SELECTED COMMERCIAL FORMULATIONS OF GLYPHOSATE - *ACCORD, RODEO, ROUNDUP* and *ROUNDUP PRO* Risk Assessment Final Report

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ACRONYMS, ABBREVIATIONS, AND SYMBOLS

a.e.	acid equivalents
a.i.	active ingredient
AEL	adverse-effect level
ACGIH	American Conference of Governmental Industrial Hygienists
AChE	acetylcholinesterase
AMPA	aminomethylphosphonate
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
bw	body weight
ChE	cholinesterase
cm	centimeter
2,4-D	dichlorophenoxyacetic acid
EC_{50}	concentration causing 50% inhibition of a process
EC_{100}	concentration causing complete inhibition of a process
F	female
F_1	first filial generation
FS	Forest Service
g	gram
GC	gas chromatography
HQ	hazard quotient
IARC	International Agency for Research on Cancer
kg	kilogram
$K_{o/c}$	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
K_p	skin permeability coefficient
L	liter
lb	pound
LC_{50}	lethal concentration, 50% mortality
LD_{50}	lethal dose, 50% mortality
LD_{95}	lethal dose, 95% mortality
LOAEL	lowest-observed-adverse-effect level
m	meter
M	male
MCS	multiple chemical sensitivity
mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
MS	mass spectrometry
MW	molecular weight
MOS	margin of safety
NCI	National Cancer Institute
NNG	N-nitrosoglyphosate
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level

ACRONYMS, ABBREVIATIONS, AND SYMBOLS (*continued*)

NRC	National Research Council
OPP	Office of Pesticide Programs
pKa	dissociation constant
POEA	polyoxyethyleneamine
ppm	parts per million
RBC	red blood cells
RfD	reference dose
RTU	ready to use
UF	uncertainty factor
U.S.	United States
U.S. EPA	U.S. Environmental Protection Agency
USDA	United States Department of Agriculture
>	greater than
≥	greater than or equal to
<	less than
≤	less than or equal to
=	equal to
≃	approximately equal to

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

To convert ...	Into ...	Multiply by ...
acres	hectares (ha)	0.4047
acres	square meters (m^2)	4,047
atmospheres	millimeters of mercurey	760
centigrade	Fahrenheit	1.8C°+32
centimeters	inches	0.3937
cubic meters (m^3)	liters (L)	1,000
Fahrenheit	centigrade	0.556F°-17.8
feet per second (ft/sec)	miles/hour (mi/hr)	0.6818
gallons (gal)	liters (L)	3.785
gallons per acre (gal/acre)	liters per hectare (L/ha)	9.34
grams (g)	ounces, (oz)	0.03527
grams (g)	pounds, (oz)	0.002205
hectares (ha) [10,000 m^3]	acres	2.471
inches (in)	centimeters (cm)	2.540
kilograms (kg)	ounces, (oz)	35.274
kilograms (kg)	pounds, (lb)	2.2046
kilograms per hectare (hg/ha)	pounds per acre (lb/acre)	0.892
kilometers (km)	miles (mi)	0.6214
liters (L)	cubic centimeters (cm^3)	1,000
liters (L)	gallons (gal)	0.2642
liters (L)	ounces, fluid (oz)	33.814
miles (mi)	kilometers (km)	1.609
miles per hour (mi/hr)	cm/sec	44.70
milligrams (mg)	ounces (oz)	0.000035
meters (m)	feet	3.281
meters/sec	miles/hour	2.237
ounces (oz)	grams (g)	28.3495
ounces per acre (oz/acre)	grams per hectare (g/ha)	70.1
ounces per acre (oz/acre)	kilograms per hectare (kg/ha)	0.0701
ounces fluid	cubic centimeters (cm^3)	29.5735
pounds (lb)	grams (g)	453.6
pounds (lb)	kilograms (kg)	0.4536
pounds per acre (lb/acre)	kilograms per hectare (kg/ha)	1.121
pounds per gallon (lb/gal)	grams per liter (g/L)	119.8
square centimeters (cm^2)	square inches (in^2)	0.155
square centimeters (cm^2)	square meters (m^2)	0.0001
square meters (m^2)	square centimeters (cm^2)	10,000
yards	meters	0.9144

Note: All references to pounds and ounces refer to avoirdupois weights unless otherwise specified.

CONVERSION OF SCIENTIFIC NOTATION

Scientific Notation	Decimal Equivalent	Verbal Expression
$1 \cdot 10^{-10}$	0.0000000001	One in ten billion
$1 \cdot 10^{-9}$	0.000000001	One in one billion
$1 \cdot 10^{-8}$	0.00000001	One in one hundred million
$1 \cdot 10^{-7}$	0.0000001	One in ten million
$1 \cdot 10^{-6}$	0.000001	One in one million
$1 \cdot 10^{-5}$	0.00001	One in one hundred thousand
$1 \cdot 10^{-4}$	0.0001	One in ten thousand
$1 \cdot 10^{-3}$	0.001	One in one thousand
$1 \cdot 10^{-2}$	0.01	One in one hundred
$1 \cdot 10^{-1}$	0.1	One in ten
$1 \cdot 10^{0}$	1	One
$1 \cdot 10^{1}$	10	Ten
$1 \cdot 10^{2}$	100	One hundred
$1 \cdot 10^{3}$	1,000	One thousand
$1 \cdot 10^{4}$	10,000	Ten thousand
$1 \cdot 10^{5}$	100,000	One hundred thousand
$1 \cdot 10^{6}$	1,000,000	One million
$1 \cdot 10^{7}$	10,000,000	Ten million
$1 \cdot 10^{8}$	100,000,000	One hundred million
$1 \cdot 10^{9}$	1,000,000,000	One billion
$1 \cdot 10^{10}$	10,000,000,000	Ten billion

EXECUTIVE SUMMARY

BACKGROUND

Three commercial formulations of the isopropylamine salt of glyphosate are used by the USDA in vegetation management programs: Accord, Rodeo, and Roundup. This document provides risk assessments for human and health and ecological effects to support the assessment of the environmental consequences of using these products in future Forest Service programs.

Glyphosate is a broad-spectrum, non-selective, post-emergence herbicide. The compound is readily soluble in water and strongly sorbed to most types of soils. The three commercial formulations of glyphosate covered by this risk assessment—Accord, Rodeo, and Roundup—all contain the isopropylamine salt of glyphosate. Two of the formulations, Accord and Rodeo, are simply aqueous solutions of the isopropylamine salt of glyphosate and contain no inert ingredients other than water. Roundup is formulated as an aqueous solution of the isopropylamine salt of glyphosate with a polyethoxylated tallow amine surfactant. Technical grade glyphosate also contains an impurity, N-nitrosoglyphosate.

Although aerial applications may be used in some instances, backpack (selective) foliar, hack and squirt, and boom spray or roadside hydraulic spraying are the most common methods for applying glyphosate in Foreset Service programs. The typical application rate used by the Forest Service is 1 lb a.i./acre, and few applications will exceed 2.5 lbs a.i./acre. The maximum allowable application rate is 7.5 lbs a.i./acre. In some instances, areas treated with glyphosate may be subject to brown-and-burn operations. In previous Forest Service vegetation management programs, glyphosate has been applied in relatively small amounts, compared with the application of other herbicides.

HUMAN HEALTH RISK ASSESSMENT

The toxicity of glyphosate is relatively well characterized in humans and experimental mammals, although the mechanism of action is not clear. The acute toxicity of glyphosate is relatively low, with oral LD_{50} values ranging from approximately 1,000 to 4,000 mg/kg. Most of the data regarding human exposure to glyphosate involves the consumption of large quantities of glyphosate during attempted suicides. The signs of toxicity are generally consistent with massive mucosal irritation and tissue degeneration. In addition, glyphosate may interfere with normal metabolic biochemical functions.

Glyphosate contains small amounts of a nitrosamine, N-nitrosoglyphosate (NNG), and is metabolized, to a small extent, to aminomethylphosphonate (AMPA). The potential effects of these compounds are encompassed by the available toxicity data on glyphosate and glyphosate formulations.

One formulation of glyphosate, Roundup, contains a surfactant, polyoxyethyleneamine (POEA). There is some uncertainty in the interpretation of the toxicity data on Roundup concerning the potential significance of POEA. For the assessment of toxic effects, this uncertainty is relatively

minor in that the available toxicity data on Roundup are adequate for the identification of toxic thresholds.

POEA contains a contaminant, 1,4-dioxane, that has been classified by U.S. EPA as a probable human carcinogen. The potential hazard associated with this effect must be addressed explicitly in the hazard characterization. The chronic toxicity of glyphosate has been well characterized in laboratory mammals. According to U.S. EPA's classification of carcinogens and assessment of the available data, glyphosate is not carcinogenic to humans. Thresholds for other toxic effects are relatively well defined. There is no evidence that glyphosate causes birth defects, and thresholds for potential reproductive effects have been defined. Glyphosate is a skin and eye irritant. This effect must be considered in the handling of commercial formulations. In addition, the toxicology of the combustion products of glyphosate has not been well characterized and this adds uncertainty to the risk assessment for brown-and-burn operations.

Two general exposure assessments are presented in section 3.2.2, job-specific assessments and incident assessments. Job-specific assessments estimate absorption associated with relatively complex job activities, such as mixing, loading, or applying glyphosate, in which multiple routes of exposure are likely. All of these assessments are given as a range based on the projected application rates, empirical observations of variability in exposure rates, and projected variations in herbicide usage [i.e., number of acres treated/hour].

Incident assessments are relatively easy to make. They estimate absorption from spilling glyphosate onto the skin or wearing contaminated clothing. All of these scenarios are extreme or accidental in nature, as discussed in sections 3.2.2 and 3.2.3.

Workers, compared with the general public, are exposed to greater levels of glyphosate and the other components in glyphosate. Exposure to glyphosate is greater for ground workers than for workers involved in aerial applications, in terms of exposure per amount of material handled; however, gross exposure to glyphosate is greater for workers involved in aerial applications because of the large quantity of material that they may handle. The average exposure rate for aerial workers is 0.014 mg/kg body weight with a range of 0.0016–0.16 mg/kg body weight. Boom spray workers may have comparable levels of exposure [0.013 (0.0016–0.11) mg/kg], and other ground workers are exposed to much less [0.006 (0.0005–0.072) mg/kg]. Members of the general public are usually exposed only to extremely low levels of glyphosate [0.00012–0.007 mg/kg], except for accidental exposures scenarios, when exposure levels may approach levels for occupational exposure [0.007–0.019 mg/kg].

The current RfD for glyphosate is 0.1 mg/kg/day (U.S. EPA 1993a), which is based on a NOAEL of 10 mg/kg/day with an uncertainty factor of 100 used to account for species-to-species extrapolation and sensitive subgroups. The RfD was reviewed by U.S. EPA on 9/1/90 and is not undergoing additional review. The Office of Pesticides of the U.S. EPA has recommended a higher RfD of 2 mg/kg/day for glyphosate (U.S. EPA 1993b). The proposed RfD has not been reviewed by the U.S. EPA RfD Work Group.

Quantitative considerations regarding the dose-response data from an epidemiology study and the dose-severity relationships in experimental mammals suggest that each of the RfDs is protective. The estimated threshold for lethality is 445 mg/kg, and the probability of observing a frank toxic effect at this dose level is about 0.04. The estimated LD_{50} for humans, based on the Taiwan poisoning experience, is approximately 3,000 mg/kg, which falls in the middle range of reported LD_{50} values for experimental mammals.

The major hazard associated with the use of glyphosate will involve accidental or incidental dermal or ocular contact. Glyphosate is an irritant to the skin and eyes. If dermal or ocular contact with undiluted or weakly diluted formulations occurs, irritation is likely to develop and will require corrective action to ameliorate the irritant effects. These irritant effects, if properly handled, will be transient.

Based on the exposure assessments discussed in section 3.2 and the dose-response assessments discussed in section 3.3, the quantitative risk assessments for workers and the general public are summarized in Tables 3-2, 3-3, and 3-4. In these tables, risk is characterized as the hazard quotient, the ratio of the anticipated level of the exposure to some index of acceptable exposure or exposure associated with a defined risk. Thus, if the hazard quotient is less than unity, concern for the exposure is minimal. As the hazard quotient increases above unity, concern also increases.

There is no substantial concern for systemic toxic effects in workers or the general public at the typical application rate of 1 lb a.i./acre or the upper range of the application rate used by the Forest Service, 2.5 lbs a.i./acre. At the maximum labelled rate of 7.5 lbs a.i./acre, there may be marginal concern for effects in some groups of workers (i.e., hazard quotients of approximately 0.6) at the upper limit of conservative exposure assumptions.

Consistent with previous assessments conducted by the Forest Service, the carcinogenic risk associated with exposure to 1,4-dioxane appears to be less than 1 in 10 million.

Given the rapid elimination of glyphosate—in the environment and from the body of mammals—as well as the very weak duration-severity relationships observed in animal studies, cummulative effects do not seem plausible. Similarly, there is no basis for identifying specific groups as being at a substantially increased risk.

ECOLOGICAL RISK ASSESSMENT

Standard toxicity bioassays have been conducted on several wildlife species, including mammals, birds, fish, and some terrestrial and aquatic invertebrates, as well as many species of aquatic and terrestrial plants. Furthermore, there are several available field studies that examine the effects of glyphosate applications comparable to those used by the Forest Service.

The toxicity studies on terrestrial animals are generally consistent with those on experimental mammals. Although the mechanism of glyphosate toxicity is unclear, glyphosate can cause toxic effects including mortality at sufficiently high dose levels. The available field studies, however, clearly

suggest that at plausible levels of ambient exposure, direct toxic effects are unlikely. The effects on terrestrial animals appear to be secondary to changes in habitat resulting from toxic effects on vegetation.

The herbicidal activity of glyphosate has been studied extensively. Glyphosate interferes with normal metabolic processes in plants, and, at sufficiently high levels of exposure, may cause cell death, tissue damage, growth inhibition, and death of the plant. The biochemical pathway that is affected is specific to plant species and does not occur in animals.

The toxicity of glyphosate to aquatic species depends on the acidity (pH) of the water. Glyphosate is more toxic in relatively highly acidic water (pH≈6) by up to a factor of about 10, compared with alkaline water (pH≈10). Generally, the reported LC_{50} values for aquatic animals range from approximately 10 to 400 mg/L, depending on the species and pH of the water.

A major qualitative difference between the effect of glyphosate and glyphosate formulations on aquatic and terrestrial organisms concerns the surfactant, POEA, used in Roundup. The surfactant is much more toxic than glyphosate to aquatic organisms. Unlike glyphosate, POEA is more toxic in alkaline water than in acidic water. Thus, the relative potency of POEA with respect to glyphosate is pH dependent.

As with the human health risk assessment, there is little indication that glyphosate will cause adverse effects in the environment at anticipated levels of exposure. The small mammal is a conservative target species for characterizing risk because small organisms, in general, will receive higher doses of an agent, compared with larger organisms, at fixed levels of exposure in environmental media (e.g., contaminated food, water, or air). Moreover, the available toxicity data do not suggest any systematic differences in sensitivity to glyphosate among species. The primary route of exposure for terrestrial animals appears to be contaminated vegetation. For this source, levels of contamination remain below those of concern even at the maximum allowable application rate, 7.5 lbs a.i./acre. At application rates anticipated by the Forest Service, levels of exposure are substantially below those of concern. This analysis is consistent with the field studies on glyphosate, which indicate that direct toxic effects are unlikely.

Glyphosate is an effective herbicide, and terrestrial plants will be affected by applications of glyphosate used to control vegetation. Non-target plants could be damaged by unintentional application or drift. The extent of drift will depend on the specific conditions under which the glyphosate is applied. As would be expected, the potential hazards of drift are greater for aerial applications, compared with ground applications. The extent of damage will depend on the plant species and time of application. Field studies involving both ground and aerial applications of glyphosate suggest that the effects of drift are likely to be most evident within 50 m of the application site.

There is not much evidence that aquatic animals or plants will be affected adversely by normal applications of glyphosate. Although glyphosate is registered for use as an aquatic herbicide, it is

only effective on aquatic plants with vegetation growing above the water level. Most species of algae and macrophytes do not appear to be more sensitive than fish or aquatic invertebrates to glyphosate. For most aquatic species, glyphosate levels of 1 mg/L are not likely to cause adverse effects. For aquatic animals, Roundup (glyphosate+POEA) is not likely to cause adverse effects at levels of 0.1 mg/L, measured as glyphosate. Furthermore, there is no evidence that Roundup is more toxic than glyphosate to aquatic plants. Some sensitive species of algae could be affected; however, the effects are likely to transient, given the rapid dispersion and removal of glyphosate from ambient water.

1. INTRODUCTION

The three commercial formulations of the isopropylamine salt of glyphosate used by the Forest Service (FS) in vegetation management programs are Accord, Rodeo, and Roundup. In 1989, the Southern Region of the Forest Service prepared a series of environmental impact statements accompanied by risk assessments covering the use of these products (USDA 1989a,b,c). The present document provides updated risk assessments for both human and health and ecological effects to support a reassessment of the environmental consequences of using these products in future Forest Service programs. An additional formulation, Roundup Pro, is being considered for use and is also included in this risk assessment.

This document has four chapters: the introduction, program description, risk assessment for human health effects, and risk assessment for ecological effects or effects on wildlife species. Each of the two risk assessment chapters has four major sections: an identification of the hazards associated with the commercial formulations of glyphosate, an assessment of potential exposure to these products, an assessment of the dose-response relationships, and a characterization of the risks associated with exposure. The sections follow the basic steps recommended by the National Research Council of the National Academy of Sciences (NRC 1983) for conducting and organizing risk assessments.

Although this is a technical support document and addresses some highly specialized technical areas, every effort has been made to ensure that the document can be understood by individuals who do not have specialized training in the chemical and biological sciences. Certain technical concepts and terms common to all parts of the risk assessment are described in as plain a language as possible in a separate document: *The Preparation of Environmental Documentation and Risk Assessments for the Forest Service* (SERA 1995a). In addition, these terms are defined in the glossary that accompanies this risk assessment. Some of the specialized terms and concepts are defined, as necessary, in the text.

This document focuses on a concise characterization of human and ecological risks associated with plausible levels of exposure to the commercial products as a result of activities contemplated by the Forest Service. Thus, the risk assessments presented in this document are not, and are not intended to be, comprehensive summaries of all of the available information.

Much of the early literature is summarized in the previously prepared chemical background statement on glyphosate (Mitre Corporation 1989), previously prepared risk assessments and environmental impact statements on glyphosate (USDA 1989a,b,c), monographs by the World Health Organization (FAO and WHO 1986), as well as a series of comprehensive reviews in *The Herbicide Glyphosate* (Grossbard and Atkinson 1985). More recently, the U.S. EPA prepared a comprehensive summary and analysis of the confidential business information (CBI) used to support the re-registration of glyphosate (U.S. EPA 1994) as well as CBI and open literature information used to support the drinking water criteria for glyphosate (U.S. EPA 1992a). Recent

reviews of the potential human health and ecological effects of glyphosate have been published by Smith and Oehme (1992) as well as WHO (1994).

Because the existing reviews provide adequate summaries of most of the available information on glyphosate, and, in the interest of economy, an updated chemical background statement was not prepared with the current risk assessment. Most of the information that would be included in an update is available in the reviews cited above. Information relevant to this risk assessment, taken from earlier reviews as well as more recent publications, is summarized in the appendices to this document.

2. PROGRAM DESCRIPTION

2.1. OVERVIEW

Glyphosate is a broad-spectrum, non-selective, post-emergence systemic herbicide. The compound is readily soluble in water and strongly sorbed to most types of soils. The three commercial formulations of glyphosate covered by this risk assessment—Accord, Rodeo, and Roundup—all contain the isopropylamine salt of glyphosate. Two of the formulations, Accord and Rodeo, are simply aqueous solutions of the isopropylamine salt of glyphosate and contain no inert ingredients other than water. Roundup is formulated as an aqueous solution of the isopropylamine salt of glyphosate with a polyethoxylated tallow amine surfactant. Technical grade glyphosate also contains an impurity, N-nitrosoglyphosate.

Although aerial applications may be used in some instances, backpack (selective) foliar, hack and squirt, and boom spray or roadside hydraulic spraying are the most common methods for applying glyphosate in Forest Service programs. The typical application rate used by the Forest Service is 1 lb a.i./acre and few applications will exceed 2.5 lbs a.i./acre. The maximum allowable application rate is 7.5 lbs a.i./acre. In some instances, areas treated with glyphosate may be subject to brown-and-burn operations. In previous Forest Service vegetation management programs, glyphosate has been applied in relatively small amounts, compared with the application of other herbicides.

2.2. GLYPHOSATE AND COMMERCIAL FORMULATIONS

Glyphosate is a broad-spectrum, non-selective, post-emergence systemic herbicide developed by Monsanto (Franz 1985). The herbicidal properties of glyphosate were first described by Baird et al. (1971). The chemical and toxicological properties of glyphosate are well studied. As of 1985, there were more than 7,000 publications on glyphosate in the literature (Franz 1985). Since 1985, more than 3,000 additional papers on glyphosate have been published.

Glyphosate is the common name for N-(phosphonomethyl)glycine:

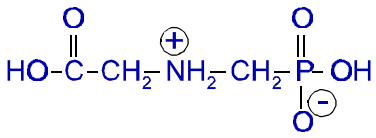


Table 2-1. Physical, chemical, and biochemical properties of glyphosate

CAS Number:	1071-83-6
Molecular weight:	169.07
Melting point (°C):	200 (Tomlin 1994)
Density (g/cm^3):	0.5 (bulk density) (Tomlin 1994)
Density (g/ml):	1.74 (WSSA 1989)
Vapor pressure (mm Hg):	1.94×10^{-7} mm Hg (45° C) (WSSA 1989)
	$< 7 \times 10^{-9}$ mm Hg (25° C) (Weber 1991)
	2.89×10^{-10} mm Hg (25° C) (SRC 1995)
	negligible (Tomlin 1994)
	practically zero (Hartley and Kidd 1985)
Water solubility:	12 g/L (25°C) (Tomlin 1994)
	1.57% (25°C) (WSSA 1989)
Henry's law constant:	insignificant (Reinert and Rodgers 1987)
	5.36×10^{-15} atm-m^3/mole (25°C) (calculated from vapor pressure and water solubility)
Log K_{ow}:	-0.70 (pH 1) (Chamberlain et al. 1994)
	-1.15 (pH 3) (Chamberlain et al. 1994)
	-1.30 (pH 5) (Chamberlain et al. 1994)
	-2.90 (pH 7) (Chamberlain et al. 1994)
	-3.05 (pH 7.5) (Chamberlain et al. 1994)
	-1.90 (pH 9) (Chamberlain et al. 1994)
	-0.80 (pH 11) (Chamberlain et al. 1994)
Soil adsorption K_{oc}:	10,000–100,000 (Weber 1991)
	554–34,000 (Piccolo et al; 1994)
	2,600–4,900 (Glass 1987)
Evaporation rate:	low (Neary et al. 1993)
Foliar half-life (days):	≈1.6 (Thompson et al. 1994)
	8–10 (Feng and Thompson 1990)
	10.6–26.6 (Newton et al. 1984)
Soil half-life (days):	20–40 (Weber 1991)
	<60 (average) (WSSA 1989)
	45–60 (Feng and Thompson 1990)
	29–40 (Newton et al. 1984)
Water half-life (days):	50–70 (U.S. EPA 1992a)
	14 (minimum rate) (Reinert and Rodgers 1987)
	42–70 (Reinert and Rodgers 1987)
	3.5–11.2 days [surface water; some glyphosate in the water column was transferred to sediment and not degraded] (Goldsborough and Brown 1993)
Air half-life (days):	5 [estimated; method of Meylan and Howard (1993)]

Some basic chemical and physical properties of glyphosate are summarized in Table 2-1. At ambient temperatures, glyphosate is a white crystal. In the crystalline form, glyphosate has both positive and negative regions of charge, indicated by the circled plus (⊕) and minus (⊖) signs in the schematic above. Such dipolar ion species are sometimes referred to as a *zwitterions*. In aqueous solutions, the hydrogen atoms of the carboxylic acid (**COOH**) and phosphate ($\mathbf{PO_2H_2}$) groups may be associated (e.g., **-COOH**) or dissociated (e.g., $\mathbf{-COO^-} + \mathbf{H^+}$) depending on the pH of the solution. The dissociation constants, or pK_a values, for these reactions are illustrated in Figure 2-1. The pH of most biological fluids range from approximately 5 to 9. Thus, within this range of pH, glyphosate has a net negative charge and is predominantly in form of H_2G^{-1} or HG^{-2}, as illustrated in Figure 2-1.

Because glyphosate has a relatively low solubility in water, about 12 g/L (see Table 2-1), the compound is usually formulated as a more soluble salt. As summarized in Table 2-2, the three commercial formulations of glyphosate covered by this risk assessment—Accord, Rodeo, and Roundup—all contain the isopropylamine salt of glyphosate. Table 2-2 gives the concentrations both as the isopropylamine salt of glyphosate (a.i.) as well as the acid equivalents of glyphosate (a.e.). Application rates are commonly expressed in units of active ingredient (a.i.), while monitoring studies and some toxicity studies are expressed in units of acid equivalents (a.e.). Unless otherwise specified, units of concentration or application rate are expressed as active ingredient and dose units are expressed as acid equivalents.

Technical grade glyphosate also contains an impurity, N-nitrosoglyphosate, which is sometimes abbreviated as NNG. The U.S. EPA has determined that 92% of technical grade glyphosate contains NNG at less than one part per million (<1 mg/L) and that this amount is toxicologically insignificant. Similarly, the surfactant used in Roundup contains 1,4-dioxane as an impurity. The upper limit of this compound in Roundup is about 0.03% (Monsanto 1990). In a previous review, the U.S. Forest Service determined that the amount of exposure to 1,4-dioxane is toxicologically insignificant (Borrecco and Neisess 1991). Both of these assessments are discussed further in the hazard identification (section 3.1).

Two of the formulations, Accord and Rodeo, are simply aqueous solutions of the isopropylamine salt of glyphosate and contain no inert ingredients other than water. Roundup is an aqueous solution of the isopropylamine salt of glyphosate with a polyethoxylated tallow amine surfactant. This material is referred to in the literature as MON 0139, with the MON presumably referring to Monsanto, or polyoxyethyleneamine (POEA) (Smith and Oehme 1992). The surfactant in Roundup is present at 15% (Hoogheem 1987; Sawada et al. 1988) or 150 g/L assuming that the 15% value refers to the level in terms of weight per unit volume. Presumably, the Roundup surfactant is a derivative of tallow, a complex mixture of fat from the fatty tissue of cattle or sheep. Tallow contains a variety of fatty acids including oleic (37–43%), palmitic (24–32%), stearic (20–25%), myristic (3–6%), and linoleic (2–3%) acids as well as small amounts of cholesterol, arachidonic, elaidic, and vaccenic acids (Budavari 1989). As discussed in the hazard identification for human health (section 3.1) and ecological effects (section 4.1), the presence of the surfactant must be considered in the risk assessments of Roundup. Roundup Pro is a recently

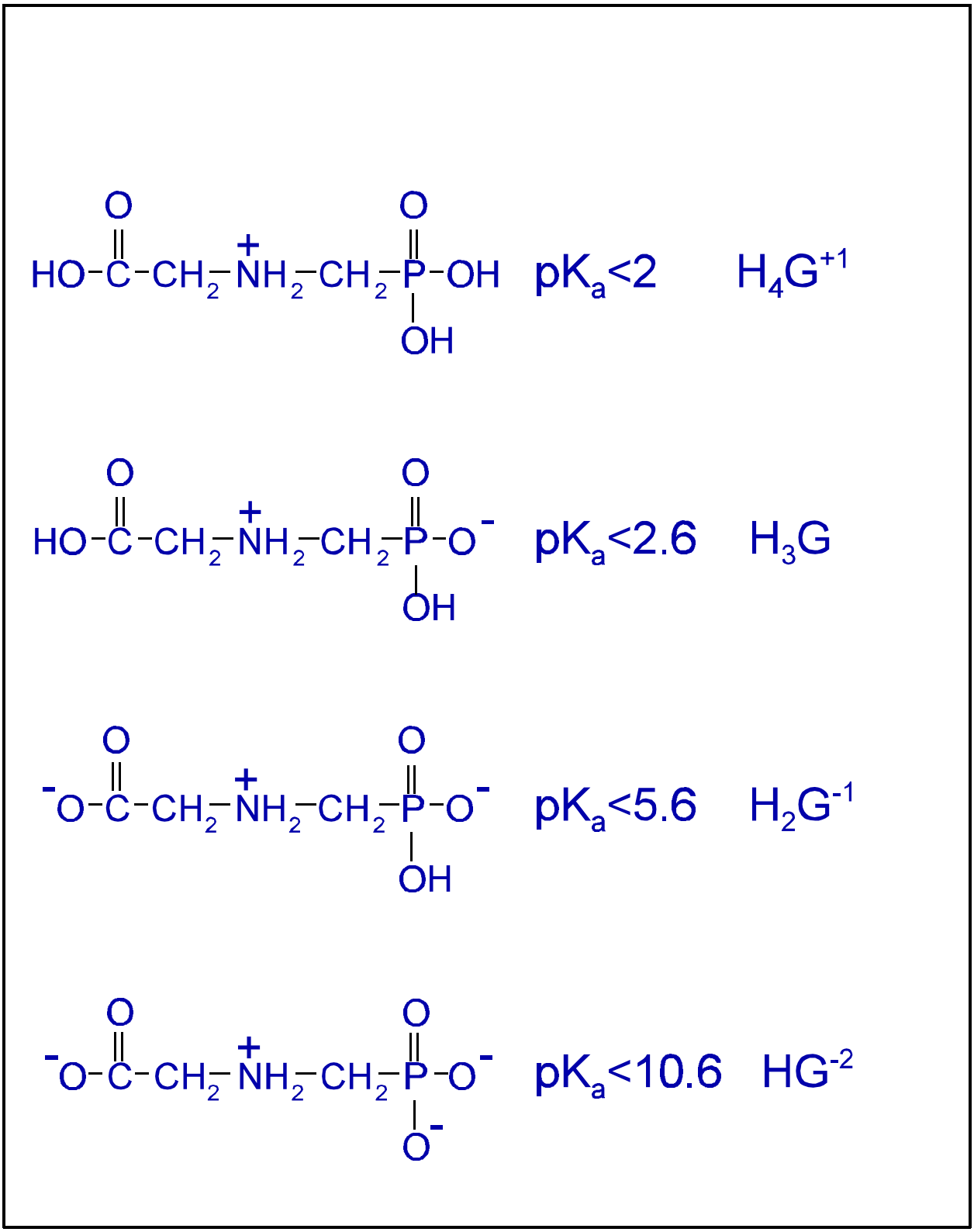


Figure 2-1. Structure and dissociation constants (pK_a) for the various forms of glyphosate.

introduced formulation of glyphosate that contains a phosphate ester neutralized polyethoxylated tallowamine surfactant at a level of 14.5% (Monsanto 1995 a,b; Monsanto 1996) or 145 g/L. Other than the specification that the tallow amine surfactant in Roundup Pro is a phosphate ester of POEA, no published information is available on the chemical differences between the surfactant in Roundup and Roundup Pro. As detailed in Sections 3 and 4, there is relatively little information available on the toxicity of Roundup Pro.

Table 2-2. Summary of commercial formulations containing glyphosate covered by this risk assessment[a]

Formulation	Ingredient	Pounds (a.i.)/gallon	Pounds (a.e.)/gallon	Grams (a.e.)/L
Accord (Monsanto)	glyphosate, isopropylamine salt (41.5%)	4	3	356
	inerts (58.5%) water			
Rodeo (Monsanto)	glyphosate, isopropylamine salt (53.8%)	5.4	4	480
	inerts (46.2%) water			
Roundup (Monsanto)	glyphosate, isopropylamine salt (41%)	4	3	356
	inerts (59%)			
	ethoxylated tallow amines (CAS No. 61791-26-2), 15%[b], and water			
Roundup Pro (Monsanto)	glyphosate, isopropylamine salt (41%)	4	3	356
	inerts (59%)			
	phosphate ester neutralized ethoxylated tallow amines, 14.5%[c], and water			

[a]Taken from Monsanto (1993, 1994a,b, 1995a) (unless otherwise specified).

[b]Hoogheem (1987) (Letter Feb 27 to Larry Gross).

[c]Monsanto 1995b.

a.e. = acid equivalents; a.i. = active ingredient

2.3. APPLICATION METHODS

Proposed application methods and vegetation management uses for glyphosate are summarized in Table 2-3. Detailed descriptions of the silvicultural uses of herbicides and the various methods of herbicide applications are available in the general literature (e.g., Cantrell and Hyland 1985) and earlier environmental impact statements conducted by the Forest Service (USDA 1989a,b,c). The following summary focuses on those aspects of application that are most germane to the exposure assessments (sections 3.2 and 4.2).

Table 2-3. Proposed uses and application methods for glyphosate

	Application Method			
	Broadcast		Selective	
Use	Aerial	Boom Spray	Backpack (Selective Foliar)	Cut Surface (Hack and Squirt)
Conifer release	O		M	F
General weeds			M	
Noxious weeds			M	
Rights-of-way	F	F	M	F
Site preparation	F		M	F
Vegetation			M	F
Wildlife habitat improvement		M	M	

M = Planned Use **F** = Potential use

O = Done commercially but not used by the Forest Service

The most commonly used application method is the backpack (selective) foliar application. In selective foliar applications, the herbicide sprayer or container is carried by backpack and the herbicide is applied to selected target vegetation. Application crews may treat up to shoulder high brush, and chemical contact with the arms, hands, or face is plausible. To reduce the likelihood of

significant exposure, application crews are directed not to walk through treated vegetation. Typically, a worker will treat approximately 0.5 acres/hour with a plausible range of 0.2–51.0 acres/hour.

