.01	Oppenhuizen (1993)
Loam	Kettula, Finland	1978	2.6	10	17	Müller et al. (1981)
Loam	Alberta, Canada	1991	4.3	15	1.08	Oppenhuizen and Goure (1993)
Loam	Manitoba, Canada	1991	4.3	15	0.80	Oppenhuizen and Goure (1993)
Loamy sand	Ontario, Canada	1991	4.2	15	0.67	Oppenhuizen and Goure (1993)
Loamy sand	California, USA	1991	9.9	15	1.94	Oppenhuizen (1993)
Sand	Georgia, USA	1991	9.0	15	3.06	Oppenhuizen (1993)
Sandy clay loam	New York, USA	1991	8.8	15	4.58	Oppenhuizen (1993)
Silt	Kettula, Finland	1978	2.6	10	3.8	Müller et al. (1981)
Silt loam	Texas, USA	1991	8.8	15	1.93	Oppenhuizen (1993)
Silty clay loam	Iowa, USA	1991	8.9	15	2.34	Oppenhuizen (1993)
Forest soils:						
Forest soil	British Columbia, Canada	1984	2.0	5	39.8	Feng and Thompson (1990)
Forest soil (under litter)	Michigan, USA	1987	4.2	15	1.40	Horner (1990)
Forest soil (under litter)	Oregon, USA	1987	4.2	15	0.07	Horner (1990)
Forest soil (under litter)	Georgia, USA	1987	4.2	15	0.14	Horner (1990)
Forest soil (no litter)	Michigan, USA	1987	4.2	15	4.67	Horner (1990)
Forest soil (no litter)	Oregon, USA	1987	4.2	15	0.15	Horner (1990)
Forest soil (no litter)	Georgia, USA	1987	4.2	15	1.87	Horner (1990)

a.e., glyphosate acid equivalents.

the soils were not given, but it is expected that the loam soil had a significantly greater moisture capacity because of its greater organic carbon content; this would result in a greater glyphosate concentration when reported on a dry weight basis.

For these reasons, the next greatest concentration from the measured values in soil was selected as the most accurate estimate of acute exposure concentrations in soil: 4.67 mg a.e./kg in forest soil, which corresponds to 15.1 mg RU/kg. This value is similar to 1.9 mg a.e./kg, which is the predicted soil concentration in the top 15 cm of soil following application at the maximum single-use rate of 4.2 kg a.e./ha. This predicted value is based on the conservative assumption that all the applied RU would reach the soil (i.e., no interception by foliage and no drift) and become uniformly distributed within the top 15 cm. The density of this soil layer was represented by a conservative estimate of 1500 kg/m^3. A range of maximum soil concentrations was derived assuming 0% and 50% interception of RU by target vegetation (Table 6).

Table 6. Predicted exposure concentrations of Roundup®, glyphosate, and POEA in environmental media following terrestrial application of Roundup®[a].

Scenario and media	Units	Media DT_{50} (days)	Foliar interception: None (bare ground)	Foliar interception: 50%
Roundup®: acute exposure concentrations				
Soil	mg RU/kg	—	15.1	7.5
Water[b]	mg RU/L	—	0.406	0.271
Sediment	mg RU/kg	—	3.5	2.3
Glyphosate: chronic exposure concentrations				
Soil	mg a.e./kg	32	0.95	0.47
Soil	mg a.e./kg	95	2.6	1.3
Water[b]	mg a.e./L	7	0.006	0.0039
Water[b]	mg a.e./L	14	0.0114	0.0076
Sediment	mg a.e./kg	28	0.19	0.13
POEA: chronic exposure concentrations				
Soil	mg POEA/kg	7	0.032	0.016
Soil	mg POEA/kg	14	0.063	0.032
Water[b]	mg POEA/L	21	0.0025	0.0017
Water[b]	mg POEA/L	42	0.005	0.0034

POEA, polyethoxylated tallowamine.
[a]See text for complete description of model.
[b]Assumes 1-ha pond, 1 m deep; 0.15-m sediment depth.

Plant Tissue. Knowledge of concentrations of glyphosate in or on plant material, including foliage, seeds, and berries, is important for predicting the potential exposure of herbivorous birds and mammals to RU and glyphosate. The ingestion of treated food is expected to be the major exposure pathway for these animals. A summary of the residue concentrations in foliage, berries, and nuts shortly after application of the herbicide is given in Table 7. To provide a conservative estimate of potential exposure, the greatest concentration of glyphosate in the different tissues was identified. The greatest concentration on foliage was 1272 mg a.e./kg (Horner 1990) and for wild berries was 19 mg a.e./kg fresh weight (Roy et al. 1989b). The greatest concentration of glyphosate in seeds was found in preharvest soybean grain (17.4 mg a.e./kg) (Kunstman 1983). Although the values are reported as glyphosate, it would be expected that the entire formulation of RU was actually present and a conversion, based on the proportion of glyphosate in RU, is appropriate to define RU exposure. In addition, because these data represent maximum concentrations of glyphosate on freshly sprayed foliage and berries, the exposures resulting from consumption of these should realistically be limited to scenarios for acute exposure. Foliage or plants containing large amounts of RU will die, thus becoming unpalatable to wildlife. Therefore, chronic exposure of wild mammals and birds to glyphosate through plant tissue is expected to be insignificant. Domestic livestock may be exposed to glyphosate residues in feed, such as hay and grain, and feeding studies that have been conducted show no effects (Williams et al., 2000).

Animal Tissue. Neither glyphosate nor RU would be expected to bioaccumulate (WHO 1994; USEPA 1993a). However, because small rodents feeding on treated foliage will acquire a level of chemical loading within their bodies, short-term food chain effects were assessed. Glyphosate residues have been measured in the viscera of mice and shrews (see Table 7). Thus, carnivorous animals such as raptors or foxes may be exposed to RU when they consume small rodents. The greatest concentration of RU in whole-body tissues of small rodents is reported as 5.1 mg a.e./kg fresh weight (Newton et al. 1984).

Channel catfish, rainbow trout, bluegill, marsh clams, and crayfish did not bioconcentrate glyphosate when exposed under laboratory conditions (WHO 1994). Bioconcentration factors (BCFs) for channel catfish, rainbow trout, and bluegill were less than 1.0 at water concentrations comparable to those expected in the field. The greatest BCF observed was 12 for crayfish and marsh clams. In depuration studies, glyphosate concentrations declined rapidly in tissues within 14–28 d after exposure ended (WHO 1994). Because glyphosate is not likely to bioconcentrate in tissues of aquatic organisms, no assessment for food chain transfer was conducted.

Surface Water. Surface waters near treated areas may be contaminated by glyphosate through runoff or drift of spray at application. In rural agricultural areas where glyphosate had been applied, glyphosate was detected in only 2 ponds of

Table 7. Concentrations of glyphosate in wildlife food sources after direct application of formulated herbicide.

Type	Location	Year	Concentration (mg a.e./kg fw)	Reference
Animal tissue				
Deer mice (viscera)	Oregon Coast Range, Canada	1978	5.1[a]	Newton et al. (1984)
Shrew (viscera)	Oregon Coast Range, Canada	1978	1.7[a]	Newton et al. (1984)
Plant tissue				
Foliage (fresh)	Carnation Creek, Canada	1984	261–448[b]	Feng and Thompson (1990)
Foliage (fresh)	Chassell, Corvallis, Cuthbert, USA	1987	650–1,272[a]	Horner (1990)
Reindeer lichen	Laukaa, Konnevesi, Finland	1976	45[a, c]	Siltanen et al. (1981)
Spartina alterniflora	Willipa Bay, Washington, USA	1992	24–66	Paveglio et al. (1996)
Topcrown foliage	Oregon Coast Range, Canada	1978	489[a]	Newton et al. (1984)
Wild berries	Laukaa, Konnevesi, Finland	1977	1.6–2.1[a, d]	Siltanen et al. (1981)
Wild berries	Harker, Lamplugh, Canada	1985	8–19	Roy et al. (1989b)
Soybean grain (following preharvest treatment)	Banks, Mississippi, USA	1982	0.71–17.4	Kunstman (1983)
Strawberry fruit (following spot/wiper treatments to weeds)	Milton, Hillsburgh, and Cambridge, Ontario, Canada	1987–1989	0.01–0.04	Cessna and Cain (1992)

a.e., glyphosate acid equivalents.
[a]It was not reported whether values were based on dry or fresh weight.
[b]Value expressed as mg a.e./kg dw.
[c]9 mon after application.
[d]6 d after application.

211. The concentration of glyphosate detected in these ponds was less than 0.15 mg a.e./L (Frank et al. 1990). Runoff or off-target drift were the primary sources of glyphosate (0.042 mg a.e./L) detected in 1 pond. To consider worst-case exposure conditions, the instantaneous concentration of RU in surface waters was estimated. The estimate was based on two assumptions, that runoff (2%) from a 10-ha field treated at the maximum single use rate entered a 1-ha pond 2 m deep and that 10% of the maximum single application rate per hectare entered the pond through drift (assuming aerial application). Two scenarios were considered for foliar interception: (1) no interception, and (2) 50% interception. The estimated maximum concentrations of RU in water based on these assumptions ranged from 0.271 to 0.406 mg RU/L (see Table 6).

When the GENEEC Tier I screening model developed by the U.S. EPA was used to estimate RU concentrations in a farm pond, estimated peak concentrations ranged from 0.024 to 0.090 mg RU/L following a single 4.2-kg a.e./ha application. The range of estimated concentrations occurs because K_{oc} and half-life values for soil and water can vary. Thus, the estimated maximum concentrations summarized in Table 6 are extremely conservative as they are an order of magnitude greater than those estimated by GENEEC. Additional conservatism was included in the risk assessment by comparing instantaneous maximum concentrations to NOEC values.

Chronic Scenario.

Soil, Surface Water, and Sediment Residue Model. To estimate chronic exposure concentrations of glyphosate and POEA, a dissipation model was used to calculate annualized daily concentrations in soil, water, and sediment. It was assumed that the concentration of glyphosate and POEA would decline in a pseudo-first-order fashion ($C_t = C_0 \cdot e^{-k \cdot t}$), where C_t is the concentration at time t, C_0 is the initial concentration at time zero, k is the dissipation rate constant in d^{-1} ($k = 0.693/DT_{50}$), and t is the elapsed time in days. Media-specific DT_{50} values were derived from empirical data (see earlier).

The initial concentration of glyphosate and POEA (C_0) in soil, sediment, or surface water was set at the concentration used in the respective acute scenario. Glyphosate acid (a.e.) was assumed to represent 31% of the acute RU value; POEA was assumed to represent 15%. Where necessary, adjustments were made to account for differences in the application rate. For the chronic exposure scenario, it was assumed that RU would be applied a total of three times over the crop growing season. The scenario involved an initial application of glyphosate at a rate of 4.2 kg a.e./ha preemergence, a second application at a rate of 1.68 kg a.e./ha 30 d later, and a final application at a rate of 0.85 kg a.e./ha preharvest (4 mon after the initial treatment). Thus, the model allowed the initial application of glyphosate to decay for 30 d and each subsequent addition of herbicide was considered by summing its contribution to the glyphosate or POEA that was already present in the medium on the day of the application. The dissipation model was then applied to this new concentration and the cumulative concentration was calculated before the next application. An example of the model output for glyphosate in surface water is given in Fig. 4. Based on the model simulation, annualized mean concentrations for glyphosate and POEA were calculated in each of the simulated media compartments. The ranges of maximum chronic concentrations of glyphosate and POEA predicted using this model are summarized in Table 6.

When the GENEEC screening model was used to estimate glyphosate concentrations in a farm pond, 56-d time-weighted average concentrations were estimated to range from 0.0005 to 0.0067 mg a.e./L following a single 4.2-kg a.e./ha application. The GENEEC model does not provide annual mean concentrations that could be compared directly with the model used in this assessment. The GENEEC estimates were significantly less than those summarized in Table 6. This difference indicates that the current assessment assumes a conservative exposure level that is greater than the level that Tier 1 screening models, such as GENEEC, would predict.

D. Environmental Concentrations from Aquatic Uses

Acute Scenario.

Concentrations of RU, glyphosate, and POEA in aquatic systems following direct application to water bodies can be greater than that resulting from agricul-

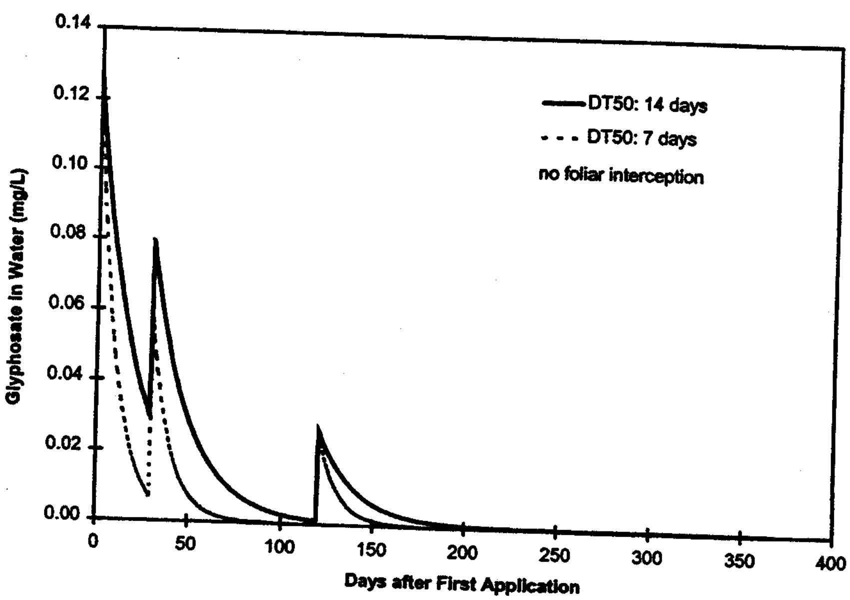


Fig. 4. Modeled glyphosate concentrations in water following terrestrial application of Roundup® at the maximum labeled use rate. For use in Hazard Quotient calculations.

tural and other nonaquatic uses. In North America, the primary use of RU is for agricultural applications; however, in some other parts of the world it is used to control emergent and floating aquatic vegetation. RU is not effective on submerged aquatic macrophytes (Barrett 1978). A number of studies have been conducted to evaluate the concentration of glyphosate in surface water and sediment after direct application (Tables 8, 9). Concentrations of glyphosate in surface water range from 0.010 to 1.700 mg a.e./L, and concentrations in sediment range from 0.11 to 19 mg a.e./kg dw.

Predicted exposure concentrations of RU following direct application to water were summarized and are consistent with field observations (Table 10). Direct application of RU at a rate of 4.2 kg a.e./ha (13.5 kg RU/ha) could result in a maximum RU concentration of 0.68 mg RU/L in a uniformly mixed, 2-m-deep water body, assuming no sorption to sediment. In a water body with a depth of 0.15 m, the maximum concentration would be 9.0 mg RU/L. Because RU is not applied directly to the water, but rather to foliage, 50% foliar interception is assumed. The maximum RU concentrations in water are 0.3 or 4.5 mg RU/L for 2-m- and 0.15-m-deep water, respectively, assuming 50% interception. The actual concentration observed is dependent on several factors including application rate, interception by target vegetation, water depth, the amount of suspended solids, and whether the water is stagnant or subject to a current, such as in a stream.

Chronic Scenario.

Glyphosate. Following direct application of RU to emergent vegetation, glyphosate dissipates through partitioning to sediments and biodegradation in the water and sediments. Maximum annualized mean concentrations of glyphosate were estimated using the first-order decay model described above, assuming water depths of 2 and 0.15 m, DT_{50} values of 7 or 14 d, and foliar interception of 0% or 50%. The resulting maximum chronic exposure concentrations range from 0.001 to 0.049 mg a.e./L (Table 10).

Polyethoxylated Tallowamine (POEA). Maximum annualized concentrations of POEA were estimated in a similar fashion, assuming that 15% of the RU formulation was composed of POEA. Dissipation was simulated as described above, using DT_{50} values for POEA of 21 or 42 d. The resulting maximum chronic exposure concentrations for POEA ranged from 0.0005 to 0.024 mg POEA/L (see Table 10).

E. Nontarget Organism Exposure Analysis

Terrestrial Uses.

Exposure of aquatic and soil organisms was based on the concentration of RU or its major components in soil or water. Interception of some herbicide by plant material was used to establish a range of maximum exposure concentrations (see Table 6).

Table 8. Concentrations of glyphosate in water after direct application of formulated herbicide.

Water type	Location	Year	Application rate (kg/ha)	Concentration[a] (mg a.e./L)	Reference
Pond	Manitoba, Canada	1986	0.89	0.141	Goldsborough and Beck (1989)
Pond	Chassell, Corvallis, Cuthbert, USA	1987	4.2	0.090–1.700	Horner (1990)
Seawater	Willipa Bay, Washington, USA	1992	2.3	0.010	Paveglio et al. (1996)
Stream	Chassell, Corvallis, Cuthbert, USA	1987	4.2	0.035–1.237	Horner (1990)
Stream	Not specified		2.2	0.020	Folmar (1978)
Surface	Drentsche Aa, Netherlands	1988–1989	nr	0.5–1	WHO (1994)
Surface	Carnation Creek, Canada	1984	2.0	0.162[b]	Feng et al. (1990)
Surface	Carnation Creek, Canada	1984	2.0	<0.001[c]	Feng et al. (1990)

nr, not reported.
[a]Measured concentrations: a.e., acid equivalent of glyphosate.
[b]Immediately after spraying.
[c]4 d after spraying.

Table 9. Concentrations of glyphosate in sediment after direct application to water.

Sediment type	Location	Year	Application rate (kg/ha)	Concentration (mg a.e./kg dw)[a]	Reference
Mudflat	Willipa Bay, Washington, USA	1992	2.3	1.16–2.82	Paveglio et al. (1996)
Pond	Chassell, Corvallis, Cuthbert, USA	1987	4.2	0.26–19	Horner (1990)
Creek	Carnation Creek, Canada	1984	2.0	6.8	Feng et al. (1990)
Stream	Chassell, Corvallis, Cuthbert, USA	1987	4.2	0.11–0.69	Horner (1990)

[a]Measured concentration: a.e., acid equivalent of glyphosate.

Table 10. Predicted exposure concentrations of Roundup®, glyphosate, and POEA in water following direct application of Roundup®[a].

Type of exposure	Units	Aquatic DT_{50} (days)	Foliar interception/water depth: None, 2 m deep	None, 0.15 m deep	50%, 2 m deep	50%, 0.15 m deep
Acute scenario[b]						
Roundup®	mg RU/L	—	0.677	9.032	0.339	4.52
Chronic scenario[c]						
Glyphosate	mg a.e./L	7	0.002	0.025	0.001	0.013
Glyphosate	mg a.e./L	14	0.014	0.049	0.002	0.025
POEA	mg POEA/L	21	0.001	0.012	0.0005	0.006
POEA	mg POEA/L	42	0.002	0.024	0.001	0.012

a.e., glyphosate acid equivalents.
[a]A single application of 4.2 kg a.e./ha was assumed.
[b]Maximum concentrations.
[c]Annualized mean concentrations.

Exposure of birds and mammals was considered to be primarily through the diet with the degree of exposure dependent on the food ingestion rate. As described earlier, a range of exposure values was established considering small animals (1 g) and large animals (5000 g) (Table 11). For herbivorous mammals, the smallest mammal considered was the meadow vole, which has a body weight range of 20–40 g, because mammals with body weights less than 20 g do not typically rely on a pure foliage diet (USEPA 1993b). The maximum

Table 11. Representative ranges of ingestion rates for birds and mammals for different types of diets.

Taxonomic group	Generic equation for ingestion rates[a]	Ingestion Rate[b,c] (g food/g bw): Body weight, 1 g	Body weight, 5000 g
Birds	$g/d = 0.648\ wt^{0.651}$ (g)	2.16	0.11
Mammals (fruit/seeds or invertebrate diet)	$g/d = 0.235\ wt^{0.822}$ (g)	0.78	0.17
Mammals (foliage diet)	$g/d = 0.235\ wt^{0.822}$ (g)	0.46[d]	0.17

[a]From Nagy (1987) for g food dw/g ww body mass: dw, dry weight; ww, wet weight.
[b]Food converted to fresh weight assuming 70% water content (e.g., 0.648 g dw = 2.16 g ww).
[c]Calculated by dividing predicted food consumption (g/d) by the body weight of the animal.
[d]Corresponds to a body weight of 20 g, because mammals with a body weight <20 g do not rely on a pure foliage diet (USEPA 1993b).

Table 12. Maximum predicted acute exposure levels of birds and mammals to Roundup® (RU) following terrestrial uses of formulated herbicide.

Taxonomic group	Type of diet	Maximum estimated roundup® concentration (mg RU/kg diet)	Acute exposure range[d] (mg RU/kg bw/d)
Birds	Fruits or seeds	145[a]	16.0–313
	Invertebrates	145[b]	16.0–313
Mammals	Fruits or seeds	145[a]	24.7–113
	Invertebrates	145[b]	24.7–113
	Foliage	2904[c]	494–1336

[a]Estimated using the seed category from Kenaga (1973) with a maximum use rate of 13.5 kg RU/ha (12.1 lb/A).
[b]Estimated using the large insect category from Kenaga (1973) with a maximum use rate of 13.5 kg RU/ha (12.1 lb/A).
[c]Estimated using the short grass category from Kenaga (1973) with a maximum use rate of 13.5 kg RU/ha (12.1 lb/A).
[d]Based on food consumption values from Table 11. Range represents large to small body weight.

RU concentration to occur in several different tissues was estimated using the nomographic method described by Kenaga (1973). The Kenaga values were larger than the measured values reported in Table 7, adding additional conservatism to the evaluation. These maximum tissue concentrations and the ranges of ingestion rates for birds and mammals were used to estimate the acute exposure range to RU (Table 12). Chronic exposure was estimated based on the glyphosate and POEA content of RU and using 6-wk residue exposures (Kenaga 1973) (Table 13).

Table 13. Maximum predicted chronic exposure levels of birds and mammals to glyphosate and surfactant following terrestrial uses.

Taxonomic group	Type of diet	Maximum estimated concentration[a] (mg/kg ww)		Chronic exposure [b,c] (mg/kg/d)	
		Glyphosate	POEA	Glyphosate	POEA
Birds	Fruits or seeds	<3.8	<1.8	0.4–8.1	0.2–3.9
	Invertebrates	<3.8	<1.8	0.4–8.1	0.2–3.9
Mammals	Fruits or seeds	<3.8	<1.8	0.6–3.0	0.3–1.4
	Invertebrates	<3.8	<1.8	0.6–3.0	0.3–1.4
	Foliage	18.8	9.1	3.2–8.6	1.5–4.2

[a]Estimated using the 6-wk typical limit for residues (Kenaga 1973) at maximum application rate of 13.5 kg RU/ha (12.1 lb/A), assuming 31% glyphosate and 15% POEA.
[b]Using food consumption values from Table 11. Range represents large to small body weights.
[c]The likelihood of these exposure levels is very small; these were used as a worst-case exposure level.

Aquatic Uses.

The intentional application of RU or other glyphosate formulations to emergent aquatic vegetation can result in greater concentrations in aquatic systems than from terrestrial uses. The range of acute exposure concentrations to RU and the chronic exposure potential for glyphosate and POEA following aquatic applications are given in Table 10.

IV. Toxicity Assessment

The objective of the toxicity assessment was to use currently available information to establish acute and chronic TRVs for RU, glyphosate, and POEA based on survival, growth, and reproduction effects on nontarget species. The first step in this process was to evaluate each study using critical assessment criteria (problem formulation section). The second step was to identify data for each potentially exposed group. The last step was to determine the most sensitive species from among each group and then to extrapolate to (1) an acute TRV for RU or (2) a chronic TRV for glyphosate or POEA. Under circumstances when data were limited, safety factors were applied to estimate threshold exposure concentrations from median data such as LC_{50} and EC_{50} (see Problem Formulation section).

A. Aquatic Organisms

Aquatic Microorganisms.

RU Acute TRV. A number of studies are available that describe the toxicity of RU, glyphosate, and AMPA to aquatic microorganisms (Table 14). EC_{50} values for effects of RU on aquatic microorganisms range from 2.1 to 189 mg RU/L. A single NOEC value based on empirical data was available for *Selenastrum capricornutum*, which was the most sensitive species based on EC_{50} data. Thus, the TRV for aquatic microorganisms was estimated based on this NOEC: 0.73 mg RU/L for growth decrease based on biomass (LISEC 1989a).

Glyphosate Chronic TRV. Glyphosate exhibits a wide range of toxicity to aquatic microorganisms. The life cycle of aquatic microorganisms is short (ranging from hours to days), so these studies represent the measurement of chronic effects (i.e., multigenerational even under relatively short exposure periods of 3–5 d). Reported EC_{50} values for effects of glyphosate on growth based on biomass range from 0.64 to 590 mg a.e./L (Table 14). NOEC values based on empirical data range from 0.28 to 33.6 mg a.e./L. The most sensitive species, *Skeletonema costatum*, exhibited a NOEC of 0.28 mg a.e./L (Malcolm Pirnie 1987b). Therefore, the chronic TRV for glyphosate for aquatic microorganisms was estimated to be 0.28 mg a.e./L.

Aquatic Macrophytes.

RU Acute TRV. EC_{50} values for effects of RU on aquatic macrophytes range from 3.9 to 15.1 mg RU/L (Table 15). NOEC values based on empirical data

Table 14. Toxicity of Roundup®, glyphosate, and AMPA to aquatic microorganisms.

Species	Test duration (days)	EC_{50} (mg/L)[a]	NOEC (mg/L)[a]	Reference
Roundup®:				
Selenastrum capricornutum				
Growth (biomass)	3	2.1	0.73	LISEC (1989a)[b]
Inhibition of growth rate	3	8.0	—	
S. capricornutum	4	2.6	—	Thomas et al. (1990)
Chlorella pyrenoidosa	7	189	—	Hernando et al. (1989)
Chlorella sorokiniana	4	3.0	2.0	Christy et al. 1981
Glyphosate (tested as acid):				
Anabaena variabilis	n.r.	2	—	Hutber et al. (1979)
Anabaena flos-aquae	7	15	9.7	Malcolm Pirnie (1987a)
Aphanocapsa strain 6308	n.r.	2	—	Hutber et al. (1979)
Aphanocapsa strain 6714	n.r.	100	—	Hutber et al. (1979)
C. pyrenoidosa	4	590	—	Maule and Wright (1984)
Chlamydomonas eugametos	4	>169	16.9	Hess 1980
Chlorococcum hypnosporum	4	68	—	Maule and Wright (1984)
Navicula pelliculosa	7	42	33.6	Malcolm Pirnie (1987c)
Nostoc sp.	n.r.	2	—	Hutber et al. (1979)
Scenedesmus acutus	4	10.2	2	Sáenz et al. (1997)

Table 14. (Continued).

Species	Test duration (days)	EC_{50} (mg/L)[a]	NOEC (mg/L)[a]	Reference
Scenedesmus quadricauda	4	7.2	0.77	Sáenz et al. (1997)
S. capricornutum	4	21.8 or 26.3		Bozeman et al. (1989)
S. capricornutum	3	485	45	NATEC (1990)
Skeletonema costatum				
Chlorophyll decrease	4	1.2	<0.6	EG & G Bionomics (1978a)
Biomass decrease	4	1.3	<0.6	
S. costatum	7	0.64	0.28	Malcolm Pirnie (1987b)[b]
S. capricornutum	7	13.8	10.6[c]	Malcolm Pirnie (1987d)
Glyphosate (tested as IPA salt):[d]				
Scenedesmus subspicatus				
Biomass	3	72.9	10.5	Dengler and Mende (1994b)
Growth rate	3	166	26.4	
Ankistrodesmus sp.	4	412	20	Gardner et al. 1997
AMPA:				
S. subspicatus E_bC_{50}	3	90	7.9	Dengler and Mende (1994a)

n.r., not reported.

[a]Units are mg RU/L, mg a.e./L, or mg AMPA/L. RU, Roundup; a.e., glyphosate acid equivalents.

[b]Reference used in setting toxicity reference value.

[c]Value from source corrected to mg RU/L or mg a.e./L.

[d] Test material was glyphosate IPA salt; LC_{50} reported as mg glyphosate IPA salt/L.

Table 15. Toxicity of Roundup®, glyphosate, and AMPA to aquatic macrophytes.