Hack and squirt applications are a form of cut surface treatment in which the bark and cambium of a standing tree is cut with a hatchet and the herbicide is then applied to the cut using a squirt bottle. This treatment is used to eliminate large trees during site preparation, conifer release operations, or rights-of-way maintenance. As with selective foliar applications, a worker usually will treat approximately 0.5 acres/hour with a plausible range of 0.25–1.0 acres/hour.

Boom spray or roadside hydraulic broadcast spraying is used primarily in rights-of-way management. Spray equipment mounted on tractors or trucks is used to apply the herbicide on either side of the roadway. Boom spray may also be used for maintenance or rehabilitation of wildlife openings, with spray equipment mounted on or towed behind tractors. Usually, about 8 acres will be treated in a 45-minute period [approximately 11 acres/hour] with approximately 200 gallons of the herbicide mixture [270 gallons/hour]. Some special truck mounted spray systems may be used to treat up to 12 acres in a 35-minute period with approximately 300 gallons of herbicide mixture [about 21 acres/hour and 510 gallons/hour] (USDA 1989b, p 2-9 to 2-10).

Aerial applications may involve the use of fixed wing aircraft (Roundup and Rodeo) or helicopters (Accord, Rodeo, and Roundup). Liquid formulations of glyphosate are applied through specially designed spray nozzles and booms. The nozzles are designed to minimize turbulence and maintain a large droplet size, both of which contribute to a reduction in spray drift. Aerial applications may only be made under meteorological conditions that minimize the potential for spray drift. In aerial applications, approximately 40–100 acres may be treated per hour.

In some instances, areas treated with glyphosate may be subject to brown-and-burn operations. As indicated in USDA (1989b), these operations involve burning a treated area 45–180 days after treatment with the herbicide.

2.4. MIXING AND APPLICATION RATES

Accord is labeled for use in forestry site preparation, utility rights-of-way maintenance, as well as conifer and hardwood release for application as a foliar spray to control or destroy most herbaceous and woody plants. For both ground and aerial applications for site preparation and rights-of-way management, the maximum labeled rate is 10 quarts/acre, which is equivalent to 7.5 lbs a.e./acre [2.5 gallons/acre · 3 lbs a.e./gallons]. The maximum amount that may be applied in a single season is 10.6 quarts/acre or approximately 8 lbs a.e./acre [10.6 quarts/acre · 0.25 gallons/quart · 3 lbs a.e./gallons]. For conifer or hardwood release, much lower application rates are used, generally 1–2 quarts/acre [0.75–1.5 lbs a.e./acre], although as many as 3 quarts/acre [2.25 lbs a.e./acre] may be used in Maine for difficult to control species. To be effective in any of these applications, Accord must be mixed with a nonionic surfactant with greater than 50% active

ingredient. The product label for Accord (Monsanto 1994a) indicates that a surfactant is required for some applications:

> *In forestry site preparation and utility rights-of-way management, this product requires use with a nonionic surfactant. Use a nonionic surfactant with greater than 50 percent active ingredient and labeled for use with herbicides. The use of this product without surfactant will result in reduced performance.*

As indicated in Table 2-2, Roundup and Roundup Pro contain the same amount of glyphosate as Accord, 3 lbs a.e./gallon. In addition, both Roundup and Roundup Pro contain a surfactant, ethoxylated tallow amine at a concentration of 15% (Roundup) or a phosphate ester neutralized polyethoxylated tallow amine (Roundup Pro). Also as with Accord, these products are applied to terrestrial vegetation for the control of undesirable plant species. Roundup, however, is registered for both crop and non-crop applications. Roundup Pro is labeled only for non-crop uses. Another Monsanto product, Roundup Ultra, appears to be identical to Roundup Pro but is labeled for agricultural uses (Matura 1996a,b). For both Roundup and Roundup Pro, the maximum labeled application rate is 5 quarts/acre or 3.75 lbs a.e./acre [1.25 gallons/acre · 3 lbs a.e./gallons]. Many weeds, however, are controlled at application rates of 1 quart/acre. As with Accord, the maximum amount of both Roundup formulations that may be applied in a single season is 10.6 quarts/acre or approximately 8 lbs a.e./acre [10.6 quarts/acre · 0.25 gallons/quart · 3 lbs a.e./gallons] (Monsanto 1994b, 1995a).

As also indicated in Table 2-2, Rodeo is essentially the same product as Accord except that glyphosate is present at a higher concentration, 4 lbs a.e./gallon in Rodeo and 3 lbs a.e./gallon in Accord. Rodeo is registered for the control of both terrestrial and aquatic plants. As with Accord, the label for Rodeo recommends the use of a nonionic surfactant. For both terrestrial and aquatic vegetation, the highest recommended application rate is 7.5 pints/acre or 3.75 lbs a.e./acre [3.75 quarts/acre · 0.25 gallons/quart · 4 lbs a.e./gallon] (Monsanto 1993). In terms of acid equivalents of glyphosate, this is the same as the maximum application rate of Roundup and 50% of the maximum application rate of Accord.

The Forest Service does not plan to use glyphosate at the highest labelled application rates. In 1995, the typical rate for glyphosate was about 1 lb a.i./acre. All but one application (2.8 lb a.i./acre) was less than 2.5 lb a.i./acre (USDA/FS 1995).

In previously conducted Forest Service vegetation management programs (USDA 1989a,b,c), glyphosate was applied in relatively small amounts, compared with the application of other herbicides. For example, in Forest Service Region 8 (comprised of Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, North California, Oklahoma, South Carolina, Tennessee, Texas, Virginia, and part of West Virginia), there are approximately 12,000,000 acres

of National Forests and Grassland, of which up to 600,000 acres are treated with various herbicides each year. In the late 1980s, glyphosate was applied to 9,700 acres/year, 0.081% of the total area and 1.6% of the treated area (USDA 1989b, p.2-4). In recent years, Forest Service use of herbicides in Region 8 has been reduced to treatment of fewer than 100,000 acres/year. In 1995, only 3,704.2 acres were treated with glyphosate (USDA/FS 1995).

3. HUMAN HEALTH RISK ASSESSMENT

3.1. HAZARD IDENTIFICATION

3.1.1. Overview. The toxicity of glyphosate is relatively well characterized in both experimental mammals and humans, although the mechanism of action is not clear. The acute toxicity of glyphosate is relatively low, with oral LD_{50} values ranging from approximately 1,000 to 4,000 mg/kg. Most of the human experience with glyphosate involves the consumption of large quantities of glyphosate during attempted suicides. The signs of toxicity are generally consistent with massive mucosal irritation and tissue degeneration. In addition, glyphosate may interfere with normal metabolic biochemical functions.

Glyphosate contains small amounts of a nitrosamine, N-nitrosoglyphosate (NNG), and is metabolized, to a minor extent, to aminomethylphosphonate (AMPA). The potential effects of these compounds are encompassed by the available toxicity data on glyphosate and glyphosate formulations.

One formulation of glyphosate, Roundup, contains a surfactant, POEA. There is some uncertainty in the interpretation of the toxicity data on Roundup concerning the potential significance of POEA. For the assessment of toxic effects, this uncertainty is relatively minor in that the available toxicity data on Roundup are adequate for the identification of toxic thresholds.

POEA, contains the contaminant 1,4-dioxane, which has been classified by U.S. EPA as a probable human carcinogen. The potential hazard associated with this effect must be addressed explicitly in the hazard characterization. The chronic toxicity of glyphosate has been well-characterized in laboratory mammals. According to the U.S. EPA classification of carcinogens and their assessment of the available data, glyphosate is not carcinogenic to humans. Thresholds for other toxic effects are relatively well-defined. There is no evidence that glyphosate causes birth defects, and thresholds for potential reproductive effects have been defined. Glyphosate is a skin and eye irritant, which must be taken into consideration when handling commercial formulations. Moreover, the toxicology of the combustion products of glyphosate has not been well characterized, which adds uncertainty to the risk assessment for brown-and-burn operations.

3.1.2. Acute Toxicity and Mechanisms of Action. The herbicidal activity of glyphosate is due primarily to the inhibition of the shikimate pathway (section 4.1). This pathway is different in plants than in animals; therefore, this mechanism of action is not likely to cause adverse effects in humans. Nonetheless, like all chemicals, glyphosate and commercial formulations of glyphosate may be toxic at sufficiently high exposure levels. In experimental mammals, acute oral LD_{50} values of glyphosate range from approximately 2,000 to 6,000 mg/kg and intraperitoneal LD_{50} values are about 10 times lower, ranging from 134 to 234 mg/kg (Appendix 1-1).

Formulations of glyphosate have been used in many suicides and attempted suicides (Appendix 1-2). By far, the most comprehensive report of human poisonings is the study by Tominack et al. (1991), which describes cases of poisoning, primarily from suicides and attempted suicides, in Taiwan. The glyphosate formulation used in Taiwan is identical to Roundup, a mixture of glyphosate and polyoxyethyleneamine. In 92 poisoning cases, the mean dose in individuals who survived was 120 mL (range of 5–500 mL) and the mean dose in individuals who died was 263 mL (range of 150–500 mL). All of the fatal cases summarized in the study involved suicide attempts; no deaths were attributed to accidental ingestion of 25 ± 22 mL. Most of this information is reported also by Talbot et al. (1991).

Similar but less detailed estimates of human exposure to glyphosate are reported in a study from Japan where there were 56 poisoning incidents involving Roundup (Sawada et al. 1988). The mean lethal dose was 206 mL, and the mean non-lethal dose was 106 mL. This study does not specify the number of surviving or fatally exposed individuals.

Taking the data from Tominack et al. (1991) and assuming an average body weight of approximately 60 kg, the average lethal dose, in terms of acid equivalents of glyphosate, is 1,560 mg/kg

$$0.263 \text{ L} \cdot 356{,}000 \text{ mg/L} \div 60 \text{ kg},$$

and the minimum lethal dose is 890 mg/kg

$$0.150 \text{ L} \cdot 356{,}000 \text{ mg/L} \div 60 \text{ kg}.$$

Based on dose-fatality relationships (Tominack et al. 1991, Table 4, p. 99), doses of 200–249 mL [approximately 1,200–1,500 mg/kg] were associated with death in 3 of 10 individuals.

The report by Tominack et al. (1991) also provides incidence data for mortality in humans after exposure to varying amounts of Roundup. In Table 4 of the Tominack et al. (1991) publication, these data are presented as a range of glyphosate quantities consumed in mL, the number of cases for the exposure range, and the number of fatalities in the exposure range. Taking the arithmetic mean of the range of exposures and using the above approach to convert these exposures to dose, the dose-response relationship for human mortality is illustrated in Figure 3-1. Figure 3-1 also plots LD_{50} values for various

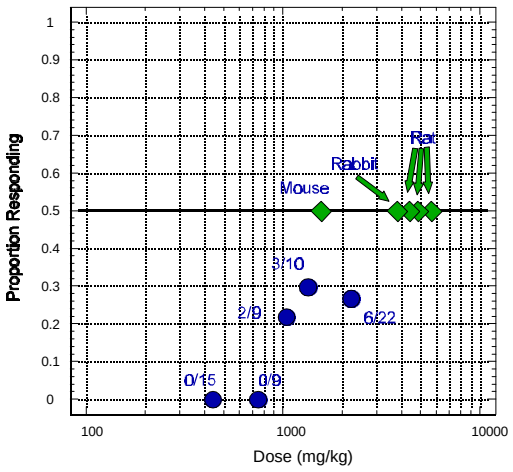


Figure 3-1: Dose-Response Data for Lethal Oral Exposures in Humans (Tominack et al. 1991) Compared with LD_{50} Values in Experimental Mammals.

experimental mammals, taken from Appendix 1-1. As illustrated in this figure, the incidence data reported by Tominack et al. (1991) are reasonably consistent with LD_{50} values in experimental mammals. It is also noteworthy that among the three species of experimental mammals—mice, rats, and rabbits—there is no apparent relationship between acute lethal potency and body weight.

As indicated in Appendix 1-1, the signs and symptoms of glyphosate or glyphosate/surfactant toxicity in humans generally include gastrointestinal effects (vomiting, abdominal pain, diarrhea), irritation, congestion, or other forms of damage to the respiratory tract, pulmonary edema, decreased urinary output sometimes accompanied by acute renal tubular necrosis, hypotension, metabolic acidosis, and electrolyte imbalances, probably secondary to the gastrointestinal and renal effects. In some cases, elevated temperatures have been noted (Tominack et al. 1991). Changes in blood enzymes have been observed and attributed to hemolysis (Sawada et al. 1988).

In experimental mammals, signs of acute toxicity after oral or intraperitoneal dosing include increased respiratory rates, elevated rectal temperature, and in some instances asphyxia convulsion. The primary pathological lesion is lung hyperemia (Bababunmi et al. 1978, Olorunsogo et al. 1977, Olorunsogo and Bababunmi 1980). Hemolysis was not noted in sheep with an inherently low erythrocyte glucose-6-phosphate activity (Geiger and Calabrese 1985).

The mechanism by which glyphosate exerts its acute toxic effects is not clear. As discussed below, the surfactant in Roundup may be a factor in some of the acute effects associated with exposure to this herbicide.

Based on a series of experiments using rat liver mitochondria exposed to the isopropanolamine salt of glyphosate without any surfactant (summarized in detail by U.S. EPA 1992a), glyphosate appears to be an uncoupler of oxidative phosphorylation (Bababunmi et al. 1979, Olorunsogo 1982, Olorunsogo and Bababunmi 1980, Olorunsogo et al. 1977, Olorunsogo et al. 1979a,b). This effect has been noted after intraperitoneal doses as low as 15 mg/kg (Olorunsogo et al. 1979a).

The uncoupling of oxidative phosphorylation effect is toxicologically significant because it unlinks the process of nutrient metabolism from the normal ability of the organism to store food energy. As a compensatory response to this effect, some biochemical processes involved in energy metabolism are increased. This can result in increased oxygen consumption, increased body temperature [because the chemical energy in the nutrients is converted to heat energy rather than chemical energy], and weight loss. Some classical uncouplers of oxidative phosphorylation—such as pentachlorophenol and dinitrophenol—have, in the past, been used as weight reducing drugs (Howard and Durkin 1973).

Many of the observations on whole animals and isolated mitochondria made by Olorunsogo and Bababunmi are consistent with an uncoupling of oxidative phosphorylation; nonetheless, others, like Tominack et al. (1991) and Talbot et al. (1991) have challenged the role of the uncoupling of oxidative phosphorylation. First, some of the details related to the observations by Olorunsogo

and Bababunmi are not consistent with the actions of classical uncoupling agents. Second, classical uncouplers generally cause abnormal respiration, increased heart rates, and extremely high fevers (e.g., >106°F), but these effects are not consistently seen in the individuals poisoned with glyphosate formulations. This clearly is the case with regard to increased body temperature. Of the 97 patients covered in the Tominack et al. (1991) report, only seven individuals had mild elevations in body temperature (>37.5°C or 99.5°F). The report does not include information on pulse rates. Abnormal respiration seems typical of the patients covered in the Tominack report as well as other reports (Appendix 1-2), but this effect is consistent with direct damage to the lungs.

Many of the effects of acute oral exposure to high doses of glyphosate or Roundup are consistent with corrosive effects on the mucosa. Summarizing studies from the Japanese literature, Talbot et al. (1991) indicate that pure glyphosate, the POEA surfactant in Roundup, as well as Roundup itself all cause corrosive effects on the gastric mucosa of dogs similar to the effects seen after exposures to high concentrations of hydrochloric acid (Mizuyama et al. in press, Sudo et al. 1987, Wakasugi et al. 1987).

3.1.3. Role of Surfactant. As summarized in section 2.2, Roundup, contains a polyethoxylated tallow amine surfactant at a level of 15% (150 g/L) and Roundup Pro contains a phosphate ester neurtalized polyethoxylated tallow amine surfactant at a level of 14.5%. The other formulations of glyphosate recommend the use of a surfactant to improve the efficacy of glyphosate. There is an extensive amount of literature on glyphosate specifically (Boerboom and Wyse 1988, Clay and Lawrie 1988, Cranmer and Linscott 1991, Sherrick et al. 1986, Turner 1985) and many other compounds (Green et al. 1992, Prasad 1989) indicating that the addition of surfactants can greatly enhance phytotoxicity of herbicides.

The potential role of the surfactant in the toxicity of Roundup was first emphasized by the Sawada et al. (1988) in their analysis of poisoning cases in humans. They indicate that the acute LD_{50} of POEA is *"less than one-third that of roundup and its active ingredient"* and reference this statement to a chapter by Atkinson (1985) in *The Herbicide Glyphosate* (Grossbard and Atkinson 1985). The Sawada reference has been quoted in turn by Martinez and Brown (1991) as indicating that *"... POEA by itself has a LD_{50} of 1-2 g/kg"*.

Atkinson (1985) does cite an LD_{50} of 4.3 g/kg for glyphosate [a rounding of the rat oral LD_{50} of 4,320 mg/kg reported in U.S. EPA (1986a) and earlier U.S. EPA reports] and indicates that this is about the same as the acute oral LD_{50} in for isopropylamine salt in rats, 4.9 g/kg. Atkinson (1985), however, does not give an acute oral LD_{50} for POEA or any other surfactant.

An acute oral LD_{50} for POEA of 1,200 mg/kg was reported in the previous EIS (USDA 1989b, p. 3-49, Coastal Plain/Piedmont Appendices). This value has been verified by Monsanto Co. and is consistent with the acute toxic potency of other surfactants: in general, acute LD_{50} values for surfactants range from several hundred to several thousand mg/kg (Kosswig 1994, Grayson and Eckroth 1983).

Although there is evidence that POEA is more toxic than glyphosate to aquatic species (section 4), the acute oral toxicity of Roundup (glyphosate and surfactant, LD_{50} in rats of 5400 mg/kg) is almost the same as that of glyphosate (LD_{50} in rats of 5,600 mg/kg) (Appendix 1-1).

Based on these LD_{50} values, the LD_{50} of the surfactant can be estimated under the assumption of dose addition (Finney 1971). This assumption requires that the components in the mixture have the same mode of action. This assumption is not certain, but it is consistent with the observation by Talbot et al. (1991) that both glyphosate and POEA may exert some of their acute toxicity via irritation of biological membranes. The assumption of dose addition is also not interactive—that is, it assumes that the components in the mixture do not influence the toxicity of one another. This assumption is conservative, compared with other non-interactive models of joint action (Mumtaz et al. 1994).

For some uniform measure of toxicity (ζ) (e.g., LD_{50}), the toxicity of any mixture (ζ_M) is predicted, under the assumption of dose addition, by:

$$\zeta_M = \frac{\zeta_1}{(B_1 + DB_2)} \qquad \textbf{(3-1)}$$

where ζ_1 is the effective exposure (e.g., LD_{50} or LD_{95} values) for one compound, π_1 and π_2 are the proportions of each compound in the mixture, and ρ is the potency defined as $\zeta_1 \div \zeta_2$. Furthermore, given the toxicity of a defined mixture (ζ_M) and one of the components (ζ_1), the potency of the second component can be calculated as:

$$D = \frac{(\zeta_1 / \zeta_M) - B_1}{B_2} \qquad \textbf{(3-2)}$$

Here, the term *defined mixture* indicates that π_1 and π_2 are known. From this relationship, the effective exposure (i.e., toxic potency) of the second component (ζ_2) can be estimated as:

$$\zeta_2 = \frac{\zeta_1}{D} \qquad \textbf{(3-3)}$$

Using the nominal LD_{50} for Roundup of 5,400 mg/kg, a π_1 of 0.356 for glyphosate (356 g/L), and π_2 of 0.15 for POEA (150 g/L), the estimated LD_{50} for POEA would be almost exactly 1,200 mg/kg, consistent with dose additivity. This approach, however, would be a misapplication of the above equations.

To estimate the toxicity of POEA from the Roundup (glyphosate+POEA) LD_{50}, this LD_{50} must be converted from units of glyphosate to total mixture mass (glyphosate+POEA). In other words, an LD_{50} of 5,400 mg glyphosate/kg bw is equivalent to a combined mass (glyphosate and POEA) of about 7,560 mg [1.4·5,400 mg], since the ratio of POEA to glyphosate is approximately 0.4 [150 g/L ÷ 356 g/L]. Similarly, the correct π_1 for glyphosate is about 0.7 [356 ÷ (150+356)] and the

correct π_2 for POEA is about 0.3 [150 ÷ (150+356)]. Using this approach, the potency of POEA relative to glyphosate is about 0.14 and the estimated oral LD_{50} in rats is for POEA is about 40,000 mg/kg [5,600 mg/kg ÷ 0.14]. This estimate is consistent with the published results of Martinez, summarized in the following paragraph, in which no mortality was noted in rats after oral doses of up to 14,286 mg/kg POEA.

Martinez and coworkers (Martinez and Brown 1991; Martinez et al. 1990) conducted a series of experiments specifically designed to assess the role of the surfactant in the acute toxicity of Roundup. In these studies, compounds were administered to groups of five rats either by gavage [direct instillation into the stomach] or direct installation into the trachea. Oral exposures to Roundup at doses of 1, 3, and 5 mL/animal caused 0%, 40%, and 100% mortality, respectively, over a 24-hour observation period. Taking an average body weight of 350 g/rat reported by Martinez and Brown (1991), the mid-dose level corresponds to approximately 3,050 mg/kg [3 mL · 356 mg a.e./mL ÷ 0.350 kg], only somewhat less than and consistent with the reported LD_{50} for Roundup of 5,400 mg/kg (Monsanto Co. 1982a,b). POEA, administered by gavage, caused no deaths at doses of 1, 3, and 5 mL/animal. Since ethoxylated surfactants generally have a density of about 1g/mL (Kosswig 1994, p. 789), the doses of POEA correspond to approximately 2,857, 8,571, and 14,286 mg/kg. The low acute oral toxicity of POEA is consistent with the similarity between the acute oral toxicity of glyphosate and Roundup, discussed above.

In the earlier study by Martinez et al. (1990), an oral dose with Roundup RTU or Roundup concentrate caused delayed (6 hours) pulmonary edema, consistent with clinical observations in humans, as summarized above. The authors concluded that *"... delayed pulmonary edema combined with blood stained weeping from the nose, diarrhea, distended GI tract, and ascites is in excellent agreement with ... The clinical picture of ... hypovolemic shock"*, as described by Sawada et al. (1988). In the individuals involved in the Taiwan studies of glyphosate poisoning, however, hematocrit, blood urea nitrogen, and central venous pressure determinations were not consistent with hypovolemia.

Intratracheal instillations in rats resulted in much more toxic effects at much lower dose levels. Roundup at doses of 0.1, 0.2, and 0.4 mg/animal caused 80% mortality at the low dose and 100% mortality at the two higher doses as well as an increase in lung weights. POEA, at the same dose levels, caused 20%, 70%, and 100% mortality as well as increases in lung weights, although the increases were less than those observed with Roundup (Martinez and Brown 1991, Table 1, p. 44). Pathological examinations indicated that both Roundup, and to a lesser extent POEA, cause hemorrhaging and congestion of the lungs after intratracheal instillations. Martinez and Brown (1991) conclude that POEA potentiates the pulmonary toxicity of glyphosate. Since, however, these investigators did not test glyphosate alone, the basis for their conclusion is not clear.

Tai et al. (1990) reported that injections of Roundup in rats led to cardiac depression caused solely by POEA and partially antagonized by glyphosate. Although this observation is interesting, it seems only marginally relevant to this risk assessment.

Based on drinking water studies of both glyphosate and Roundup (i.e., glyphosate with POEA), the surfactant does not affect the rapid elimination rate of glyphosate (NTP 1992).

Pertinent data regarding the subchronic or chronic toxicity of POEA were not located in the published literature. In a letter to the Forest Service concerning a review of the previous EIS, Monsanto provides single page summaries of two studies conducted by Bionetics on what is characterized as the *Roundup surfactant* (Long 1987, letter to Larry Gross dated March 12, 1987). In the first study, dietary concentrations of 0, 1,250, 2,500, or 5,000 ppm (0, 1.6, 3.8, or 6.5 mg/kg/day) were fed to Sprague-Dawley rats for 13 weeks. The study is identified as LBI Project No. 2290. The results of the study are summarized as follows:

> *No toxic signs were observed except for slow acclimation to the highest dosage in the first three weeks. Clinical laboratory tests failed to reveal important differences between controls and test animals. Microscopic examination of organs collected at necropsy revealed only histiocytic infiltrations of the lamina propria of the small intestines and sinusoids of mesenteric lymph nodes at all dosage levels.*

In the second study, the surfactant was fed to dogs via gelatin capsules at doses of 0, 10, 20, and 30 mg/kg, 3 times/day, over the final 10 weeks of a 14-week study. Dosing during the first 4 weeks, if any, is not specified, and the LBI project number is not specified. The summary states:

> *The material showed no effect with regard to survival, general appearance, behavior, neurologic, electrocardiographic or histopathological parameters. Changes observed during the study included decreased food consumption, depressed growth rate, reduced serum calcium and total protein, increased relative and absolute renal and cardiac weights, and increased relative adrenal weights. None of the observed changes would preclude the use of Roundup surfactant as an adjuvant in a herbicide formulation.*

No further information is provided. Superficially, these studies increase rather than lessen concern about the presence of the surfactant.

Very little information is available on the surfactant in Roundup Pro. The surfactant in Roundup Pro is described as a *phosphate ester neutralized polyethoxylated tallowamine* (Monsanto 1996). As with the polyethoxylated tallowamine in Roundup, this surfactant is presumably produced by the ethoxylation of tallow amine with ethylene glycol. Since tallow is a complex and variable mixture of fatty acids and other minor components and since the nature and extent of ethoxylation

can vary with different conditions during synthesis, the significance of the difference between the surfactants used in Roundup and Roundup Pro is not apparent from the available chemical descriptions of these two surfactants.

Based on a comparison of the limited toxicity data on Roundup Pro with corresponding data on Roundup, it appears that the surfactant in Roundup Pro may be less irritating than than in Roundup. As summarized in Appendix 1-6, apparently comparable studies of dermal irritation in the rabbit (Monsanto Co. 1982b vs Kirk 1993a), eye irritation in the rabbit (Monsanto Co. 1982a,b vs Kirk 1993d) and dermal sensitization in the guinea pig (Monsanto Co. 1983c vs Kirk 1993f) suggest that Roundup Pro is less active than Roundup. Since the only difference between Roundup and Roundup Pro is the surfactant and since glyphosate itself does not appear to be a irritant, these differences in biological activity between Roundup and Roundup Pro probably reflect differences in the activity of the surfactants. These differences, however, do not seem to be reflected strongly in the available summaries of acute toxicity studies. Roundup and Roundup Pro have comparable acute oral and dermal LD_{50}s in experimental mammals (Appendix 1-1) and birds (Appendix 2-1). In addition, as indicated in section 4, the acute toxicity of Roundup and Roundup Pro to aquatic species is similar.

Based on the apparent similarities in the biological activity of Roundup and Roundup Pro, the extensive data Roundup are used in this risk assessment to characterize the risks of exposures to Roundup Pro, recognizing that this may be a somewhat conservative approach at least with respect to irritant effects.

3.1.4. Subchronic or Chronic Systemic Toxic Effects. As indicated in Section 1, several reviews are available that cover the majority of the available literature on subchronic and chronic effects of glyphosate. Much of the published literature is discussed in U.S. EPA's *Drinking Water Criteria Document for Glyphosate* (U.S. EPA 1992a). Unpublished studies submitted by chemical manufacturers are reviewed in the IRIS sheet on glyphosate (U.S. EPA 1993) as well as U.S. EPA's *Re-registration Eligibility Decision (RED) Document on Glyphosate* (U.S. EPA 1993a). The most recently published review covering the subchronic and chronic toxicity of glyphosate is by Smith and Oehme (1992). The only studies not covered by these reviews are NTP's subchronic toxicity studies in rats and mice (NTP 1992) and a very recent publication on subchronic toxicity in male rabbits (Yousef et al. 1995). The most relevant information from the available reviews and more recent publications is summarized in Appendix 1-3.

One of the more consistent signs of subchronic or chronic exposure to glyphosate is loss of body weight. This effect has been noted in mice (U.S. EPA 1986a, NTP 1992), rats (NTP 1992, Stout and Ruecker 1990), and rabbits (Yousef et al. 1995). This observation is consistent with the work of Olorunsogo and coworkers, summarized in section 3.1.2, indicating that glyphosate may be an uncoupler of oxidative phosphorylation (U.S. EPA 1992a, NTP 1992). Loss of body weight, particularly in studies using dietary exposure, can be secondary to decreased food consumption. In the NTP bioassay using mice, however, weight loss was noted at the two higher

dose levels but there were no significant differences in food consumption between any of the treated groups and the control group. Similarly, in rabbits, the weight loss was not associated with a decrease in food consumption (Yousef 1995). In the NTP study using rats (NTP 1992), a slight decrease in food consumption was observed in the high dose group (50,000 ppm in the diet), which amounted to 91% of control values for females and 88% of control values for males. This behavior may account for the weight decrease in females, 95% of controls, and possibly for the weight decrease in males, 82% of controls.

There are relatively few histopathological changes associated with exposure to glyphosate. Salivary gland lesions [cytoplasmic changes in the parotid and submandibular glands of rats and the parotid salivary glands in mice] were observed in both rats and mice in the NTP (1992) study. These changes could be blocked by propanolol, an adrenergic antagonist, which suggests that this effect is mediated by an adrenergic mechanism. Given the lack of any clear signs that glyphosate causes specific neurotoxic effects, the significance of this observation to potential effects in humans is unclear. The implications for potential carcinogenic effects are discussed below.

The only other specific and consistent effect of glyphosate involves effects on the testicles. In the NTP (1992), relative testicular weights in mice were increased. In rats, there was a 20% decrease in sperm counts at the two highest dose levels, 1,678 and 3,398 mg/kg/day. Given the absence of specific testicular pathology in either species, the NTP concluded that: *"There was no evidence of adverse effects on the reproductive system of rats or mice"* (NTP 1992, p. 5). This finding is consistent with the bulk of the other studies summarized in Appendix 1-3, in which no adverse effects on the testes are reported, although an increase in testicular weight—relative and absolute—was observed in mice at 3,465–7,220 mg/kg/day (U.S. EPA 1986). The recent study by Yousef et al. (1995) suggests that more serious effects are plausible. Substantial decreases in libido, ejaculate volume, sperm concentrations, semen initial fructose and semen osmolality as well as increases in abnormal and dead sperm were observed in rabbits. All of the effects were statistically significant at $p<0.05$. A limitation of the study is that the authors report the doses as 0.1 and 0.01 of the LD_{50} but do not specify the actual doses. Using the reported rabbit LD_{50} of 3,800 mg/kg (Appendix 1-1), the doses correspond to 38 and 380 mg/kg. The toxicological significance of the observed effects described by Yousef et al. (1995) is clear. Nonetheless, in multi-generation reproduction studies (section 3.1.4), no effects on reproductive performance have been observed at dietary levels equivalent to doses of 1,500 mg/kg/day.

Other signs of toxicity seem general and non-specific. A few studies report changes in liver weight, blood chemistry that would suggest mild liver toxicity, or liver pathology (U.S. EPA 1986, NTP 1992, Stout and Ruecker 1990). Changes in pituitary weight have also been observed (Monsanto Co. 1985). Signs of kidney toxicity, which might be expected based on the acute toxicity of glyphosate, have not been reported consistently and are not severe (Monsanto Co. 1987, NTP 1992, U.S. EPA 1986). As summarized by NTP (1992), various hematological changes have been observed but are not considered severe and are attributed to mild dehydration.

Jensen (1989) has published a self-report suggesting that glyphosate exposure has caused immunological and neurological effects that *"... No doctor has been able to accurately diagnose and treat..."* The *in vivo* studies on experimental animals (Appendix 1-3) were not designed specifically to test neurotoxic or immunological effects. Nonetheless, pronounced effects on either the nervous system or immunologic competence could have been detected by these tests secondary to general changes in the health or behavior of the animals. Similarly, the extensive human experience with glyphosate (Appendix 1-2) does not support the assertion that glyphosate is specifically toxic to the immune system or nervous system. Moreover, *in vitro* assays using human immunocompetent cells—natural killer cells and cytotoxic T cells—do not indicate that exposure to glyphosate or Roundup at concentrations ranging from 0.01 to 10 μmoles affects the nervous system or the immune system (Flaherty et al. 1991).

As discussed further in the dose-response assessment (section 3.3), dose levels expressed in mg/kg/day cause comparable effects over broad periods of exposure, which is consistent with the rapid elimination of and lack of toxic metabolites from glyphosate (Brewster et al. 1991, Monsanto Co. 1993a,b, NTP 1992). In the kinetic study conducted by NTP (1992), the elimination of glyphosate followed a two-compartment model with an alpha (distribution) phase of 0.5 hours and a beta (elimination phase) of 13 hours. This finding is reasonably consistent with an oral study in rats, in which whole body elimination followed first order kinetics, with an elimination half-time of approximately 48 hours (Brewster et al. 1991).

3.1.5. Reproductive and Teratogenic Effects. Glyphosate has been subject to two multi-generation reproduction studies as well as three teratology studies (Appendix 1-4). There is no indication from these studies that glyphosate induces teratogenic effects (i.e., birth defects).