Species	Test duration (days)	EC_{50} (mg/L)[a,b]	NOEC (mg/L)[a]	Reference
Roundup®				
Lemna gibba	7	15.1	—	Perkins (1997)
Myriophyllum sibiricum	14	3.9	0.78[c]	Perkins (1997)[d]
Lemna minor	14	4.9	—	Hartman and Martin (1984)
L. minor	14		56	Lockhart et al. (1989)
Potamogeton pectinatus	14		24	Hartman and Martin (1985)
Glyphosate				
Myriophyllum sibiricum	14	1.6	0.08[e]	Perkins (1997)[d]
Lemna gibba	7	10	—	Perkins (1997)
L. gibba	14	25.5	16.6	Malcolm Pirnie (1987e)

[a]Units are mg RU/L or mg a.e./L. RU, Roundup; a.e., glyphosate acid equivalents; AMPA, aminomethylphosphonic acid.
[b]EC_{50} endpoint is for growth inhibition.
[c]Derived from an acute EC_{50}/acute NOEC ratio of 5.
[d]Reference used in setting toxicity reference value.
[e]Derived from an acute EC_{50}/chronic NOEC ratio of 20.

range from 24 to 56 mg RU/L. The acute TRV for aquatic macrophytes was estimated based on the derived NOEC from the most sensitive species. The most sensitive species was *Myriophyllum sibiricum* with an EC_{50} of 3.9 mg RU/L based on root length (Perkins 1997). A NOEC of 0.78 mg RU/L was derived by dividing the EC_{50} by a five-fold application factor.

Glyphosate Chronic TRV. EC_{50} values for effects of glyphosate on aquatic macrophytes range from 1.6 to 25.5 mg a.e./L (Table 15). A single NOEC value based on empirical data was available: 16.6 mg a.e./L for *Lemna gibba*. The chronic TRV for effects of glyphosate on aquatic macrophytes was based on change in root length EC_{50} for the most sensitive species, *Myriophyllum sibiricum* (Perkins 1997). The chronic TRV for glyphosate was estimated to be 0.08 mg a.e./L by dividing this EC_{50} (1.6 mg a.e./L) by a 20-fold application factor.

Aquatic Invertebrates.

RU Acute TRV. The acute toxicity of RU to aquatic invertebrates ranges from practically nontoxic to moderately toxic (Table 16). EC_{50} and LC_{50} for effects of RU on invertebrates range from 9.7 to 200 mg RU/L; NOEC values based on empirical data range from 4.4 to 7.8 mg RU/L. The most sensitive species was *Daphnia magna*, for which the 48-hr EC_{50} was 9.7 mg RU/L (Folmar et al. 1979). An acute NOEC value of 1.9 mg RU/L was derived by use of an LC_{50}/acute NOEC application factor of 5.

Table 16. Acute toxicity of Roundup®, glyphosate, AMPA, and POEA to aquatic invertebrates.

Species	Test duration (days)	EC_{50} or LC_{50} (mg/L)[a]	NOEC (mg/L)[a]	Reference
Roundup®: freshwater species				
Scapholeberis kingi	0.125	61	—	Sun (1987)
Anopheles quadrimaculatus larvae (mosquito)	1	673.4	—	Holck and Meek (1987)
Chironomus plumosus	2	58.1[c]	—	Folmar et al. (1979)
Daphnia magna	2	9.7[c]	1.9[e]	Folmar et al. (1979)[f]
D. magna	2	24	7.8	EG & G Bionomics (1980f)
D. magna	2	12.9[c]	4.6[c]	EG & G Bionomics (1980e)
Daphnia pulex	2	19	—	Hartman and Martin (1984)
Gammarus pseudolimnaeus	2	42	4.4	ABC Inc. (1982b)
G. pseudolimnaeus	2	200[c]	—	Folmar et al. (1979)
D. pulex	4	25.5	—	Servizi et al. (1987)
G. pseudolimnaeus	4	138.7[c]	—	Folmar et al. (1979)
Orconectes nais	4	7	—	Mayer and Ellersieck (1986)
Procambarus clarkii	4	47.3	—	Holck and Meek (1987)
Glyphosate: freshwater species (tested as acid)				
Daphnia magna	2	780	560	ABC Inc. (1978a)
Pseudosuccinea columella	n.r.	98.9	—	Thompson (1989)
Glyphosate: freshwater species (tested as IPA salt)[b]				
D. magna	2	930	320	ABC Inc. (1981a)
Chironomus plumosus	2	55	—	Folmar et al. (1979)
Chironomus riparius	2	5600	—	Buhl and Faerber (1989)
Hyalella azteca[d]	10	>530	265	Beyers (1993)
Chrionomus tentans[d]	10	>530	265	Beyers (1993)
Glyphosate: marine species (tested as acid)				
Crassostrea virginica, eggs	2	>10	10	Bionomics (1973a)
Palaemonetes vulgaris	4	281	210	Bionomics (1973b)
Uca pugilator	4	934	650	Bionomics (1973b)
Mysidopsis bahia	4	>1000	—	EG & G Bionomics (1978c)
Tripneustese esculentes	4	>1000	1000	EG & G Bionomics (1978d)
AMPA: freshwater species				
D. magna	2	690	320	ABC Inc. (1991a)
POEA: freshwater species				
C. plumosus	2	13.0	—	Folmar et al. (1979)
D. magna	2	2.0	0.32	ABC Inc. (1980b)
D. pulex	2	4.1	—	Moore et al. (1987)
D. pulex	4	2.0	—	Servizi et al. (1987)

[a]Units are mg RU/L, mg a.e./L, mg AMPA/L, or mg POEA/L. RU, Roundup; a.e., glyphosate acid equivalents. [b]Test material was glyphosate IPA salt; LC_{50} reported as mg glyphosate IPA salt/L. [c]Value from data source corrected to mg RU/L. [d]Sediment/water test. [e]Derived from an acute EC_{50}/acute NOEC ratio of 5. [f]Reference used in setting acute toxicity reference value.

Glyphosate Chronic TRV. The chronic TRV for aquatic invertebrates was based on chronic toxicity to the most sensitive species for either RU or glyphosate a.e. (Table 17). The most sensitive species was *Daphnia magna*, for which the NOEC value was 50 mg a.e./L (ABC Inc. 1982d) and 3.2 mg RU/L (0.99 mg a.e./L; ABC Inc. 1989b). An additional application factor of 2 was applied to the 0.99 mg a.e./L NOEC value because toxicity data were only available for one species. Consequently, the chronic TRV selected for effects of glyphosate on aquatic invertebrates was 0.50 mg a.e./L.

POEA Chronic TRV. As no chronic toxicity data were available for aquatic invertebrates, the chronic TRV was estimated from the acute toxicity to the most sensitive species (see Table 16). EC_{50} for effects of POEA on invertebrates range from 2.0 to 13.0 mg/L, with a single reported NOEC value based on empirical data of 0.32 mg/L (*Daphnia magna*). A chronic NOEC was derived from the lowest EC_{50} by use of an acute EC_{50}/chronic NOEC application factor of 20; this resulted in a derived chronic NOEC of 0.1 mg POEA/L.

Fish.

RU Acute TRV. Acute toxicity values of RU were used to estimate the acute TRV because exposure to the intact formulation cannot be excluded for acute exposures. Acute LC_{50} values were available for 12 species of fish (Table 18), and range from 4.2 to 52 mg RU/L. NOEC values based on empirical data range from 1.0 to 23 mg RU/L. Rainbow trout (*Oncorhynchus mykiss*) were the most sensitive based on LC_{50} values (Folmar et al. 1979). Because a measured NOEC was unavailable for the study that had reported the LC_{50} of 4.2 mg RU/L, an acute NOEC value was derived by use of an acute LC_{50}/acute NOEC application factor of 5, resulting in an estimated acute NOEC of 0.84 mg RU/L.

RU is more acutely toxic to aquatic animals than glyphosate (Table 18).

Table 17. Chronic toxicity of Roundup® and glyphosate to aquatic freshwater invertebrates.

Species	Test duration (days)	NOEC (mg/L)[a]	Reference
Roundup®			
Daphnia magna	21	3.2	ABC Inc. (1989b)[b]
Tubifex tubifex	28	>89	Perkins (1997)
Glyphosate			
D. magna	21	100	ABC Inc. (1989c)
D. magna	21	50	ABC Inc. (1982d)

[a]Units are mg RU/L or mg a.e./L. RU, Roundup; a.e., glyphosate acid equivalents.
[b]Reference used in setting chronic toxicity reference value.

Table 18. Acute toxicity of Roundup®, glyphosate, AMPA, and POEA to fish.

Species	Test (days)	LC_{50} (mg/L)[a]	NOEC (mg/L)[a]	Reference
Roundup®:				
Bluegill sunfish, *Lepomis macrochirus*	4	5.8	2.2	ABC Inc. (1982a)
Bluegill sunfish, *L. macrochirus*	4	16.1[b]	—	Folmar et al. (1979)
Bluegill sunfish, *L. macrochirus*	4	34[b]	21	EG & G Bionomics (1980b)
Carp, *Cyprinus carpio*	4	10	5.6	Liong et al. (1988)
Carp, *C. carpio*	4	26	—	Sun (1987)
Carp, *C. carpio*	4	15	—	Tooby et al. (1980)
Channel catfish, *Ictalurus punctatus*	4	39	23	EG & G Bionomics (1980a)
Channel catfish, *I. punctatus*, fry	4	10.6[b]	—	Folmar et al.(1979)
Channel catfish, *I. punctatus*, adult	4	42.0[b]	—	Folmar et al. (1979)
Chinook salmon, *Oncorhynchus tshawytsha*	4	20	—	Mitchell et al. (1987)
Chinook salmon, *O. tshawytsha*	4	27, 17[c]	—	Wan et al. (1989)
Chum salmon, *Oncorhynchus keta*	4	19, 11[c]	—	Wan et al. (1989)
Coho salmon, *Oncorhynchus kisutch*	4	22	—	Mitchell et al.(1987)
Coho salmon, *O. kisutch* fry	4	42.0	—	Servizi et al. (1987)
Coho salmon, *O. kisutch*	4	27, 13[c]	—	Wan et al. (1989)
Fathead minnow, *Pimephales promelas*	4	7.4[b]	—	Folmar et al. (1979)
Fathead minnow, *P. promelas*	4	23	13.6	EG & G Bionomics (1980d)
Mosquitofish, *Gambusia affinis*	2	15	—	Sun (1987)
Pink salmon, *Oncorhynchus gorbuscha*	4	31, 14[c]	—	Wan et al. (1989)
Rainbow trout, *Oncorhynchus mykiss*	4	8.2	6.4	ABC Inc. (1982c)
	4	22	8.0	EG & G Bionomics (1980g)
	4	27	6.75	Morgan et al. (1991)
	4	27[b]	21.4[b]	EG & G Bionomics (1980c)
Fingerling	4	4.2–27[b]	0.84[d]	Folmar et al. (1979)[e]
Natural waters	4	52	—	Hildebrand et al. (1982)
Natural waters	4	15	—	Mitchell et al. (1987)

Table 18. (Continued).

Species	Test (days)	LC_{50} (mg/L)[a]	NOEC (mg/L)[a]	Reference
Natural waters	4	15, 14[c]	—	Wan et al. (1989)
	4	33.6[b]	—	Morgan and Kiceniuk (1992)
Sockeye salmon, *Oncorhynchus nerka*	4	26.7	—	Servizi et al. (1987)
Tilapia sp.	4	7.4	1.0	Liong et al. (1988)
Fish (mean of several species)	Acute	13	—	WHO (1994)
Glyphosate (tested as acid):				
Bluegill sunfish, *Lepomis macrochirus*	2	>24	24	Bionomics (1973c)
Bluegill sunfish, *L. macrochirus*	4	120	100	ABC Inc. (1978c)
Chinook salmon, *Oncorhynchus tshawytsha*	4	30, 211[c]	—	Wan et al. (1989)
Chum salmon, *Oncorhynchus keta*	4	22, 148[c]	—	Wan et al. (1989)
Coho salmon, *Oncorhynchus kisutch*	4	36, 174[c]	—	Wan et al. (1989)
Flagfish (juvenile), *Jordanella floridae*	4	>30	—	Holdway and Dixon (1988)
Harlequin fish, *Rasbora heteromorpha*	4	168	<100	HRC (1977)
Pink salmon, *Oncorhynchus gorbuscha*	4	23, 190[c]	—	Wan et al. (1989)
Rainbow trout, *Oncorhynchus mykiss*	4	22, 197[c]	—	Wan et al. (1989)
Rainbow trout, *O. mykiss*	4	86	42	ABC Inc. (1978b)
Sheepshead minnow, *Cyprinodon variegatus*	4	>1000	1000	EG & G Bionomics (1978b)
Fish (several species)	Acute	94		WHO (1994)
Glyphosate (tested as IPA salt):[f]				
Bluegill sunfish, *Lepomis macrochirus*	4	140–220	—	Folmar et al. (1979)
Bluegill sunfish, *L. macrochirus*	4	>1000	560	ABC Inc. (1981b)
Channel catfish, *Ictalurus punctatus*	4	130	—	Folmar et al. (1979)
Fathead minnow, *Pimephales promelas*	4	97	—	Folmar et al. (1979)
Fathead minnow, *P. promelas*	4	>648	648	Beyers (1995)
Plains minnow, *Hybognathus placitus*	4	>648	648	Beyers (1995)

Table 18. (Continued).

Species	Test (days)	LC_{50} (mg/L)[a]	NOEC (mg/L)[a]	Reference
Rainbow trout, *Oncorhynchus mykiss*	4	>1000	1000	ABC Inc. (1981c)
Rainbow trout, *O. mykiss*	4	140–240	—	Folmar et al. (1979)
AMPA:				
Rainbow trout, *Oncorhynchus mykiss*	4	520	33	ABC Inc. (1991b)
POEA:				
Bluegill sunfish, *Lepomis macrochirus*	4	1.3	0.56	ABC Inc. (1980a)
Bluegill sunfish, *L. macrochirus*	4	1.0–3.0	—	Folmar et al. (1979)
Channel catfish, *Ictalurus punctatus*	4	13	—	Folmar et al. (1979)
Chinook salmon, *Oncorhynchus tshawytsha*	4	2.8, 1.7[c]	—	Wan et al. (1989)
Chum salmon, *Oncorhynchus keta*	4	2.4, 1.4[c]	—	Wan et al. (1989)
Coho salmon, *Oncorhynchus kisutch* fry	4	3.5	—	Servizi et al. (1987)
Coho salmon, *O. kisutch*	4	3.2, 1.8[c]	—	Wan et al. (1989)
Fathead minnow, *Pimephales promelas*	4	1.0	—	Folmar et al. (1979)
Pink salmon, *Oncorhynchus gorbuscha*	4	2.8, 1.4[c]	—	Wan et al. (1989)
Rainbow trout, *Oncorhynchus mykiss*	4	4.2	3.2	ABC Inc. (1980c)
Rainbow trout, *O. mykiss*	4	0.65–7.4	—	Folmar et al. (1979)[g]
Rainbow trout, *O. mykiss*	4	2.5, 1.7[c]	—	Wan et al. (1989)
Rainbow trout, *O. mykiss* fry	4	3.2	—	Servizi et al. (1987)
Sockeye salmon, *Oncorhynchus nerka* fry	4	2.6	—	Servizi et al. (1987)

[a]Units are mg RU/L, mg a.e./L; or mg POEA/L. RU, Roundup; a.e., acid equivalents.
[b]Value from data source corrected to mg RU/L.
[c]Values for soft (creek) and hard (lake) water, respectively.
[d]Derived from an acute LC_{50}/acute NOEC ratio of 5.
[e]Reference used in setting acute toxicity reference value.
[f]Test material was glyphosate IPA salt; LC_{50} reported as mg glyphosate IPA salt/L.
[g]Reference used in setting chronic toxicity reference value, assuming an acute LC_{50}/chronic NOEC ratio of 20.

RU LC_{50} values range from 4.2 to 52 mg RU/L, compared with glyphosate values that range from 22 to >1000 mg a.e./L. The results of several studies on glyphosate appear in the literature but were not included in Table 18 because several of the criteria for inclusion were not met. One study reported glyphosate LC_{50} of 5.5 mg a.e./L for carp (*Cyprinus carpio*) and 7.9 mg a.e./L for *Tilapia* sp. (Wang et al. 1994). Fundamental water chemistry values and survival of control organisms were not reported in these studies. In another study (Wan et al. 1989), several values for glyphosate LC_{50} less than 22 mg a.e./L were reported for nonnatural waters (dechlorinated city water). These values were not included in this evaluation because it was not clear if the pH observed for the city water, and possibly other water quality parameters, were artifacts of the water treatment process. Exclusion of these glyphosate values did not affect the acute TRV because the TRV was based on RU that has greater toxicity deriving from the surfactant.

The greater aquatic toxicity of RU compared to glyphosate is attributed to POEA. For rainbow trout, the 96-hr RU LC_{50} value was 8.3 mg RU/L (2.6 mg a.e./L), whereas the glyphosate LC_{50} from the same study was 140 mg a.e./L (Folmar et al. 1979). The same authors reported a POEA 96-hr LC_{50} of 1.6 mg/L, which suggests that POEA is the main source of aquatic toxicity.

Glyphosate Chronic TRV. Glyphosate exhibited little chronic toxicity to fish (Table 19). No effects on survival, growth, or reproduction of adult fathead minnow or progeny were observed when exposed to concentrations as great as 26 mg a.e./L for up to 8 mon (EG & G Bionomics 1975). A prolonged study with RU (21 d) was conducted with rainbow trout to determine effects on growth or survival (ABC Inc. 1989d). The NOEC for that study was 2.4 mg RU/L (0.74 mg a.e./L). Because the latter NOEC is protective of all glyphosate chronic values, 0.74 mg a.e./L was selected as the chronic TRV for fish.

Table 19. Chronic toxicity of Roundup® and glyphosate to fish.

Species	Test duration (days)	NOEC[a] (mg/L)	Reference
Roundup®			
Rainbow trout, *Oncorhynchus mykiss*	21	2.4	ABC Inc. (1989d)[b]
Glyphosate			
Fathead minnow, *Pimephales promelas*	255	26	EG & G Bionomics (1975)
Rainbow trout, *O. mykiss*	21	52	ABC Inc. (1989a)

[a]Units are mg RU/L or mg a.e./L; RU, Roundup; a.e., acid equivalents.
[b]Reference used in setting toxicity reference value.

POEA Chronic TRV. Because no chronic toxicity data were available for fish, the chronic TRV was based on the acute toxicity to the most sensitive species (see Table 18). The LC_{50} for the most sensitive species, *Oncorhynchus mykiss*, was 0.65 mg/L (Folmar et al. 1979). A chronic NOEC was derived by assuming an acute LC_{50}/chronic NOEC application factor of 20. This resulted in a derived chronic NOEC of 0.03 mg POEA/L, which was selected as the chronic TRV.

Amphibians.

In acute toxicity studies with amphibians, RU is slightly to moderately toxic, whereas glyphosate is practically nontoxic to slightly toxic (Table 20). This difference in toxicity is similar to that observed for fish and aquatic invertebrates and is likely related to the presence of POEA in RU. The acute TRV for amphibians was based on the species most sensitive to RU, which was *Litoria moorei*, for which the 48-hr LC_{50} value is 8.1 mg RU/L (Mann and Bidwell 1999). As described earlier, a five-fold application factor was applied to the LC_{50} value to predict a no-mortality concentration of 1.6 mg RU/L, which was estimated to be the acute TRV for amphibians. Because fish in acute studies tend to be equally sensitive or more sensitive than tadpoles to glyphosate, the chronic TRV for fish (0.74 mg a.e./L) was used for amphibians.

B. Soil Organisms

Soil Microorganisms.

The toxicity of RU and glyphosate to soil microorganisms and soil processes varies considerably among taxa (Table 21). Because the life cycle of soil microorganisms is of short duration relative to metazoans (ranging from hours to days), these studies are multigenerational even for exposure periods of 3–5 d, and any effects are considered chronic effects. Therefore, separate TRVs for acute and chronic exposures were not derived. Instead, a chronic TRV for RU was used to assess risk from acute as well as chronic exposures.

The chronic TRV for soil microbes was selected based on the most sensitive soil parameter for which information was available. *In vitro* studies utilizing artificial substrates such as agar were included in the table but were excluded from the risk assessment because several researchers have indicated that it is difficult to extrapolate from the artificial substrates to natural soil ecosystems (Estok et al. 1989; Wan et al. 1998). Soil nitrification/urea hydrolysis was the most sensitive to the effects of glyphosate and was inhibited at concentrations of glyphosate greater than 5.0 mg a.e./kg dry weight of soil. Glyphosate did not affect any of the other parameters measured under laboratory conditions except at greater concentrations (Martens and Bremner 1993). Thus, the chronic TRV for soil microbes/processes was estimated to be 5.0 mg a.e./kg soil (equivalent to 16 mg RU/kg soil). This NOEC is approximately 10-fold conservative as the next greater concentration tested was 50 mg a.e./kg.

Table 20. Acute toxicity of Roundup® and glyphosate to amphibians.

Species	Test duration (days)	LC_{50} (mg/L)[a]	NOEC (mg/L)[b]	Reference
Roundup®:				
Crinia insignifera, tadpole	2	10	—	Mann and Bidwell (1999)
C. insignifera, newly emerged	2	144	—	Mann and Bidwell (1999)
C. insignifera, adult	2	137	—	Mann and Bidwell (1999)
Heleioporus eyrei, tadpole	2	17.5	—	Mann and Bidwell (1999)
Limnodynastes dorsalis, tadpole	2	8.3	—	Mann and Bidwell (1999)
Litoria moorei, tadpole	2	8.1	1.6[c]	Mann and Bidwell (1999)[d]
L. moorei, tadpole	2	32.2	—	Mann and Bidwell (1999)
C. insignifera, tadpole	2	<54.9	—	Bidwell and Gorrie (1995)
C. insignifera, adult	4	96.8	54	Bidwell and Gorrie (1995)
L. moorei, tadpole	4	18.7	5.5	Bidwell and Gorrie (1995)
L. moorei, adult	4	>165	165	Bidwell and Gorrie (1995)
Xenopus laevis, embryo	4	72	—	Perkins (1997)
Glyphosate (tested as acid):				
C. insignifera, newly emerged	2	83.6	—	Mann and Bidwell (1999)
L. moorei, tadpole	2	121	—	Mann and Bidwell (1999)
L. moorei, tadpole	2	81.2	—	Mann and Bidwell (1999)
C. insignifera, adult	4	78	45	Bidwell and Gorrie (1995)
L. moorei, tadpole	4	111	—	Bidwell and Gorrie (1995)
L. moorei, adult	4	>180	180	Bidwell and Gorrie (1995)
Glyphosate (tested as IPA salt):[e]				
C. insignifera, tadpole	2	>466	—	Mann and Bidwell (1999)
Heleioporus eyrei, tadpole	2	>373	—	Mann and Bidwell (1999)
Limnodynastes dorsalis, tadpole	2	>400	—	Mann and Bidwell (1999)
L. moorei, tadpole	2	>343	—	Mann and Bidwell (1999)

[a]Units are mg RU/L or mg a.e./L; RU, Roundup; a.e., acid equivalents.
[b]Derived from raw data; not reported by study authors.
[c]Derived from an acute EC_{50}/acute NOEC ratio of 5.
[d]Reference used in setting toxicity reference value.
[e]Test material was glyphosate IPA salt; LC_{50} reported as mg glyphosate a.e./L.

It has been reported that glyphosate can have effects on nodule formation and root weights of *Rhizobium trifolii* at 2 mg a.e./kg soil (Eberbach and Douglas 1983, 1989). The soil used in that study had less than 0.5% organic matter and greater than 75% sand. The tests were conducted in the laboratory and did not simulate field spray methods. These data were not considered representative of the major agricultural soils and were not utilized in calculating the TRV for soil microbes.

Soil Invertebrates.

Effects of both RU and glyphosate on the earthworm, *Eisenia foetida*, have been investigated in standard 14-d laboratory soil assays (IBR 1991a,b; Table 22). No mortality was observed at the maximum exposure of 5000 mg RU/kg soil or 3750 mg a.e./kg soil. A dose-related effect on weight was not observed in either study. Worms were slack and soft in several test concentrations in both studies. The NOEC values based on slack and soft worms were 500 mg RU/kg dry weight soil and 118.7 mg a.e./kg. An additional application factor of 2 was applied to these NOEC values because toxicity data were only available for one species. Thus, the acute TRV was estimated to be 250 mg RU/kg soil, and the chronic TRV was estimated to be 59.4 mg a.e./kg soil.

Two other studies have reported greater RU toxicity to earthworms but did not meet the criteria for inclusion in this analysis. In the first study, glyphosate applied to soil at the prescribed rate was reported to have caused significant reductions in the rate of growth and maturation of earthworms at lesser concentrations than that selected for the TRV (Springett and Gray 1992). Only six worms were included per treatment, and variability among worms and treatments was not thoroughly characterized. Results of the assay indicated that RU was as toxic or more toxic than several insecticides, which is not consistent with other literature. For these and other methodological reasons, the study was not included in this analysis. In the second study, the effects of glyphosate and other pesticides on the earthworm *Aporrectodea caliginosa* were reported. The LC_{50} for RU was reported to be 218 mg RU/kg in soil (Mohamed et al. 1995). This LC_{50} value was not used in this analysis because the study did not meet the criteria established for inclusion. Actual mortality levels were not reported, which precluded verification of LC_{50}. This point is important because the LC_{50} reported for glyphosate after 17 d was greater than the greatest concentration tested, which indicates that the value had been extrapolated.

C. Terrestrial Organisms

The TRVs for birds and mammals for RU and glyphosate were calculated by first determining the safe level in the diet and then converting the dietary concentration to a daily exposure rate, such as mg RU/kg bw/d. This method allows for TRVs to reflect differences in food consumption rates for different animals. For the purpose of this assessment, nontarget arthropods were separated into pollinators (honeybees) and beneficial arthropods (predators and parasites). TRVs were not established for the risk assessment of these groups. Because of the lack of data regarding toxicity, POEA TRVs were not derived for birds or arthropods.

Table 21. Toxicity of Roundup® and glyphosate to terrestrial microorganisms.

Soil process	Test duration (days)	NOAEC[a]	Units	Reference
Roundup®:				
Effects observed in soil				
Nitrification	25	21.4	mg a.e./kg	Stratton (1990)
Nitrification	3	230	mg a.e./kg	Carlisle and Trevors (1986)
Nitrification	42	50	mg a.e./kg	Preston and Trofymow (1989)
Nitrification	28	24	mg a.e./kg	Todt (1991)
Nitrification	7–21	5.0	mg a.e./kg	Martens and Bremner (1993)
Dehydrogenase activity	28	24	mg a.e./kg	Todt (1991)
Nitrogen fixation	7	12.7	mg a.e./kg	Carlisle and Trevors (1986)
Immobilization of ammonium	42	50	mg a.e./kg	Preston and Trofymow (1989)
Urea hydrolysis	42	50	mg a.e./kg	Preston and Trofymow (1989)
Urea hydrolysis	n.r.	11.5	mg a.e./kg	Lethbridge et al. (1981)
Urea hydrolysis	1	5.0	mg a.e./kg	Martens and Bremner (1993)[b]
Glyphosate:				
Effects observed in soil				
Nitrogen fixation	7	12.7	mg a.e./kg	Carlisle and Trevors (1986)
Nitrification	3	76.7	mg a.e./kg	Carlisle and Trevors (1986)

Table 21. (Continued).

Soil process	Test duration (days)	NOAEC[a]	Units	Reference
Nitrification	21	10	mg a.e./kg	Tu (1994)[c]
Nitrification	14	<10	mg a.e./kg	Tu (1994)[c]
Denitrification	7,14	10	mg a.e./kg	Tu (1994)[c]
Ammonification	7	10	mg a.e./kg	Tu (1994)[c]
Degradation of cellulose, starch, protein	84	25	mg a.e./kg	ABC Inc. (1978d)
Degradation of leaf litter	84	25	mg a.e./kg	ABC Inc. (1978d)
Effects observed *in vitro*:				
Pseudomonas aerugionosa	n.r.	10[d]	mg a.e./dm^3	Vassilev (1982)
Salmonella typhurium murium	n.r.	10[d]	mg a.e./dm^3	Vassilev (1982)
Escherichia coli	n.r.	10[d]	mg a.e./dm^3	Vassilev (1982)
Nitrogen fixation, EC_{50} *Azotobacter chroococcum*	n.r.	399	mg a.e./L	Santos and Flores (1995)
Nitrogen fixation, EC_{50} *Aztoobacter vinelandii*	n.r.	300	mg a.e./L	Santos and Flores (1995)

[a]NOAEC unless specified. Effects may have been observed at higher levels, but they were not judged to be adverse (e.g., stimulatory effects). All concentrations are expressed as glyphosate acid equivalents (a.e.).
[b]Reference used in setting toxicity reference value.
[c]Only one concentration (10 mg a.e./kg) was tested in this study.
[d]Tests without aeration.