In the teratology studies, the observed signs of toxicity—respiratory and gastrointestinal effects—were similar to those observed in acute toxicity studies and occurred at dose levels that were also comparable. In a multi-generation reproduction study in rats (Appendix 1-4, Bio/Dynamics, Inc. 1981b), unilateral focal tubular dilation of the kidney was observed in male F_{3b} pups at 30 mg/kg/day but not at 10 mg/kg/day. In a subsequent study, no such effects were observed at doses up to 1,500 mg/kg/day. As discussed in section 3.3, the U.S. EPA has classified 30 mg/kg/day as the LOAEL and has based the RfD for glyphosate on the 10 mg/kg/day NOAEL for this effect. This effect is consistent with the acute toxicity of glyphosate (see section 3.1.1), rather than a specific reproductive effect.

The dose levels used in the multi-generation reproduction studies are either within the range of the estimated doses in the Yousef et al. (1995) study or substantially greater—38 and 380 mg/kg. As indicated in Appendix 1-4, the multi-generation reproductions studies found no effect on reproductive capacity. The studies, however, did not specifically look at semen or testicular pathology.

3.1.6. Carcinogenicity and Mutagenicity. Information regarding the mutagenicity and carcinogenicity of glyphosate has been reviewed in detail by U.S. EPA (U.S. EPA 1992a, 1993b). Carcinogenicity studies on glyphosate are summarized in Appendix 1-3, and *in vitro* mutagenicity studies are summarized in Appendix 1-5.

Although Roundup has been shown to cause an increase in chromosomal aberrations in a plant (*Allium sp.*) associated with cell abnormalities in spindle fiber (Rank et al. 1993), the screening studies for mutagenicity (Appendix 1-5) are largely negative. None of the *in vivo* studies using mammalian species or mammalian cell lines have reported mutagenic activity (i.e., NTP 1992, Rank et al. 1993). Two studies (Vyse and Vigfusson 1979, Vigfusson and Vyse 1980) report a significant increase in sister chromatid exchanges in human lymphocytes *in vitro*. The authors of these studies conclude from their results that glyphosate is, at most, slightly mutagenic. Based on the weight of evidence of all available studies at the time of the assessments, U.S. EPA (U.S. EPA 1992a, 1993b) concluded that glyphosate is not mutagenic. More recent studies do not provide data that challenges the U.S. EPA assessment.

Tumors have been observed in some of the chronic toxicity studies (Appendix 1-2). As discussed in U.S. EPA (1992a), the studies conducted before 1990 were judged by U.S. EPA as insufficient for evaluating the potential carcinogenicity of glyphosate because the observed responses were equivocal or the dose levels were inappropriate (i.e., the highest dose used was not the maximum tolerated dose). U.S. EPA requested the study by Stout and Ruecker (1990) and judged it to be adequate. Although the study indicated increases in some tumor types (pancreatic islet cell adenomas in low dose male rats, hepatocellular adenomas in male rats, and C-cell adenomas of the thyroid males and females), the effects were not dose related.

The current entry in IRIS, which is U.S. EPA's on-line reference source for risk assessments, for glyphosate indicates that the weight of evidence for the carcinogenicity of the compound is equivocal (U.S. EPA Cancer Assessment Group D: Not classifiable as to human carcinogenicity). This entry was made on October 1, 1993. The classification is also given in U.S. EPA (1992a). The Re-registration Eligibility Decision document on glyphosate (U.S. EPA 1993b, dated September 1993) indicates that the classification has been changed to Group E: Evidence of non-carcinogenicity for humans. This classification is also indicated in U.S. EPA's most recent publication of tolerances for glyphosate (U.S. EPA 1995).

Given the marginal mutagenic activity of glyphosate and the failure of several chronic feeding studies to demonstrate a dose-response relationship for carcinogenicity, the Group E classification is appropriate. There is no indication that glyphosate presents a risk of carcinogenicity to humans.

The potential hazards posed by contaminants in glyphosate or the POEA surfactant are discussed in section 3.1.10.

3.1.7. Irritation and Sensitization. Glyphosate has been shown to cause irritation to the gastrointestinal tract and lungs has been demonstrated in *in vivo* toxicity studies as well as human poisoning incidents (section 3.1.2).

Smith and Oehme (1992) have reviewed most of the published and unpublished studies regarding the irritant effects of glyphosate and glyphosate formulations. As summarized in Appendix 1-6, glyphosate can cause irritation to the skin and eyes. Glyphosate may be classified as a mild to moderate irritant to the skin and eyes. Although glyphosate is an irritant, there are no data indicating that the compound causes sensitization in animals (Auletta 1986a,b) or humans (Maibach 1986). The studies by Branch (1981) and Heenehan (1979a,b) suggest that the surfactant in Roundup may be more irritating than or may enhance the irritant properties of glyphosate. As discussed in section 3.1.3, surfactants are, by definition, surface active agents and may cause general irritation to tissue. As also discussed in Section 3.1.3, Roundup Pro appears to be less active as an eye or skin irritant than Roundup.

Hindson and Diffey (1984a) reported that a formulation of glyphosate used in the United Kingdom, Tumbleweed, could cause photosensitization. The effect, however, was subsequently attributed to an adjuvant, benzisothiazolone (Hindson and Diffey 1984b). Benzisothiazolone is not used in the glyphosate formulations covered by this risk assessment. Based on the study by Maibach (1986) using volunteers, there is no evidence that glyphosate itself causes photoirritation or photosensitization.

3.1.8. Systemic Toxic Effects from Dermal Exposures. As discussed in section 3.2, most plausible exposure scenarios for workers and the general public involve dermal exposure. As summarized in Appendix 1-1, no mortality occurred in experimental mammals exposed to dermal concentrations of glyphosate that exceeded the oral LD_{50}. This suggests that dermal concentrations of glyphosate are absorbed less readily than the oral doses.

Consistent with this relationship between oral and dermal LD_{50} values, the available experimental studies indicate that glyphosate may not be completely absorbed after oral administration and may be very poorly absorbed after dermal applications.

After oral exposure, urinary excretion of glyphosate in rats and rabbits was less than 36% (Smith and Oehme 1992, p. 356). Similar results are reported by Brewster et al. (1991) in a study in which urinary elimination was less than 36%. Since any compound eliminated in the urine after oral administration must have been absorbed, these studies indicate that at approximately 35%, at least, of the glyphosate was absorbed after oral administration. No data are available for assessing the biliary excretion of glyphosate. Nonetheless, given the rapid fecal elimination of glyphosate after oral administration, it is likely that glyphosate is incompletely absorbed from the gastrointestinal tract (Smith and Oehme 1992).

Two dermal absorption studies have been conducted using glyphosate, and both of them indicate that glyphosate is very poorly absorbed across the skin. In an unpublished study by Maibach

(1981), monkeys absorbed <2% of a dermally applied dose over a 7-day period. In the first 24 hours, only 0.4% of the applied dose was eliminated in the urine. This summary seems to be identical to the published study by Wester et al. (1991) of which Dr. Maibach is a coauthor. Using ^{14}C-labelled glyphosate with Roundup (glyphosate and POEA surfactant), *in vitro* absorption across human cadaver skin was <2% when applied at 0.5 to 154 μg/cm^2 of skin. *In vivo* percutaneous absorption in monkeys was 0.8±0.6% at a dose of 25 μg/cm^2 and 2.8±0.8% at a dose of 270 μg/cm^2. Very little binding to skin was noted. The glyphosate/surfactant mixture did not partition into powdered human stratum corneum ($\leq$0.05% after 24 hours in 1:20 or 1:32 dilutions). *In vivo*, washing with soap and water effectively removed 90±4% of the applied dose in monkeys.

Similar results are reported by Dirks (1983a) in an unpublished summary of a study by Dr. Thomas Franz at the University of Washington. Monkeys absorbed approximately 1.8% of glyphosate over a 7-day exposure period. The maximum daily absorption rate was approximately 0.4%.

Although the dermal absorption of glyphosate appears to be much less than the oral absorption and *in vivo* dermal toxicity studies have not produced signs of systemic toxicity in experimental mammals or humans, the potential significance of dermal absorption cannot be disregarded and this route is considered in the exposure assessments for workers and the general public (section 3.2).

3.1.9. Inhalation Exposures [including Brown-and-Burn Operations]. There is very little information regarding the inhalation toxicity of glyphosate. Because of the low volatility rate for glyphosate (Table 2-1), the U.S. EPA waived the requirement of an acute inhalation study in the re-registration of this compound (U.S. EPA 1993b, p. 10).

Monsanto sponsored an unpublished study regarding the inhalation toxicity of glyphosate, which is summarized in Smith and Oehme (1992). Sprague-Dawley rats were exposed to Roundup as a one-third dilution at 0.05–0.36 mg/L of air 6 hours/day, 5 days/week for 22 days. The only effects noted were irritation to the nasal turbinates, trachea, and lungs. This finding is consistent with the effects observed after acute oral exposures (see section 3.1.2). The inhalation LC_{50} of Roundup Pro in rats is about 4.2 mg/L. As with Roundup, Roundup Pro caused irritation to the nasal turbinates (Kirk 1993c).

One published study (Jamison et al. 1986) has suggested a potential affect associated with glyphosate after inhalation exposures to flax dust. In this study, human volunteers were exposed to two different types of flax dust: one derived from glyphosate treated flax and the other derived from flax not treated with glyphosate. The glyphosate treated flax consistently caused a greater depression in respiratory function than the glyphosate treated flax dust. As noted by the authors, the glyphosate was applied to the flax six weeks prior to testing and it is likely that there was very little glyphosate residue on the flax. The authors also note that particles size distribution of the

two dusts used in the study was not significantly different. Based on particle size distribution data presented in this publication (Hamison et al. 1986, Table 1, p. 810), however, the glyphosate treated flax dust contained about 25% more particles in the 0-1µ range. Particles in this range typically penetrate to the alveolar sacs (Razman and Klassen 1996). Thus, even though the distributions in the particle sizes for the two forms of flax may not be statistically significantly different, the higher concentration of respirable particles in the glyphosate treated flax may be contributed to the apparent difference in biological activity.

Although inhalation of glyphosate is not a typical route of exposure, it may occur during brown-and-burn operations. As discussed in section 2.3 on application methods, brown-and-burn operations are conducted 45–180 days after treatment with the herbicide. As discussed by Bush et al. (1987), the combustion of several herbicides does not result in exposure to toxic air concentrations of herbicides. These investigators, however, did not look specifically at glyphosate and did not take toxic combustion products into consideration. Dost (1986) discusses the general problem of exposure to PAH from the combustion of wood or other vegetation but does not discuss the formation of toxic combustion products from the combustion of herbicides.

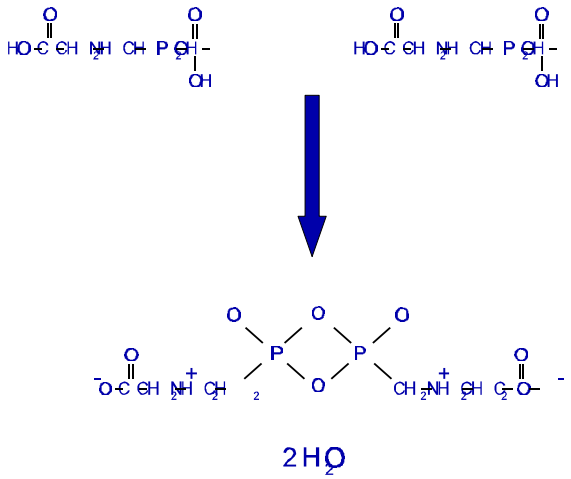


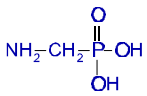
Figure 3-2: Polycondensate formed during incineration of glyphosate (redrawn from Smith and Oehme 1992).

The thermal degradation of glyphosate has been studied by Flora and Simon (1981). During combustion at temperatures ranging from 200°C to 240°C, glyphosate forms a polycondensate, as illustrated in Figure 3-2. This range of temperatures is typical of slow combustion but is far less than the 800–1,000°C temperatures of an actively burning wood stove or fireplace (Bush et al. 1987).

No information is available regarding the toxicological properties of the combustion product shown in Figure 3-2. Furthermore, no information is available regarding the identity or toxicity of other potential combustion products. As discussed in section 3.4, this lack of data limits the ability to characterize the potential hazard of brown-and-burn operations involving glyphosate.

3.1.10. Impurities and Metabolites.

3.1.10.1. Aminomethylphosphonate (AMPA) **--** The primary metabolite of glyphosate in mammals and other organisms is aminomethylphosphonate (AMPA):



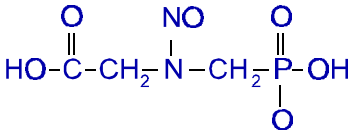
AMPA

which is formed together with glyoxylate (HCO-COOH). In mammals, only very small amounts of AMPA, less than 1% of the absorbed dose, are formed (U.S. EPA 1992a, Brewster et al. 1991). Relatively little is known about the biological activity of this compound. AMPA is poorly absorbed from the gastrointestinal tract (about 20%) and is eliminated primarily in the urine (Smith and Oehme 1992).

The relative paucity of information on this metabolite does not have a significant impact on this risk assessment. The toxicity studies on which the hazard identification and subsequent dose-response assessment are based involve *in vivo* exposure to glyphosate and presumably the subsequent formation of and exposure to AMPA. Therefore, the toxicological effects, if any, of the formation of AMPA are likely to be captured by animal toxicology studies involving whole-body exposure to glyphosate.

The approach of examining the potential importance of the metabolites of a chemical agent is common in the risk assessment of xenobiotics, which generally involve the formation of one or more metabolites, some of which may be more toxic than the parent compound. Usually, the parent compound is selected as the agent of concern because the toxicology studies and monitoring studies provide information about the agent. Thus, the dose metameter for the risk assessment is most clearly expressed as the parent compound. In cases where a toxic metabolite is known to be handled differently by humans, this simple approach may be modified. There is no indication that such a modification is necessary for glyphosate.

3.1.10.2. N-nitrosoglyphosate (NNG) -- Glyphosate also contains N-nitrosoglyphosate (NNG) as an impurity:



NNG

Nitroso compounds are characterized by the *N=O* group, a double bond between a nitrogen and oxygen. Nitrosamines are nitroso compounds in which the nitroso group is attached to a nitrogen atom, *N-N=O*. NNG contains the nitrosoamine group. Certain groups of nitrosoamines have served as model compounds in some of the classical studies on chemical carcinogenicity (e.g., Druckrey 1967). While there is a general concern for the carcinogenic potential of nitroso

compounds, the contribution of specific nitroso compounds to carcinogenic risk is difficult to quantify (Mirvish 1995).

The EPA re-registration document for glyphosate states:

> *Technical grade glyphosate contains N-nitrosoglyphosate (NNG) as a contaminant. Carcinogenicity testing of nitroso contaminants is normally required only in those cases in which the level of nitroso compounds exceeds 1.0 ppm. Analyses showed that greater than 92% of the individual technical glyphosate samples contained less than 1.0 ppm NNG. The Agency concluded that the NNG content of glyphosate was not toxicologically significant.*

The rationale for this policy decision is unclear. Cancer risk is normally expressed as a product of exposure or dose with potency, where potency is expressed in units that are the reciprocal of the exposure or dose. For example, if the potency of a carcinogen is 0.01 $(mg/kg/day)^{-1}$, the risk associated with exposure to 0.2 mg/kg/day is two in one thousand or one in five hundred [$0.01 \cdot 0.2 = 0.002$]. From this relationship, it follows that a carcinogen with a potency of 1 $(mg/kg/day)^{-1}$ present as a contaminant at 0.1% presents a greater hazard than a carcinogen with a potency of 0.01 $(mg/kg/day)^{-1}$ present at a concentration of 5% [$1 \cdot 0.001 > 0.05 \cdot 0.01$ or $0.001 > 0.005$]. Thus, while this policy decision may be consistent with EPA's mandate, it has little impact on this hazard identification.

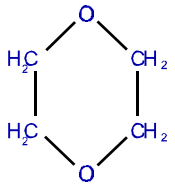
Monsanto has conducted an apparently extensive series of tests on NNG. A summary of the studies is available (Roundup Herbicide Information Sheet October, 1986 - R, entitled N-Nitrosoglyphosate), Monsanto). This summary states:

> *A series of studies were conducted by a number of separate laboratories to evaluate the health characteristics of N-nitrosoglyphosate. The results of these studies indicate that NNG is relatively non-toxic, is rapidly excreted without undergoing any chemical change, does not bioaccumulate, is not mutagenic and does not cause birth defects or cancer in laboratory test species.*

Details of these studies have not been published and have not been reviewed as part of this risk assessment. Analyses of mutagenicity and carcinogenicity studies are subject to wide variation based on the interpretation of the data and evaluation of the study design; nonetheless, the summary quoted above is taken as presumptive evidence that NNG is not likely to pose a significant hazard that would not be encompassed by the available toxicity studies on glyphosate. Thus, NNG will be treated similarly to AMPA in this risk assessment.

An additional concern with NNG, however, is the potential formation of NNG from glyphosate under ambient conditions in the environment. This possibility has been examined by Young and Khan (1978). NNG can be formed from glyphosate by nitrosatation involving a third-order process. The maximum reaction occurs at 20°C and a pH of 2.5 with a rate of 2.43 M^{-2} sec^{-1}. At a pH <5, typical of most ambient conditions, the reaction rate is negligible. Based on this analysis, the formation of NNG from reactions with soil or water nitrates is likely to be negligible. This is consistent with the field studies that failed to detect NNG in soil with high levels of nitrate nitrogen (2 ppm) and glyphosate (5 ppm) (Khan and Young 1977, Khan 1981). This information together with the Monsanto summary indicates that NNG is not a substantial concern for the current risk assessment.

3.1.10.3. 1,4-Dioxane -- 1,4-Dioxane, is a contaminant in POEA. U.S. EPA (1992b) considers dioxane to be a carcinogen, Class B2: Probable human carcinogen and has derived a cancer potency factor (referred to by U.S. EPA as a slope factor) of 0.011 $(mg/kg/day)^{-1}$. This assessment has been reviewed by and is in concordance with the analysis by the Agency for Toxic Substances and Disease Registry (DeRosa et al. 1996). As indicated in section 2, the dioxane is present in Roundup at a level of approximately 0.03% (Monsanto Co. 1990) or 300 mg/L (300 ppm). This is about a factor of 0.00084 [300 mg/L ÷ 356,000 mg/L] less than the level of glyphosate in Roundup.



As discussed in sections 3.1.3. and 3.1.4, data regarding the chronic toxicity of Roundup or POEA are not sufficient for determining whether these mixtures are carcinogenic. Even if negative studies were available, however, it could be argued that the carcinogenic activity of dioxane was masked or diluted by the herbicide or other components in the surfactant. In an update to the last Forest Service risk assessment on glyphosate (USDA 1989a,b,c) (Heydens 1989 appended to Borrecco and Neisess 1991), it was demonstrated that the upper limit of risk associated with contamination was extremely low (e.g., $<1 \cdot 10^{-7}$). The cancer potency factor used in the previous risk assessment was 0.0076 $(mg/kg/day)^{-1}$, almost the same as the value currently recommended by U.S. EPA (i.e., both round to 0.01). A brief update of the previous risk assessment is presented in section 3.4.

Borrecco and Neisess (1991) derived toxicity based criteria for 1,4-dioxane and use the information to calculate margins of safety for exposure to 1,4-dioxane. According to the available toxicity data, dioxane does not present unique toxic effects; therefore, its toxicity is likely to be encompassed by the available toxicity data on Roundup.

3.2. EXPOSURE ASSESSMENT

3.2.1. Overview. Two general exposure assessments are presented in this section, job-specific assessments and incident assessments. Job-specific assessments estimate absorption associated with relatively complex job activities, such as mixing, loading, or applying glyphosate, in which multiple routes of exposure are likely. All of these assessments are given as a range based on the projected application rates, empirical observations of variability in exposure rates, and projected variations in herbicide usage [i.e., number of acres treated/hour].

Incident assessments are relatively easy to make. They estimate absorption from spilling a solution onto the skin or wearing contaminated clothing. All of these scenarios are extreme or accidental in nature, as discussed in sections 3.2.2 and 3.2.3.

Workers are exposed to far more glyphosate and other components in glyphosate than are members of the general public. Workers involved in aerial applications are exposed to glyphosate at a lesser rate than ground workers in terms of exposure per amount of material handled; however, the gross exposure is greater for workers involved in aerial applications because of the large quantity of material that they may handle. The average exposure for workers involved in aerial applications is 0.014 mg/kg body weight with a range of 0.0016–0.16 mg/kg body weight. Boom spray workers may have comparable levels of exposure [0.013 (0.0016–0.11) mg/kg], and other ground workers are exposed to much less [0.006 (0.0005–0.072) mg/kg]. Members of the general public are likely to be exposed only to very low levels of glyphosate [0.00012–0.007 mg/kg], except in the case of accidental exposure when levels may approach those of occupational exposure levels [0.007–0.019 mg/kg].

3.2.2. Workers.

3.2.2.1. ***Job Categories*** **--** As outlined in the program description (see section 2), this risk assessment is concerned with aerial applications and three types of ground applications: backpack, cut surface, and boom spray. As discussed in SERA (1995a), occupational exposure generally involves inhalation and dermal exposure, with the dermal route generally contributing far more to exposure than the inhalation route. Several studies have been conducted in which the absorbed dose can be estimated as a function of the amount of material handled and the chemical specific exposure factors, which are expressed as mg agent/kg bw · lb a.i handled.

Much the literature regarding occupational exposure rates involves exposure to 2,4-D (SERA 1993, 1995b). For ground applications of 2,4-D, plausible estimates and ranges of exposure rates are $9.6x10^{-5}$ ($4.9x10^{-6}$ to $1.9x10^{-3}$) mg/kg/lb a.i. for roadside hydraulic spraying and $1.4x10^{-3}$ ($4.4x10^{-5}$ to $4.2x10^{-2}$) mg/kg/lb a.i. for cut surface, streamline, and directed foliar applications (see Table 3-2 in SERA 1995b).

Generally, exposure rates for workers involved in aerial applications are much less than those for ground workers (SERA 1993). For 2,4-D, exposure rates ranging from $2x10^{-5}$ to $4x10^{-5}$ mg/kg/lb a.i. are typical for pilots and mixer/loaders. Exposure rates for flaggers are only about 1–2% of

those for pilots and mixer/loaders. As with ground workers, exposure rates for workers involved in aerial applications vary widely, with the upper and lower limits of exposure spanning about an order of magnitude (SERA 1993, see Table 11). Thus, for workers involved in the aerial application of 2,4-D, except for flaggers, a typical rate with plausible ranges for exposure would be $3x10^{-5}$ ($3x10^{-6}$ to $3x10^{-4}$) mg/kg/lb a.i.

The rate of dermal absorption of glyphosate seems to be less than that of 2,4-D. In humans, approximately 5.8% of a dermal dose of 2,4-D was eliminated in the urine over a 5-day observation period (Feldman and Maibach 1974). As summarized in section 3.1.7, monkeys eliminated only 0.8–2.8% of the applied dose of glyphosate over a 7-day observation period. Consequently, the dermal absorption of glyphosate seems to about 33% of the dermal absorption of 2,4-D, and the occupational exposure rates for glyphosate also should be about 33% of those for 2,4-D. Two occupational exposure studies on glyphosate (Lavy et al. 1992, Jauhiainen et al. 1991) are useful for assessing the adequacy of this supposition.

The Lavy et al. (1992) involves the exposure of nursery workers to glyphosate. Workers applied Roundup to small weeds in a nursery bed by placing a 290 mL (2.5x3.5 cm) cylindrical metal shield surrounding the spray nozzle over the weed—to protect adjacent conifer seedlings—and then spraying the weed with Roundup. Using passive monitoring (i.e., hand washes, skin patches, and clothing patches) and assuming a dermal absorption rate of 1.8% (Dirks 1983b), exposure rates ranging form $5.8 \cdot 10^{-4}$ to $2.8 \cdot 10^{-2}$ mg/kg bw/kg a.i. were estimated for 11 workers (Lavy et al. 1992, Table 3, p. 10). The geometric mean of these estimates was $2.9 \cdot 10^{-3}$ mg/kg bw/kg a.i. Converting the mean estimate to units of mg/kg bw/lb a.i [by multiplying by 0.4536 kg/lb] yields $1.3 \cdot 10^{-3}$ ($2.6 \cdot 10^{-4}$ to $1.27 \cdot 10^{-2}$) mg/kg bw/lb a.i. These estimates are very close to the estimated rates for ground applications (excluding roadside hydraulic spraying) of 2,4-D summarized above: $1.4x10^{-3}$ ($4.4x10^{-5}$ to $4.2x10^{-2}$) mg/kg/lb a.i.

In addition to estimates based on passive monitoring, Lavy et al. (1992) also conducted biological monitoring by collecting complete 5-day urine samples. In a total of 355 urine samples, no glyphosate was detected (limit of detection = 0.01 μg/g). As discussed by Lavy et al. 1992 (p. 11, column 1), this suggests that the exposure rates based on passive monitoring may overestimate true exposure.

The magnitude of this overestimate can be examined further by reassessing the data on the most exposed individual. This individual (worker #4) weighed 63.5 kg and handled, on average, 0.54 kg [1.18 lbs] of glyphosate per day. Assuming that the concentration of glyphosate in the urine was just below the limit of detection of 0.01 μg/g or approximately 0.01 μg/mL and assuming a relatively high urinary output of 2,000 mL (Taylor 1988), the total absorbed dose would be 20 μg or 0.02 mg. This corresponds to 0.0003 mg/kg bw [0.02 mg ÷ 63.5 kg] and $2.5 \cdot 10^{-4}$ mg/kg bw/lb a.i. handled [0.0003 ÷ 1.18]. The rate for this individual estimated by passive monitoring was approximately 40 times greater: $2.8 \cdot 10^{-2}$ mg/kg bw/kg a.i. (Lavy et al. 1992, Table 3, last column, third entry down) or $1.1 \cdot 10^{-2}$ mg/kg bw/lb a.i.

In the study by Jauhiainen et al. (1991), biological monitoring was conducted on five workers using Roundup in *brush saw spraying*. This activity seems comparable to selective foliar applications using a backpack or cut surface treatments. Each worker handled an average of 9.8 L of an 8% solution of Roundup. The amount of glyphosate handled each day was approximately 0.279 kg,

$$9.8 \text{ L} \cdot 0.08 \cdot 0.356 \text{ kg/L}$$

(Jauhiainen et al. 1991, p. 62, column one, top of page).

Urine samples [not total daily urine] were collected at the end of each work day for 1 week during the application period, and one sample was taken 3 weeks after the applications. The urine samples were assayed for glyphosate using gas chromatography/electron capture with a limit of detection of 0.1 ng/μL or 0.1 mg/mL. No glyphosate was detected in any of the urine samples using this method. One urine sample—not otherwise described—was assayed for glyphosate by gas chromatography/mass spectroscopy (GC/MS), and glyphosate was detected at a level of 0.085 ng/μL, which is equivalent to 0.085 μg/mL. Again assuming a relatively high rate of urine output to provide an upper limit of the absorbed dose, assuming that this urine sample was representative, and using the default body weight of 70 kg (U.S. EPA 1989a), the absorbed dose would be 0.17 mg or 170 μg [0.085 μg/mL · 2,000 mL] or 0.0024 mg/kg [0.17 mg ÷ 70 kg]. The corresponding exposure rate would be 0.0086 mg/kg bw · kg a.i. [0.0024 mg/kg bw ÷ 0.279 kg a.i.]. This value corresponds to approximately $9 \cdot 10^{-3}$ mg/kg bw · kg a.i. and is about a factor of 5 less than the upper limit of the 2,4-D exposure rate (4.2×10^{-2} mg/kg bw · kg a.i.) and relatively close to the calculated dose of $2.5 \cdot 10^{-4}$ mg/kg bw/lb a.i. for the most exposed individual in the Lavy et al. (1992) study. This estimate is also consistent with the available data indicating that the absorption of glyphosate (0.8-2.4%) is about a factor of five less than that of 2,4-D (5.6%).

Because of these relatively consistent relationships, the rates of $2.5 \cdot 10^{-4}$ mg/kg bw/lb a.i. to $9 \cdot 10^{-3}$ mg/kg bw · lb a.i. will be taken as a plausible range of exposure rates for glyphosate, with 0.0015 mg/kg bw/lb a.i.—the geometric mean of this range—used as the central estimate. This range will be applied to backpack and cut surface treatments in this risk assessment.

By analogy to 2,4-D exposure rates on roadside hydraulic spraying (summarized above), rates used for boom spray treatments will be taken as a factor of 0.07 less ($9.6 \times 10^{-5} \div 1.4 \times 10^{-3}$). Thus, for this activity the exposure rates will be estimated as 0.00011 ($1.8 \cdot 10^{-5}$ to $6.3 \cdot 10^{-4}$) mg/kg bw/lb a.i.

There are no data regarding the exposure workers involved in the aerial application of glyphosate. As with ground boom spray treatments, application rates for aerial workers will be estimated as the rate for other ground workers applying glyphosate, $1.5 \cdot 10^{-3}$ ($2.5 \cdot 10^{-4}$ - $9 \cdot 10^{-3}$) mg/kg bw/lb a.i. multiplied by the ratio of the central estimates of the 2,4-D rates for aerial application (3×10^{-5} mg/kg bw/lb a.i.) divided by the 2,4-D rate for ground workers (other than roadside hydraulic

spraying), $1.4x10^{-3}$ mg/kg bw/lb a.i. Using this ratio of 0.02, the rates for workers involved in aerial applications is taken as $3 \cdot 10^{-5}$ ($5 \cdot 10^{-6}$ - $2 \cdot 10^{-4}$) mg/kg bw/lb a.i.

Table 3-1. Quantitative summary of occupational exposure to glyphosate, excluding accidental or incidental exposure

Treatment method	Treatment rate (acres/hour)	Exposure rate (mg/kg/lb a.i.)	Daily dose (mg/kg bw)[a]
Boom spraying	15 11-21	$1.1 \cdot 10^{-4}$ $1.8 \cdot 10^{-5}$ - $6.3 \cdot 10^{-4}$	0.013 0.0016-0.11
Backpack and cut surface	0.5 0.25-1	$1.5 \cdot 10^{-3}$ $2.5 \cdot 10^{-4}$ - $9 \cdot 10^{-3}$	0.006 0.0005-0.072
Aerial applications (pilots and mixer/loaders)	60 40-100	$3 \cdot 10^{-5}$ $5 \cdot 10^{-6}$ - $2 \cdot 10^{-4}$	0.014 0.0016-0.16

[a]Assuming an application rate of 1 lb/acre and an 8-hour work day.

All of the above application rates are summarized in Table 3-1. In this table, plausible levels of exposure for ground and aerial applications are estimated as the product of the typical application rate currently used by the Forest Service (1lb/acre), the area treated per hour (acres treated/hour by a worker), and the exposure rate (mg/kg bw/lb a.i.). All calculations assume that the worker applies the product for 8 hours/day. This is a reasonably conservative estimate for workers on an extended 10-hour day but an overestimate for workers on a standard 8-hour day. This potential overestimate is a relatively minor factor, given the variability in exposure rates among individuals.

Estimated daily doses are presented as a central value and a range. The central value is based on the approximate geometric mean of the anticipated range of treatment rates and mean exposure rate. The lower range of the daily dose is based on the lower range of the treatment rates and the lower range of the exposure rate. The upper range of the daily dose is based on the upper range of treatment rates and the upper range of the exposure rate.

There is a linear relationship between exposure the application rate. As discussed in section 2.4, the Forest Service may use lesser or greater application rates. The consequences of differing rates of application are discussed in the risk characterization (section 3.4).

3.2.2.2. ***Immersion or Contaminated Clothing*** **--** Incidental occupational exposure may occur from improper handling or use of the herbicide, or from accidental contamination of the skin or clothing by a spill. All of these scenarios can be modelled using Fick's first law. As

discussed in Durkin et al. (1995), scenarios that use Fick's first law require an estimate of the permeability coefficient, K_p, expressed in cm/hour. There is not an experimentally determined K_p value for glyphosate in the available literature. Based on structure-activity relationships (U.S. EPA 1992c), a K_p of $1.2 \cdot 10^{-6}$ cm/hour for an aqueous solution of glyphosate can be calculated from the following equation:

$$\log K_p = -2.7 + 0.71 \log K_{o/w} - 0.0061\, MW \qquad \textbf{(3-4)}$$

where $\mathbf{K_{ow}}$ is the octanol water partition coefficient and **MW** is the molecular weight. For glyphosate, the molecular weight is 169.07 (see Table 2-1). The K_{ow} for glyphosate varies with pH (Chamberlain et al. 1994). At a neutral pH, the log of the K_{ow} is approximately -2.9 (see Table 2-1), and this value is used to estimate the dermal penetration rate (K_p). Based on equation 3-4, the estimated K_p for glyphosate is $1.2 \cdot 10^{-6}$ cm/hour.

The commercial formulations of glyphosate covered by this risk assessment contain glyphosate at levels of 360 g/L (Accord and Roundup) and 480 g/L (Rodeo) (see Table 2-2). The water solubility of glyphosate, however, is only 12 g/L (see Table 2-1). This apparent inconsistency is related to the effect of pH on water solubility. In general, ionizable molecules are more soluble in water when they are in an ionized state. As indicated in Figure 2-1, glyphosate is a mixture of H_2G^{-1} and HG^{-2} at a neutral pH. Thus, as the undissociated form of glyphosate, H_4G^{+} is added to water it disassociates to H_2G^{-1} and HG^{-2} with the release of charged protons, H^{+}, causing an increase in the pH. This, in turn, limits the solubility of glyphosate in water. The commercial formulations of glyphosate, however, use the isopropylamine salt ($(CH_3)_2CHNH_3^{+}$) of glyphosate. The salt essentially acts as a buffer, maintaining the pH of the solution as more glyphosate is added. Thus, for the isopropylamine salt of glyphosate as for salts of ionizable organics in general, the salt formulation has a much higher solubility in water than the nominal value for the molecule. For this risk assessment, the nominal concentrations of the acid equivalents of glyphosate are used for the exposure assessment. Because the isopropylamine salt is almost completely dissociated in water, the K_p for glyphosate, as derived in the previous paragraph, is used to estimate absorption.