Table 22. Toxicity of Roundup® and glyphosate to terrestrial and soil invertebrates.

Species	Route	Test duration (days)	Endpoint[a]	Concentration (mg/kg soil or µg/bee)	Reference
Roundup®:					
Earthworm, *Eisenia foetida*	Diet	14	NML	5000	IBR (1991b)
Earthworm, *E. foetida*	Diet	14	NOEC	500	IBR (1991b)[b]
Honeybee, *Apis mellifera*	Diet	2	LD_{50}	>100	HRC (1972)
Honeybee, *A. mellifera*	Contact	2	LD_{50}	>100	HRC (1972)
Glyphosate:					
Earthworm, *E. foetida*	Diet	14	NML	3750[c]	IBR (1991a)
Earthworm, *E. foetida*	Diet	14	NOEC	118.7[c]	IBR (1991a)[b]
Honeybee, *A. mellifera*	Diet	2	LD_{50}	100	HRC (1972)
Honeybee, *A. mellifera*	Contact	2	LD_{50}	>100	HRC (1972)

[a]NML, no-mortality level. NOEC is for behavior.
[b]Reference used in setting toxicity reference value.
[c]Test material was glyphosate IPA salt; LC_{50} reported as mg glyphosate a.e./L.

Birds.

RU Acute TRV. RU and glyphosate are considered to be practically nontoxic to birds on the basis of results of acute and chronic tests (Table 23). In most cases, the greatest dose administered was insufficient to elicit significant deleterious effects. For mature male zebra finches, *Poephila guttata*, the 5-d LC_{50} was greater than 8064 mg RU/kg diet (Evans and Batty 1986). Bobwhite quail, *Colinus virginianus*, and mallard, *Anas platyrhynchos*, exposed to 5620 mg RU/kg in the diet for 5 d exhibited no effect on the growth or survival. When converted to a dietary intake of RU based on the food ingestion rate of the quail (0.093 kg diet/kg bw/d) (USEPA 1993b), the acute TRV was estimated to be 523 mg RU/kg bw/d.

Glyphosate Chronic TRV. Chronic effect levels of glyphosate were estimated based on long-term (20-wk) feeding studies. Chronic studies for both the mallard and bobwhite quail were available. The NOAEC value from each study was used as the chronic TRV (1000 mg a.e./kg of feed). As for the acute TRV, the chronic TRV was converted to 93 mg a.e./kg/d, based on the dietary intake of the smaller bobwhite quail (0.093 kg diet/kg bw/d).

Wild Mammals.

RU Acute TRV. Acute oral LD_{50}s for mammals range from 4860 to >5000 mg RU/kg/d for RU and from 2047 to 5700 mg a.e./kg/d for glyphosate (Table 24).

Table 23. Acute and chronic toxicity of Roundup®, glyphosate, and AMPA to birds.

Species	Route	Test duration	LC_{50}	NOEC	Units[a]	Reference
Roundup®:						
Bobwhite quail, *Colinus virginianus*	Diet	8 d	>5620	5620	mg RU/kg diet	Wildlife International (1990b)[b]
Mallard, *Anas platyrhynchos*	Diet	8 d	>5620	5620	mg RU/kg diet	Wildlife International (1990a)[b]
Zebra finch, *Peophila guttata*	Diet	5 d	>8064	8064	mg RU/kg diet	Evans and Batty (1986)
Glyphosate (tested as acid):						
Bobwhite quail, *Colinus virginianus*	Oral	Single dose	>3851	3851	mg a.e./kg bw	Wildlife International (1978c)
Bobwhite quail, *Colinus virginianus*	Diet	8 d	>4640	4640	mg a.e./kg diet	Hazleton Lab (1973a)
Mallard, *Anas platyrhynchos*	Diet	8 d	>4640	4640	mg a.e./kg diet	Hazleton Lab (1973b)
Mallard, *Anas platyrhynchos*	Diet	16 wk	—	1000[c]	mg a.e./kg diet	Wildlife International (1978a)[b]
Bobwhite quail, *Colinus virginianus*	Diet	17 wk	—	1000[c]	mg a.e./kg diet	Wildlife International (1978b)[b]
AMPA:						
Mallard, *Anas platyrhynchos*	Diet	8 d	>5620	5620	mg/kg diet	Wildlife International (1991c)
Bobwhite quail, *Colinus virginianus*	Diet	8 d	>5620	5620	mg/kg diet	Wildlife International (1991b)
Bobwhite quail, *Colinus virginianus*	Oral	Single dose	>2250	1350	mg/kg diet	Wildlife International (1991a)

[a]RU, Roundup; a.e., glyphosate acid equivalents.
[b]Reference used in setting toxicity reference value.
[c]NOEC for reproductive effects.

Table 24. Acute and chronic toxicity of Roundup®, glyphosate, and AMPA to terrestrial mammals.

Species	Route	Test duration	Endpoint	Level	Units[a]	Reference
Roundup®:						
Goat	Oral	Acute	LD_{50}	4,860	mg RU/kg/d	Rowe (1987a)[b]
			NML	2,100		
Mouse	Oral	n.r.	LD_{50}	>5,000	mg RU/kg/d	IET (1986)
			NML	2,500		
Rat	Oral	n.r.	LD_{50}	5,000	mg RU/kg/d	Blaszcak (1988a)
Hopping mouse						
Notomys mitchelli	Diet	4 d	NOAEL	>16,000	mg RU/kg diet	Evans and Batty (1986)
(*N. mitchelli*)	Diet	4 d	NOAEL	>16,000	mg RU/kg diet	Evans and Batty (1986)
Marsupial						
Sminthopsis macroura	Diet	4 d	NOAEL	>16,000	mg RU/kg diet	Evans and Batty (1986)
Glyphosate:						
Rabbit	Oral	21 d	NOAEL[c]	175	mg a.e./kg/d	Tasker (1980)
Rat	Oral	Acute	LD_{50}	2,047	mg a.e./kg/d	Knapek et al. (1986)
Rat	Oral	Acute	LD_{50}	>5,000	mg a.e./kg/d	(See footnote d.)
Goat	Oral	Acute	LD_{50}	3,500	mg a.e./kg/d	Rowe (1987c)
Goat	Oral	Acute	LD_{50}	5,700	mg a.e./kg/d	Rowe (1987b)[e]
Mouse	Diet	13 wk	NOAEL	507	mg a.e./kg/d	NTP (1992)
Mouse	Diet	13 wk	NOAEL	1,890	mg a.e./kg/d	Bio/Dynamics (1979)
Mouse	Diet	24 mon	NOAEL	814	mg a.e./kg/d	Bio/Dynamics (1983a)

Table 24. (Continued).

Species	Route	Test duration	Endpoint	Level	Units[a]	Reference
Rat	Diet	13 wk	NOAEL	>12,500	mg a.e./kg diet	Tauchi (1979)
Rat	Diet	13 wk	NOAEL	205	mg a.e./kg/d	NTP (1992)
Rat	Diet	13 wk	NOAEL	1,267	mg a.e./kg/d	Stout and Johnson (1987)
Rat	Diet	24 mon	NOAEL	410	mg a.e./kg/d	Stout and Ruecker (1990)[b]
Rat	Diet	3 generations	NOAEL	>30	mg a.e./kg/d	Bio/Dynamics (1981b)
Rat	Diet	2 generations	NOAEL	666	mg a.e./kg/d	Reyna (1990)
Rat	Diet	Chronic	NOAEL	>31	mg a.e./kg/d	Bio/Dynamics (1981a)
Dog (capsule dosing)	Oral	52 wk	NOAEL	>500	mg a.e./kg/d	Reyna and Rucker (1985)
AMPA:						
Rat	Dietary	90 d	NOEL	400	mg/kg/d	Estes (1979)
Rat	Dietary	90 d	LOEL	1,200	mg/kg/d	Estes (1979)
Rat (pregnant)	Dietary	21 d	NOEL	400	mg/kg/d	Holson (1991)
Dog	Dietary	90 d	NOEL	263	mg/kg/d	Tompkins (1991)
POEA:						
Rat	Diet	1 mon	NOEL	52	mg/kg/d	Ogrowsky (1989)
Rat	Diet	3 mon	NOEL	33	mg/kg/d	Stout (1990)[b]
Rat (pregnant)	Oral	21 d	NOEL	15	mg/kg/d	Holson (1990)

[a]RU, Roundup; a.e., glyphosate acid equivalents; NML, no-mortality level.
[b]Reference used in setting toxicity reference value.
[c]Based on maternal toxicity.
[d]FDRL (1988); Inveresk Research (1989a); NOTOX (1988); Blaszcak (1988b); Branch (1981).
[e]Test material was glyphosate IPA salt; LD_{50} reported as glyphosate a.e.

LD_{50} values greater than 2000 mg a.e./kg are considered to be practically non-toxic (USEPA 1985d). AMPA was also observed to cause little toxicity. The acute toxicity data for mammals was in the form of single oral doses rather than feeding studies. The acute exposure level was derived for mammals using the least empirical no-mortality level (NML). The acute TRV was estimated to be 2100 mg RU/kg, based on the NML for the goat (Rowe 1987a).

Glyphosate Chronic TRV. Because glyphosate is rapidly degraded in the environment, chronic exposures in the wild are unlikely. Despite little likelihood of prolonged exposure, laboratory studies have been conducted, and these studies employ sustained levels of glyphosate concentrations much greater than would be observed in the natural environment (see Table 24). A detailed discussion of these studies can be found in WHO (1994). A two-generation rat study produced a no-observed-effect level (NOEL) of 205 mg a.e./kg/d (NTP 1992). However, this NOEL was not based on survival, growth, or reproduction, but rather on slight histopathological changes that could not be directly related to these endpoints. Therefore, the chronic TRV was estimated to be 410 mg a.e./kg/d based on survival, growth, and reproduction endpoints in another 24-mon rat study (Stout and Ruecker 1990). This value was applied to both small and large animals.

POEA Chronic TRV. The chronic TRV for POEA to mammals was based on the NOEL for the longest duration toxicity study (see Table 24). The NOEL for a 3-mon dietary toxicity study in rats was 33 mg/kg/d (Stout 1990). An additional application factor of 2 was applied to this NOEC value because toxicity data were only available for one species (rat). Thus, the chronic TRV for POEA to mammals was estimated to be 16.5 mg/kg/d.

Nontarget Arthropods.

The acute toxicity values observed for RU and glyphosate to honeybees (*Apis mellifera*) are given in Table 22. Tests using honeybees exposed to 100 µg/bee of RU or glyphosate in the diet and applied topically were designed to mimic secondary exposure of bees through the consumption of pollen or direct exposure resulting from inadvertent overspray, respectively (HRC 1972). The dietary and contact LD_{50}s were >100 µg RU/bee. For glyphosate, the dietary and contact LD_{50}s were 100 and >100 µg a.e./bee, respectively. A general guideline has been suggested for assessing toxicity of pesticides to honeybees (Felton et al. 1986). A hazard ratio is derived from the formula (g active ingredient/ha)/(LD_{50} for bees in µg/bee). Depending on the ratio, the risk to bees is as follows:

Hazard ratio <50	Low risk
Hazard ratio 50–2,500	Moderate risk (further assessment needed)
Hazard ratio >2,500	High risk (further assessment or mitigation needed)

Based on these data, the hazard ratio would be 4300/100 (for a 4.3-kg a.e./ha application rate), or 43; this puts glyphosate in a low-risk category. No chronic assessment was conducted for honeybees because of the large safety margin in the acute assessment and the expected rapid decline in environmental exposure to this species.

Laboratory toxicity tests have been developed that can be used to evaluate the toxicity of pesticides to beneficial arthropods, except bees, and other nontarget arthropods (SETAC 1994). Initial laboratory screening tests used artificial exposure scenarios in which the pesticide was applied at the maximum use rate onto artificial substrates such as glass or synthetic soil and the organisms remained in contact with the pesticide film for several days. Depending on results of these screening tests, subsequent tests such as extended laboratory tests, semifield and field tests may be needed. These "higher-level" tests incorporate more realistic environmental exposure conditions.

The effects of RU have been investigated in the screening-level assay with 18 different beneficial predators and parasites (Hassan et al. 1988). RU was found to be harmless to 13 species, slightly harmful to 4 species and moderately harmful to 1 species (carabid beetle). The authors of the study did not believe that sufficient toxicity potential existed to warrant semifield and field tests that were performed for some of the other compounds tested in the same program. However, a subsequent semifield test with a similar glyphosate formulation was conducted with carabid beetles (Mead-Briggs 1990). Even when beetles were directly oversprayed at the maximum use rate, no mortality was observed. The reason for the discrepancy between the results of laboratory screening tests and semifield studies is not known, but is possibly related to the artificial nature of the laboratory glass plate assays, such as potential stickiness of the formulation on the glass substrate. As the tests were not designed to determine an EC_{50} or NOEC value, no TRV was estimated for beneficial arthropods.

Beneficial arthropod tests are currently being conducted on several glyphosate formulations used in the European Union. Ground beetle, ground spider, parasitic wasp, and predatory mite are being tested in screening assays. The formulations at the maximum use rate caused no effects on beetles or spiders. Some effects were observed on parasitic wasps and predatory mites at the maximum use rate in the screening tests. The effects were reduced or eliminated when realistic exposure conditions and substrates were included. Risk evaluation is further discussed in the Risk Characterization section.

Nontarget Terrestrial Plants.