During the handling process, an individual may immerse a part of the body into the formulation for a short time, either through mischance or imprudent handling. The worst case scenario would involve a worker who places both hands in the concentrated formulation of Rodeo (480 g a.e./L). For this risk assessment, the surface area of the hands will be estimated at 0.084 m^2 (U.S. EPA 1992c). Concentrations of 480 g/L are equivalent to 480 mg/mL, which, in turn, is equivalent to 480 mg/cm^3.

For this scenario, the estimated absorbed dose, using Fick's first law, is approximately 0.00012 mg/kg

$$1.2 \cdot 10^{-6} \text{ cm/hour} \cdot 480 \text{ mg/cm}^3 \cdot 1/60 \text{ hour} \cdot 840 \text{ cm}^2 \div 70 \text{ kg}.$$

Estimated doses for other immersed areas and durations can be calculated in a similar way. If, however, the scenario involves contaminated clothing (e.g., the chemical spilled inside of gloves), which might be worn for a long time, absorbed doses could be much higher. For example, contaminated gloves worn for 1 hour would lead to an exposure 60 times greater than that described for the immersion scenario [i.e., 0.0069 mg/kg].

Much less severe and probably more typical scenarios could be derived. With the exception of individuals involved in preparing field mixtures (i.e., mixer/loaders) most individuals will not come into contact with undiluted commercial formulations. As discussed in the risk characterization (section 3.4) the worst-case scenarios described above do not approach exposures of concern. Consequently, there would be little purpose in deriving the more typical exposure scenarios for immersion or contaminated clothing.

3.2.2.3. ***Accidental Spills*** **--** In accidental spill scenarios, it is important to estimate the amount of liquid adhering to the surface of the skin. In one study, as much as 4 mg liquid/cm^2 of skin surface was retained on hands removed immediately from beakers containing water or ethanol (Mason and Johnson 1987). When beakers containing light paraffin oil were used, approximately twice this amount was retained. In most instances, using these values should result in a plausible upper estimate of retention because chemical loss from the skin surface due to moving or washing are not considered. Thus, the amount of chemical transferred to the skin after a spill may be calculated as:

$$D_{Skin} = RF \cdot P \cdot A \quad \textbf{(3-5)}$$

where:

D_{Skin} = dose remaining on surface of skin (µg)
RF = retention factor (µg/cm^2) (for example, 4,000–8,000µg/cm^2)
P = proportion of agent in the liquid
A = skin area exposed (cm^2)

Any person handling a concentrated formulation or located near the area where the handling takes place may be subject to an accidental spill. This is different from immersion in that most of the liquid will run off the surface of the skin immediately after the spill unless the material is kept in contact with the skin by saturated clothing. If the clothing is saturated, the scenario outlined above applies. If the chemical spills on the skin but is not kept in contact with the skin, the exposure will be much less.

Consider the effects of spilling glyphosate over the lower legs. The surface area of the lower legs is taken as 2,070 cm^2 (U.S. EPA 1992c). The upper limit of the amount of liquid adhering to the surface of the skin is taken as 8 mg/cm^2 of skin (Mason and Johnson 1987). Assuming a density of 1.0 for the aqueous solution, this is equivalent to 0.008 mL/cm^2. Hence, the volume of liquid adhering to the skin is 16.56 mL [2070 cm^2 · 0.008 mL/cm^2]. For concentrations of 360-480 mg/mL, the amount of glyphosate adhering to the skin can be estimated as approximately 6,000–8,000 mg [16.56 mL · 360–480 mg glyphosate/mL].

To estimate the absorbed dose, some estimate of absorption rate as percent of applied dose/hour is necessary. No human data are available regarding the absorption of glyphosate. Based on the study by Wester et al. (1991), absorption rates of 0.8–2.8% over a 7-day observation period were observed. The maximum absorption rates per day were 0.45–1% and occurred on the first day of exposure. These rates correspond to rates of 0.0002–0.0004 h^{-1} if the daily rates are simply divided by 24.

Assuming that the skin is washed thoroughly after 1 hour ,the absorbed dose can be estimated as 0.017–0.046 mg/kg

$$6{,}000\text{–}8{,}000 \text{ mg} \cdot 0.0002\text{–}0.0004 \text{ h}^{-1} \div 70 \text{ kg}.$$

These exposures assume a contaminated skin surface of 2070 cm^2. The exposure estimate using Fick's first law in section 3.2.2.2. involves a surface area of 840 cm^2 and yields estimated absorbed doses of 0.00012 mg/kg for a 1-hour exposure. Using the absorption rates of 0.0002–0.0004 h^{-1} and a surface area of 840 cm^2, the estimated doses are 0.007–0.019 mg/kg. Thus, for comparable exposure conditions, the method based on simple absorption rates yields estimates that are approximately 60–160 times greater than estimates based on Fick's first law.

This discrepancy may be similar to the one noted by Lavy et al. (1992) in the worker exposure study. Based on the absorption rate of 1% used in that study, detectable levels of glyphosate should have been, but were not, present in the urine of workers. An explanation discussed by the investigators is that the absorption rates in monkeys may be less than the absorption rate in humans. The structure activity relationships proposed by U.S. EPA (1992c) are intended to estimate the K_p in humans. The lower estimates of absorbed dose based on Fick's first law are consistent with Lavy's speculation. As discussed in section 3.5, this uncertainty has relatively little impact on this risk assessment because none of these estimates of absorbed dose approach a level of toxicological concern.

3.2.3. General Public.

3.2.3.1. ***Scenarios and Assumptions*** **--** Under normal conditions, members of the general public should not be exposed to substantial levels of glyphosate. During application, members of the general public are excluded from treatment areas. In cases of accidental spills, exclusion zones are established and members of the general public are not permitted to enter the area.

Nonetheless, any number of exposure scenarios could be constructed for the general public, based on varying assumptions concerning application rates, dispersion, canopy interception, and human activity. For this risk assessment, several very conservative scenarios are developed. As discussed below, most of these scenarios should be regarded as extreme, some to the point of limited plausibility.

Many of the exposure scenarios for the general public involve a child. This is because the relationships of surface area and consumption rates to body weight result in estimated doses (mg agent/kg body weight) for young children that are higher than those for adults (U.S. EPA 1989a). Consumption-specific values are taken from U.S. EPA (1989a,b). The chemical-specific assumptions for glyphosate are the same as those used for workers.

Dermal exposure scenarios that involve children use the same set of assumptions: the child is 2- to 3-years old, weighs 10–11 kg, and has a total body surface area of 0.6 m^2 or 6,000 cm^2 for a body weight of 11 kg (U.S. EPA 1992c). For most scenarios, the child is assumed to be naked, maximizing the surface area of the body in contact with the chemical. In all cases, there are linear relationships among the exposed surface area of the body, the estimated absorbed dose, and the subsequent risk.

3.2.3.2. ***Direct Spray*** **--** For this exposure scenario, it will be assumed that a naked child is sprayed directly with glyphosate, during right-of-way maintenance, with a hydraulic sprayer. Assuming that the child is completely covered (that is, 100% of the surface area of the body is exposed). The highest spray solution recommended for any of the commercial formulations is 10% (Roundup, USDA 1995) which corresponds to 36 g (a.e.)/L or 36 mg (a.e.)/mL. Thus, the dose deposited on the child will be 1,728 mg

$$0.008\ mL/cm^2 \cdot 36\ mg/mL \cdot 6{,}000\ cm^2$$

Taking the range of absorption rates of 0.0002–0.0004 h^{-1} and assuming that the child is washed completely 1 hour after being sprayed, the absorbed dose is estimated as approximately 0.031–0.061 mg/kg,

$$1{,}728\ mg \cdot 0.0002–0.0004\ h^{-1} \div 11\ kg.$$

For a young woman, it will be assumed that the feet and legs [2,915 cm^2] are sprayed directly with a 36 mg/L solution of glyphosate. The dose deposited on the woman would be approximately 840 mg,

$$0.008\ mL/cm^2 \cdot 36\ mg/mL \cdot 2915\ cm^2.$$

Assuming a 1-hour exposure period, the absorbed dose is estimated as approximately 0.0026–0.0053 mg/kg,

$$840\ mg \cdot 0.0002–0.0004\ h^{-1} \div 64\ kg.$$

As discussed in section 3.2.2.3., these doses are probably overestimated because of the reliance on data regarding absorption rates in monkeys. While other less severe scenarios could be created, the dose estimates from the very conservative scenarios described above, using very

conservative exposure assumptions, do not result in exposure levels of concern, as discussed in section 3.4.

3.2.3.3. ***Dermal Exposure from Contaminated Vegetation*** **--** In this exposure scenario, it is assumed that the herbicide is sprayed at a given application rate and that an individual comes in contact with sprayed vegetation or other contaminated surfaces at some period after the spray operation. As discussed in Durkin et al. (1995), some estimate of dislodgeable residue of the herbicide must be available. This information is not available for glyphosate. Moreover, empirical estimation methods for relating dislodgeable residue to application rates and chemical/physical properties have not been published.

Immediately after the spray application, levels of exposure may approximate those involving contact with direct spray, as estimated above. Generally, after the liquid carrier dries, exposure levels are expected to decrease. For example, in a study by Harris and Solomon (1992), 2,4-D was applied to turf at a nominal rate of 11 μg/cm^2. Immediately after the liquid carrier dried, the dislodgeable residue of 2,4-D was 0.92 μg/cm^2, about a factor of 10 less than the nominal rate.

As discussed above, the typical application rate for glyphosate is 1 lb a.i./acre or approximately 0.0112 mg a.i./cm^2 (11.2 μg a.i./cm^2). This application rate corresponds to glyphosate levels of about 8.4 μg a.e./cm^2, which is relatively close to the foliar deposition rates observed by Thompson et al. (1994) after glyphosate applications of approximately 1 lb a.i./acre (15–30 μg a.e./cm^2). If the dislodgeable residue for glyphosate follows a pattern similar to that of 2,4-D, the dislodgeable residue immediately after the liquid carrier dries will be approximately 0.00084 mg/cm^2 or approximately 1 μg/cm^2. Following the methods provided by Durkin et al. (1995, equation 4, p. 68), the transfer rate would be about 1.1 μg/(cm^2·hour) [$10^{0.05}$]

$$[(1.09 \cdot \log(1\ \mu g/cm^2)] + 0.05 = 0.05.$$

The exposed dose for an individual, wearing shorts and a short-sleeved shirt, in contact with the contaminated vegetation for 1 hour would be 5,830 μg or approximately 5.8 mg

$$1.1\ \mu g/(cm^2 \cdot hour) \cdot 5{,}300\ cm^2 \cdot 1\ hour.$$

Taking the dermal absorption rates of 0.0002–0.0004 h^{-1}, which are equivalent to 0.005–0.01 day^{-1}, and assuming a 64 kg body weight for a young woman, the absorbed dose would be 0.0005–0.0009 mg/kg

$$5.8\ mg \cdot 0.005–0.01\ day^{-1} \cdot 1\ day \div 64\ kg.$$

These estimated doses should be regarded as crude approximations at best. There are no analyses regarding the relationship between application rates and dislodgeable residues in the literature. Nonetheless, the estimated doses associated with 1 hour of contact with contaminated vegetation (0.0005–0.0009 mg/kg), are much less than the estimated doses associated with accidental

exposure to direct spray (0.0026–0.0053 mg/kg) (see section 3.2.3.2), and this relationship appears to be reasonable.

3.2.3.4. ***Contaminated Water*** **--** Water can be contaminated from runoff, leaching from contaminated soil, from a direct spill, or unintentional contamination from aerial applications. Although glyphosate is chemically stable in pure aqueous solutions (Anton et al. 1993), it is degraded relatively fast by microbial activity, and water levels are further reduced by the binding of glyphosate to suspended soil particulates in water (Zaranyika and Nyandoro 1993) and dispersal (Bowmer 1982, Comes et al. 1976, Goldsborough and Beck 1989, Goldsborough and Brown 1993).

There are several relevant monitoring studies that are useful for estimating exposure to glyphosate in water. After an aerial application of Roundup at a rate of 2 kg a.i./ha [about 1.8 lb a.i./acre] over a 10 km^2 area in Vancouver Island, British Columbia, maximum concentrations in streams that were intentionally oversprayed reached about 0.16 mg a.e./L and rapidly dissipated to <0.04 mg a.e./L after 10 minutes. After a storm event, peak concentrations in stream water were <0.15 mg a.e./L, rapidly dissipating to ≤0.02 mg a.e./L before the end of the storm event (Feng et al. 1990, Kreutzweiger et al. 1989). At the same application rate, another Canadian study noted maximum stream concentrations of 0.109–0.144 mg a.e./L, occurring 7–28 hours after aerial application. Similar results were noted in a study conducted in Oregon, in which forest streams were oversprayed at a rate of 3.3 kg a.i./ha [2.9 lb a.i./acre]. Maximum water levels in streams reached 0.27 mg a.e./L (Newton et al. 1984). When normalized for application rates, the maximum levels in stream water from these three studies range from 0.088 to 0.093 mg a.e./L·lb a.i. applied.

These rates can be used to estimate exposure levels associated with accidental direct spray of a stream. Because the range in rates is so narrow, only the higher end of the range will be used. Thus, at the typical application rate of 1 lb a.i./acre, the maximum anticipated concentration would be 0.093 mg a.e./L. Assuming that a 10 kg child consumes 1 L of the contaminated water, the dose of glyphosate (a.e.) would be 0.0093 mg/kg/day:

$$0.093 \text{ mg/L} \cdot 1 \text{ L} \div 10 \text{ kg}.$$

This dose would increase linearly with the application rate. This is discussed further in the risk characterization (section 3.4).

Concentrations of glyphosate in ponds that are over sprayed appear to be somewhat less than those found in streams. For example, in three forest ponds oversprayed at 2.1 kg a.i./ha [1.9 lb a.i./acre], maximum initial glyphosate concentrations were <0.1 mg a.e./L or 0.05 mg a.e./L·lb a.i. applied (Goldsborough and Brown 1993).

There is no information suggesting that glyphosate applications will result in significant levels of the compound in water over prolonged periods of time. In the study by Reynolds et al. (1993),

levels of glyphosate in stream water ranged from not detectable (<0.1 μg/L) to a trace (<1.0 μg/L) following storm events that occurred 20–150 days after application. Similar levels, 0.1–1.0 μg/L, were detected in ponds 70 days after applications (Goldsborough and Brown 1993).

For estimating the effects of chronic exposure, a concentration range of 0.1–1.0 μg/L will be used, based on the above monitoring studies cited above. Assuming that a 10 kg child consumes 1 L of water/day, the estimated dose for prolonged exposures is 0.01–0.11 μg/kg/day or 0.00001–0.0001 mg/kg/day

$$0.1–1\ \mu g/L \cdot 1\ L/day \div 10\ kg.$$

3.2.3.5. ***Oral Exposure from Contaminated Fish*** **--** Glyphosate has a relatively low potential for bioconcentration. In a bioconcentration study using ^{14}C-glyphosate, bioconcentration in carp exposed to levels in water of 5–50 μg/L ranged from about 10 after 1 day of exposure to about 40 after 14 days of exposure (Wang et al. 1994). These estimates of bioconcentration, however, are based on total radioactivity rather than the identification of glyphosate residues. Consequently, the apparent bioconcentration may simply reflect the binding of glyphosate metabolites to fish tissue. As discussed above, peak levels of glyphosate in ambient water are not likely to exceed 0.093 mg/L·lb applied and will dissipate rapidly.

For estimating glyphosate residues in fish shortly after application, the 1-day bioconcentration factor (BCF) of 10 will be used as a conservative approximation. Thus, residues in fish of 0.93 mg/kg fish·lb applied would be expected. Assuming that a 70 kg man consumes a maximum of 158 g of fish per day (Ruffle et al. 1994, Table II, p. 397), the resulting dose associated with the typical application rate of 1 lb/acre would be 0.002 mg/kg

$$0.93\ mg/kg\ fish \cdot 0.158\ kg \div 70\ kg.$$

For estimating glyphosate residues in fish for prolonged periods after application, the 14-day BCF of 40 will be used. As discussed above, typical levels in ambient water are not likely to exceed 1 μg/L·lb applied. Thus, residues in fish would be estimated at a rate of 40 μg/kg·lb applied. Using the same maximum value for fish consumption as above, the resulting dose associated with the typical application rate of 1 lb/acre would be 0.00009 mg/kg

$$0.04\ mg/kg\ fish \cdot 0.158\ kg \div 70\ kg.$$

As with other exposure scenarios in this section, the consequences of higher application rates are discussed in the risk characterization (section 3.4).

3.2.3.6. ***Oral Exposure from Contaminated Vegetation*** **--** After ground or aerial applications, glyphosate will be deposited on vegetation. Although members of the general public are excluded from the area while treatments are being conducted, it is conceivable that

contaminated vegetation could be consumed by individuals shortly after treatment. The most plausible scenario involves the consumption of contaminated berries.

The most relevant publication for assessing exposure from such a scenario is that of Siltanen et al. (1981). These investigators monitored levels of glyphosate on cowberries and bilberries after backpack sprays of Roundup at an application rate of 0.25 and 0.75 kg a.i./ha [0.22 and 0.67 lb a.i./acre]. At 6 days after treatment with 0.67 lb/acre, residues on cowberries were 1.6 mg/kg. At 7 days after treatment, residues on bilberries were 2.1 mg/kg. The residue data plotted over a 70-day post-application observation period are illustrated in Figure 3-3.

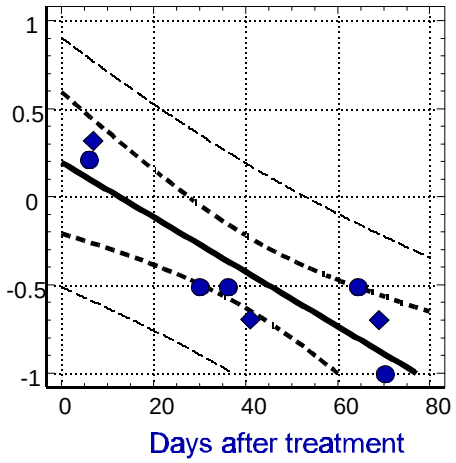


Figure 3-3: Residues of glyphosate on cowberries (**M**) and bilberries (♦) after the application of glyphosate at 0.67 lb/acre [data from Siltanen et al. 1981].

These data fit a first order model (p=0.004) with a dissipation rate of 0.015 day^{-1}, which corresponds to a half-time of about 46 days. This model is indicated by the thick solid line in Figure 3-3. The thick dashed lines represent the 95% confidence interval, and the thin outer lines represent the 95% prediction interval. Although the data fit a simple one-compartment first order model, visual inspection of the data suggests that a two-compartment first order model could also be applied.

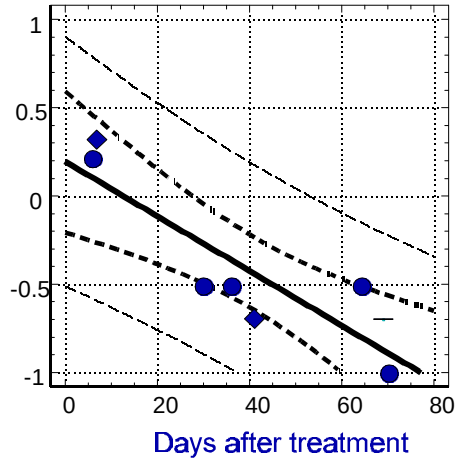


Figure 3-3: Residues of glyphosate on cowberries (**M**) and bilberries (♦) after the application of glyphosate at 0.67 lb/acre [data from Siltanen et al. 1981].

For this exposure assessment, the residues immediately after application will be estimated at about 3 mg/kg or 4.5 mg/kg berry · lb a.i applied. This is approximately the 95% upper confidence limit using the one-compartment model as well as the apparent value at t_0 using a two-compartment model (eye fit). This is also consistent with the monitoring data at days 6 and 7.

For this exposure assessment, it will be assumed that a 64 kg woman (U.S. EPA 1985) consumes 1 pound (0.454 kg) of contaminated berries. Based on these assumptions, the estimated dose is 0.032 mg/kg

$$4.5 \text{ mg/kg} \cdot 0.454 \text{ kg} \div 64 \text{ kg}.$$

Longer-term exposure to contaminated vegetation will be based on the average of the residue levels between time zero and day 20 using the first order model. This clearly is an arbitrary approach. Using different time periods might result in higher or lower estimates of exposure. As discussed in the risk characterization (section 3.4), the residue levels are sufficiently far removed from levels of concern that the time period selected makes little difference to this risk assessment. At time zero, the estimated residue level is about 1.6 mg/kg ($10^{0.2}$); at day 20, the residue level is about 0.4 mg/kg ($10^{-0.4}$). In a first order model, the time-weighted average between two time periods is simply the geometric mean of the values. Thus, over the initial 20-day period, the time-weighted average concentration is 0.8 mg/kg $[(1.6 \cdot 0.4)^{0.5}]$. Using the same assumptions as those for acute exposure, the estimated dose is 0.006 mg/kg

$$0.8 \text{ mg/kg} \cdot 0.454 \text{ kg} \div 64 \text{ kg}.$$

These estimates apply to the typical application rate of 1 lb/acre and would increase in a linear fashion as the application rate increases. The consequences of increased application rates are discussed in the risk characterization (section 3.4).

3.3. DOSE-RESPONSE ASSESSMENT

3.3.1. Overview. The current RfD for glyphosate is 0.1 mg/kg/day (U.S. EPA 1993a). This is based on a NOAEL of 10 mg/kg/day with an uncertainty factor of 100 used to account for species-to-species extrapolation and sensitive subgroups. This RfD was reviewed by U.S. EPA on 9/1/90 and is not currently under additional review. The Office of Pesticides of the U.S. EPA has recommended a higher RfD of 2 mg/kg/day for glyphosate (U.S. EPA 1993b). This proposed RfD has not been reviewed by the Agency RfD Work Group.

A quantitative consideration of the dose-response data in humans from the Taiwan study as well as a consideration of the dose-severity relationships in experimental mammals suggests that both of these RfDs are protective. The estimated threshold for lethality is 445 mg/kg. The probability of observing a frank toxic effect at this dose level is about 0.04. The estimated LD_{50} for humans, based on the Taiwan poisoning experience is about 3000 mg/kg, in the mid-range of reported LD_{50} values for experimental mammals.

3.3.2. Existing Guidelines.

The RfD for glyphosate is based on a study cited by U.S. EPA as Monsanto Co. (1981) which appears to be identical to the study cited as Bio/Dynamics, Inc. (1981b) in Appendix 1-4. The study is summarized as follows by U.S. EPA (1993a) in the documentation of the RfD:

> *Rats (CD Sprague-Dawley) were administered glyphosate continuously for three successive generations. Dietary*

concentrations of glyphosate were adjusted weekly during growth, and between mating rest periods to achieve dose levels of 0, 3, 10, and 30 mg/kg/day. Each generation (F0, F1, F2) consisted of 12 male and 24 female rats. Each parent generation was mated to produce two litters. Offspring from the second litters of the F0 and F1 parents (F1b and F2b litters, respectively) were selected to be parents for subsequent generations. Offspring not included in the selection procedure and offspring from the first litter intervals of each generation (F1a, F2a, F3a) were given a gross postmortem examination and discarded. Randomly selected offspring from the second litters of the F2 generation (F3b litters) were given a gross postmortem examination and selected tissues taken and saved. Subsequently tissues from control and high-dose F3b offspring were evaluated microscopically (10/sex/group). Tissues from control and high-dose parent generations parent generations (F0, F1, and F2) were also evaluated.

No treatment-related effects on fertility were noted, nor were any systemic effects in adult rats apparent. Male pups from the F3b mating of the high dose group (30 mg/kg/day) showed an increase in the incidence of unilateral renal tubular dilation. Based on this finding, the NOEL and LEL for this study are 10 and 30 mg/kg/day, respectively.

WHO/FAO (1986) has recommended a somewhat higher value, 0.3 mg/kg/day, as an acceptable daily intake (ADI). This is based on a 26 month feeding study in rats in which the NOAEL was 31 mg/kg/day. This study is summarized in Appendix 1-3 as Bio/Dynamics, Inc. (1981a). As with the U.S. EPA RfD, an uncertainty factor of 100 was used.

The Office of Drinking Water (U.S. EPA 1992a) has proposed a 10-day health advisory for glyphosate of 17.5 mg/L and a longer-term health advisory of 1 mg/L. The longer-term health advisory is base on 0.1 mg/kg/day reference dose, as summarized above. The 10-day health advisory is based on the NOAEL of 175 mg/kg/day in the rabbit teratogenicity study, summarized in appendix 1-4 as Monsanto Co. (1980). As with a the RfD, an uncertainty factor of 100 was applied to this RfD to account for species-to-species extrapolation and sensitive subgroups. This 10-day exposure limit of 1.75 mg/kg/day was multiplied by 10 kg, the default weight for a child, and divided by 1 L, the default amount of water consumed by a child.

The U.S. EPA Office of Pesticides recommends an RfD of 2 mg/kg/day for glyphosate (U.S. EPA 1993b). This is based on the same study (Monsanto Co. 1980), described in the previous

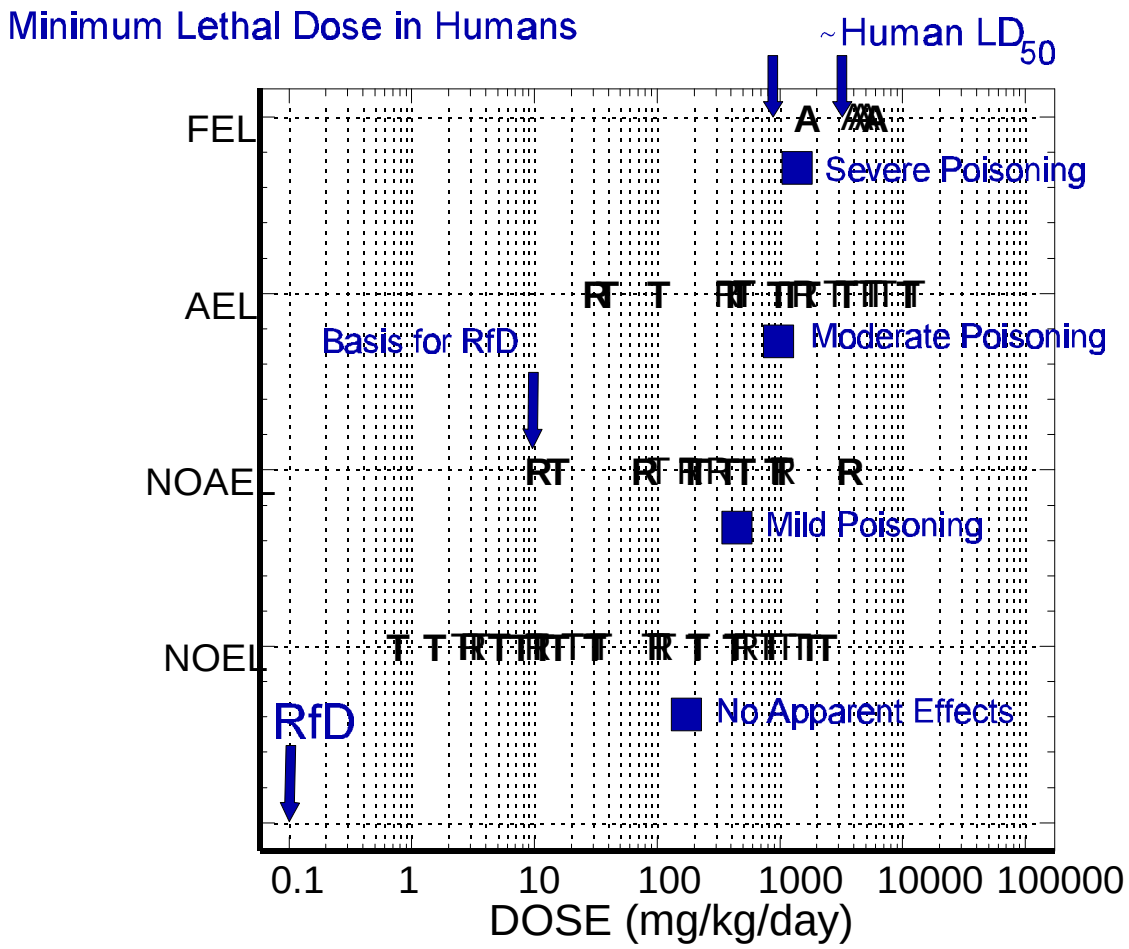


Figure 3-4: Dose-severity Relationships for Glyphosate. [See text for details.]

paragraph, used by the Office of Drinking Water to derive the 10-day exposure limit of 1.75 mg/kg/day. The proposed RfD of 2 mg/kg/day is simply a rounding of the 1.75 mg/kg/day value. This proposed RfD has not been reviewed by the Agency RfD Work Group.

3.3.3. Dose-Response and Dose-Severity Relationships. As summarized in section 3.2, all exposure scenarios for the general public and most exposure scenarios for workers yield estimates that are below the current RfD of 0.1 mg/kg/day. In some instances, the upper ranges of some estimates are slightly above 0.1 mg/kg/day. All of these exposure estimates, however, are based on the typical application rate of 1 lb a.i./acre. In some cases, the Forest Service uses treatments above 2 lbs a.i./acre. In addition, the maximum label rate for glyphosate is 7.5 lbs/acre. Thus, depending on specific program needs in the future, higher application rates could be considered. Adequate data are available on glyphosate to estimate the potential impact of such exposures by considering dose-response and dose-severity relationships.

There is a striking concordance between the available human and animal data. As illustrated earlier in Figure 3-1, the dose-mortality data in humans is consistent with estimates of oral LD_{50} values in experimental mammals. Several different dose-response models can be use to quantitatively compare the lethality data on humans with those available on experimental mammals. In general, different dose-response models yield similar results in the region of observed responses but may differ substantially in the low dose region. To estimate the LD_{50} in humans, variants of the multistage model were used, one non-threshold and one with a threshold. Both models yielded virtually identical estimates of the LD_{50}, approximately 3000 mg/kg, somewhat less than the reported values for rats and rabbits and substantially above the reported value for mice.

For systemic toxic effects, it is generally assumed that population thresholds exist. In other words, below a certain dose, no individual in the population will respond. This assumption is fundamental to risk assessment methods for systemic toxic effects. For cancer, population thresholds are not generally assumed and non-threshold models are considered appropriate. The threshold version of the multi-stage model used in this analysis yielded an estimate of the threshold at about 445 mg/kg.

The dose-severity relationships for experimental mammals and humans are also similar, as illustrated in Figure 3-4. In this figure, the animal data are taken from Appendices 1-3 and 1-4. In cases where dietary exposure levels were not converted to units of dose in mg/kg/day, such conversions were made using the methods presented in U.S. EPA (1986b, Reference Values for Risk Assessment). The animal data are categorize using four standard severity levels: NOEL (no observed effect level), NOAEL (no observed adverse effect level), AEL (adverse effect level), and FEL (frank effect level), as discussed in SERA (1995a). Three different groups of end-points are presented: general systemic toxic effects (***T***), reproductive or developmental effects (***R***), and acute LD_{50} values (***A***). The estimated human oral LD_{50}, as estimated above, is plotted as a FEL with the LD_{50} values from experimental mammals.

As indicated in Appendices 1-3 and 1-4, these studies span exposure periods ranging from 1 day to more than 2 years. The exposure axis is not presented in this figure. As discussed below, the duration of exposure is not an important variable in the toxicity of glyphosate. The study on which the RfD is based as well as the RfD itself are plotted and labelled with arrows.

Figure 3-4 also includes human data from the study by Tominack et al. (1991). These investigators report mean dose levels associated with four levels of severity. Patients in the least severe category were asymptomatic. The average amount of Roundup consumed by these patients was 31 mL. Assuming an average body weight of 60 kg and using the concentration of 356 g of glyphosate a.e./L, this corresponds to an average dose of 184 mg/kg. This is plotted as a box just below the NOEL line in Figure 3-4 and labelled as *"No apparent effects"*. Patients with transient signs or symptoms localized to the oral mucosa or gastrointestinal tract had, on average, consumed 72 mL (≃427 mg/kg). This point is labelled as *"Mild poisoning"* in Figure 3-4. Patient with *"Moderate poisoning"* had consumed on average 176 mL (≃1,044 mg/kg). These patients

evidenced gastrointestinal tract irritation lasting less than 24 hours, transient decreases in blood pressure or decreased urinary output, transient hepatic or renal damage, acid-base disturbances, or pulmonary dysfunction which did not require intubation. *"Severe poisoning"*, which included fatal cases, occurred in patients who had on average consumed 216 mL (≈1,282 mg/kg). The publication by Tominack et al. (1991) also reports the variability of the doses associated with each of these severity levels.

For experimental mammals, the dose-severity relationships can be assessed using categorical regression analyses (Durkin et al. 1992; Hertzberg 1989; McCullagh 1980). This approach correlates categorical responses—such as NOELs, NOAELs, AELs, and FELs—with factors that may influence the response such as dose and duration of exposure. The method results in estimates of the probability of a group of animals subjected to a given exposure being classified into a given category. For the statistical analyses, data on NOELs and NOAELs were combined. This was done for two related reasons. First, the primary concern for this risk assessment is the delineation between regions of adverse and non-adverse effects. Thus, the distinction between a NOEL and NOAEL is not critical. Second, many reported NOELs could be artifacts of the level of detail at which the animals are examined. For example, simply because there are no adverse effects based on gross examination of organs does not mean that effects might not be seen if all organs were examined microscopically. Consequently, analyses were conducted using both four categories (NOELs, NOAELs, AELs, and FELs) as well as two categories (NOELs and NOAELs combined as well as AELs and FELs combined).

Initially, the categorical regression was conducted on both dose and duration of exposure. The effect of duration was not statistically significant ($p=0.7267$). This seems reasonable given the data on the influence of duration of exposure on toxicity. For example, all of the LD_{50} values shown in Figure 3-4 involved single doses. Many of the AELs, some of which are doses at or above reported LD_{50} values, involved exposure periods of up 2 years. This apparently anomalous result can be explained by two factors. First, all of the LD_{50} studies involved intubations: the animal was given the total dose by stomach tube at one time. Most of the subchronic and chronic studies involved dietary exposures, in which the daily dose was spread out over the course of the day depending on the animals eating habits. Thus, the animals who were intubated in LD_{50} studies received essentially more severe exposures for a given dose. Secondly, for chemicals that are eliminated rapidly and do not cause cumulative damage, there is often very little relationship between the duration of exposure and the severity of response for a fixed dose level.

Because of the lack of significance of duration, the analysis was re-run using only dose as the independent variable. The results of this analysis indicate that the probability of an adverse effect at the RfD of 0.1 mg/kg/day is 0.0005. A doses of 1 mg/kg/day the probability of observing an adverse effect is 0.003. At a dose that is 100 fold above the RfD (i.e., 10 mg/kg/day) the probability of an adverse effect is 0.12. This analysis suggest that the current RfD is highly protective and that the proposed alternative RfD of 2 mg/kg/day is also protective. At this higher level, the probability of an adverse effect is 0.006.

All of the above estimates are based on the two category analysis - the segregation of any adverse effect from non-adverse effects. They indicate the probability of a group of animals exposed at the specified dose level evidencing responses sufficiently, albeit perhaps minimally, severe to classify the dose level as *adverse* based on the responses observed in the group of animals.

The four category analysis can be used to estimate the probability of observing effects that would be classified as *frank signs of toxicity*. These effects are sufficiently severe that they can be observed in the whole organism without the use of invasive methods. The probability of a frank toxic effect at the RfD of 0.1 mg/kg/day is 0.00005. At the proposed alternative RfD of 2 mg/kg/day, the probability increases to only 0.0006.

The consistency between the categorical analysis using data on groups of experimental animals and dose-response analyses of the human lethality data using the multi-stage model is relatively good. At the estimated threshold for lethality, 445 mg/kg, the probability of observing a frank toxic effect is about 0.04. At this dose, the non-threshold version of the multi-stage model estimate the probability of mortality at about 0.02. At the estimated human LD_{50} of about 3000 mg/kg, the categorical regression using two categories (NOELs and NOAELs combined as well as AELs and FELs combined) indicates the probability of observing an AEL or FEL of 0.7. The four category model, however, substantially underestimates the probability of observing a FEL, 0.13. Visual inspection of Figure 3-4 suggests that this is attributable to the relatively small number of FELs in experimental mammals and the overlap of FELs with AELs. As discussed above, this overlap may be related to the rapid elimination and lack of cumulative damage in longer-term studies.

A somewhat more detailed analysis could be conducted on data collected by Tominack et al. (1991) that would provide information on the probabilities of individuals rather than groups being classified as adverse responders to given doses of glyphosate. The necessary data for such an analysis [amount consumed, body weight or sex/age, and severity classification] is not presented in the Tominack publication.

The significance of the categorical regression on animals and the available human data relates to the use of the uncertainty factor. As summarized in the previous section, the current RfD as well as the proposed U.S. EPA Office of Pesticides alternative use an uncertainty factor of 10 for species to species extrapolation (i.e., extrapolating from experimental animals to humans). This is a common default procedure. For glyphosate, however, the available data suggest that humans are no more sensitive to glyphosate than experimental mammals. This in turn suggests that the current and proposed RfD may be overly protective by a factor 10 or greater. In other words, the RfDs suggest that no adverse effects are anticipated at doses of 0.1-2 mg/kg/day. The human data suggest that no frank adverse effects are likely at doses substantially above 10 mg/kg/day.

3.4. RISK CHARACTERIZATION

3.4.1. Overview. The major hazard associated with the use of glyphosate will involve accidental or incidental dermal or ocular contact. Glyphosate is an irritant to the skin and eyes. If dermal or ocular contact with undiluted or weakly diluted formulations occurs, irritation is likely to develop and will require corrective action to ameliorate the irritant effects. These irritant effects, if properly handled, will be transient.

Based on the exposure assessments discussed in section 3.2 and the dose-response assessments discussed in section 3.3, the quantitative risk assessments for workers and the general public are summarized in Tables 3-2, 3-3, and 3-4. In these tables, risk is characterized as the hazard quotient, the ratio of the anticipated level of the exposure to some index of acceptable exposure or exposure associated with a defined risk. Thus, if the hazard quotient is less than unity, concern for the exposure is minimal. As the hazard quotient increases above unity, concern also increases.

There is no substantial concern for systemic toxic effects in workers or the general public when glyphosate is used at the typical application rate of 1 lb a.i./acre or at the upper range of the application rate used by the Forest Service, 2.5 lbs a.i./acre. At the maximum labelled rate of 7.5 lbs a.i./acre, there would be marginal concern for effects in some groups of workers (i.e., hazard quotients of about 0.6) at the upper limit of conservative exposure assumptions.

Consistent with previous assessments conducted by the Forest Service, the carcinogenic risk associated with exposure to 1,4-dioxane appears to be less than 1 in 10 million.

Given the rapid elimination of glyphosate—in both the environment and from the body of mammals—as well as the very weak duration-severity relationships reported in the animal data, cumulative effects do not seem plausible. Similarly, there is no basis for identifying specific groups at substantially increased risk.

Table 3-2. Summary risk characterization for occupational exposure to glyphosate, excluding accidental or incidental exposure

Treatment Method	Daily Dose (mg/kg bw)[a]	Hazard Quotient for Specific Application Rate		
		1 lb/acre	2.5 lbs/acre	7.5 lbs/acre
Boom spraying	0.013 0.0016-0.11	0.007 (0.0008-0.06)	0.02 (0.002-0.1)	0.05 (0.006-0.4)
Backpack and cut surface	0.006 0.0005-0.072	0.003 (0.0003-0.04)	0.008 (0.0006-0.09)	0.02 (0.002-0.3)
Aerial applications (pilots and mixer/loaders)	0.014 0.0016-0.16	0.007 (0.0008-0.08)	0.02 (0.002-0.2)	0.05 (0.006-0.6)

[a]Assuming an application rate of 1 lb/acre. [See Table 3-1 for details of exposure estimate.]

[b]Based on the proposed RfD of 2 mg/kg/day (U.S. EPA 1993b).

3.4.2. Workers. A quantitative summary of the risk characterization for each of the job categories covered in this risk assessment is presented in Table 3-2. In this table, the hazard quotients are based on the RfD of 2 mg/kg/day proposed by the U.S. EPA Office of Pesticides. As discussed in the dose-response assessment (section 3.3), the current RfD of 0.1 mg/kg/day for lifetime exposures is 20 times less than the RfD recommended by the U.S. EPA Office of Pesticides. Hence, if the lower RfD were used, the hazard quotients given in Table 3-2 would be 20 times greater and some hazard quotients would exceed unity (i.e., a level of concern).

The selection of the higher or lower RfD has relatively little impact on the substance of the risk characterization. When an RfD is exceeded, an attempt must be made to assess the health consequences. As discussed in the dose-response and dose-severity relationships (section 3.3.3), there is no evidence that significant health effects should occur from exposure to glyphosate levels <10 mg/kg/day, which is the NOAEL on which the lower RfD is based. This assessment is supported by the categorical regression of the animal toxicity data and the available human dose-severity data from poisoning incidents. Thus, the higher RfD is selected because the resulting hazard quotients more clearly reflect the toxicological significance of the projected exposures.

As indicated in Table 3-2, there is no basis for concern at the typical application rate (1 lb a.i./acre) or the upper range of application rates currently used by the Forest Service (2.5 lbs

a.i./acre). Similarly, no central estimates or upper limits of the hazard quotients exceed unity at the maximum labelled application rate (7.5 lbs a.i./acre).

Table 3-3. Quantitative summary of risks for workers after accidental or incidental exposure[a]

Activity	Scenario	Dose (mg/kg/day)	HQ[b]
Immersion of hands	1 minute	0.00012	0.00006
Wearing contaminated gloves	1 hour	0.0069	0.003
Accidental spill on lower legs	effective washing after 1 hour (best estimate of dermal absorption)	0.007-0.019	0.004-0.01

[a] See sections 3.2.2.2. and 3.2.2.3. for details regarding the exposure assessment.

[b] Based on the proposed RfD of 2 mg/kg/day (U.S. EPA 1993b).

The accidental scenarios for workers, as summarized in Table 3-3, result in hazard quotients that are comparable to those associated with exposure scenarios for application rates of 1 lb/acre. This is consistent with the fact that the accidental scenarios are not dependent on application rates. In other words, the accidental scenarios are on based on mischance or misuse of the concentrated formulation over a relatively short period of time. As with the analysis of job categories, the accidental or incidental exposures do not suggest a substantial level of concern for systemic toxic effects.

As summarized in section 3.1.6, glyphosate and glyphosate formulations are skin and eye irritants. Quantitative risk assessments for irritation are not normally derived, and, for glyphosate specifically, there is no indication that such a derivation is warranted. As discussed by Maibach (1986), glyphosate with the POEA surfactant, is about as irritating as standard dish washing detergents, all purpose cleaners, and baby shampoos.

The only area of remarkable uncertainty concerns brown-and-burn operations. Glyphosate, like Roundup, does not appear to be very toxic by inhalation (i.e., irritant effects in rats at levels of 0.05–0.36 mg/L [50–360 mg/m^3] of air for 6 hours/day, 5 days/week for 22 days (Smith and Oehme 1992). During application, air concentrations of glyphosate are generally a factor of 60 below this level [2.8–15.7 μg/m^3 or 0.0028–0.0157 mg/m^3 (Jauhiainen et al. 1991)]. Residues of glyphosate in air during brown-and-burn operations have not been measured but certainly would be much lower, given that brown-and-burn operations take place about 45–180 days after treatment with the herbicide. Consequently, there is no evidence to suggest that toxic levels of glyphosate are likely to be encountered.

As reviewed by Dost (1986, 1987), the generation of carcinogenic PAH, a generic concern with brown-and-burn operations, is associated with extremely low levels of risk [ranging from about 1 in 1 million to 1 in 10 million]. This is the only identifiable concern with brown-and-burn operations and does not pertain to the use of glyphosate. Nevertheless, as discussed in section 3.2, glyphosate forms a polycondensate on combustion at temperatures ranging from 200 to 240°C. It is likely that other combustion products are formed under different combustion conditions. No information is available regarding the inhalation toxicity of the polycondensate or other possible combustion products.

The significance of this data gap should not be subject to over interpretation. With the exception of some plastics, the combustion products known to pose a risk to fire fighters, the combustion products of most xenobiotics have not been examined in detail. The necessity of addressing this data gap must be weighed against the need to address other data gaps on glyphosate and other chemicals. The combustion products of burning wood and vegetation are respiratory irritants as well as carcinogens, and exposure to these combustion products should be avoided. There is no basis for believing that the presence of low or even high levels of glyphosate residues will have a significant impact on this hazard.

Table 3-4. Quantitative summary of risks for the general public[a]

Activity	Scenario	Dose (mg/kg/day)	Hazard Quotient[b]
Direct spray	naked child, entire body surface, wash after 1 hour.	0.031-0.061	0.02-0.03
	young woman, feet and legs, wash after 1 hour	0.0026–0.0053	0.001-0.003
Walking through treated area	dermal Absorption, contaminated vegetation	0.0005-0.0009	0.0002-0.0005
Contaminated water	10 kg child consuming 1 L immediately after spraying.	0.0093	0.005
	0.1-1.0 μg/L in ambient water.	0.00001 -0.0001	0.0001-0.001[c]
Consumption of contaminated fish	shortly after spraying.	0.002	0.001
	over prolonged periods	0.00009	0.0009[c]
Consumption of contaminated vegetation	berries shortly after spraying.	0.032	0.02
	berries, time zero to day 20	0.006	0.06[c]

[a] Application rate of 1 lb a.i./acre. See section 3.2.3. for details regarding the exposure assessment.

[b] Based on U.S. EPA 10-day health advisory = 2 mg/kg/day unless otherwise noted.

[c] Based on verified U.S. EPA RfD for lifetime exposures = 0.1 mg/kg/day.

3.4.3. General Public. The quantitative hazard characterization for the general public is summarized in Table 3-4. Most of the exposure scenarios involve relatively short-term exposures. For these scenarios, hazard quotients are derived using the same toxicity value, 2 mg/kg/day, as was used by the U.S. EPA (1992a) in deriving the 10-day health advisory. For the longer-term exposures involving contaminated water, fish and vegetation, the current RfD of 0.1 mg/kg/day is used. As discussed in the previous section, the higher value of 2 mg/kg/day, proposed by the U.S. EPA Office of Pesticides, could be justified for these longer-term exposures. As summarized in Table 3-4, however, this would have no impact on the characterization of risk.

Using this very conservative approach for quantitatively characterizing risk, no hazard quotients for the general public exceed 0.06 for the typical application rate of 1 lb/acre. For all scenarios except direct spray, the estimated exposures and consequent hazard quotients will increase linearly as the application rate increases. For an application rate of 2.5 lbs a.i./acre, the highest rate currently used by the Forest Service, the highest hazard quotient would be 0.15. At the

maximum labelled rate of 7.5 lbs a.i./acre, the highest hazard quotient would be 0.45. Thus, under current or foreseeable program uses, no hazards are apparent for the general public.

3.4.4. Sensitive Subgroups. No reports were encountered in the literature leading to the identification of sensitive subgroups. There is no indication that glyphosate causes sensitization or allergic responses, which does not eliminate the possibility that some individuals with multiple chemical sensitivity might be sensitive to glyphosate as well as many other chemicals.

3.4.5. Connected Actions. There is very little information available on the interaction of glyphosate with other compounds. As summarized in section 3.1, the available data do not suggest a synergistic interaction between glyphosate and the POEA surfactant from plausible routes of exposure. One report (Tai et al. 1990) suggests that glyphosate administered by injection may antagonize/reduce the toxic effect of POEA.

3.4.6. Cumulative Effects. As noted above, this risk assessment specifically considers the effect of repeated exposures and no adverse effects are anticipated. As discussed in the dose-response and dose-severity relationships (see section 3.3.3), the daily dose rather than the duration of exposure determines the toxicological response. Consequently, repeated exposure to levels below the toxic threshold should not be associated with cumulative effects.

3.4.7. Carcinogenic Effects of 1,4-Dioxane. As summarized in section 3.1.9.3, 1,4-dioxane is a contaminant in POEA and has been classified as a probable human carcinogen by U.S. EPA. The cancer potency factor derived by U.S. EPA is 0.011 $(mg/kg/day)^{-1}$. This is a 95% upper limit on potency from a cancer bioassay using mammals.

The level of 1,4-dioxane in Roundup is a factor of 0.00084 of the level of glyphosate (as acid equivalent). Heydens (1990 appended to Borrecco and Neisess 1991) estimated that the upper limit of cancer risk associated with this contamination was extremely low (e.g., $<1 \cdot 10^{-7}$ or 1 in 10 million).

The current analysis, while based on somewhat different exposure assumptions and a slightly different cancer potency factor, supports this assessment. For workers involved in the aerial application of glyphosate, the mean estimate of exposure is 0.014 mg/kg/day (see Table 3-2). This is the most highly exposed occupational group. Using this dose as a lifetime average exposure and correcting for the proportion of 1,4-dioxane relative to glyphosate, the cancer risk is $1 \cdot 10^{-7}$,

$$0.014 \text{ mg/kg/day} \cdot 0.00084 \cdot 0.011 (\text{mg/kg/day})^{-1}$$

identical to the upper limit derived by Heydens (1990). Assuming a 30 year working period in a 70 year life span and making the very conservative assumption that the product would be handled 100 days/year, risk would be reduced by a factor of about 0.1,

$$(100 \div 365) \cdot (30 \div 70).$$

With these adjustments, the estimated risk would be $1 \cdot 10^{-8}$ or 1 in 100 million.

Other groups of workers would be at less risk. The only chronic source of glyphosate exposure to the general public is through drinking water. The upper limit of the daily dose associated with this route is about 0.001 mg/kg/day. Using the methods discussed above and not adjusting for occupational use, the cancer risk would be about $7 \cdot 10^{-10}$, or 7 in 10 billion.

As with the conclusions previously reached by Heydens (1990), the cancer risk associated with exposure to 1,4-dioxane secondary to the use of Roundup is negligible for workers or members of the general public.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview. Standard toxicity bioassays have been conducted on several wildlife species, including mammals, birds, fish, and some terrestrial and aquatic invertebrates, as well as many species of aquatic and terrestrial plants. In addition, a number of field studies have been conducted on effects of glyphosate applications that are comparable or almost the same as those used by the Forest Service.

The toxicity studies on terrestrial animals are generally consistent with those on experimental mammals. Although the mechanism of glyphosate toxicity is unclear, glyphosate can cause toxic effects including mortality at sufficiently high dose levels. The available field studies, however, clearly suggest that at plausible levels of ambient exposure, direct toxic effects are unlikely. The effects on terrestrial organisms appear to be secondary to changes in habitat resulting from toxic effects on vegetation.

The herbicidal activity of glyphosate has been studied extensively. Glyphosate interferes with normal metabolic processes in plants, which in sufficiently high exposures, may result in cell death, tissue damage, growth inhibition, and death of the plant. The biochemical pathway that is affected is specific to the plant species and does not occur in animals.

The toxicity of glyphosate to aquatic species depends on the acidity (pH) of the water. Glyphosate is more toxic in relatively acidic waters (pH≈6) by as much as a factor of 10, compared with alkaline waters (pH≈10). In general, the reported LC_{50} values for aquatic animals range from approximately 10 to 400 mg/L, depending on the species and pH of the water.

A major qualitative difference between the effect of glyphosate and glyphosate formulations on aquatic and terrestrial organisms concerns a polyethoxylated tallow amine surfactant (POEA) used in Roundup. For aquatic organisms, the surfactant is much more toxic than glyphosate. Unlike glyphosate, POEA is more toxic in alkaline water than in acid water. Thus, the relative potency of POEA with respect to glyphosate is pH dependent.

4.1.2. Toxicity to Terrestrial Animals. As summarized in the human health risk assessment (see section 3), the inhibition of the shikimate pathway by glyphosate in plants (section 4.1.3) is not a consideration in assessing potential toxic effects in humans. Nonetheless, glyphosate may be associated with acute or longer-term toxic effects as discussed in section 3.1. Information regarding the toxicity of glyphosate to birds is summarized in Appendix 2-1.

Inhibition of oxidative phosphorylation has been implicated as a possible mechanism by which glyphosate causes adverse effects in experimental mammals (see section 3.1.2); however, there is not adequate information about terrestrial wildlife from which to make a further assessment about

the importance of this mechanism. As in the human health risk assessment, the potential significance of non-specific toxic effects can be assessed from the available toxicity studies (Appendix 2-1).

Most of the acute toxicity studies summarized in Appendix 2-1 involve the use of birds to assess either the gross toxic potency of glyphosate or Roundup by oral exposure or the toxicity to eggs after immersion in glyphosate solutions. Consistent with the apparent lack of teratogenic activity in experimental mammals, there is no indication that glyphosate or Roundup causes birth defects in birds (Batt et al. 1980, Hoffman and Albers 1984).

The study by Hoffman and Albers (1984) is somewhat difficult to interpret because of the way in which doses are expressed—lb/acre at 100 gallons/acre. In this study, eggs were immersed in various concentrations of several pesticides, including glyphosate, for approximately 30 seconds and observed throughout development. The reported LC_{50} for glyphosate from Roundup is 178 lbs/acre at 100 gallons/acre. This probably corresponds to a concentration of 80.1 kg ÷ 378.5 L

$$(178 \text{ lbs} \cdot 0.45 \text{ kg/lb}) \div (100 \text{ gallons} \cdot 3.785 \text{ L/gallon})$$

or approximately 200 g/L, which corresponds to a solution of about 20% (w/v). This LC_{50} is consistent with the NOEL reported by Batt et al. (1980), which involved a less severe exposure-immersion in a 5% solution for 5 seconds.

Information on the acute lethal potency of glyphosate is considered quantitatively in section 4.3. As discussed in section 4.3, the available toxicity data on birds, snails, and honey bees indicate that these species are no more sensitive than experimental mammals and humans are to glyphosate.

In addition to these laboratory bioassays, there are several field studies that have assessed the effects of glyphosate on terrestrial organisms (Appendix 2-2). These studies indicate that at application rates comparable to or greater than those contemplated by the Forest Service effects on terrestrial mammals will be secondary to effects on vegetation. This has been demonstrated for moose (Santillo 1994), small mammals (Anthony and Morrison 1985, D'Anieri et al. 1987, Ritchie et al. 1987, Santillo et al. 1989a, Sullivan 1990), rabbits (Hjeljord et al. 1988), birds (Cayford 1988, Linz et al. 1994, MacKinnon and Freedman 1993, Solberg and Higgins 1993), carabid beetles (Brust 1990), and various other invertebrates (Byers and Bierlein 1984, Moldenke 1992, Santillo et al. 1984, Yokoyama and Pritchard 1984). In some cases, the effects noted in these studies appeared to be beneficial to the species under study [e.g., increased use by water fowl associated with an increase in open water after treatment with Rodeo (Solberg and Higgins 1993)]. In most cases, the effects noted were changes in population density that reflected changes in food availability or suitable habitat.

Very few studies suggest the potential for toxic effects. In a laboratory study in which isopods were exposed to leaf litter at levels equivalent to application rates of 2.1 kg/ha, the effect on litter

degradation depended on the tree species. Direct toxic effects—evidenced by increased mortality—could not be ruled out but were not statistically significant (Eijsackers 1992). In a laboratory study, effects on earthworm cultures treated at levels equivalent to application rates of 0.7–2.8 g/ha included decreased growth rates and early mortality (Springett and Gray 1992). The direct relevance of this study is limited, however, because the exposure conditions (i.e., spraying twice weekly on culture dishes) do not closely approximate field conditions.

Glyphosate residues or perhaps residues of adjuvants used with glyphosate have been shown to affect grazing preference in cattle (Jones and Forbes 1984) but not sheep (Kisseberth et al. 1986). Consistent with results in experimental mammals and aquatic species, glyphosate is not bioconcentrated by terrestrial mammals (Newton et al. 1984).

4.1.2. Toxicity to Terrestrial Plants. As reviewed by Smith and Oehme (1992), glyphosate causes a variety of toxic effects in plants, including the inhibition of photosynthesis, respiration, and nucleic acid synthesis. The primary mechanism of action, however, appears to be an inhibition of the shikimic acid pathway in plants that affects the metabolism of certain phenolic compounds and the synthesis of aromatic amino acids. At the biochemical level, this affects the formation of plant proteins and tissue. At the level of the whole plant, this inhibition leads to an inhibition or cessation of growth, cellular disruption, and, at sufficiently high levels of exposure, plant death. The time course for these effects can be relatively slow, depending on the plant species, growth rate, climate, and application rate. Gross signs of toxicity, which may not be apparent for 2–4 days in annuals or for more than 7 days in perennials, include wilting and yellowing of the vegetation, followed by browning, breakdown of plant tissue, and, ultimately, root decomposition.

Glyphosate is absorbed primarily through the foliage, and the absorption is rapid. Approximately 33% of the applied glyphosate is absorbed within a few hours after application. Because glyphosate is strongly adsorbed to soil, relatively little if any absorption occurs through the roots (Smith and Oehme 1992).

In actively growing plants, translocation involves cell to cell transport through the cuticle followed by long distance transport via vascular tissue. In dormant plants, transport is much slower and may be negligible. Glyphosate is not extensively metabolized or detoxified in plants. In plants that share a common seedpiece or propaglue node, such as sugar cane, translocation from plant to plant can result in injury to plants that are not treated directly (Dal Piccolo et al. 1980).

Glyphosate can reduce the emergence and weights of progeny seedlings on crops such as corn, soybeans, and johnson grass (Jeffery et al. 1981). It is not clear whether this effect is caused by direct toxic action on the seeds or simply reduced vigor in the parent plant as the seeds develop.

4.1.3. Toxicity to Aquatic Organisms. As with terrestrial species, the acute lethal potency of glyphosate and glyphosate formulations has been relatively well defined. Furthermore, as discussed in section 4.3, several NOECs (no observable effect concentrations) have been determined for various durations of exposure.

The primary qualitative difference in the hazard characterization for aquatic and terrestrial species involves the importance of the surfactant in Roundup. As discussed in section 3.1.3., POEA is included in Roundup at a concentration of 150 g/L and the other formulations of glyphosate recommend the use of surfactants. Two aquatic toxicity studies (Folmar et al. 1979, Wan et al. 1989) have been conducted on glyphosate, POEA, and Roundup which permit a quantitative assessment of the relative toxicities of glyphosate and POEA as well as the effects of combined exposures to these agents. Both of these studies indicate that POEA is substantially more toxic than glyphosate and is the primary toxic agent of concern.

The study by Folmar et al. (1979) is summarized in Table 4-1. As indicated in the first column of this table, these investigators conducted bioassays on four species of fish and one invertebrate (midge larvae). The following three columns give the LC_{50} values for glyphosate, POEA, and Roundup, respectively. For fish, the 96-hour LC_{50} values are given in the table. Folmar et al. (1979) report LC_{50} values for 24 and 48 hours but these values are not substantially different from those at 96 hours. The fifth column calculates the relative potency (ρ) of POEA with respect to glyphosate as the LC_{50} of glyphosate divided by the corresponding LC_{50} for POEA. In other words, for rainbow trout at pH 6.5, the LC_{50} for POEA is 7.4 mg/L and the corresponding LC_{50} for glyphosate is 140 mg/L. Thus, the relative potency of POEA with respect to glyphosate is about 19 [$140 \div 7.4 = 18.92$].

In mixtures, the concept of relative potency provides an explicit tool for identifying the most significant toxic agent(s) in a mixture as well as for assessing potential interactions among agents in a mixture (Durkin 1981, Mumtaz et al. 1994). For example, for a mixture of two agents with the same potency present in a mixture in proportions of π_1 and π_2, the fractional contribution of each agent to the toxicity of the mixture is simply the proportion (π_1 or π_2) of the agent in the mixture. When the potencies differ, both agents contribute equally to the toxicity of the mixture when π_1 is equal to $\rho\pi_2$. As above, ρ is defined here as the LC_{50} of component 1 divided by the LC_{50} of component 2.

In Roundup, glyphosate is present at 356 g/L and POEA is present at 150 g/L. The proportion of glyphosate in Roundup (π_G), ignoring the only other constituent which is water, is about 0.7 [$356 \div (356+150)$]. Similarly, the proportion of POEA (π_S for proportion of surfactant) in the mixture is about 0.3 [$150 \div (356+150)$]. Both constituents would contribute equally to the mixture if the relative potency of POEA was about 2.3 [$0.7 \div 0.3$]. The relative potency of POEA with respect to glyphosate is much greater than 2.3, at least for fish species (Table 4-1). Thus, POEA is the more significant toxic agent in the mixture.

Table 4-1. Estimates of relative potency and toxicological interaction of glyphosate and POEA[a]

Species/Assay/Study	Observed LC_{50} values					
	Glyphosate	POEA	Roundup[b]	ρ	Predicted LC_{50}	Pred.÷ Obs.
			pH 6.5			
Rainbow trout	140	7.4	10.8	19	22	2.0
Bluegills	140	1.3	6.0	108	3.1	0.5
			pH 7.2, 96 hr unless specified			
Midge larvae, 48 hr.	55	13	25	4.2	28	1.1
Rainbow trout	140	2	11.8	70	6.5	0.6
Fathead minnow	97	1.0	3.2	97	3.2	1.0
Channel catfish	130	13	18	10	35	1.9
Bluegills	140	3.0	7.1	47	9.5	1.3
			pH 9.5			
Rainbow trout	240	0.65	2.0	369	2.1	1.1
Bluegills	220	1.0	2.6	220	3.3	1.3

[a]Data from Folmar et al. (1979).

[b]Value reported by Folmar as mg a.i multiplied 1.42 to account for added mass of surfactant.

ρ = LC_{50} of glyphosate ÷ LC_{50} of POEA.

The magnitude of the difference can be expressed in various ways, the simplest of which is the ratio of the concentrations or equivalently the ratios of the proportions adjusted for the difference in potency:

$$\frac{D \cdot B_2}{B_1} \qquad \textbf{(4-1)}$$

For example, if the relative potency is 70, as it is in Table 4-1 for rainbow trout at pH 7.2, POEA may be said to contribute 30 [70 · 0.3 ÷ 0.7] times more than glyphosate to the toxicity of the mixture.

This method of describing relative toxic contribution is based on the assumption that the components in the mixture do not affect one another (i.e., there are no toxicological interactions). For terrestrial plants, such interactions have been clearly documented. One method for assessing whether or not similar interactions are plausible in aquatic species is to compare the observed LC_{50} values for Roundup to the LC_{50} values that would be predicted by one model of non-

interactive joint action, simple similar action (Finney 1971, Durkin 1981). Using this assumption, the expected LC_{50} can be calculated as:

$$LC_{50_{Roundup}} = \frac{LC_{50_{Glyphosate}}}{(B_G + DB_S)} \qquad \textbf{(4-2)}$$

where π and ρ are as defined above.

The predicted LC_{50} values for Roundup based on this assumption are presented in the second to the last column of Table 4-1, and the ratio of the predicted to observed LC_{50} values are given in the last column. Ratios >1 suggest some form of greater than additive toxicity, and, conversely, ratios <1 indicate less than additive toxicity. Note also that the observed LC_{50} values for Roundup are presented as the total concentration of glyphosate and POEA. In other words, the LC_{50} values for Roundup reported in Folmar et al. (1979) are multiplied by 1.42 ((352+150)÷352) and give the LC_{50} values in units of weight of both glyphosate and POEA. These units are required for the above equation 4-2.

As indicated in Table 4-1, there is a tendency for the toxicity of glyphosate to decrease (i.e., the LC_{50} values increase—as the pH increases), although the changes are not substantial. The effect of pH on POEA is also not substantial but the effect seems to be the opposite of the effect that pH has on glyphosate. In all of the bioassays, the surfactant is more toxic than glyphosate. Because of the effect of pH on toxicity, the relative potency of POEA increases as pH increases. At all pH levels, the ratio of predicted to observed LC_{50} values for Roundup does not deviate remarkably or systematically from unity, suggesting that no substantial interactions take place between these two compounds.

A similar analysis of the results presented by Wan et al. (1989) are summarized in Table 4-2. In general, this study agrees well with the earlier study by Folmar et al. (1979). In all cases, the surfactant is substantially more toxic than glyphosate. The effect of pH is more consistent and more substantial: the toxicity of glyphosate decreases and the toxicity of the surfactant increases with increasing pH. Consequently, the relative potency of the surfactant to glyphosate also increases with increasing pH. The LC_{50} values reported in Wan et al. (1989) for Roundup are expressed as "*mg product/L*." In calculating the expected LC_{50} values for Roundup in Table 4-2, it is assumed that these LC_{50} values include the concentrations of both glyphosate and the surfactant. As indicated in the last column of this table, the ratio of the predicted to observed LC_{50} values for Roundup are consistently <1, indicating a less than additive interaction.

The significance of this information to the current risk assessment is that much of the toxicity and all of the available monitoring data used in the risk assessment for aquatic species is on glyphosate rather than the surfactant. Because POEA is the toxic agent of primary concern in Roundup, the monitoring data used in the exposure assessment and toxicity data used in the dose response assessment must be adjusted, as discussed below, to consider the differences in potency between these two agents.

Table 4-2. Estimates of relative potency and toxicological interaction of glyphosate and POEA in five species of salmonids[a]

Species/Assay/Study	Observed 96-hour LC_{50} Values					
	Glyphosate	POEA	Roundup[a]	ρ	Predicted LC_{50}	Pred.÷ Obs.
			Soft Water pH 6.3			
Coho	27	4.6	32	5.9	10.9	0.34
Chum	10	2.7	20	3.7	5.5	0.28
Chinook	19	2.8	33	6.8	6.9	0.21
Pink	14	4.5	33	3.1	8.5	0.26
Rainbow	10	2	33	5	4.5	0.13
			Soft Water pH 7.2			
Coho	36	3.2	27	11.3	8.8	0.33
Chum	22	4.2	19	5.2	9.7	0.51
Chinook	30	2.8	27	10.7	7.5	0.28
Pink	23	2.8	31	8.2	7.2	0.23
Rainbow	22	2.5	15	8.8	6.6	0.44
			Hard Water pH 8.2			
Coho	210	1.8	13	117	5.9	0.45
Chum	202	1.4	11	144	4.6	0.41
Chinook	220	1.7	17	129	5.6	0.32
Pink	380	1.4	14	261	4.6	0.33
Rainbow	220	1.7	14	129	5.6	0.40

[a]Data from Wan et al. (1989)

[b]As reported by Wan et al. (1989) in units of mg product/L.