RU is a broad-spectrum herbicide and will affect many types of nontarget plants if applied to the foliage. Therefore, a detailed risk assessment on the impact of foliar application of RU in the treated area was not conducted. However, effects on areas adjacent to the treated site must be considered. To assess risk to nontarget plants, a study of vegetative vigor was conducted with glyphosate in accordance with U.S. EPA guidelines (Chetram and Lucash 1994). Ten species of plants were treated post emergence with different levels of glyphosate mixed with Triton surfactant/emulsifier. The NOEC values, based on plant dry weight and plant height, ranged from 39 to 628 g a.e./ha. In another study, reported EC_{50} values based on shoot dry weight for 14 species of wild plants ranged from 0.7 to 93 µg a.e./plant (Breeze et al. 1992). The results were not reported as a use rate, nor was information provided that would allow calculation of a field application rate. Based on these two studies, the acute TRV for terrestrial plants was estimated to be 39 g a.e./ha. No chronic TRV was derived.

Another route by which RU could potentially affect plant growth is through soil residues and their effect on plant germination. Once in contact with the soil, the toxicity of RU to plants decreases rapidly to levels that are not phytotoxic. Glyphosate is strongly adsorbed to soil particles (Franz et al. 1997). This property significantly restricts the movement of the active ingredient into the plant. No effects of RU on seed germination in forests were reported at 976 mg a.e./kg dry weight soil (Morash and Freedman 1989). This value was used as the chronic TRV for plant germination in soil treated with RU. However, this is an overestimate of toxicity under most conditions. Normally, there is no toxicity of glyphosate adsorbed from soils by plants.

D. Summary of Toxicity Reference Values

TRVs for each potentially exposed group of organisms are summarized in Table 25. The acute TRV was typically based on RU and the chronic TRV on glyphosate and/or POEA. AMPA was evaluated for toxicity to algae, aquatic inverte-

Table 25. Summary of acute and chronic toxicity reference values (TRVs) for aquatic and terrestrial wildlife for Roundup® and glyphosate.

Taxa	Roundup®: acute toxicity reference value[a]	Glyphosate: chronic toxicity reference value[a]
Aquatic microorganisms	0.73 mg RU/L	0.28 mg a.e./L
Aquatic macrophytes	0.78 mg RU/L	0.08 mg a.e./L
Aquatic invertebrates	1.9 mg RU/L	0.50 mg a.e./L[b]
Fish	0.84 mg RU/L	0.74 mg a.e./L[b]
Amphibians	1.6 mg RU/L	Same as fish
Soil microorganisms[c]	16 mg RU/kg soil	5.0 mg a.e./kg
Soil invertebrates	250 mg RU/kg soil	59.4 mg a.e./kg
Birds	5620 mg RU/kg in diet	1000 mg/kg in diet
	523 mg RU/kg bw/d	93 mg a.e./kg bw/d
Mammals	2100 mg RU/kg bw/d	410 mg a.e./kg bw/d

[a]RU, Roundup; a.e., glyphosate acid equivalents. See Table 27 for POEA toxicity reference values.

[b]Values derived from chronic RU studies to add additional conservatism to the evaluation (see text).

[c]Chronic values for the formulation relevant to microorganisms.

brates, fish, birds, and mammals and was found to have very low toxicity. Because little toxicity of AMPA was observed in any studies, no TRVs were derived in this assessment.

V. Risk Characterization

Potential risk to nontarget organisms exposed to RU, glyphosate, and/or POEA was estimated by comparing the TRVs derived in the Toxicity Assessment section with the respective maximum level of exposure estimated in the Exposure Assessment section. AMPA was found to cause little toxicity to nontarget organisms and does not bioaccumulate in the environment. Therefore, this metabolite was assumed to pose only minimal risk to the environment and is not discussed in detail in this section.

A. Hazard Quotient Analyses

Risk was expressed in the form of a hazard quotient (HQ):

HQ = exposure level/toxicity reference value (TRV) (acute or chronic)

Interpretation of the HQ values is a key element of the risk characterization phase. The value of the HQ dictates whether there is a need for a further, more quantitative evaluation of the potential risk. As stated previously, the HQ methodology applied in a Tier I assessment is structured to be a conservative one-tailed test that can only be used to rebut the presumption of a risk of adverse effects. If the HQ is less than 1.0, it can be concluded with great certainty that there is essentially no probability of population- or community-level effects occurring in nontarget organism populations. The magnitude of the HQ is more a function of the assumptions of the assessment and cannot be used to determine the magnitude of risk or safety. If HQ values exceed 1.0, the potential for adverse effects is indicated but not demonstrated. Within the context of the risk assessment, a HQ > 1.0 indicates the need for further evaluation of the specific issues surrounding chemical exposure and toxic potency before making a final decision regarding the level of risk. Because the slope of the concentration response is generally unknown, the magnitude by which the HQ exceeds 1 is not a good indicator of the risk. However, if the HQ is greater than 100 or 1000, margins of safety inherent in the conservative assumptions used to derive the HQ are likely to be exceeded and a potential risk to the organism and the ecosystem is indicated. This level of exceedance of the HQ would generally trigger field-level trials and monitoring or exposure and effects at the population and community level.

Based on the results of this assessment, no acute or chronic HQs greater than 1 were observed for aquatic, soil, or terrestrial organisms for nonaquatic uses such as agriculture and forestry (Tables 26, 27). Therefore, no higher-tier analysis was indicated. Aquatic uses in shallow water bodies (≤ 0.15 m) can yield HQs greater than 1 (Table 28). However, examination of conservative exposure

Table 26. Summary of acute hazard characterization for terrestrial use of Roundup®.

Potentially exposed taxa	Exposure units	Toxicity reference value	Range of maximum predicted exposure concentrations: foliar interception			Hazard quotient (HQ) range: foliar interception		
			50%		None	50%		None
Aquatic organisms:								
Microorganisms	mg RU/L	0.73	0.271	—	0.406	0.37	—	0.56
Macrophytes	mg RU/L	0.78	0.271	—	0.406	0.35	—	0.52
Invertebrates	mg RU/L	1.9	0.271	—	0.406	0.14	—	0.21
Fish	mg RU/L	0.84	0.271	—	0.406	0.32	—	0.48
Amphibians	mg RU/L	1.6	0.271	—	0.406	0.17	—	0.25
Soil organisms:								
Soil Microorganisms	mg RU/kg	16	7.5	—	15.1	0.47	—	0.94
Soil Invertebrates	mg RU/kg	250	7.5	—	15.1	0.03	—	0.06
			Size of animal					
			Large		Small	Large		Small
Terrestrial organisms:[a]								
Birds, fruit/seed diet	mg RU/kg bw/d	523	16.0	—	313	0.03	—	0.60
Birds, invertebrate diet	mg RU/kg bw/d	523	16.0	—	313	0.03	—	0.60
Mammals, fruit/seed diet	mg RU/kg bw/d	2,100	24.7	—	113	0.01	—	0.05
Mammals, invertebrate diet	mg RU/kg bw/d	2,100	24.7	—	113	0.01	—	0.05
Mammals, foliage diet	mg RU/kg bw/d	2,100	494	—	1,336	0.24	—	0.64

[a]Hazard characterization for honeybees and beneficial arthropods was based on use rates rather than concentrations in media. See text for hazard characterization discussion.

assumptions reduces the risk to manageable levels within the context of the desired weed management objectives.

B. Terrestrial Uses

Aquatic Organisms.

Laboratory-Based Risk Characterization. The range of maximum water concentrations following application of RU for agricultural or other terrestrial uses was estimated to be 0.271–0.406 mg RU/L. The most sensitive species were aquatic microorganisms and aquatic macrophytes, with maximum HQ values ranging from 0.35 to 0.56 (Table 26). These values are considerably less than 1.0, indicating that RU poses minimal risk to aquatic organisms following terrestrial use. Chronic exposure to glyphosate or POEA following terrestrial use of RU yields HQ values less than 1.0, indicating minimal chronic risk to aquatic organisms.

Discussion and Field Observations. As described earlier, GENEEC estimated peak concentrations in surface water following a single terrestrial application of 4.2 kg RU/ha ranged from 0.024 to 0.09 mg RU/L. The model used in this assessment, which is extremely conservative, resulted in a maximum predicted exposure concentration for RU of 0.403 mg RU/L (0.13 mg a.e./L) for a static water body receiving drift and runoff from an adjacent field. All these exposure levels are considerably greater than have been observed in surface waters in agricultural areas (Frank et al. 1990). The maximum observed value associated with offsite movement of RU was 0.042 mg a.e./L (0.135 mg RU/L). Therefore, it can be concluded that a likely maximum exposure concentration for RU in surface water is at least threefold less than the concentration estimated by the simulation models used in this risk assessment.

There are a number of reasons why the observed concentrations of glyphosate are less than those predicted by the conservative model used to derive the estimated environmental concentration (EEC). To afford extra margins of safety, the exposure level for aquatic organisms is intentionally overestimated by the modeling approach that was employed in the risk assessment. The model assumed that spray drift and surface runoff would both occur simultaneously and immediately following application. In reality, it is unlikely that surface runoff would occur at the same time as the spray drift because the former is dependent on a storm event to wash residues into nearby watercourses. The risk assessment applied additional conservatism by using as assumption that both glyphosate and POEA move through the environment as a mixture at the same rate. Sorption rates of POEA by foliage indicate that the surfactant adsorbs to plant material more strongly than glyphosate (Sherrick et al. 1986). This differential binding suggests that the proportion of surfactant present would likely be less than glyphosate at offsite locations. It can, therefore, be concluded that the exposure of aquatic organisms to the more toxic component of the RU formulation, the

Table 27. Summary of chronic hazard characterization for terrestrial use of Roundup®.

Potentially exposed taxa	Exposure units	Toxicity reference value	Range of maximum predicted exposure concentrations:[a] foliar interception			Hazard quotient (HQ) range: foliar interception		
			50%		None	50%		None
Aquatic organisms:								
Glyphosate								
Microorganisms	mg a.e./L	0.28	0.0039	—	0.0114	0.01	—	0.04
Macrophytes	mg a.e./L	0.08	0.0039	—	0.0114	0.05	—	0.14
Invertebrates	mg a.e./L	0.50	0.0039	—	0.0114	0.01	—	0.02
Fish	mg a.e./L	0.74	0.0039	—	0.0114	<0.01	—	0.02
Amphibians	mg a.e./L	0.74	0.0039	—	0.0114	<0.01	—	0.02
POEA								
Invertebrates	mg POEA/L	0.1	0.0017	—	0.005	0.02	—	0.05
Fish	mg POEA/L	0.03	0.0017	—	0.005	0.06	—	0.17

Table 27. (Continued).

Potentially exposed taxa	Exposure units	Toxicity reference value	Range of maximum predicted exposure concentrations:[a] foliar interception			Hazard quotient (HQ) range: foliar interception		
			50%		None	50%		None
Soil organisms:								
Glyphosate								
Soil microorganisms	mg a.e./kg	5	0.47	—	2.6	0.09	—	0.52
Soil invertebrates	mg a.e./kg	59.4	0.47	—	2.6	0.01	—	0.04
Terrestrial organisms:[b]								
Glyphosate								
Birds, fruit/seed diet	mg a.e./kg bw/d	93	0.41	—	8.1	<0.01	—	0.09
Birds, invertebrate diet	mg a.e./kg bw/d	93	0.41	—	8.1	<0.01	—	0.09
Mammals, fruit/seed diet	mg a.e./kg bw/d	410	0.64	—	3.0	<0.01	—	0.01
Mammals, invertebrate diet	mg a.e./kg bw/d	410	0.64	—	3.0	<0.01	—	0.01
Mammals, foliage diet	mg a.e./kg bw/d	410	3.2	—	8.6	0.01	—	0.02
POEA								
Mammals, fruit/seed diet	mg POEA/kg bw/d	16.5	0.31	—	1.4	0.02	—	0.09
Mammals, invertebrate diet	mg POEA/kg bw/d	16.5	0.31	—	1.4	0.02	—	0.09
Mammals, foliage diet	mg POEA/kg bw/d	16.5	1.5	—	4.2	0.09	—	0.25

[a]Using conservative DT_{50} values in dissipation prediction.
[b]Hazard characterization for honeybees and beneficial arthropods was based on use rates rather than concentrations in media. See text for hazard characterization discussion.

Table 28. Summary of acute and chronic hazard characterization for aquatic use of Roundup®.

Potentially exposed taxa	Exposure units	Toxicity reference value	Range of maximum exposure concentrations[a]: water depth			Hazard quotient (HQ) range: water depth		
			2 m		0.15 m	2 m		0.15 m
Acute Scenario:								
Roundup®								
Microorganisms	mg RU/L	0.73	0.339	—	4.52	0.46	—	6.19
Macrophytes	mg RU/L	0.78	0.339	—	4.52	0.43	—	5.79
Invertebrates	mg RU/L	1.9	0.339	—	4.52	0.18	—	2.38
Fish	mg RU/L	0.84	0.339	—	4.52	0.40	—	5.38
Amphibians	mg RU/L	1.6	0.339	—	4.52	0.21	—	2.82
Chronic Scenario:								
Glyphosate								
Microorganisms	mg a.e./L	0.28	0.001	—	0.025	<0.01	—	0.09
Macrophytes	mg a.e./L	0.08	0.001	—	0.025	0.08	—	0.31
Invertebrates	mg a.e./L	0.50	0.001	—	0.025	<0.01	—	0.05
Fish	mg a.e./L	0.74	0.001	—	0.025	<0.01	—	0.03
Amphibians	mg a.e./L	0.74	0.001	—	0.025	<0.01	—	0.03
POEA								
Invertebrates	mg POEA/L	0.1	0.0005	—	0.012	<0.01	—	0.12
Fish	mg POEA/L	0.03	0.0005	—	0.012	0.02	—	0.40

[a]Assumes 50% interception by target vegetation.

surfactant, would be reduced in any offsite exposure scenario. Another highly conservative assumption used was that all the RU applications are at the maximum use rate, and by aerial application. In actual experience, aerial applications make up a small proportion of total RU applications; in addition, aerial applications in agricultural crops have a maximum application rate of 0.84 kg a.e./ha, which is one-fifth the maximum rate for hand- or ground-based spraying of 4.2 kg a.e./ha.

The acute risk characterization compared the peak concentration in water to a toxicity value that was derived over an extended duration, such as 2 or 4 d. It has been observed that concentrations of glyphosate and associated surfactant in the water column dissipate rapidly as the result of degradation and sorption to particulates as well as dilution and advection. These properties affect the general bioavailability of the product, suggesting the actual dose may be considerably less. For example, in a forestry application in Chassell, MI, the initial concentration of glyphosate measured in a pond was 1.7 mg a.e./L. One day later this concentration had decreased to 0.307 mg a.e./L, and by day 3 the concentration of glyphosate was 0.172 mg a.e./L, about 1/10th of the initial concentration (Horner 1990).