4.2. EXPOSURE ASSESSMENT

4.2.1. Terrestrial Animals. Terrestrial animals may be exposed to any applied herbicide from direct spray; the ingestion of contaminated media (vegetation, prey species, or water); grooming activities; indirect contact with contaminated vegetation; or inhalation.

As would be expected from the pharmacokinetics behavior of glyphosate in experimental mammals, the available field studies indicate that glyphosate is not bioaccumulated in terrestrial species including carnivores (shrews and weasels), herbivores (woodrat, squirrel, vole, and chipmunk), or omnivores (deer mice) (Newton et al. 1984). In these species, whole body residues remained substantially less than residues found on vegetation after an initial application of glyphosate at 3.3 kg/ha. Initial residues on vegetation ranged from 5 mg/kg (litter) to 489 mg/kg (top crown). Whole body residues were <0.5 mg/kg, and the highest residue in the viscera of these species, was 5 mg/kg. Over a 55-day post-application collection period, the investigators noted that body residues were not detectable, even when residues on vegetation remained detectable, which indicates that glyphosate is eliminated rapidly by these species.

In this exposure assessment, estimates of oral exposure are expressed in the same units as the available toxicity data (i.e., oral LD_{50} and similar values). As in the human health risk assessment, these units are usually expressed as mg of agent per kg of body weight and abbreviated as mg/kg body weight. For dermal exposure, the units of measure usually are expressed in mg of agent per cm of surface area of the organism and abbreviated as mg/cm^2. In estimating dose, however, a distinction is made between the exposure dose and the absorbed dose. The *exposure dose* is the amount of material on the organism (i.e., the product of the residue level in mg/cm^2 and the amount of surface area exposed), which can be expressed either as mg/organism or mg/kg body weight. The *absorbed dose* is the proportion of the exposure dose that is actually absorbed by the animal. Inhalation exposure is calculated, in a similar way, as the proportion of the compound retained in the animal after exposure. Sometimes, it is appropriate to combine oral, dermal, or inhalation exposure in order to estimate the total impact on the organism, as discussed further in the risk characterization (section 4.4).

For the exposure assessments discussed below, general allometric relationships are used to model exposure. In the biological sciences, allometry is the study of the relationship of body size or mass to various anatomical, physiological, or pharmacological parameters (e.g., Boxenbaum and D'Souza 1990). Allometric relationships take the general form:

$$y = aW^x \qquad \textbf{(4-3)}$$

where W is the weight of the animal, y is the variable to be estimated, and the model parameters are **a** and **x**.

For most allometric relationships used in this exposure assessment, such as the relationship of body weight to surface area as well as the consumption of food and water, **x** ranges from

approximately 0.65 to 0.75. These relationships dictate that, for a fixed level of exposure (e.g., levels of a chemical in food or water), small animals will receive a higher dose, in terms of mg/kg body weight, than large animals will receive.

For many compounds, allometric relationships for interspecies sensitivity to toxicants indicate that for exposure levels expressed as mg toxicant per kg body weight (mg/kg body weight), large animals, compared with small animals, are more sensitive. Glyphosate is an exception to this general pattern. As discussed in section 4.3., larger animals, including humans, appear to be no more sensitive than small animals are to glyphosate. Consequently, for the exposure assessment, generic estimates of exposure are given for a small mammal. A body weight of 20 g is used for a small animal, which approximates the body weight of small mammals such as mice, voles, shrews, and bats. All body weight values are taken from U.S. EPA (1989a) unless otherwise specified. In some scenarios, the available toxicity data support specific assessments for other species, such as birds or invertebrates. Examples of such assessments are discussed below.

4.2.1.1. ***Direct Spray*** **--** In the broadcast application of any herbicide, wildlife species may be sprayed directly. This is similar to the accidental exposure scenarios for the general public discussed in section 3.2.2.

In a scenario involving exposure to direct spray, the extent of dermal contact depends on the application rate and the surface area of the organism. As discussed in section 2, the Forest Service usually uses glyphosate at an application rate of 1 lb a.e./acre or approximately 0.0112 mg a.e./cm^2. Most application rates will not exceed 2.5 lbs a.e./acre or 0.0280 mg/cm^2 and the maximum allowable application rate is 7.5 lbs a.e./acre or 0.0840 mg/cm^2.

For mammals, surface area (*SA*) can be calculated as a function of body weight (Boxenbaum and D'Souza 1990):

$$SA\ (cm^2) = 1110 \cdot BW(kg)^{0.65}$$

where:

SA = surface area (cm^2)
BW = body weight (kg)

(4-4)

Thus, the calculated surface area of a 20 g mammal is approximately 87 cm^2 [$1110 \cdot 0.020^{0.65}$] or 4.4 cm^2/g body weight. At the typical application rate of 0.0112 mg/cm^2 (1 lb a.e./acre), the animal would be exposed to approximately 25 mg/kg

$$0.5 \cdot 87\ cm^2 \cdot 0.0112\ mg/cm^2 \div 0.020\ kg = 24.36\ mg/kg.$$

Here, surface area is divided by 0.5, assuming that only 50% of the body surface is exposed to the direct spray. Higher application rates would result in exposures that increase linearly with the application rate: about 60 mg/kg at 2.5 lbs a.e./acre and 180 mg/kg at the maximum allowable rate of 7.5 lbs a.e./acre.

The above estimates represent the exposure dose, the amount deposited on the organism. For most organisms, the risk characterization must be based on estimates of absorbed dose which is then compared to oral toxicity data (e.g., NOAELs and LD_{50} values). To estimate the absorbed dose from the exposure dose requires estimates of dermal absorption rates. As in the human health risk assessment (see section 3.2.2.3), absorption rates of 0.005–0.01 day^{-1} will be used. These values are based on dermal absorption studies in monkeys (Wester et al. 1991) using glyphosate and the POEA surfactant. Thus, at an application rate of 1 lb a.e./acre, the absorbed dose after a direct spray would be approximately 0.1–0.2 mg/kg/day

$$24.36 \text{ mg/kg} \cdot 0.005\text{–}0.01 \text{ day}^{-1}$$

and about 0.3–0.6 mg/kg/day at an application rate of 2.5 lbs a.i./acre. At the maximum allowable application rate of 7.5 lbs a.i./acre, the estimated absorbed dose would be approximately 1–2 mg/kg/day. All of these estimates apply to the amount absorbed during the first 24-hour period following the direct spray event.

These estimates of absorbed doses may bracket plausible levels of exposure for small mammals. Some animals, particularly birds, groom frequently, and grooming may contribute to the total absorbed dose by the direct ingestion of the compound during grooming of fur or feathers. Furthermore, other vertebrates, particularly amphibians, may have skin that is far more permeable than the skin of most mammals (Moore 1964). Quantitative methods for considering the effects of grooming or increased dermal permeability have not been located in the available literature. As discussed in section 4.4, even if instantaneous and complete dermal absorption is assumed, the exposure doses derived are of minimal concern. Consequently, this exposure assessment uses the assumption of complete and instantaneous absorption as an upper limit of exposure to account for the effects of grooming or unusually high dermal permeability.

Limited contact toxicity data are available for the honey bee (Appendix 2-1). As summarized in U.S. EPA (1993b), the contact LD_{50} for glyphosate is >0.100 mg/bee. Using a body weight of 0.093 g for the honey bee (USDA 1993) and the equation above for body surface area, the estimated surface area for the honey bee is 2.6 cm^2. Thus, an application rate of 0.0112 mg a.i./cm^2 would correspond to approximately 0.03 mg/bee

$$0.0112 \text{ mg a.i./cm}^2 \cdot 2.6 \text{ cm}^2 = 0.02912 \text{ mg.}$$

Similarly, the upper range of anticipated application rates (2.5 lbs a.i./acre) would correspond to a dose of approximately 0.07 mg/bee, and the highest allowable rate, 7.5 lb/acre, would correspond to a dose of approximately 0.2 mg/bee.

4.2.1.2. ***Indirect Contact*** **--** As in the human health risk assessment (see section 3.2.3.3), the only approach for estimating the potential significance of indirect dermal contact is to assume a relationship between the application rate and dislodgeable foliar residue. The study by Harris and Solomon (1992), discussed in section 3.2.3.3, is used to estimate that the dislodgeable

residue will be approximately 100 times less than the nominal application rate. Thus, at an application rate of 1 lb a.i./acre or approximately 0.0112 mg/cm^2, the estimated dislodgeable residue will be 0.0001 mg/cm^2.

Unlike the human health risk assessment, however, no transfer rates are available for wildlife species. As discussed in Durkin et al. (1995), the transfer rates for humans are based on brief (e.g., 0.5–1 hour) exposures that measure the transfer from contaminated soil to uncontaminated skin. Wildlife, compared with humans, may spend much longer periods of time in contact with contaminated vegetation. It is reasonable to assume that for prolonged exposures an equilibrium may be reached between levels on the skin and levels on contaminated vegetation, although there are no available data regarding the kinetics of such a process. The available bioconcentration data on glyphosate discussed in section 4.1 suggests that glyphosate is not likely to partition from the surface of contaminated vegetation to the surface of skin, feathers, or fur. Thus, a plausible partition coefficient is unity (i.e., the residue on the animal will be equal to the dislodgeable residue on the vegetation).

The exposure dose may be estimated in a manner similar to that for direct dermal exposure (section 4.2.2.1). For a 20 g mammal with a surface area of 87 cm^2, the exposure dose is 0.44 mg/kg

$$87 \text{ cm}^2 \cdot 0.0001 \text{ mg/cm}^2 \div 0.020 \text{ kg}.$$

Unlike the calculation for direct dermal exposure, this calculation assumes that 100%, rather than 50%, of the body surface is exposed to the contamination.

As with the direct contact scenario, the estimates of exposure dose are the upper limits of absorbed dose and may apply to animals that groom extensively or animals that have highly permeable skin. Because the exposure doses are far below any level of concern for direct toxic effects, as discussed in the risk characterization (section 4.4), this exposure scenario will not be expanded to consider the distinction between exposure dose and absorbed dose.

4.2.1.3. ***Ingestion of Contaminated Vegetation or Prey --*** As in the human health risk assessment, the consumption of contaminated vegetation is a plausible route of exposure. In the human health risk assessment, residues on berries of 4.5 mg/kg berry associated with the application of 1 lb a.i./acre were used. This estimate could also be applied to wildlife species that might consume berries. As indicated in several field studies (Appendix 2-2), however, much higher concentrations may be encountered on other types of vegetation, such as leaves near the top of the canopy.

The most extensive study relating foliar residues to application rates is that of Thompson et al. (1994). In this study, VISION, a glyphosate formulation equivalent to Roundup, as well as two other formulations of glyphosate, were applied to plots of sugar maple using backpack sprayers at

rates ranging from about 0.25 to 2 kg/ha. Foliar residues at various times after application fit the general exponential (first-order) decay model:

$$Y = \alpha \cdot e^{-\beta \cdot t} \qquad \textbf{(4-5)}$$

where ***Y*** is the mass of glyphosate per unit mass of vegetation, ***t*** is time in days, and and are model parameters. At an application rate of 1.1 kg/ha (1 lb a.i./acre), the average residue on vegetation immediately after spraying was about 287 mg/kg. By comparison, initial residues in the top crown of a forest canopy immediately after an aerial application of 3.3 kg/ha (≈2.94 lbs a.i./acre) were about 489 mg/kg (Newton et al. 1984). Normalized for an application rate of 1 lb a.i./acre, this rate corresponds to residues of about 170 mg/kg. Both of these values are somewhat greater than the empirical estimate of 125 mg/kg·lb a.i. provided by these investigators for extreme estimates of residues on leaves and leafy crops but are very close to the estimate of 240 mg/kg·lb a.i. given by Hoerger and Kenaga (1972) for extreme estimates of residues on range grass.

As a conservative approach, a residue rate of 300 mg/kg·lb a.i. applied will be used. This encompasses the highest rate reported in the study by Thompson et al. (1994) but is not implausibly above the lower rates noted in other studies.

Allometric relationships and species specific data (U.S. EPA 1989a) suggest that the amount of food consumed per day by a small mammal (i.e., approximately 20 g) is equal to about 15% of the mammal's total body weight. Using this estimate with a residue rate of 300 mg/kg·lb a.i. yields a dose estimate of 45 mg/kg

$$0.15 \cdot 300 \text{ mg/kg·lb a.i} \cdot 1 \text{ lb/acre}.$$

Higher application rates would yield correspondingly high dose estimates (e.g., approximately 110 mg/kg at 2.5 lbs a.i./acre and 340 mg/kg at 7.5 lbs a.i./acre).

All of these estimates are based on the assumption that 100% of the diet is contaminated. Under the assumption that only 10% of the diet is contaminated, the dose estimates decrease by a factor of 10. All of these dose estimates apply to levels on vegetation immediately after application.

As discussed in section 4.4, the above exposure estimates are of minimal concern for acute exposure. For estimating the effects of longer-term exposures, time-weighted average concentrations will be used, similar to the approach taken in the human health risk assessment (see section 3.2.3.6). In the study by Thompson et al. (1994), the average decay parameter ( ) for three blocks treated with VISION was approximately 0.4 $days^{-1}$, corresponding to a residue half-time of about 1.6 days [0.693÷0.4]. Consequently, by day 20, the residues would be about 0.0003 [$e^{-20 \cdot 0.4} = 0.000335$] of those at time zero. Therefore, the geometric mean concentration over the 20-day period would be approximately 0.02 [$(1 \cdot 0.0003)^{0.5}$=0.018] of the time zero level. Taking 300 mg/kg·lb a.i as the residue at time zero, the time-weighted average over the first 20

days after application would be 6 mg/kg/day [0.02 · 300 mg/kg·lb a.i]. Using a 15% value for food consumption, this would correspond to a time-weighted average dose of approximately 1 mg/kg/day per lb a.i.

$$6 \text{ mg/kg/day} \cdot 0.15 = 0.9 \text{ mg/kg bw}$$

Thus, for each pound of glyphosate applied per acre, average doses over the first 20 days after application would be 1 mg/kg/day.

Other time periods—10 or 90 days—would yield different estimates of intake rates. Periods of <20 days would yield estimates close to those associated with acute exposure studies. A period of 20 days is selected as a reasonably conservative period (i.e., one that leads to relatively high estimates of average daily exposure). As discussed in the risk characterization, the dose estimates over this period are not likely to be of ecological concern. Consequently, corresponding calculations are not made for longer exposure periods.

4.2.1.4. ***Ingestion of Contaminated Water*** **--** Estimates of acute and chronic exposure to contaminated waters will be identical to those used in the human health risk assessment. For acute exposures at the typical application rate of 1 lb a.i./acre, the maximum anticipated concentration is 0.093 mg/L. For longer-term exposures, the average level in water associated with an application rate of 1 lb a.i./acre is 1.0 μg/L. The basis for these estimates is discussed in section 3.2.3.4.

There are well-established relationships between body weight and water consumption across a wide range of mammalian species [e.g., U.S. EPA (1989a)]. Mice, weighing about 0.02 kg, consume approximately 0.005 L of water/day (i.e., 0.25 L/kg body weight/day). Thus, for the assessment of acute toxic effects, the estimated dose for a small mammal is 0.02 mg/kg,

$$0.093 \text{ mg/L} \cdot 0.005 \text{ L} \div 0.02 \text{ kg}.$$

For estimating the potential for chronic toxic effects in a small mammal, the estimated dose is 0.00025 mg/kg

$$0.001 \text{ mg/L} \cdot 0.005 \text{ L} \div 0.02 \text{ kg}.$$

4.2.2. **Terrestrial Plants.** The primary hazard to non-target terrestrial plants is from unintended direct deposition or spray drift. Unintended direct spray will result in exposure equivalent to the application rate. As discussed in the dose-response assessment for terrestrial plants (section 4.3.3), such exposures are likely to result in adverse effects to a number of plant species.

The potential for spray drift has been investigated in a number of field studies. Yates et al. (1978) studied the kinetics of glyphosate drift over a flat field after ground and aerial applications

involving the use of various nozzles and various spray application rates. During the application, wind speeds were about 2–4 m/second, which is about 4.4–8.8 miles/hour. Glyphosate deposition 25 m (about 83 feet) downwind from the application site ranged from approximately $5 \cdot 10^{-6}$ to $7 \cdot 10^{-4}$ of the nominal application rate, based on drift deposited on Mylar fallout sheets (Yates et al. 1978, p. 600, Figure 1). In addition, this study demonstrates that the deposition between 25 and 800 m generally followed a double log linear relationship (i.e., the log of the distance down wind plotted against the log of the deposition yielded a straight line for most applications, although curvelinear relationships were noted for some applications).

Substantially greater drift was found by Riley et al. (1991). In this study, glyphosate was applied using a Bell 206B helicopter with a 13.1 m mid-mounted boom and operating a 21.3 m swath. The average emission rate was 4.5 L/nozzle/minute under a pressure of 207 kPa. During the three applications, wind speeds ranged from 1.5–4.2 m/second (3.4–9.4 miles/hour). Glyphosate deposition 30 m downwind from the application site was less than 0.1 of the nominal application rate. At 200 m down wind, the deposition was less than 0.05 of the nominal application rate.

Information on the off-site deposition of glyphosate after aerial application has also been presented in a series of publications by Payne and coworkers (Payne 1992, Payne 1993, Payne et al. 1990, Payne and Thompson 1992, Payne et al. 1989). Like the results reported by Riley et al. (1991), the downwind depositions noted by Payne and coworkers tend to be higher than those noted by Yates et al. (1978). Payne (1993) attributes this difference primarily to difference in release heights: 2 m (Yates et al. 1978) and 10 m (Payne 1992, Payne 1993, Payne et al. 1990, Payne and Thompson 1992, Payne et al. 1989). Based on a statistical analysis of deposit rates using an double log model (i.e., mathematically equivalent to the model used in allometric relationships), Payne and Thompson (1992) have reported decay coefficients (in the allometric equation) ranging from 1.7 to 4.3 for aerial applications using a release height of 10 m with wind speeds ranging from 2.2 to 5.7 m/second (4.9–12.8 miles/hour) with stable, neutral, unstable boundary layers. Similar to the results of Riley et al. (1991), glyphosate deposition 50 m downwind from the application site was about 0.1 of the nominal application rate. At 200 m down wind, the deposition was less than 0.002–0.005 of the nominal application rate (Payne 1993).

4.2.3. Aquatic Organisms. As discussed in section 3.2.3.4, fields studies indicate that maximum initial concentrations of glyphosate in water after aerial or ground applications can be estimated, based on application rates, at 0.088–0.093 mg/L·lb applied. Glyphosate concentrations in natural water will diminish rapidly due to microbial degradation, binding to suspended particulate, or dispersion.

While these estimates can be used directly to estimate the effects of glyphosate alone, the Roundup formulation of glyphosate contains a surfactant, and, as discussed in section 4.1.3, the surfactant is the primary toxicant of concern for the toxic effects of Roundup on fish. Unpublished studies conducted by Monsanto (Hoogheem 1987, Letter Feb 27 to Larry Gross with attachments) indicate that the surfactant, like glyphosate, will have a relatively short

residency time in ambient water. Nonetheless, because of the acute toxic effects of the surfactant, the added toxic burden of the surfactant must be considered in the risk assessment. As discussed in the following section, however, there are adequate data regarding the toxicity of Roundup to aquatic species to support separate dose-response relationships. Consequently, the exposure rates given above will be applied to all glyphosate formulations, and the fact that Roundup has a relatively greater degree of toxicity will be considered in the dose-response assessment.

4.3. DOSE-RESPONSE ASSESSMENT

4.3.1. Terrestrial Animals. As summarized in Appendices 1-1 and 2-1, glyphosate has a low order of acute toxicity to mammals, birds, and invertebrates, with acute oral LD_{50} values ranging from about 1,500 to >5,000 mg/kg. Based on the available human data, the acute oral LD_{50} for Roundup appears to be about 3,000 mg/kg (see section 3.3).

For many chemicals, systematic differences in species sensitivity are apparent and generally indicate that small animals are less sensitive (i.e., have higher LD_{50} values) than large animals. This general pattern is the basis for the uncertainty factor of 10 used for animal-to-human extrapolation in the derivation of the RfD (Dourson and Stara 1983) and is often used to extrapolate across species (e.g., Davidson et al. 1986) based on the general allometric relationship:

$$LD_{50} = aW^{b} \qquad \textbf{(4-6)}$$

where W is the body weight and a and b are model parameters. When small species are less sensitive than larger species, the slope parameter, b, is negative.

Glyphosate appears to be an exception to this general pattern: no substantial or systematic differences are apparent in the acute oral toxicity of glyphosate to large and small animals. The data supporting this assertion are summarized in Table 4-3. In this table, the LD_{50} values for experimental mammals (rats and mice) are taken from Appendix 1-1. All of these LD_{50} values are for technical grade glyphosate (i.e., no surfactants or other additives).

The LD_{50} for humans is based on human poisoning episodes (Tominack et al. 1991) and the statistical analysis of the dose-response relationships (see section 3.1.2). These incidents involved exposure to Roundup (i.e., glyphosate with the POEA surfactant). As discussed in section 3.1.2., the reported acute oral LD_{50} values in rats for glyphosate and Roundup are almost the same (5,500 versus 5,400 mg/kg). It seems reasonable, therefore, to use the estimated human LD_{50} for Roundup to assess interspecies patterns of sensitivity to glyphosate. As discussed below, using

Table 4-3. Toxicity data used to illustrate the variability of interspecies sensitivity to glyphosate

Species	LD_{50} (mg/kg)	Body weight[a] (kg)
Humans	3000	70[b]
Rabbit[d]	3800	1.35
Mallard duck[d]	>1000	1.0
Bobwhite quail[d]	>1000	0.178
Mouse[c]	1568	0.020
Rat[c]	5600 4873 4320	0.400
Snail[d]	>4994	0.00025
Honey bee[d]	>1075	0.000093

[a] All data from USDA (1993), unless otherwise specified

[b] U.S. EPA (1989a)

[c] Data taken from Appendix 1-1.

[d] Data taken from Appendix 2-1.

this data point does not substantially affect the analysis.

All of the data on wildlife (rabbits, birds, snails, and honey bees) are taken from Appendix 2-1. Except for the LD_{50} for bees, all of the LD_{50} values reported in Table 4-3 are derived from LC_{50} values that represent levels of glyphosate in the diet. These values are converted to LD_{50} values using estimates of dietary intake taken from USDA (1993). With the exception of the LD_{50} for rabbits, the LD_{50} values for these species are all expressed as greater than a particular value (e.g., >1,000 mg/kg for quail). For snails, the dose of 4,994 mg/kg was associated with no mortality (Schuytema et al. 1994). Thus, the dose of 4,994 mg/kg is actually an LD_0. For the other species, it is not clear in the publications from which these data are taken whether any mortality was observed.

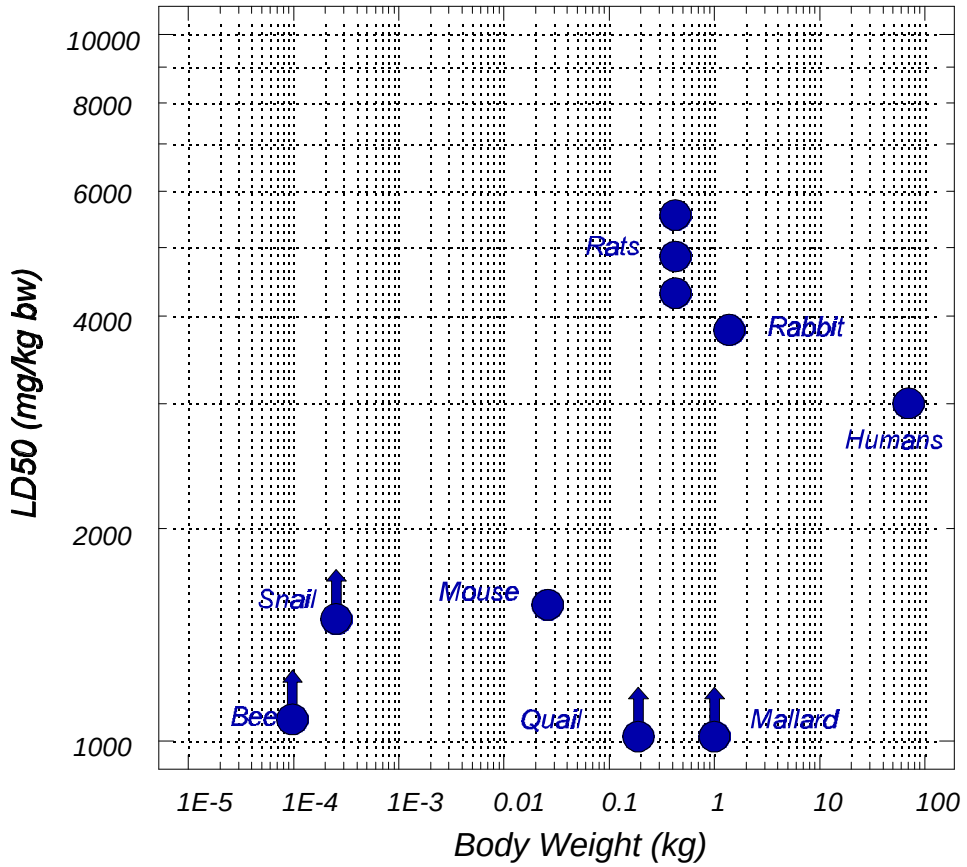


Figure 4-1. Variability of interspecies sensitivity to glyphosate (see Table 4-1 for data and text for discussion).

The data summarized in Table 4-3 are illustrated in Figure 4-1. In this figure, the LD_{50} values are plotted as circles for the various species as labeled. Points plotted with an arrow pointing upward indicate those LD_{50} values that are expressed as greater than (>) a particular value. In other words, the true LD_{50} is higher than the plotted value. Visual inspection of this data clearly indicates no basis for assuming that larger organisms are more sensitive than smaller organisms are to glyphosate. Although the data on rats, rabbits, and humans fit the typical allometric relationship the differences among these LD_{50} values are not substantial. More importantly, if the standard allometric relationship held, the expected LD_{50} for the mouse would be about 9,000 mg/kg rather than the reported value of 1,568 mg/kg. Including only those points that represent true LD_{50} values, the allometric relationship suggests that smaller animals may be somewhat less sensitive than larger animals ($\boldsymbol{b}$ = 0.04) but the relationship is not statistically significant (p=0.64). Thus, the weight of the evidence supports the assertion that there does not appear to be systematic differences among species.

For non-target terrestrial species the LD_{50} of 1,569 mg/kg, rounded downward to 1,500 mg/kg, will be used directly to assess the potential for acute lethal effects. An estimate of 400 mg/kg will be used for the acute dose which is not likely to be lethal or associated with frank adverse effects. This is rounded down from the estimated threshold of 485 mg/kg, derived in section 3.3.3. Based on the categorical regression analysis, the likelihood of a group of animals exposed to this dose being classified as evidencing frank adverse effects is approximately 3%. These benchmarks will be applied to the exposure of the 20 g mammal, derived in section 4.2.

To assess the potential for longer-term toxic effects, the NOAEL of 10 mg/kg/day will be used, consistent with the derivation of the RfD for the protection of human health (see section 3.3.2). The consequences of exceeding this level of exposure will be assessed using the categorical regression of the dose-severity data as summarized in section 3.3.3.

4.3.2. Terrestrial Plants. As discussed in the hazard identification for terrestrial plants (see section 4.1.2), there are three types of exposure to be considered: direct contact (i.e., either direct spray or drift), vaporization, and soil contamination. Because glyphosate has a low rate of volatility and a high affinity for soil, volatilization and soil contamination present negligible hazards. Moreover, direct contact with glyphosate from either drift or unintentional application is plausible.

For direct spray or drift, the relevant exposure metameter is the application rate or functional rate of deposition expressed in units of toxicant weight per unit area [e.g., lb a.i./acre]. In some respects, the product labels for glyphosate (USDA 1995) provide useful information on effective levels of application and suggests differences in species or life-stage sensitivity. For example, the maximum broadcast application rate, 7.5 lbs. a.i./acre, is effective against most species and life stages of terrestrial plants. Conversely, application levels <0.5 lbs a.i./acre, are less than those recommended for the control of most plant species.

The dose-response assessment for direct spray can also be developed based on field studies with glyphosate (Appendix 2-2). The effect of glyphosate on non-target vegetation can be influenced by both the application rate and timing of application. Neal and Skroch (1985) assayed the effect of glyphosate on 13 species of woody ornamental at application rates ranging from 0.8 to 3 kg/ha and at six different application times apportioned over the course of 1 year (March, April, June, August, September, and November). Injury was assessed by visual rating as well as the weight of fresh shoots harvested. All observations were made on the first June following treatment. In general, all application rates caused some level of damage in all species tested. This is consistent with the results of other field studies summarized in Appendix 2-2 indicating that application rates $\geq$0.75 kg/ha cause direct toxic effects in a variety of plant species (Anthony and Morrison 1985, Boyd et al. 1995, Cain 1991, Lund-Hoie and Rognstad 1990, MacKinnon and Freedman 1993, Newton et al. 1992, Ogner 1987 a,b,c,d, Santillo 1994, Santillo et al. 1989a). In the study by Neal and Skroch, the different plant species were classified into four groups depending on how application timing affected the degree of damage. The most sensitive species, ajuga, azalea, and

variegated liriope, were damaged at all application dates. Other species, such as juniper, were less sensitive to glyphosate and most damaged by summer applications. As discussed by Neal and Skroch (1985), different species-to-specific temporal patterns have been observed for other species, such as deciduous fruit trees and evergreen species, which may be most severely affected by applications made during the fall.

There are two studies, Marrs et al. (1991) and Breeze et al. (1992), that try specifically to assess the potential for damage from glyphosate drift. The study by Marrs et al. (1991) used a simple bioassay design in which pots containing five perennial species of two age groups (classified as young and old) were placed at various distances downwind of plots treated with glyphosate using a tractor-mounted sprayer at a rate of 2.4 kg/ha (≈2.1 lbs/acre). The treated plots were surrounded by grasses of varying heights. The mean wind speed during all applications ranged from 2 to 4 m/second (4.4–9 miles/hour). After the application, the potted plants were removed to a greenhouse and observed for 20 weeks. In plants located 4 m downwind, treatment caused a modest reduction (12% decrease in yield, compared with controls) in only one species, mature *Leontodon hispidus*. In three other trials, a slight stimulation of growth was observed in young *Leontodon hispidus* as well as young *Digitalis* and *Primula*.

The study by Breeze et al. (1992) consisted of a series of bioassays in which the ED_{50} and ED_{10} values (in units of μg/plant based on a reduction in shoot weight) of glyphosate were assayed in 14 species of wild plants. The reported ED_{50} values range from 19 μg/plant (*Cardamin pratensis*) to >1,000 μg/plant (five species). An empirical exposure model was used by Breeze et al. (1992) to estimate droplet deposition:

$$97 + 31.9x - 35.13x^2 + 17.13x^3 - 3.975X^4 + \quad \textbf{(4-7)}$$

where ***y*** is the common log of the number of droplets deposited per m^2 and ***x*** is the distance downwind in meters. The exposure model assumes a 100 μm droplet diameter at an application rate of 200 L/ha, and a release height of 0.5 m above a crop in unstable conditions. A comparison of the bioassay results to the modelled exposures is presented and discussed in section 4.4.

4.3.3. Aquatic Organisms.

4.3.3.1. Fish -- As summarized in Appendix 2-3 and reviewed by U.S. EPA (1993b, RED) as well as Smith and Oehme (1992), glyphosate is relatively non-toxic to fish, with 24- to 96-hour LC_{50} values ranging from approximately 10 mg/L at a relatively acidic pH (≈6) to >200 mg/L at alkaline pH (≈10). As noted in Appendix 2-3, much higher LC_{50} values have been reported for glyphosate in some species. Specific reasons for these discrepancies have not been identified. The results of acute aquatic bioassays can be highly variable depending on experimental conditions and the initial state of the organisms assayed.

As discussed in section 4.1.3, Roundup and the surfactant used in Roundup are substantially more toxic and relationship of pH to toxicity is the opposite of that for glyphosate. At a relatively

acidic pH, LC_{50} values for Roundup range from about 6 mg/L to about 30 mg/L for various species. At an alkaline pH, LC_{50} values approach 1 mg/L.

There is a very weak duration-response relationship for glyphosate, like the pattern seen for the effect of time on the response of experimental mammals (see section 3.3.3). This has been demonstrated clearly in the bioassays conducted by both Folmar et al. (1979) and Wan et al. (1989), each of which presents 24-, 48-, and 96-hour LC_{50} values for several species of fish. A comparison of the 24- and 48-hour LC_{50} values from these studies is given in Appendix 2-3. In no case does the difference vary by more than a factor of 2.

In addition to a weak time-response relationship, glyphosate appears to exhibit a relatively steep dose-severity relationship. In other words, the threshold for toxicity seems to be relatively close to levels that cause substantial lethality. This has been demonstrated both by Anton et al. (1994) for three formulations of glyphosate. In this study, the ratio of 96-hour LC_{50} values to 96-hour NOECs (no observed effect concentrations) ranged from about 6 to 3. In other words, a decrease in concentration by only a factor of 3–6 reduced the toxic response from substantial lethality to no apparent effect.

Both the weak dose-response relationship and strong dose-severity relationship are consistent with the available long-term studies in fish. As summarized by U.S. EPA (1993b, p. 40-41), the 96-hour LC_{50} for glyphosate in the fathead minnow is 97 mg/L, and the chronic NOEC from a full life cycle study in this species was only about 4 times less, 25.7 mg/L. For Roundup, a 10-day NOEC of 2.78 mg/L at pH 5.7 has been reported for Coho salmon (Mitchell et al. 1987a). Higher concentrations were not tested. The NOEC, however, is only a factor of about 10 less than the LC_{50} reported by Wan et al. (1989) for this species at a pH of 6.3.

For assessing the potential for toxic effects in fish, a reference concentration of 1 mg/L will be used for glyphosate. This is about a factor of 10 less than the lowest reported LC_{50}. For Roundup, a 10-fold lower value will be used, 0.1 mg/L. Again, this is about a factor of 10 less than the lowest reported LC_{50}. At these levels, there is no reason to anticipate acute or long-term effects in fish. As these levels are exceeded, effects might be seen in some organisms depending on the pH of the water.

4.3.3.2. Aquatic Invertebrates -- The toxicity of glyphosate and Roundup to aquatic invertebrates is summarized in Appendix 2-4. As with fish, highly variable results have been reported. Where comparable data are available, however, the patterns appear to be similar to those observed in fish. For example, LC_{50} values using daphnia are comparable for glyphosate (780 mg/L) and Rodeo (930 mg/L) but much lower for Roundup (5.3 mg/L) (Monsanto Co. 1982a, Appendix 3-3).

Table 4-4. Acute toxicity of glyphosate (Rodeo) and adjuvants*

Organism	Duration (hours)	LC_{50} Values (mg/L)	
		Glyphosate	X-77 Spreader
Daphnia magna, water flea	48	218	2.0
Hyalella azteca, amphipod	96	720	5.3
Chironomus riparius, midge	48	1,216	10
Nephelopsis obscura, bait leech	96	1,177	14

*Source: Henry et al. (1994)

The toxicity of glyphosate and a surfactant, X-77 Spreader, used with Rodeo has been examined by Henry et al. (1994), using several species of aquatic invertebrates (Table 4-4). This study also provides data regarding the toxicity of Chem-Trol, another adjuvant that was used with Rodeo. Chem-Trol was virtually non-toxic (LC_{50} >28,000 mg/L) and information about this agent is not reviewed further in this risk assessment. All bioassays were conducted at a pH of 8.1–8.2. Like the results reported by Folmar et al. (1979) and Wan et al. (1989) for fish, the surfactant was much more toxic than glyphosate, with the relative potencies of the surfactant ranging from about 83 to 135. These relative potencies are only somewhat less than those reported by Folmar et al. (1979) for bioassays conducted at pH 9.5 (see Table 4-1) and overlap with those reported by Wan et al. (1989) for bioassays conducted at pH 8.2 (see Table 4-2). Daphnia were significantly more sensitive than the other invertebrates to glyphosate. The LC_{50} for daphnia, 218 mg/L, is about the same as that reported for fish at a comparable pH. Henry et al. (1994) also conducted a series of experiments on mixtures of glyphosate, the surfactant, and Chem-Trol. Like the results of the earlier studies on fish, no remarkable deviations from additivity were noted.

In a study of avoidance behavior, Folmar (1978) noted that mayflies avoided Roundup at concentrations of 10 mg/L; however, no effect was noted at concentrations of 1 mg/L.

Because the available data on aquatic invertebrates are similar to those with fish, reference concentrations for fish will be used also for invertebrates.

4.3.3.3. Aquatic Plants -- The toxicity of glyphosate and Roundup to aquatic plants is summarized in Appendix 2-5. As indicated in this appendix, the available data suggests that glyphosate is not preferentially toxic to aquatic plants when exposures occur via contaminated water. Although glyphosate is registered for use in the control of aquatic vegetation, it is not effective if all or most of the foliage is under water (Monsanto Co. 1993, Rodeo product). As with the effect on terrestrial plants, direct foliar absorption is the primary route of absorption.

The only substantial inconsistency in the available literature concerns the inhibition of *Anabaena flosaquae*. U.S. EPA (1993b) reports an LC_{50} of 11.7 mg/L, and an LC_{50} of 304 mg/L is reported by Maule and Wright (1984). As noted above, the results of bioassays on the same species can differ remarkably with differences in experimental conditions. The reasons for the differences in the results reported by Maule and Wright (1984) and U.S. EPA (1993b) are not apparent. The U.S. EPA summary does not provide detailed information about experimental conditions.

The study by Peterson et al. (1994) is specifically designed to assess the impact of glyphosate at ambient levels. As summarized in Appendix 2-5, these investigators assessed the inhibition of carbon fixation in various species of green algae and cyanobacter as well as one macrophyte, *Lemna minor*, from exposure to glyphosate in water at a concentration of 2.8 mg a.i./L. This concentration was selected because, following the exposure assumptions used by these investigators [application on to a 15 cm deep body of water], a concentration of 2.8 mg a.i./L could be associated with an application rate of 4.272 kg/ha (≈3.8 lbs a.i./acre). As discussed in section 4.2.2.3, this risk assessment assumes an exposure factor of 0.093 mg/L·lb applied. At an application rate of 3.8 lb/acre, this would be associated with an exposure level of 0.35 mg/L. The nearly 10-fold difference is due to the fact that the exposure assumptions used by Peterson et al. (1994) do not consider dispersion, particulate binding, or other removal processes. At a concentration of 2.8 mg/L, substantial (>20%) inhibition was observed in only two species of green algae. No effect was seen on *Lemna minor*.

The only information regarding the effect of Roundup on aquatic vegetation comes from the study by Goldsborough and Brown (1989) in which the EC_{50} values for the inhibition of photosynthesis in a mixed population of algae from several different ponds ranged from 35.4 to 44.4 mg/L. The NOEC for this effect was 0.89 mg/L. These values seem to be consistent with those for glyphosate.

A concentration of 1 mg/L would not be sufficiently protective for most algal species. The lowest EC_{50} is 0.85 mg/L or about 1 mg/L. The relationship between EC_{50} values and NOECs has not been studied extensively for glyphosate. The study by Goldsborough and Brown (1989) indicates that the values differ by a factor of about 40. Thus, the reference concentration for sensitive algal species would be about 0.02 mg/L [0.85 mg/L ÷ 40]. Based on the study by Peterson et al. (1994), it is apparent that some species of algae would be unaffected by concentrations more than 100 times greater than this level.

4.4. RISK CHARACTERIZATION

4.4.1. Overview. As with the human health risk assessment, there is very little indication that glyphosate will cause adverse effects in the environment at anticipated levels of exposure. The small mammal is used as a conservative target species for characterizing risk because small organisms, compared with large organisms, generally receive higher doses of an agent at fixed levels of exposure in environmental media (e.g., contaminated food, water, or air). In addition, the available toxicity data do not suggest the existence of systematic differences in sensitivity to glyphosate among species. As in the human health risk assessment, the primary route of exposure for terrestrial animals appears to be contaminated vegetation. For this source, levels of contamination remain below those of concern even at the highest allowable application rate, 7.5 lbs a.i./acre. At application rates anticipated by the Forest Service, levels of exposure are substantially below those of concern. This analysis is consistent with the field studies on glyphosate that indicate the unlikelihood of direct toxic effects.

Glyphosate is an effective herbicide, and terrestrial plants will be affected by applications of glyphosate used to control vegetation. Non-target plants could be damaged by unintentional application or drift. The extent of drift will depend on the specific conditions under which the application occurs. As would be expected, the potential hazards of drift are greater for aerial than ground applications. The extent of damage will depend on the species of plant and the time of application. Field studies involving both ground and aerial applications of glyphosate suggest that the effects of drift are likely to be most evident within 50 m of the application site.

There is little evidence to suggest that aquatic animals or plants will be adversely affected by normal applications of glyphosate. Although glyphosate is registered for use as an aquatic herbicide, it is only effective on aquatic plants whose vegetation is above the water level. Most species of algae and macrophytes do not appear to be more sensitive than fish or aquatic invertebrates are to glyphosate. For most aquatic species, glyphosate levels of 1 mg/L are not likely to cause detectable adverse effects. For aquatic animals, Roundup (glyphosate+POEA) is not likely to cause adverse effects at levels of 0.1 mg/L, measured as glyphosate. There is no reason to suggest that Roundup is more toxic than glyphosate to aquatic plants. Some sensitive species of algae, however, could be affected. Given the rapid dispersion or removal of glyphosate from ambient waters, these effects would most likely be transient.

4.4.2. Terrestrial Animals. The risk characterization for terrestrial animals is summarized in Table 4-5. The top part of table 4-5 summarizes each of the quantitative exposure assessments made in section 4.2.1. for the small (20 g) mammal. The bottom part of the table summarizes the dose-response relationships discussed in section 4.3.1. For each of the exposure assessments, the last column in the table gives the highest hazard quotient relevant to the exposure assessment. The derivation of each of these hazard quotients and an explanation of the term *relevance* is provided in the following paragraphs. Because the individual components of the exposure assessments are pathway specific, this section ends with a discussion of concern for multi-pathway exposures.

There are two risk characterizations for dermal exposures. One involves direct spray, and the other involves dermal contact with contaminated vegetation. For the direct spray scenario, absorption rates of 0.5–1%/day are used as plausible estimates for most species, and a rate of 100% per day is used as a conservative upper limit intended to account for the effects of grooming or unusually high skin permeability. As might be expected, the exposure scenario involving direct dermal spray results in higher levels of exposure, for which the most relevant endpoint of concern is the potential for an acutely lethal effect. In other words, this is an accidental exposure scenario, likely to occur only infrequently. Thus, it is not reasonable to evaluate the potential hazard of a one-time direct spray with a subchronic NOEL. Consequently, for the direct spray scenarios, the relevant hazard quotients are based on a dose estimate that is not likely to cause death after acute exposure.

Table 4-5. Summary risk characterization for a 20 g terrestrial mammal at an application rate of 1 lb a.i./acre

Media/Scenario	Exposure Estimates (mg/kg)	Highest Relevant Hazard Quotient
Direct spray, dermal		
7% dermal absorption	0.1-0.2	0.0003[a]
100% dermal absorption	25	0.06[a]
Indirect dermal contact		
100% dermal absorption	0.44	0.04[b]
Consumption of vegetation		
Extreme exposure assumptions (t_0)		
10% of diet contaminated	4.5	0.01[b]
100% of diet contaminated	45	0.1[a]
Typical exposure assumptions (t_{20})		
10% of diet contaminated	0.1	0.01[b]
100% of diet contaminated	1	0.1[a]
Consumption of water		
Typical ambient levels	0.00025	0.00003[b]
Max. anticipated conc. (0.093 mg/L)	0.02	0.00005[a]
ESTIMATES OF PLAUSIBLE EFFECT/NO-EFFECT LEVELS		
LD_{50}	1500	
Non-lethal acute dose	400	
Long-term NOEL	10	

[a] Hazard quotient based on nonlethal acute dose.

[b] Hazard quotient based on long-term NOEL.

In contrast, indirect dermal contact is a typical exposure scenario. Although glyphosate is not extremely persistent in the environment, the foliar half-times are measured in days, and animals inhabiting the treated area may be in contact with substantial amounts of residue on vegetation for several days. Consequently, hazard quotients for this scenario are based on the estimated long-term NOEL. Therefore, the relevant hazard quotients are higher for the exposure scenario involving indirect dermal contact than for the exposure scenario involving direct dermal contact. Table 4-5 includes only the highest relevant hazard quotient, which is presented in the last column.

Both of the dermal exposure scenarios are conservative. The assumption of 100% dermal absorption is only marginally plausible. Although grooming is a reasonable concern, there is no evidence in the literature to suggest that grooming will substantially enhance exposure to glyphosate among wildlife species or experimental mammals. Furthermore, the study by Gaines (1969) suggests that grooming is not significant in the toxic response of small mammals.

The same assessments can be made for scenarios based on the consumption of contaminated vegetation or water. It is conceivable that 100% of the diet or consumed water is contaminated in a given day; however, it is far less plausible that this rate of contamination would occur for several days. Consequently, hazard quotients derived from this assumption are evaluated using the estimate of the nonlethal acute dose. That 10% of the diet would be contaminated and might remain so for several days seems far more plausible. Consequently, these scenarios are evaluated with the estimate of the long-term NOEL. The most representative hazard quotient is probably the one based on the assumption that 10% of the diet or water is contaminated.

All of the hazard quotients summarized in Table 4-5 apply to application rates of 1 lb a.i./acre. The relationship of these hazard quotients to application rates is linear. Reciprocal of the highest allowable application rate, 7.5 lbs a.i./acre, is 0.133. Thus, hazard quotients $\geq$0.133 given in Table 4-5 would be a marginal cause for concern. The only hazard quotients approaching this level are those for contaminated vegetation, making the very conservative assumption that 100% of the diet is contaminated.

In the environment, organisms are exposed to compounds by more than one pathway. Taking the worst case scenarios for each of the pathways in Table 4-5 results in a dose estimate of about 70 mg/kg [25 + 0.44 + 45 + 1 + 0.02] for the 20 g mammal. This exceeds the long-term NOEL by a factor of 7; is a factor of about 6 less than the estimated nonlethal dose; and is less than the LD_{50} by a factor of about 20. Based on the categorical regression analysis, the probability that this dose would be associated with a frank toxic effect is approximately 1%. This dose estimate is applicable to an exposure scenario in which an animal is sprayed directly with the herbicide, consumes a day's worth of water immediately after spraying, eats highly contaminated vegetation or prey, and remains in the area for a 24-hour period in contact with contaminated vegetation. This is not proposed as a plausible scenario; its purpose is to illustrate that even with very conservative assumptions, the levels of glyphosate that terrestrial mammals and birds are likely to encounter are not likely to constitute a hazard.

This assessment is consistent with the available field studies summarized in Appendix 2-2 and discussed in section 4.1.3. If any effects are seen in terrestrial mammals after the application of glyphosate, they are most likely to be associated with changes in habitat rather than direct toxic effects. The specific changes will depend on the nature of the existing vegetation and species under consideration but are not likely to differ dramatically from changes that would be caused by mechanical clearing.

4.4.3. Terrestrial Plants. As discussed in section 4.2.2, there are three pathways of exposure for plants that are potentially significant in herbicide applications: direct deposition, volatilization, and soil transport. For glyphosate, the potential effects of volatilization and soil contamination are likely to be marginal. Glyphosate has a very low volatility and is tightly bound to and degraded in soil. Consequently, these routes of exposure are not quantitatively addressed in this risk assessment.

Direct deposition, either through unintentional direct spraying or spray drift does present a plausible hazard. If plants are accidentally sprayed at the application rates used by the Forest Service, they are likely to be damaged, particularly in the upper ranges of anticipated application rates. This kind of exposure may be regarded as an accidental scenario, which is relatively easy to control with proper management and application. The extent and duration of damage will depend on the time of application and plant species.

The extent of drift will depend on conditions during application, such as wind speed, wind direction, topography, the distance from the ground at which the herbicide is applied, and the droplet size of the herbicide spray. Aerial applications are likely to generate greater drift than ground applications, as illustrated in the study by Yates et al. (1978). Nonetheless, even for aerial applications conducted under relatively unfavorable conditions, off-site deposition at 30–50 m is likely to be less than 0.1 of the nominal application rate. Thus, at the high range of application contemplated by the Forest Service, 2.5 lbs a.i./acre, the deposition at 30–50 m would be $\leq$0.25 lbs a.i./acre. Consistent with the analyses presented by Marrs et al. (1991) and Breeze et al. (1992), this could damage some sensitive plant species.

4.4.3. Aquatic Organisms. As discussed in section 4.3., the reference concentration for fish and aquatic invertebrates based on the results of laboratory bioassays is 1 mg glyphosate/L. Roundup is much more toxic because of the presence of the surfactant. When expressed as concentrations of glyphosate, the reference concentration is 0.1 mg/L. At these reference levels, no adverse effects would be anticipated in the most sensitive species. As concentrations increase above these levels, adverse effects would be anticipated. Most algal species are no more sensitive than fish or invertebrates are to glyphosate. Some species, however, might show evidence of marked growth inhibition at glyphosate concentrations of about 1–3 mg/L. The reference concentration for sensitive algal species is 0.02 mg/L.

As discussed in section 4.2, an exposure rate of 0.088–0.093 mg glyphosate/L·lb a.i. applied can be derived primarily from monitoring studies. For the risk characterization, this value will be rounded to 0.1 mg/L·lb a.i. applied per acre.

At the typical application rate of 1 lb a.i./acre, the anticipated levels in water initially after exposure would be about 0.1 mg/L. At this level, no adverse effects on fish, aquatic invertebrates, macrophytes, or most species of algae would be anticipated from the application of Accord, Rodeo, or Roundup.

At the maximum labelled application rate, 7.5 lbs a.i./acre, concentrations of glyphosate would be expected to reach about 0.75 mg/L. At this level, no effects on fish, aquatic invertebrates, macrophytes, or most species of algae would be expected from the application of Accord or Rodeo. This concentration of glyphosate after the application of Roundup, however, would be close to the LC_{50} for some species of fish and aquatic invertebrates at a pH ranges of about 7–9. At the highest anticipated application rate, the reference level for glyphosate from applications of Roundup would be exceeded by a factor of 2.5. It is not clear whether this level of exposure would cause observable effects in fish or aquatic invertebrates.

The gross responses of most species of algae are likely to resemble those of fish and aquatic invertebrates. Sensitive species, however, might show evidence of marked growth inhibition even at the lowest application rate. The effects of Roundup or other glyphosate formulations, however, would probably be similar (i.e., Roundup does not seem to be more toxic than other glyphosate formulations are to algae).

These conclusions, for the most part, are consistent with available field studies, as summarized in Table 4-6. At or near the typical application rate of 1 lb a.i./acre, Rodeo has not been associated with adverse effects in aquatic invertebrates (Henry et al. 1994, Solberg and Higgins 1993). As indicated in Table 4-6, the cause of the decreased abundance of aquatic invertebrates noted by Solberg and Higgins (1993) could not be determined. Field studies at higher application rates have not been encountered.

At an application rate 1.8 lbs a.i./acre, Roundup has been associated with signs of irritation in caged trout and an increase in stream drift of some invertebrates (Kreutzweiser et al. 1989, Reynolds et al. 1993). No frank signs of toxicity were noted. The peak level of glyphosate noted in this study was about 0.1 mg/L, the reference concentration for glyphosate from Roundup.

The study by Sullivan et al. (1981) is difficult to interpret. The investigators monitored the population of several different species of algae over a relatively long period and could not differentiate between the effects of treatment and seasonable changes. Nonetheless, this study is consistent with the above risk characterization in that substantial effects attributed to treatment could not be detected and would not be expected from the dose-response and exposure assessments.

Table 4-6. Field studies on glyphosate useful for the risk assessment of aquatic species

Formulation/ Application	Observations	Reference
Roundup, 2 lbs a.i./acre, aerial or manual spray boom	No clear effects associated with treatment over a 47-day observation period. Several variations in different species of algae were noted in a pond and in streams. Changes occurring in the streams were attributed to seasonal changes rather than treatment. Changes seen in algal species in the pond could not be clearly associated with treatment.	Sullivan et al. 1981
Roundup, 2.0 kg/ha [1.8 lbs a.i./acre], aerial	None of the post-spray drift volumes of most invertebrates were significantly higher than pre-spray values. A transient increase was seen for *Gammarus* (scud)—2-fold—and *Paraleptophlebia* (mayfly)—11-fold—species. This effect could not be unequivocally linked to treatment. Stressed behavior in caged coho salmon and an apparent decrease in the abundance of juvenile coho salmon were observed.	Kreutzweiser et al. 1989, Reynolds et al. 1993
Rodeo, 2.8 L/ha [1.3 kg/ha or 1.2 lbs a.i./acre], aerial	Treatment was effective in killing cattails on the treated wetland. The abundance of aquatic invertebrates decreased, the cause of which (migration or mortality) could not be determined.	Solberg and Higgins 1993
Rodeo, 0.1531 kg a.i./L ·5.8 L/ha [0.9 kg/ha or 0.8 lbs a.i./acre], aerial	Water pH of 6.4-10.7 (mean=8). No significant differences in mortality rates for aquatic invertebrates (leeches, amphipods, snail, and midges) were observed over a 1-21 day observation period.	Henry et al. 1994

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6. GLOSSARY

Absorption -- The process by which the agent is able to pass through the body membranes and enter the bloodstream. The main routes by which toxic agents are absorbed are the gastrointestinal tract, lungs, and skin.

Acetylcholine -- A naturally occurring chemical responsible for the transmission of impulses between nerve cells or between nerve cells and an effector cell (such as a muscle cell). Broken down to inactive compounds by acetylcholinesterase.

Acetylcholinesterase -- An enzyme responsible for the degradation of acetylcholine to acetic acid and choline. The inhibition of this enzyme leads to an excess of acetylcholine in nerve tissue. This can lead to a broad spectrum of clinical effects (Table 7-2).

Acute exposure -- A single exposure or multiple exposure occurring within a short time (24 hours or less).

Additive Effect -- A situation in which the combined effects of two chemicals is equal to the sum of the effect of each chemical given alone. The effect most commonly observed when two chemicals are given together is an additive effect.

Adenosine Diphosphate (ADP) -- A molecule used as a substrate in metabolism of nutrients in which the chemical energy in the nutrient is converted to ATP.

Adenosine Triphosphate (ATP) -- A molecule used as an energy source in many biochemical reactions in living things. During the energy transfer process, the ATP is converted to ADP and inorganic phosphorous.

Adjuvant(s) -- Formulation factors used to enhance the pharmacological or toxic agent effect of the active ingredient.

Adrenergic -- A type of nerve which uses an adrenaline like substance as a neurotransmitter.

Adsorption -- The tendency of one chemical to adhere to another material.

Adverse-Effect Level (AEL) -- Signs of toxicity that must be detected by invasive methods, external monitoring devices, or prolonged systematic observations. Symptoms that are not accompanied by grossly observable signs of toxicity. In contrast to Frank-effect level.

Aerobes -- Organisms that require oxygen.

Allelopathic Effects -- Literally *reciprocal pathology*. In plant pathology, the term is used to describe the release of substances from one plant that may have an adverse effect on another plant.

Allometric -- pertaining to allometry, the study and measure of growth. In toxicology, the study of the relationship of body size to various physiological, pharmacological, pharmacokinetic, or toxicodynamic processes among species.

Anaerobes -- Organisms that do not require oxygen.

Ascites -- The accumulation of fluid in the peritoneal cavity. Tis condition may be caused by increased venous pressure or decreased plasma albumin and is often associated with cardiac failure, cirrhosis of the liver, or renal deficiency.

Assay -- A kind of test (noun); to test (verb).

Biologically Sensitive -- A term used to identify a group of individuals who, because of their developmental stage or some other biological condition, are more susceptible than the general population to a chemical or biological agent in the environment.

Broadleaf weed -- A nonwoody dicotyledonous plant with wide bladed leaves designated as a pest species in gardens, farms, or forests.

Cancer Potency Parameter -- A model-dependent measure of cancer potency $(mg/kg/day)^{-1}$ over lifetime exposure. [Often expressed as a q_1^* which is the upper 95% confidence limit of the first dose coefficient (q_1) from the multistage model.]

Carcinogen -- A chemical capable of inducing cancer.

Carcinoma -- A malignant tumor.

Carrier -- In commercial formulations of insecticides or control agents, a substance added to the formulation to make it easier to handle or apply.

Cholinergic -- Refers to nerve cells that release acetylcholine.

Chronic Exposure -- Long-term exposure studies often used to determine the carcinogenic potential of chemicals. These studies are usually performed in rats, mice, or dogs and extend over the average lifetime of the species (for a rat, exposure is 2 years).

Confounders -- A term used in discussions of studies regarding human populations (epidemiology studies) to refer to additional risk factors that if unaccounted for in a study, may lead to erroneous conclusions.

Conifer -- An order of the Gymnospermae, comprising a wide range of trees, mostly evergreens that bear cones and have needle-shaped or scalelike leaves; timber commercially identified as softwood.

Connected Actions -- Exposure to other chemical and biological agents in addition to exposure to the control agent during program activities to control vegetation.

Contaminants -- For chemicals, impurities present in a commercial grade chemical. For biological agents, other agents that may be present in a commercial product.

Controls -- In toxicology or epidemiology studies, a population that is not exposed to the potentially toxic agent under study.

Cumulative Exposures -- Exposures that may last for several days to several months or exposures resulting from program activities that are repeated more than once during a year or for several consecutive years.

Cytosolic -- Found in the cytoplasm of a cell.

Dams -- Females.

Degraded -- Broken down or destroyed.

Dermal -- Pertaining to the skin.

Dislodgeable Residues -- The residue of a chemical or biological agent on foliage as a result of aerial or ground spray applications, which can be removed readily from the foliage by washing, rubbing or having some other form of direct contact with the treated vegetation.

Dose-response Assessment -- A description of the relationship between the dose of a chemical and the incidence of occurrence or intensity of an effect. In general, this relationship is plotted by statistical methods. Separate plots are made for experimental data obtained on different species or strains within a species.

Drift -- That portion of a sprayed chemical that is moved by wind off a target site.

EC_{50} -- A concentration that causes 50% inhibition or reduction. As used in this document, this values refers to a 50% inhibition of growth.

EC_{100} -- A concentration that causes complete inhibition or reduction. As used in this document, this values refers to a complete inhibition of growth.

Empirical -- Refers to an observed, but not necessarily fully understood, relationship in contrast to a hypothesized or theoretical relationship.

Enzymes -- A biological catalyst; a protein, produced by an organism itself, that enables the splitting (as in digestion) or fusion of other chemicals.

Epidemiology Study -- A study of a human population or human populations. In toxicology, a study which examines the relationship of exposures to one or more potentially toxic agent to adverse health effects in human populations.

Exposure Assessment -- The process of estimating the extent to which a population will come into contact with a chemical or biological agent.

Extrapolation -- The use of a model to make estimates outside of the observable range.

Fibroma -- A benign tumor composed mainly of fibrous or fully developed connective tissue.

Formulation -- A commercial preparation of a chemical including any inerts or contaminants.

Frank effects -- Obvious signs of toxicity.

Frank-effect Level (FEL) -- The dose or concentration of a chemical or biological agent that causes gross and immediately observable signs of toxicity.

Gavage -- The placement of a toxic agent directly into the stomach of an animal, using a gastric tube.

Genotoxic -- Causing direct damage to genetic material. Associated with carcinogenicity.

Geometric Mean -- The measure of an average value often applied to numbers for which a log normal distribution is assumed.

Gestation -- The period between conception and birth; in humans, the period known as pregnancy.

Half-time or Half-life -- For compounds that are eliminated by first-order kinetics, the time required for the concentration of the chemical to decrease by one-half.

Hazard Quotient (HQ) -- The ratio of the estimated level of exposure to the RfD or some other index of acceptable exposure.

Hazard identification -- The process of identifying the array of potential effects that an agent may induce in an exposed human population.

Hematological -- Pertaining to the blood.

Hematology -- One or more measurements regarding the state or quality of the blood.

Henry's law constant -- An index of the tendency of a compound to volatilize from aqueous solutions.

Herbaceous -- A plant that does not develop persistent woody tissue above the ground (annual, biennial, or perennial, but whose aerial portion naturally dies back to the ground at the end of a growing season. They include such categories as grasses and grass-like vegetation.

Herbicide -- A chemical used to control, suppress, or kill plants, or to severely interrupt their normal growth processes.

Histopathology -- Signs of tissue damage that can be observed only by microscopic examination.

Hydrolysis -- Decomposition or alteration of a chemical substance by water.

Hydroxylation -- The addition of a hydrogen-oxygen or hydroxy (-OH) group to one of the rings. Hydroxylation increases the water solubility of aromatic compounds. Particularly when followed by conjugation with other water soluble compounds in the body, such as sugars or amino acids, hydroxylation greatly facilitates the elimination of the compound in the urine or bile.

Hyperemia -- An increase in the amount of blood in an organ or region of the body with distention of the blood vessels. This may be caused either by an increase in dilation of the blood vessels (active hyperemia) or a hindance of blood drainage from the site (passive hyperemia).

Hypoactivity -- Less active than normal.

Hypovolemia -- Low or decreased blood volume. If this condition is sufficiently severe, the individual may go into shock and die.

In vivo -- Occurring in the living organism.

In vitro **--** Isolated from the living organism and artificially maintained, as in a test tube.

Inerts -- Adjuvants or additives in commercial formulations of glyphosate that are not readily active with the other components of the mixture.

Interpolation -- The use of mathematical models within the range of observations

Intraperitoneal -- Injection into the abdominal cavity.

Invertebrate -- An animal that does not have a spine (backbone).

Irritant Effect -- A reversible effect, compared with a corrosive effect.

Larva (pl. larvae) -- An insect in the earliest stage after hatching.

Lethal Concentration$_{50}$ (LC$_{50}$) -- A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{50}$ (LD$_{50}$) -- The dose of a chemical calculated to cause death in 50% of a defined experimental animal population over a specified observation period. The observation period is typically 14 days.

Lowest-Observed-Adverse-Effect Level (LOAEL) -- The lowest dose of a chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Malignant -- Cancerous.

Margin of safety (MOS) -- The ratio between an effect or no effect level in an animal and the estimated human dose.

Metabolite -- A compound formed as a result of the metabolism or biochemical change of another compound.

Metameter -- Literally, the unit of measure. Used in dose-response or exposure assessments to describe the most relevant way of expressing dose or exposure.

Microorganisms -- A generic term for all organisms consisting only of a single cell, such as bacteria, viruses, and fungi.

Microsomal -- Pertaining to portions of cell preparations commonly associated with the oxidative metabolism of chemicals.

Minimal Risk Level (MRL) -- A route-specific (oral or inhalation) and duration- specific estimate of an exposure level that is not likely to be associated with adverse effects in the general population, including sensitive subgroups.

Mitochondria -- Subcellular organelles involved in the conversion of food to stored chemical energy.

Most Sensitive Effect -- The adverse effect observed at the lowest dose level, given the available data. This is an important concept in risk assessment because, by definition, if the most sensitive effect is prevented, no other effects will develop. Thus, RfDs and other similar values are normally based on doses at which the most sensitive effect is not likely to develop.

Multiple Chemical Sensitivity -- A syndrome that affects individuals who are extremely sensitive to chemicals at extremely low levels of exposure.

Mutagenicity -- The ability to cause genetic damage (that is damage to DNA or RNA). A mutagen is substance that causes mutations. A mutation is change in the genetic material in a body cell. Mutations can lead to birth defects, miscarriages, or cancer.

Myeloma -- primary tumor of the bone marrow.

Myotonic -- pertaining to muscle spasms.

Neuropathy -- Damage to the peripheral nervous system.

Neurotransmitter -- A substance used by a nerve cell in the transmission of impulses between nerve cells or between nerve cells and an effector cell.

Non-target -- Any plant or animal that a treatment inadvertently or unavoidably harms.

No-Observed-Adverse-Effect Level (NOAEL) -- The dose of a chemical at which no statistically or biologically significant increases in frequency or severity of adverse effects were observed between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

No-Observed-Effect Level (NOEL) -- The dose of a chemical at no treatment-related effects were observed.

Normal Distribution -- One of several standard patterns used in statistics to describe the way in which variability occurs in a populations.

Octanol-Water Partition Coefficient (K_{ow}) -- The equilibrium ratio of the concentrations of a chemical in n-octanol and water, in dilute solution.

Ocular -- Pertaining to the eye.

Oxidative phosphorylation -- An metabolic process in which the metabolism of molecules in or derived from nutrients is linked to the conversion (phosphorylation) of ADP to ATP, a major molecule for storing energy in all living things.

Parenteral -- Any form of injection.

Partition -- In chemistry, the process by which a compound or mixture moves between two or more media.

Pasquill-Gifford vertical dispersion parameter -- A term which mathematically describes the upward dispersion of a gas as it travels downwind.

Pasquill stability category -- A method of classifying air stability based on a set of general descriptions such as wind speed and cloud cover.

Pathway -- In metabolism, a sequence of metabolic reactions.

Perennial -- A plant species having a lifespan of more than 2 years.

pH -- The negative log of the hydrogen ion concentration. A high pH (>7) is alkaline or basic and a low pH (<7) is acidic.

pK_a -- The negative log of the hydrogen ion concentration or pH at which 50% of a weak acid is dissociated.

pK_b -- The negative log of the hydrogen ion concentration or pH at which 50% of a weak base is dissociated.

Pharmacokinetics -- The quantitative study of metabolism (i.e., the processes of absorption, distribution, biotransformation, elimination).

Plasma Cholinesterase -- Another term for **Pseudocholinesterase**. The normal physiological role of this cholinesterase is not known. Inhibition of this enzyme is considered an index of exposure to many organophosphate insecticides.

Plasma -- The fluid portion of the blood in which particulates are suspended.

Precommercial thinning -- Cutting in immature stands to improve the quality and growth of the remaining stand.

Prospective -- looking ahead. In epidemiology, referring to a study in which the populations for study are identified prior to exposure to a presumptive toxic agent, in contrast to a retrospective study.

Pseudocholinesterase -- A term for cholinesterase found in the plasma. The normal physiological role of this cholinesterase is not known. Inhibition of this enzyme is considered an index of exposure to many organophosphate insecticides.

Release -- A work done to free desirable trees from competition with overstory trees, less desirable trees or grasses, and other forms of vegetative growth.

Reference Dose -- Oral dose (mg/kg/day) not likely to be associated with adverse effects over lifetime exposure, in the general population, including sensitive subgroups.

Reproductive Effects -- Adverse effects on the reproductive system that may result from exposure to a chemical or biological agent. The toxicity of the agents may be directed to the reproductive organs or the related endocrine system. The manifestations of these effects may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions dependent on the integrity of this system.