Hazard quotients based on concentrations of glyphosate in sediment were not used to characterize potential hazards to aquatic organisms. In accordance with recommendations of Maund et al. (1997), potential risk to sediment organisms is indicated if all the following criteria are met:

1. *Daphnia magna* 96-hr EC_{50} is less than 1 mg/L or 21-d NOEC is less than 0.1 mg/L
2. Soil partition coefficient (K_{oc}) is greater than 1000
3. 50% dissipation time (DT_{50}) in water is greater than 30 d

If any of the criteria are not met, then the product is likely to pose minimal risk to sediment-dwelling organisms. RU, glyphosate, and POEA do not meet the first or third criterion. The second criterion would be met for glyphosate and POEA. Collectively, only one of the criteria is met, and thus additional data are not necessary to assess hazard to sediment organisms. As with the fate of RU in surface water, RU in the sediment is also subject to rapid biodegradation. This suggests initial concentrations would decline quickly and, assuming that the initial maximum concentration persisted for 4 d, would represent an overestimate of approximately 10 fold. Based on these observations, minimal risk from the application of RU would be expected for sediment-dwelling organisms.

Soil Organisms.

Laboratory-Based Risk Characterization. Maximum concentrations of RU in soil immediately after application can range from 7.5 to 15.2 mg RU/kg soil. Soil microorganisms varied considerably in their sensitivity to RU. Based on the most sensitive soil microbial process, the HQ range is 0.47 to 0.94 (see Table 26). The HQ range for soil invertebrates is 0.03 to 0.06. Based on the conservative assumptions in this analysis, minimal acute hazard is predicted for populations of soil organisms. HQ values for soil organisms under chronic exposure conditions are less than 0.52 (see Table 27).

The assumptions used in the derivation of the exposure level for soil organisms are extremely conservative. The potential soil concentrations used to define the exposure to terrestrial microorganisms were based on a worst-case scenario, defined as RU applied to bare ground at the maximum agricultural use rate. This scenario assumed that all the herbicide entered the soil with none being intercepted by vegetation. Because RU is only effective when applied to foliage, it is unlikely that large quantities of the herbicide would ever be used on areas devoid of vegetation. The measured concentration of glyphosate and RU in soil immediately following the application of RU can show considerable variability (see Table 5). The maximum value of 4.7 mg a.e./kg soil (15.2 mg RU/kg soil) was used in the exposure model to derive the final soil concentrations, but other studies support the use of values as small as 0.07 mg a.e./kg soil (0.2 mg RU/kg soil). A more typical soil concentration resulting from agricultural applications would be more than twofold less than those used to calculate the hazard quotient, and at lesser concentrations, no significant impacts are expected on soil microorganisms or soil processes.

Discussion and Field Observations: Soil Microorganisms. A number of studies have not detected effects of glyphosate on soil organisms under field use conditions. When soil was treated at concentrations between 19.8 and 29.3 mg a.e./kg, as well as at levels up to 200 times greater, it was concluded that the use of glyphosate at label use rates in agriculture and forestry should have no adverse effects on nitrification in soil (Stratton 1990). Similarly, an evaluation of nitrogen fixation, denitrification, and nitrification in soil, with and without glucose added as an energy source for microorganisms, indicated that there was no effect on nitrogen fixation under aerobic conditions, even at concentrations of formulation up to 635 mg a.e./kg (Carlisle and Trevors 1986). Denitrification, based on N_2O consumption, was slightly affected at 12.7 mg a.e./kg, but not at greater concentrations. N_2O production was strongly stimulated at all but the least concentration of glyphosate (12.7 mg a.e./kg) in nonamended soils. In soil amended with glucose at concentrations up to 635 mg a.e./kg, RU had no effect on nitrite reduction. Nitrate and nitrite formation were affected at the greatest concentrations, but not at 76.7 mg a.e./kg. It was concluded that application at recommended rates of RU would cause no effects on soil nitrogen cycling (Carlisle and Trevors 1986).

Similarly, after a comprehensive evaluation of the available information, it was concluded that effects of glyphosate and RU on microorganisms have been minor and reversible in most cases. Furthermore, several authors have noted that direct toxic effects of field-applied glyphosate could not be separated from changes in habitat (WHO 1994; Müller et al. 1981; Gomez and Sagardoy 1985; Preston and Trofymow 1989; Mekwatanakarn and Sivasithamparam 1987a; Santillo et al. 1989b; Chakravarty and Chatarpaul 1990a,b; Stratton and Stewart 1992). Additional field studies not cited in WHO (1994) also confirmed that application of glyphosate and RU at label-recommended rates does not have long-term negative effects on mycorrhizae, actinomycetes, or bacteria (Heinonen-Tanski et al. 1985; Mekwatanakarn and Sivasithamparam 1987b; Chaterpaul et al. 1989), nitrification (Martens and Bremner 1993; Todt 1991), dehydrogenase activity (Todt 1991), or decomposition (Fletcher and Freedman 1986).

Other researchers have reported minor effects at or near the normal use rates. In a study in which sandy soil was treated with RU at 1.5 L/ha (0.54 kg a.e./ha), decreases in fungal and bacterial populations were observed after 2 mon, with a return to normal densities after 6 mon (Chakravarty and Chatarpaul 1990a). The overall metabolic activity did not change at any time. Effects on rhizobial bacteria have been observed at 2 mg a.e./kg soil (Eberbach and Douglas 1983, 1989), which approaches levels that may be found after application. However, it could not be established whether the effect was on the bacteria directly or on the plants they depend on for energy. Glyphosate has been evaluated for toxicity to ectomycorrhizial fungi in agar culture, and one of the three species showed nearly 50% inhibition of growth at a concentration of 1 mg a.e./L (Estok et al. 1989). The other two species were not affected at 10 mg a.e./L. In another study, no effects on the *in vitro* growth of five species of ectomycorrhizal fungi were observed in agar medium at concentrations up to 3.59 mg a.e./L (Chakravarty and Chatarpaul 1990a). At higher concentrations, some effects on growth were observed. However, in both studies, the authors qualified the findings with a caution that agar medium represents a very different condition from the forest floor or soil environment.

Microbial systems are complex; thus, considerable variation can be expected among tests and among soil types. The weight of evidence for effects of RU on soil microorganisms indicates that adverse effects would be unlikely as a result of application at normal field rates. Any minor effects to communities would be expected to disappear rapidly (WHO 1994). This conclusion is further substantiated by studies that have monitored microbial communities and processes over 12–17 yr of annual use. Two field studies have evaluated the effect of glyphosate, presumably applied as the RU formulation as this was an efficacy trial. In the first study, glyphosate was applied to a heavy clay soil as a single application at a rate of 1.12 kg a.e./ha for 5 yr, then at a rate of 0.70 kg/ha for 13 yr (Biederbeck et al. 1997). Populations of microorganisms were assessed at three time points: once before the yearly herbicide application, once 3 wk after the final application, and again about 2 mon later. Numbers of bacteria, actinomycetes, fungi, nitrifiers, and denitrifiers were not deleteriously affected by application of the herbicide. Similarly, microbial biomass and carbon or nitrogen mineralization were generally unaffected by the herbicide application. In the second study, glyphosate (presumably in the form of RU as this was an efficacy trial) was applied at a rate of 1.5 kg a.e. /ha to the same plots of silty clay loam soil for 12 yr (Hart and Brookes 1996). Soils were sampled 4 wk after herbicide application and again after 6 mon. No long-term effect on soil biomass or on carbon or nitrogen mineralization (conversion of soil organic nitrogen to ammonia or nitrate) was observed as a result of herbicide application.

Discussion and Field Observations: Soil Invertebrates. Earthworms are predicted to be at minimal risk from the use of RU or glyphosate. The effects of glyphosate on earthworms within an agricultural setting have been reviewed (Edwards and Bohlen 1996). Glyphosate was ranked as 0 on a scale from 0 (relatively nontoxic) to 4 (extremely toxic). Glyphosate applied at label-recommended rates did not affect growth or survival of earthworms (Dalby et al. 1995). Further evidence for the lack of earthworm effects from RU was observed in conservation tillage research for erosion control (Lamarca 1996). No-till farming is a type of conservation tillage in which fields are not plowed before seeding. This practice leaves the stubble of the previous crop to prevent soil from eroding into streams or being blown away by wind. Before planting, an application of herbicide is used to kill weeds. Then, seed drills are used to plant through the dead weeds and old crop stubble. RU is the herbicide most frequently used to prepare no-till fields for seeding. In a tillage effects study, no impact on earthworm populations was observed following application of RU to pastureland before no-till planting of barley (Guo et al. 1999).

A recent study has been completed investigating chronic effects of glyphosate and AMPA on earthworms. No effects on growth, survival or reproduction were observed after 56 d of exposure to concentrations up to 21.31 mg a.e./kg soil and 28.12 mg AMPA/kg soil (Hayward and Mallett 2000).

Terrestrial Organisms.

Laboratory-Based Risk Characterization. Birds and mammals are exposed to RU or glyphosate mainly through their diet. This assessment assumed that animals would enter treated areas and consume only vegetation containing maximum concentrations of RU. Even considering small animals with large food ingestion rates relative to body weight, all acute HQs were less than 1.0 (see Table 26). The actual HQs will be considerably less than the values presented as many of the TRVs were set based on maximum threshold doses from toxicity tests where no effects were observed. Chronic HQs for both glyphosate and POEA were also less than 1.0, also indicating minimal risk.

Beneficial arthropods serve as pollinators or as biological control agents for pest species and are thus important components of terrestrial ecosystems. A general guideline has been suggested for assessing toxicity of pesticides to honeybees (Felton et al. 1986). A hazard ratio is derived from the formula [(g active ingredient/ha)/(LD_{50} for bees in μg/bee)]. The hazard ratio for honeybees is <50, indicating minimal risk. Most species of beneficial arthropods are not affected by exposure to RU. However, on-field effects in certain species cannot be excluded.

To adequately characterize risk for nontarget arthropods, separation of the risk analysis into on-field and off-field is appropriate (SETAC 1994). On-field risk assessments are focused on maintaining biological control capabilities in treated fields. The assessment of on-field risks of herbicides is complicated by ecological effects that are associated with changes in vegetation (see "Discussion and Field Observations: Nontarget Arthropods"). As vegetation dies, the habitat changes and the abundance of invertebrates change. Consequently, rather large changes in beneficial arthropod abundance may be expected to occur on-field. Off-field risk assessment is based on the amount of downwind drift of the RU after application. At a maximum use rate of 4.2 kg a.e./ha and 4% drift at 1 m (from ground application), a conservative exposure value for nontarget arthropods off-site is 0.17 kg a.e./ha (Ganzelmeier et al. 1995). Data available for RU and other formulations indicate minimal risk to arthropods in offsite areas.

Nontarget terrestrial plants in the treated area are at acute risk from the application of RU. The degree of risk off-field depends on the movement of RU off the treated field and the sensitivity of the different species and different life-styles present. More detail on effects of spray drift on nontarget plants is discussed in the following field observation section.

Discussion and Field Observations: Birds. Several comprehensive field studies have observed birds in forest plots treated with RU (Eggestad et al. 1988; Santillo et al. 1989b; Freedman 1991; MacKinnon and Freedman 1993; Woodcock et al. 1997; Lautenschlager et al. 1998). Decreases in bird populations have been observed following treatment of their habitat with RU. However, this has been attributed to habitat change associated with removal of vegetation and subsequent suppression of vegetation growth by the herbicide (MacKinnon and Freedman 1993). The forest plots were recolonized by different bird species that favored conifer-dominated stands. For instance, the female adult black grouse (*Tetrao tetrix*) favored the vegetation that occurred 4–6 yr after glyphosate treatments but the cocks did not (Eggestad et al. 1988). The abundance of certain species in areas treated with RU is directly a function of the vegetation present (Lautenschlager et al. 1998). In general, seed-eating species favor glyphosate-treated areas because early successional species of plants (high seed producers) are more abundant in these areas (Lautenschlager et al. 1998). The habitat changes associated with the application of RU can affect bird populations causing both increases and decreases of individual populations. In no case was there evidence of direct toxicity of RU or glyphosate to birds.

Discussion and Field Observations: Wild Mammals. Numerous field studies have been conducted to investigate the effects of glyphosate treatment on mammals (Sullivan and Sullivan 1981; Santillo et al. 1989a; Hjeljord et al. 1988; Sullivan 1990; Hjeljord 1994; Cumming et al. 1996; Cole et al. 1998). Mammals generally do not take up residence on treated plantations during the first couple of years after spray because of limited habitat. Slowly, these areas are repopulated as the vegetation develops. Reduction of the mountain hare (*Lepus timidus*) was observed the first year after spraying a forest plantation with glyphosate, but populations recovered after the second year (Hjeljord et al. 1988). Similar observations that habitat changes are the cause of population changes in moose (*Alces alces*) in areas treated with glyphosate have been reported (Hjeljord 1994). It has been concluded that there is minimal risk to small mammals from the application of glyphosate products and that the effects observed in the field studies are a result of changes in habitat (Freedman 1991).

Discussion and Field Observations: Nontarget Arthropods. A field study was conducted to investigate the effects of aerial application on honeybee hives (Burgett and Fisher 1990). Beehives and blooming vegetation in the immediate vicinity (1.5 A) were oversprayed at a rate of about 5% RU in 60 gal of water (6 lb a.e./A or 6.8 kg a.e./ha). No acute or chronic effects were observed for adult honeybees or for brood production. These findings were further supported by the results from direct feeding trials in the field. No effects to bees were observed as a result of direct RU exposure in sucrose syrup from a hive feeder.

Many field studies have investigated the effects of glyphosate formulations on beneficial arthropods other than honeybees. No substance- or dose-related effects on mites or springtails were observed in a sandy soil in an Argentine semiarid region up to 96 d after application of RU herbicide at rates up to 2.8 kg a.e./ha (Gomez and Sagardoy 1985). No consistently measurable changes in number of nematodes or springtails located in the upper 3 cm of ferrohumic podsols were observed after treatment with RU (Preston and Trofymow 1989). Arthropod populations have been examined in soils covered with alder trees (*Alnus rubra*) in British Columbia (Canada). The only effect observed was a measurable reduction in the number of both oribatid and nonoribatid mites on one of the treated sites, approximately 20 d after application. No difference in the number of mites on this site was observed at the end of the study. In this experiment of approximately 180 d, the herbicide formulation was hand sprayed at a rate of 2 kg a.e./ha.

Reduced populations of herbivorous insects and ground invertebrates were observed in a 4- to 5-yr-old clearcut planted with spruce seedlings (*Picea sp.*) up to 3 yr after treatment with RU herbicide (Santillo et al. 1989a,b). During this 3-yr study, the vegetation did not recover completely, which suggests that the majority of effects on invertebrates were mainly caused by habitat change. On the other hand, no changes in populations of predatory insects were observed in a clearcut located in Maine (U.S.A.) that was sprayed with 1.7 kg glyphosate a.e./ha (Whitehouse and Brown 1993). The authors of the study concluded that insect communities would not be affected by the use of glyphosate applied to the base of trees in farm forestry.

The effects of glyphosate on beneficial insects has also been investigated by studying indigenous carabid beetle populations after field application (Brust 1990). Both relative densities and movement of carabid beetles in and out of treated areas were monitored. Direct toxicity was not observed in the field, and there was no repellency observed. Shifts in population densities were observed in the weeks following treatment, but these changes were more closely associated with changes in the vegetation of the plots rather than direct toxicity following the glyphosate treatment. The absence of direct toxic affects was confirmed in the laboratory. Other researchers concluded that the primary factors influencing the changes in carabid beetle and spider populations were deprivation of a particular species of suitable food and severe changes in habitat (Asteraki et al. 1992).

Several studies have found that the application of glyphosate can increase populations of beneficial insects. In laboratory experiments to simulate treatment of cotton fields, numbers of the western bigeyed bug, *Geocoris pallens*, increased (Yokoyama and Pritchard 1984). However, the authors of this study did not measure behavioral effects and cautioned that responses might differ under field conditions. No effects on the diversity of common butterfly species were observed when glyphosate was used to control trees, shrubs, and blackberry in wire zones, but numbers of individuals did increase (Bramble et al. 1997).

In summary, the literature supports the conclusion that nontarget arthropods are at minimal risk from glyphosate and its formulations in offsite areas. Within treated areas, applications of the herbicide can produce changes in species diversity and in population size and structure for beneficial insects through modifications of available food sources and habitat.

Discussion and Field Observations: Nontarget Plants. Downwind drift levels from ground application equipment have been well characterized by Ganzelmeier et al. (1995). Predicted rates of drift are 4.0% at 1 m, 0.9% at 4 m, 0.6% at 5 m, and 0.3% at 10 m for boom sprayers. Based on these rates, a buffer distance of 4 m for a field treated with a 4.2 kg a.e./ha would yield a deposition rate of 0.037 kg/ha. This level is almost identical to the TRV for nontarget plants of 39 g a.e./ha (0.039 kg/ha). These predictions are consistent with field observations, in which a buffer distance of 8 m was found to be protective of all species tested in a field mesocosm study in fields treated with RU, mecoprop, or MCPA (Marrs and Frost, 1997). Moreover, the data indicated that few effects were observed within the buffer zone.

RU had no effect on seed germination in forests when applied at a rate of 976 mg a.e./kg dw soil (Morash and Freedman 1989). The absence of effect observed at this concentration probably reflects a lack of bioavailability rather than resistance of the germinating seedling to glyphosate. The fact that RU is biologically unavailable allows the use of RU in preparing wildlife food plots. Plant-back of desired plant species can occur as soon as is practical. There are several other studies that provide quantitative information regarding the bioavailability of glyphosate in soil. One study indicated that plants grown in soil containing glyphosate did not take up the herbicide, as evidenced by the absence of residues in plants (Ragab et al. 1985). Another study reported that soil residues of glyphosate are not easily extracted by water, reflecting the tight association with soil (Soulas 1992). These data indicate that RU or glyphosate poses minimal risk to the germination of nontarget plants when applied in accordance with the label.

C. Aquatic Uses

Glyphosate-based herbicides are used throughout the world for control of various emergent and floating weeds. In some countries, RU is used for aquatic weed control, whereas in the U.S. the product used is Rodeo®, which is a formulation containing only water and the IPA salt of glyphosate (54%). The U.S. Rodeo® label specifies that a surfactant be added to Rodeo® for a final tank mix up to 0.05%.

RU or Rodeo® is used to control unwanted vegetation. The plants to be controlled are often alien species that have disturbed the natural plant and animal communities. As discussed earlier, the ecological risk assessment framework (USEPA 1998) requires that the assessment endpoints be defined for each risk assessment. For aquatic weed control, the assessment endpoint would be different than the general assessment endpoint defined earlier. The assessment endpoint for aquatic weed control would need to be case specific and focus on the key elements of the rehabilitation process. In some cases, short-term declines in populations may be anticipated because of changes in habitat. The assessment endpoints need to reflect the long-term goal of the rehabilitation process.

It is inevitable that some short-term population level effects on plants and associated animals should occur in the pursuit of a long-term goal characteristic of restoration/rehabilitation projects. For example, Willapa Bay, in the state of Washington, is a large estuary that supports a commercial oyster fishery (Simenstad et al. 1996). In addition, the mudflats are an important staging ground for waterfowl on the Pacific Flyway. *Spartina* sp. (cordgrass) from the eastern coast of the U.S. was introduced into this estuary and has spread across the mudflats. Spreading of *Spartina* sp. has decreased the amount of habitat available for invertebrate populations that are necessary sustenance for migrating waterfowl and to sustain an energy-rich fishery. Control of *Spartina* sp. is difficult, and any vegetation control method will have significant effects on the ecosystem. In this case, meeting the stated goal for the rehabilitation project is paramount, and assessment endpoints should be changed to reflect this goal.

Applications of RU to control emergent aquatic macrophytes can produce greater localized concentrations of RU or glyphosate in water than those from runoff from terrestrial uses. Direct addition of RU to a 2-m-deep water body at the maximum use rate of 4.2 kg a.e./ha yields an instantaneous exposure concentration of 0.68 mg RU/L (0.21 mg a.e./ha). At this level, no TRVs would be exceeded indicating minimal risk. If added to a 1-m-deep water body, the concentration would be 1.4 mg RU/L (0.42 mg a.e./L). The TRVs for aquatic invertebrates and fish were set at 1.9 mg RU/L and 0.84 mg RU/L, and therefore an exceedence of effect values may occur. In shallower waters, the concentration would become greater, as would the hazard quotient. These estimated concentrations for aquatic uses assume no interception by plant material and no dissipation of POEA or glyphosate. Under actual field conditions, these factors and others would significantly mitigate exposure to varying degrees.

Glyphosate has been used extensively to control aquatic weeds and restore ecosystems affected by introductions of exotic weeds. During this period of use, there have been no documented cases of adverse effects on fish or aquatic invertebrates associated with glyphosate use for this purpose. Several field studies have investigated effects of aquatic weed control applications on aquatic animals (Solberg and Higgins 1993; Findlay and Jones 1996; Simenstad et al. 1996; Linz et al. 1997). No measurable increases in effects on density, abundance, or survival of aquatic invertebrates have been reported from the direct effects of glyphosate in field studies (Haag 1986; Henry et al. 1991; Gardner and Grue 1996; Simenstad et al. 1996, Linz et al 1999). Fewer aquatic invertebrates were observed in Rodeo-treated wetlands, but more aquatic invertebrates were observed in the cattail regrowth areas of Rodeo-treated wetlands relative to controls (Solberg and Higgins 1993). Those authors suggested that the initial decrease in habitat was most likely responsible for the changes observed. Birds that require wetlands for breeding purposes include numerous species of ducks, black terns (*Chlidonias niger*), and American coots (*Fulica americana*). These birds favor the open water produced by glyphosate treatment (Linz et al. 1994, 1996, 1997).

VI. Uncertainty Analysis

The data available on RU, glyphosate, AMPA, and the surfactant (POEA) provide a substantial database for the characterization of risk of these components as well as commercial formulations and degradation products to ecological systems. However, because of the complex nature of ecosystems and the infinite variety of circumstances, the database can never be complete. Therefore, an assessment of particular uncertainties where significant improvement in the quality and quantity of information will lead to more accurate hazard assessments should be considered.

The amount of environmental fate and effects data available for the POEA surfactant is limited. Soil and aquatic degradation rates were similar to those of glyphosate, but details of how POEA binds to soil and other environmental particulates is less well understood. Although toxicity information is available for POEA in fish and aquatic invertebrates, toxicity data for birds, terrestrial arthropods, and aquatic plants are limited. The lack of data for aquatic plants may be important in aquatic restoration programs where the safety margins can become small in shallow water.

In some cases, the assessment of risk was confounded by the effects of RU on the habitat of organisms in the treated areas. For example, one researcher measured the migration of beetles out of a treated field (Brust 1990). The migration was not related to avoidance of RU or other toxicological effects; rather, the preferred habitat of the beetles was destroyed. A better understanding of the ecological effects of vegetation management programs will provide a better basis on which to judge effects of herbicides. In addition, many of the tests used to assess toxicity to nontarget arthropods use highly artificial exposure conditions, such as a glass plate, which may not relate well to natural exposure conditions. More research is needed in lab-to-field extrapolation in these studies.

Spray drift is a potential source of exposure for nontarget plants downwind from the treated site. The amount of drift has been extensively studied and is largely a function of environmental conditions, use rate, and application equipment. Regarding effects of drift on nontarget plants, Marrs and Frost (1997) noted that field responses were not easily predicted based on extrapolation from laboratory data. Using the most sensitive species from laboratory evaluations can lead to prediction of more severe effects than are actually observed in the field. Some factors that contribute to mitigating effects in the field may include variation in life-stage sensitivities and interception by vegetation. More research is needed to better understand effects of spray drift on nontarget vegetation under field conditions.

Summary

Roundup® Herbicide (RU) and its active ingredient glyphosate have been extensively investigated in ecological toxicity studies to support registrations in various countries and also in many scientific investigations independent of regulatory considerations. The purpose of this study was to review the current state of knowledge on the ecological toxicity of Roundup and glyphosate and to consider this information in a comprehensive ecological risk assessment. A conservative hazard quotient method was used to evaluate risk. The hazard quotient (HQ) was calculated by dividing the maximum environmental exposure concentration derived from modeling or environmental monitoring data by the greatest level of Roundup or glyphosate found to have no effect on survival, growth, or reproduction of the most sensitive nontarget organisms. Roundup, which contains glyphosate and a polyethoxylated tallowamine surfactant (POEA), was used for acute HQ determination, because exposure to the total formulation was a reasonable assumption. Because of differential rates of dissipation, the individual components of the formulation (either glyphosate or the surfactant) were considered separately for chronic HQs. The risk assessment evaluated both terrestrial and aquatic uses of Roundup. Conclusions of the analysis are as follows.

1. Roundup® Herbicide contains the isopropylamine salt of glyphosate as the active ingredient and a surfactant (typically polyethoxylated tallowamine) to facilitate plant uptake of the active ingredient. Roundup® is a broad-spectrum herbicide and will control many types of herbacious and woody plants. Several formulations of Roundup® are used worldwide and may have different amounts of the components.
2. Glyphosate in the environment tends to bind tightly to soil and particulate matter and is essentially unavailable to plants or other soil organisms. As a result, glyphosate has little activity in soil, and the herbicidal effects are lim-

ited to foliar contact. In addition, glyphosate is very water soluble and does not partition into animal fats. Consequently, glyphosate does not bioconcentrate in fish or other animals. Less is known about the environmental fates of surfactants, but rates of degradation appear to be similar to glyphosate.

3. The risk assessment described here considered terrestrial and aquatic uses of Roundup®. Acute risk characterization assumed that organisms were potentially exposed to the intact formulation, whereas chronic risk characterization considered potential exposure to the components of Roundup® (glyphosate and the surfactant). This approach allowed the acute assessment to consider acute effects of the surfactant that are greater than glyphosate alone for aquatic animals.
4. For terrestrial uses of Roundup® (agricultural, forestry, rights-of-way, residential, etc.), minimal acute and chronic risk was predicted for potentially exposed nontarget organisms. This conclusion is based on the conservative hazard quotient analysis that resulted in no HQ values greater than 1. The following taxa were evaluated using the hazard quotient method: aquatic microorganisms, aquatic macrophytes, aquatic invertebrates, warm water and cold water fish, amphibians (tadpoles), soil microorganisms, soil invertebrates, birds, and mammals. Honeybees and other beneficial arthropods and nontarget terrestrial plants were not evaluated using the hazard quotient method.
5. Honeybees are not affected by glyphosate formulations, either by ingestion or direct overspray, at maximum use rates. The majority of other beneficial arthropods are unaffected by Roundup®. Although screening tests under extreme exposure conditions indicate toxicity of glyphosate formulations to some beneficial arthropods at the maximum use rates, these effects were reduced or eliminated when more realistic exposure conditions were used. These data demonstrate minimal risk to beneficial arthropods in areas adjacent to treated fields. Within treated fields, vegetation changes resulting from herbicide use can lead to significant changes in beneficial arthropod populations.
6. Nontarget terrestrial plants in areas adjacent to the treated areas may be exposed to Roundup®. From ground applications, small amounts of herbicide may move downwind from treated areas to adjacent nontarget areas via spray drift. However, as glyphosate is not active in soil, potential herbicidal effects will be limited to only those plants very near the treated area that are in a sensitive growth stage at the time of treatment. Aerial applications can result in increased drift relative to ground applications, but recent technological advances have significantly reduced aerial spray drift.
7. Greater exposure of aquatic organisms to Roundup® is likely during use to control emergent aquatic macrophytes compared to terrestrial uses. Acute and chronic HQ values are less than 1.0 (minimal risk) for all taxa for direct addition of Roundup® to 2-m-deep water at the maximum use rate. Acute HQ values can approach or exceed 1.0 in shallow water (0.15 m). Examination of assumptions reveals that degradation, sorption, and interception by target

vegetation of greater than 50% will mitigate the potential for effects in shallow waters. Even in shallow waters, the chronic HQ did not exceed 1. Use of Roundup® for aquatic habitat restoration can be safely carried out, but requires consideration of items such as application rate, depth of water, and vegetation coverage.

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