Resorption -- Removal by absorption. Often used in describing the unsuccessful development and subsequent removal of post-implantation embryos.

Retrospective -- looking behind. In epidemiology, referring to a study in which the populations for study are identified after exposure to a presumptive toxic agent, in contrast to a prospective study.

RfD -- A daily dose which is not anticipated to cause any adverse effects in a human population over a lifetime of exposure. These values are derived by the U.S. EPA.

Right-of-way -- a corridor of low growing shrubs or grasses that facilitate the maintenance and protection of utility power lines and provide transport pathways for humans or wildlife.

Route of Exposure -- The way in which a chemical or biological agent enters the body. Most typical routes include oral (eating or drinking), dermal (contact of the agent with the skin), and inhalation.

Scientific Notation -- The method of expressing quantities as the product of number between 1 and 10 multiplied by 10 raised to some power. For example, in scientific notation, 1 kg = 1,000 g would be expressed as 1 kg = 1 x 10^3 g and 1 mg = 0.001 would be expressed as 1 mg = 1 x 10^{-3}.

Sensitive subgroup -- Subpopulations that are much more sensitive than the general public to certain agents in the environment.

Site preparation -- The removal of competition and conditioning of the soil to enhance the survival and growth of seedlings or to enhance the seed germination.

Species-to-Species Extrapolation -- A method involving the use of exposure data on one species (usually an experimental mammal) to estimate the effects of exposure in another species (usually humans).

Subchronic Exposure -- An exposure duration that can last for different periods of time, but 90 days is the most common test duration. The subchronic study is usually performed in two species (rat and dog) by the route of intended use or exposure.

Substrate -- With reference to enzymes, the chemical that the enzyme acts upon.

Synapse -- The space between two nerve cells or a nerve cell and an effector cell such as muscle.

Synergistic Effect -- A situation is which the combined effects of two chemicals is much greater than the sum of the effect of each agent given alone.

Systemic Toxicity -- Effects that require absorption and distribution of a toxic agent to a site distant from its entry point at which point effects are produced. Systemic effects are the obverse of local effects.

Teratogenic -- Causing structural defects that affect the development of an organism; causing birth defects.

Teratology -- The study of malformations induced during development from conception to birth.

Threshold -- The maximum dose or concentration level of a chemical or biological agent that will not cause an effect in the organism.

Toxicity -- The inherent ability of an agent to affect living organisms adversely.

Uncertainty Factor (UF) -- A factor used in operationally deriving the RfD and similar values from experimental data. UFs are intended to account or (1) the variation in sensitivity among members of the human population; (2) the uncertainty in extrapolating animal data to the case of humans; (3) the uncertainty in extrapolating from data obtained in a study that is less than lifetime exposure; and (4) the uncertainty in using LOAEL data rather than NOAEL data. Usually each of these factors is set equal to 10. See table 2-4 for additional details.

Urinalysis -- Testing of urine samples to determine whether toxic or other physical effects have occurred in an organism.

Vehicle -- A substance (usually a liquid) used as a medium for suspending or dissolving the active ingredient. Commonly used vehicles include water, acetone, and corn oil.

Vertical dispersion parameter -- A term which mathematically describes the upward dispersion of a gas as it travels downwind.

Vertebrate -- An animal that has a spinal column (backbone).

Volatile -- Referring to compounds or substances that have a tendency to vaporize. A material that will evaporate quickly.

Xenobiotic -- A chemical that does not naturally occur in an organism.

Zwitterion -- A molecule with regions of both positive and negative charges.

Appendix 1-1. Acute toxicity of glyphosate to mammals

Formulation	Species	Exposure Route	Exposure Level	Effect	Reference
Glyphosate	rat	oral	5600 mg/kg	LD_{50}	Monsanto Co. 1982a,b, 1983a
Roundup	rat	oral	5400 mg/kg	LD_{50}	Monsanto Co. 1982a,b, 1983a
Roundup Pro	rat	oral	>5000 mg/kg	LD_{50}	Kirk 1993a
Rodeo	rat	oral	>5000 mg/kg	LD_{50}	Monsanto Co. 1982a,b, 1983a
Glyphosate (NOS)	rat	oral	4873 mg/kg	LD_{50}	Bababunmi et al. 1978
Glyphosate	rat	oral	4320 mg/kg	LD_{50}	U.S. EPA 1986
Glyphosate (NOS)	mouse	oral	1568 mg/kg	LD_{50}	Bababunmi et al. 1978
Glyphosate	rabbit	oral	3800 mg/kg	LD_{50}	U.S. DOE 1983a
Glyphosate (NOS)	rat	intraperitoneal	238 mg/kg	LD_{50}	Bababunmi et al. 1978
Glyphosate (NOS)	mouse	intraperitoneal	134 mg/kg	LD_{50}	Bababunmi et al. 1978
Glyphosate	rabbit	dermal	>7940 mg/kg	MLD	Monsanto Co. 1982a,b, 1983b
Roundup	rabbit	dermal	>5000 mg/kg	LD_{50}	Monsanto Co. 1982a,b, 1983b
Roundup Pro	rat	dermal	>5000 mg/kg	LD_{50}	Kirk 1993b
Rodeo	rabbit	dermal	>5000 mg/kg	LD_{50}	Monsanto Co. 1982a,b, 1983b

LD_{50} = Lethal dose (50% kill); LC_{50} = lethal concentration (50% kill); MLD = minimum lethal dose; NOS = not otherwise specified.

Appendix 1-2. Case reports of poisoning by glyphosate formulations

Number (N), formulation, [Location]	Average Dose	Symptoms, Outcome, and post mortum pathology	Reference
2, Roundup [New Zealand]	200-250 ml [fatal]	Vomitting and acidosis. Both individuals died. Ulcerated oropharynx, congested lungs and airway mucossa, petechial submucosal hemorrhages and gastric funduc, acute pulmonary edema, and acute tubular necrosis of the lungs in on individual. Edema of the bronchi and lungs in the other individual.	Dickson et al. 1988
1, Roundup [U.S.]	N.S.	A self report of "*nervous system and immune system problems*" that "*no doctor has been able to accurately diagnose and treat...* "	Jensen 1989
1, Roundup [Japan]	N.S.	Foam and fluid in the trachea and bronchi. Death attributed to infalation of vomitus into the lungs	Kageura et al. 1988
N.S., Roundup and others, [France]	N.S.	Estimated lethal dose of about 1 g/kg.	Kammerer 1995
4, Roundup [New Zealand]	50 -1,000 ml [non-fatal]	Abdominal pain, diarrhea and vomitting. Decreased urinary output. Estimates of non-fatal doses: 85 g for 27 year old male, 18-36 g for 15 year old female, "*up to 1 liter*" for a 38 year old male. About 72-91 g for a 43 year old woman.	Menkes et al. 1991
	200-250 ml [fatal]		
56, Roundup [Japan]	104 ml [non-fatal] 206 ml [fatal]	Hypovolemic shock. Sore throat, abdominal pain, and vomiting. Pulmonary edema (3 cases) and severe pneumonia (2 cases). Oliguria, anuria, and hypotension in all fatal cases. Increases serum amylase and WBC count, some with increased bilirubin and LDH activity, probably attributable to hemolysis.	Sawada et al. 1988
1, Roundup [New Zealand]	200-250 ml [fatal]	Hypotension, metabolic acidosis, and vomiting, and hyperkalemia. Death due to respiratory and cardiac arrest. Pulmonary edema and acute renal tubular necrosis.	Temple and Smith 1992
92, Roundup [Taiwan]	120 ml (range of 5-500 ml) [non-fatal]	Irritation and pain in the throat and mouth, some with oral mucosal ulceration. Gastritis, esophagitis, and mucosal edema. Vomiting and diarrhea. Abdominal or epigastric pain. Diffuse pulmonary damage, non-cardiogenic pulmonary edema. Intensive therapy failed to reverse hypoxemia in fatal cases. Oliguria or anuria in 10 patients, perhaps related to hypotension. Metabolic acidosis. Mild temperature elevations in 7 patients.	Tominack et al. 1991
	263 ml (range of 150-500 ml) [fatal]		

Appendix 1-3. Effects on mammals of long-term exposure to glyphosate[a]

Species/ Strain/ Sex/No.	Route/ Exposure Level (Estimated Dose) and Duration	Effects	Reference
Rats/ F344/N 10/sex/ dose	3125, 6250, 12500, 25000, 50000 ppm in diet for 13 weeks. (205, 410, 811, 1678, 3393 mg/kg/day for males) (213, 421, 844, 1690, 3393 mg/kg/day for females)	Decrease in body weight in males (20%) and females (5%) at the highest dose level. In males, small increases in relative liver, kidney, and testicle weights and a decrease in relative thymus wieght. No significant organ weight changes in females. Hematologic changes (increased hematocrit, RBC) at the three higher dose levels and increased hemoglobin at the two higher dose levels in males. The hematologic effects are unremarkable and attributed to mild dehydration. Treatment related increases in alkaline phosphatase in both sexes at all time points suggestive of mild liver toxicity. In males at the two higher dose levels, a 20% decrease in sperm counts. In females, a longer estrous cycle at the highest dose. Salivary gland lesions in both sexes at all dose levels with increasing incidence and severity with increasing dose. The effect could be blocked by isoproterenal, indicating an adrenergic mechanism.	NCI 1992
Mice/ $B6C3F_1$/ 10/sex/ dose	3125, 6250, 12500, 25000, 50000 ppm in diet for 13 weeks. (507, 1065, 2273, 4776, 10780 mg/kg/day for males) (753, 1411, 2707, 5846, 11977 mg/kg/day for females)	Body weight depression at the two highest dose levels for both sexes. Increases in relative heart, kidney, liver, lung, thymus, and testis for male mice. No differences in food consumption between the dosed and control groups. No effects on sperm motility or estrous cycle length. Salivary gland lesions.	NCI 1992
Rabbits/ New Zealand white/ male/ 4/dose	$1/10^{th}$ and $1/100^{th}$ of the LD_{50} orally in geletin capsul for 6 weeks with an additional 6 week recovery period. [?? LD_{50} for rabbit ≃ 3800 mg/kg. Doses of about 38 mg/kg and 380 mg/kg]	Decreased body weight, libido, ejaculate volume, sperm concentrations, semen initial fructose and semen osmolality. Increases in abnormal and dead sperm.	Yousef et al. 1995

Appendix 1-3. Effects on mammals of long-term exposure to glyphosate[a]

Species/ Strain/ Sex/No.	Route/ Exposure Level (Estimated Dose) and Duration	Effects	Reference
Rat/NS/ NS/NS	200, 600, or 2000 ppm in diet for 90 days	No significant abnormalities. Parameters included body weights, food consumption, behavioral reactions, mortality, hematology, blood chemistry, urinalyses, gross pathology and histopathology	USDA 1981, WSSA 1993
Rat/ Sprague Dawley	1000, 5000, or 10,000 ppm in diet for 90 days for 2 years	Increased serum inorganic phosphorus and potassium values in both sexes at all tested doses; increased serum glucose in males at mid- and high-dose levels; increased BUN and alkaline phosphatase in males at the high dose. Slight changes observed in hematological, clinical chemistry, and urinalysis parameters not considered dose or compound related. Study did not establish an effect level or MTD.	Monsanto Co. 1987
Rat/NS/ 50/sex/ group	0, 30, 100, or 300 ppm in diet for 2 years	Increased incidence of cytoplasmic vacuolation and lipid content in livers of high-dose rats. Endpoints included food consumption, weight gain, final body weight, organ weights, organ to body weight and organ to brain weight ratios, hematology, clinical chemistry, and urine analyses. NOEL = 31 mg/kg/day.	Monsanto Co. 1983b, USDA 1981
Rat/ Sprague Dawley6 0/sex/ group	2000, 8000, or 20,000 ppm in diet for 24 months (89, 362, or 940 mg/kg/day for males) (113, 45, or 1183 mg/kg/day for females)	Significant decrease in body weight gain in high-dose females (day 51-month 20); significant increases in cataracts and lens abnorm-alities in high-dose males; significant decrease in urinary tract pH in high-dose males; increased relative liver weights; significantly in-creased incidence of inflam-mation of the gastric mucosa in mid-dose females. This study reports a NOAEL of 8000 ppm based on decreased body weight data. Increased incidence of pan-creatic islet cell adenomas (low-dose males) and C-cell adenomas in the thyroid of mid- and high-dose males and females; slight increase in hepatocellular adenomas in males. Due to the high incidence of pancreatic cell adenomas, the EPA recommended that the carcinogenic potential of glyphosate be evaluated by the Peer Review Committee.	Stout and Ruecker 1990
Rat/ Sprague Dawley5 0/sex/ group	0, 30, 100, or 300 ppm in diet for 26 months (3.1, 10.3, or 31.5 mg/kg/day for males) (3.4, 11.3, or 34.0 mg/kg/day for females)	No significant changes in body weight gain, organ weights, organ/body weight ratios, or hematological and clinical chemistry parameters. Increased rate of interstitial cell tumors of the testes in high-dose males. Systemic NOAEL for nonneoplastic effects = 31 mg/kg/day. Authors concluded that HDT not carcinogenic to rats. EPA concluded that since the HTD was not an MTD, study was not a valid carcinogenicity study under EPA guidelines.	Bio/dynami cs, Inc. 1981a
Mouse/N S/NS	100 or 300 ppm in diet for 18 months	No evidence of increase in incidence of cytoplasmic vacuolation or lipid content	USDA 1981

Appendix 1-3. Effects on mammals of long-term exposure to glyphosate[a]

Species/ Strain/ Sex/No.	Route/ Exposure Level (Estimated Dose) and Duration	Effects	Reference
Mouse/CD/50/sex/group	1000, 5000, or 30,000 ppm in diet for 24 months (111–250, 519–1264, or 3465–7220 mg/kg/day for males) (129–288, 690–1322, or 4232–9859 mg/kg/day for females)	Lower mean body weights (as much as 11% at week 102) among high-dose males; elevated mean absolute and relative weights of testes in high-dose males. Histopathological changes included hepatic centrilobular hypertrophy and necrosis of hepatocytes in high-dose males and chronic interstitial necrosis and proximal tubule epithelial cell basophilia and hypertrophy of the kidneys in high-dose females. Sporadic occurrence (not dose related) of lymphoreticular tumors in treated females and renal tubular adenomas in males. The NOAEL for nonneo-plastic chronic effects from this study is 5000 ppm, which corresponds to a dose of 750 mg/kg/day. The oncogenic response in this study (occurrence of renal adenomas in male mice) is considered equivocal. **NOTE: U.S. EPA 1995 [Federal Register July 7, Vol 60, No. 130 indicates that the exposure duration was 18 months, not 24 (*cf* #5, pg. 35366)].**	U.S. EPA 1986
Dog/Beagle/NS/NS	200, 600, or 2000 ppm in diet for 90 days	No significant differences in absolute organ weights, organ to body weight ratios, and organ to brain weight ratios between treated dogs and controls. Parameters included body weights, food consumption, behavioral reactions, mortality, hematology, blood chemistry, urinalyses, gross pathology and histopathology.	USDA 1981, WSSA 1993
Dog/NS/6/sex/group[b]	20, 100, or 500 mg/kg/day in gelatin capsules for 1 year	At 3 months, slight but toxicologically important decrease in serum sodium and potassium concentrations in males at mid- and high-dose levels and in females at high-dose level. Apparent decreases in absolute and relative weights of pituitaries in mid- and high-dose males not correlated with histopathological effects. Systemic NOAEL >500 mg/kg/day.	Monsanto Co. 1985
Dog/NS/4/sex/group	0, 30, 100, or 300 ppm in diet for 2 years	No treatment related abnormalities; no evidence of gross or histopathological liver changes. Endpoints included food consumption, weight gain, final body weight, organ weights, organ to body weight and organ to brain weight ratios, hematology, clinical chemistry, and urine analyses.	USDA 1981

[a] Adaped from U.S. EPA 1992, except for NCI 1992 and Youssef 1995, which are taken from the primary references.

[b] Although U.S. EPA 1992 indicates that the strain of dogs is not specified, the title of the study title indicates that Beagle dogs were used.

NS = Not specified; M = male; F = female; NOAEL = no-observed-adverse-effect level; MTD = maximum tolerated dose

Appendix 1-4. Assays for reproductive/teratogenic effects in mammals after exposure to glyphosate

Species/Strain	Sex/No.	Exposure Route	Exposure Level (Estimated Dose)	Exposure Duration	Effects	Reference
Rat/Sprague-Dawley	12 M 24 F	diet	0, 3, 10, or 30 mg/kg/day	60 days[a]	An increase in unilateral focal tubular dilation of the kidney in the male F_{3b} pups (7/10 in treated animals compared with 2/10 in concurrent controls) of dams treated with 30 mg/kg/day. No compound-related effects were observed on fetal, pup, and adult survival; mean parental and pup body weight and food consumption; and mating, pregnancy, fertility, and gestation length. The authors of this study noted that the historical control indices of tubular lesions varied markedly in male weanling rat, and on the basis of the data from this 3-generation study concluded that the highest dose tested (30 mg/kg/day) had no adverse reproductive effects. Nonetheless, in view of the observed kidney lesions in the male F_{3b} pups of dams treated with the highest dose, U.S. EPA 1992 concludes that a more appropriate systemic NOAEL for this study is 10 mg/kg/day, and that the LOAEL is 30 mg/kg/day based on renal effects observed in male F_{3b} weanlings.	Bio/dynamics Inc. 1981b
Rat/Sprague-Dawley	30/sex/ group	diet (*ad libitum*)	2000, 10,000, or 30,000 ppm	throughout premating, mating, gestation, and lactation	Groups of pregnant Charles River COBS CD rats (25/dose) were administered glyphosate orally by gavage as a single daily dose on days 6 through 19 of gestation. A definite reduced mean maternal body weight gain was noted in the 3500 mg/kg/day dose group over the treatment period due to mean maternal body weight loss during the first 3 days of treatment. At 3500 mg/kg/day a statistically significant increase in the mean number of early resorptions resulted in a slight increase in mean postimplantation loss. A statistically significant decrease in the mean number of total implantations, viable fetuses, and mean fetal body weight and a slight decrease in the mean number of corpora lutea was noted in this group. Based on these findings, the NOEL and LEL for maternal toxicity are 1000 and 3500 mg/kg/day, respectively. An increase in the number of litters and fetuses with unossified sternebrae was noted in the 3500 mg/kg/day dose group. Based on this finding, the NOEL and LEL for developmental toxicity are 1000 and 3500 mg/kg/day, respectively	Bio/dynamics 1981b
Rat/NS	F/NS	diet	0, 100, 500, or 1500 mg/kg/day	2-generation	Treatment-related effects were observed only in the high-dose group and included soft stools in F0 and F1 males and females, decreased food consumption and body weight gain of the F1a, F2a, and F2b male and female pups during week 2 and 3 of lactation. Systemic and developmental NOELs of 500 mg/kg/day; reproductive NOEL of 1500 mg/kg/day. **NOTE: This is probably the same study as above but has *different* NOELs.**	U.S. EPA 1995

Appendix 1-4. Assays for reproductive/teratogenic effects in mammals after exposure to glyphosate

Species/Strain	Sex/No.	Exposure Route	Exposure Level (Estimated Dose)	Exposure Duration	Effects	Reference
Rat/CD	F/NS	gavage	0, 300, 1000, or 3500 mg/kg/day	days 6–19 of gestation	Breathing difficulty, reduced activity, diarrhea, stomach hemorrhages, weight gain deficits,altered physical appearance, and mortality during treatment in high-dose dams; unossified sternebrae in fetuses from high-dose dams. The NOAELs for fetotoxicity and maternal toxicity are each 1000 mg/kg/day and the NOAEL for teratogenicity is 3500 mg/kg/day (HDT).	Monsanto Co. 1980, U.S. EPA 1986
Rabbit/NS	F/NS	NS	10 or 30 mg/kg body weight	during fetal organogenesis	No teratogenic effects	USDA 1981
Rabbit/Dutch Belted	F/NS	gavage	75, 175, or 350 mg/kg/day	days 6-27 of gestation	Nasal discharge, diarrhea, altered physical appearance and death among dams in the high-dose group; no evidence of fetal toxicity or birth defects in offspring. The NOAEL for maternal toxicity is 175 mg/kg/day and the NOAEL for fetotoxicity is 350 mg/kg/day. The NOAEL for teratogenicity is 350 mg/kg/day (HDT).	Monsanto Co. 1980, U.S. EPA 1986

[a] This is a 3-generation study in which the F_0 generation received the test diet for 60 days prior to breeding. Glyphosate administration was continued through mating, gestation, and lactation for two successive litters (F_{1a}, F_{1b}).

NS = Not specified; M = male; F = female; NOAEL = no-observed-adverse-effect level; LOAEL = lowest-observed-adverse-effect level; HTD= highest dose tested.

Appendix 1-5. Studies assessing the mutagenicity of glyphosate

Formulation	Organism	Exposure Level	Nature of Exposure	Effects	Reference
Glyphosate	*Bacillus subtillis*	≤2000 µg/plate	rec assay	no effects	Monsanto Co. 1982a, U.S. EPA 1982
Glyphosate	*Salmonella typhimurium*	≤2000 µg/plate	reverse mutation	no effects	U.S. EPA 1982
Glyphosate	*Salmonella typhimurium*	NS	NS	no effects	Monsanto Co. 1982a
Glyphosate	*Bacillus subtilis*	NS	bacterial cell (NOS)	no effects	Monsanto Co. 1982a
Glyphosate	*Saccharomyces cerevisiae*	NS	yeast cells	no effects	Monsanto Co. 1982a
Glyphosate	*Salmonella typhimurium*	10, 50, 100, 1000, 5000 µg/plate	reversion assay with and without the presence of S9	no statistically significant induction of revertant above solvent control levels; no significant dose-response relationship observed	Li and Long, 1988
Glyphosate	*Escherichia coli*	10, 50, 100, 1000, 5000 µg/plate	WP2 reversion assay	no statistically significant induction of revertant above solvent control levels; no significant dose-response relationship observed	Li and Long, 1988
Glyphosate	*Bacillus subtilis*	10, 50, 100, 1000, 5000 µg/plate	rec-assay	differential inhibition of growth of the recombination devicient strain (M45) vs. recombination proficient strain (H17); no growth inhibition observed for either strain at concentrations of 20-2000 µg/disk	Li and Long, 1988
Glyphosate	*Salmonella typhimurium*	≤5000 µg/plate	reversion assay	no effects	Moriya et al. 1983
Glyphosate	*Escherichia coli*	≤5000 µg/plate	reversion assay	no effects	Moriya et al. 1983
Glyphosate	*Salmonella typhimurium*	≤10,000 µg/plate	plate incorporation assay in the absence or presence of Aroclor 1254-induced male Sprague-Dawley rat of Syrian hamster liver S9	no mutagenicity	NTP Working Group 1992
Glyphosate	mouse	100, 150, 200 mg/kg bw	mice bone marrow micronucleus assay	no clastogenicity	Rank et al. 1993

Appendix 1-5. Studies assessing the mutagenicity of glyphosate

Formulation	Organism	Exposure Level	Nature of Exposure	Effects	Reference
Glyphosate	mouse	5 or 10 mg/kg bw	dominant lethal test in males	no effects	USDA 1981
Glyphosate	mouse	≤2000 mg/kg	dominant lethal test	no effects	Monsanto Co. 1982a, U.S. EPA 1982
Glyphosate	mouse	1/2 LD_{50} (NOS)	production of polychromatic erythrocytes with micronuclei	no evidence of mutagenicity	Benova et al. 1989
Glyphosate	rat	1000 mg/kg	chromosomal aberrations in bone marrow cells	no clastogenic effects[a]	Monsanto Co. 1982a, U.S. EPA 1982
Glyphosate	rat	0.125 mg/ML[b]	rat hepatocyte primary culture/DNA repair assay	no DNA effects	Monsanto Co. 1983d
Glyphosate	hamster	2-25 mg/mL	forward mutation in cultured CHO cells with or without presence of Aroclor-1254 activating system	cytotoxicity to CHO cells at concentrations >10 mg/mL; no mutagenicity	Monsanto Co. 1983d
Glyphosate	rat	5, 17.5, 22.5 mg/mL	CHO/HGPRT gene mutation in the absence or presence of 1, 2, 5, or 10% S9	significant cytoxicity (>50% cell killing) at 22.5 mg/mL in the absence of S9 and 17.5 mg/mL in the presence of 10% S9; no statistically significant mutagenic response at any S9 levels	Li and Long 1988
Glyphosate	rat	2, 5, 10, 15, 20 mg/mL	CHO/HGPRT gene mutation in the absence of S9	significant cytotoxicity at 20 mg/mL; no statistically significant higher mutant frequency than solvent control; no statistically significan dose-response relationship	Li and Long 1988
Glyphosate	rat	10, 15, 20, 25 mg/mL	CHO/HGPRT gene mutation in the presence of 5% S9	significant cytotoxicity at 25 mg/mL; no statistically significant higher mutant frequency than solvent control; no statistically significan dose-response relationship	Li and Long 1988

Appendix 1-5. Studies assessing the mutagenicity of glyphosate

Formulation	Organism	Exposure Level	Nature of Exposure	Effects	Reference
Glyphosate	rat	$1.25x10^{-5}$, $6.25x10^{-5}$, $1.25x10^{-4}$, $6.25x10^{-4}$, $1.25x10^{-3}$, $1.25x10^{-2}$, $1.25x10^{-1}$	hepatocyte unscheduled DNA repair synthesis assay	no cytotoxicity or statistically significant increase in net grains/nucleus above solvent control	Li and Long 1988
Glyphosate	rat	1 g/kg	in vivo rat bone marrow cytogenetics	chromatid type aberrations in treated and control rats at low frequencies; approximately 1% frequency of chromatid deletions (most frequent category); no statistically signficant increases in chromosomal aberrations or achromatic lesions	Li and Long 1988
Glyphosate	*Vicia faba*	35, 70, 105, 140, 350, 700, 1050, 1400 µg/g soil[c]	frequency of micronucleated cells	no genotoxicity	De Marco et al. 1992
Glyphosate	Allium	1440, 2880 µg/L	Allium anaphase-telophase assay	no effect	Rank et al. 1993
Roundup	*Salmonella typhimurium*	360, 720, 1081, 1440 µg/plate	plate incorporation assay in the absence or presence of Aroclor induced S9 mix	slight but significant number of revertants at 360 µg/plate for TA98 (without S9) and at 720 µg/plate for TA100 (with S9)	Rank et al. 1993
Roundup	Allium	1440, 2880 µg/L	Allium anaphase-telophase assay	statistically significant increase in chromosome aberrations	Rank et al. 1993
Roundup	mouse	133, 200 mg/kg bw	mice bone marrow micronucleus assay	no clastogenicity	Rank et al. 1993
Roundup	human	0.25, 2.5, 25 mg/mL	SCE in human lymphocytes *in vitro*	statistically significant increase ($p<0.001$) in SCE at 0.25 and 2.5 mg/mL; no lymphocyte growth at highest dose	Vyse and Vigfusson 1979, Vigfusson and Vyse 1980

[a]This study not considered adequate for assessing endpoint of concern.
[b]Highest nontoxic concentration.
[c]Used as an emulsifiable liquid in Solado trading formulation (SIAPA) containing 21% active ingredient
CHO = Chinese hamster ovary; SCE = sister-chromatid exchange, NOS = not otherwise specified

Appendix 1-6. Dermal and ocular irritation by glyphosate or glyphosate formulations

Forumulation	Species	Exposure Route	Exposure Level	Effect	Reference
Roundup	rabbit	dermal	not reported	moderate skin irritation (FHSA score = 4.3)[a]	Monsanto Co. 1982b
Roundup Pro	rabbit	dermal	0.5 ml	slight edema only at 1 hour. No irritation after 7 days.	Kirk 1993a
Glyphosate and Rodeo	rabbit	dermal	not reported	no irritation (FHSA score = 0.1)[a]	Monsanto Co. 1982a, 1983b
Roundup	human	dermal	not reported	no visible skin changes	Monsanto Co. 1982c
Roundup	guinea pig	dermal	not reported	mild to severe irritation, erythema, edema, and necrosis[b]	Monsanto Co. 1983c
Roundup Pro	guinea pig	dermal	100% formulation	minimsl irritation and no edema. No evidence of sensitizaiton.	Kirk 1993f
Glyphosate	rabbit	ocular	not reported	slight irritation (FHSA score = 6.9)[c]	Monsanto Co. 1982a,b, 1983b
Roundup	rabbit	ocular	not reported	moderate irritation (FHSA score = 18.4)[c]	Monsanto Co. 1982a,b, 1983b
Roundup Pro	rabbit	ocular	0.1 ml	slight iritis at 1 and or 24 hours. Slight transient corneal epithelial ulceration in 2 animals at one hour. No effects by day 7.	Kirk 1993d
Rodeo	rabbit	ocular	not reported	no irritation (FHSA score = 0.0)[c]	Monsanto Co. 1982a,b, 1983b

[a]Based on Federal Hazardous Substance Act skin irritation scores where 0 represents no irritation and 8.0 represents maximum irritation.
[b]These effects were observed beginning with sixth exposure. Although the results suggest some cumulative irritation potential, the response to a challenge dose indicated no sensitization.
[c]Based on Federal Hazardous Substance Act ocular irritation scores where 0 represents no effect and 110 represents maximum irritation.

Appendix 2-1. Bioassays of glyphosate relevant to risk assessment of wildlife

Species	Nature of Exposure	Effects	Reference
		Roundup	
broiler chickens	dietary levels of 60.8, 608, and 6080 ppm from days 1 to 21 of age.	No effects at the two lower levels. At the highest level, there was a substantial (≈50%) decrease in body weight of both sexes.	Kubena et al. 1981
zebra finches	dietary level of 5,000 ppm	Death in 3-7 days after body weight losses of about 30-60%. Food consumption was decreased by 20-30%.	Evans and Batty 1986
domestic chicken	eggs immersed in 0, 1, or 5% solution. Immersion for 5 seconds. Eggs immersed at 0, 6, 12, or 18 days of embryonic development.	No significant differences regarding hatchability or time to hatch compared with controls	Batt et al. 1980
mallard eggs	egg immersion assays in which dose/concentration was expressed in units of lb/A at 100 gal/A.	LC_{50} of 178 lb/A at 100 gal/A. This is stated by the authors to be equivalent to 59 times the field application rate. No indication of reduced growth or abnormal survivors.	Hoffman and Albers 1984
		Roundup Pro	
duck	dietary study	LD_{50} greater than 5620 ppm.	Matura 1996a
quail	dietary study	LD_{50} greater than 5620 ppm.	Matura 1996a
		Glyphosate	
mallard	ingestion, 8 days	LC_{50} >4640 ppm	Monsanto Co. 1982a
bobwhite quail	ingestion, 8 days	LC_{50} >4640 ppm	Monsanto Co. 1982a
mallard	ingestion, no effects on reproduction up to 1000 ppm with 83% pure technical grade and no effects up to 30 ppm with 94% pure technical grade. Duration of exposure not specified.		U.S. EPA 1993b
bobwhite quail	ingestion, no effects on reproduction up to 1000 ppm with 83% pure technical grade. Duration of exposure not specified.		U.S. EPA 1993b

Appendix 2-1. Bioassays of glyphosate relevant to risk assessment of wildlife

Species	Nature of Exposure	Effects	Reference
white rock chicken	injection into air sac of egg on day 10	LD_{50} = 12.88 mg/100 g egg	Olorunsogo et al. 1978
white rock chicken	injection into yolk sac of egg on day 10	LD_{50} = 24.74 mg/100 g egg	Olorunsogo et al. 1978
white leghorn chicken	injection into air sac of egg on day 10	LD_{50} = 13.12 mg/100 g egg	Olorunsogo et al. 1978
white leghorn chicken	injection into yolk sac of egg on day 10	LD_{50} = 25.44 mg/100 g egg	Olorunsogo et al. 1978
chicken (adult hens)	oral, 1250, mg/kg twice/day, 3 consecutive days; repeated for a total dose of 15,00 mg/kg	no behavioral or microscopic treatment-related effects	Monsanto Co. 1982a
Brown graden snail, *Helix aspersa*	ingestion of contaminated food for 14 days.	No mortality at dietary concentration of up to 4,994 ppm. [Dose ≃1,500 mg/kg body weight assuming 30% food consumption factor (APHIS 1993).]	Schuytema et al. 1994
Honey bee	contact toxicity and oral exposure	LD_{50} > 100 μg/bee. [>1075 mg/kg using a body weight of 0.093 g (APHIS 1993)]	U.S. EPA 1993b

Appendix 2-2. Summary of field or field simulation studies on glyphosate formulations

Application	Observations	Reference
Glyphosate (NOS), 0.75 lbs/acre, aerial application. Less than 7 year post cutting clear cut. Comparable are uses as control.	**Vegetation:** Mortality in only about 5% of shrubs (primarily salmonberry and thimpleberry). Defoliation in about 50% of shrubs one year post-spray with increase in herbaceous (grass) cover. **Small Mammals:** No marked changes in diversity and evenness of small-mammal communities over two year post-application observation period. Transient increase in *Microtus oregoni* associated with increase in grasses.	Anthony and Morrison 1985
Roundup, 1 ml applied in drilled holes around root collar of treated pine trees. Untreated trees served as controls.	Increased attack success as well as egg and larval development of mountain pine beetle (MPB). Corresponding increases observed in MPB predators and parasites.	Bergvinson and Borden 1991
Roundup, applied in drilled holes around root collar at doses ranging from about 0.006 to 0.6 g/tree.	Increased predation by woodpeckers on mountain pine beetles (MPB) over a 1 year observation period.	Bergvinson and Borden 1992
Roundup, 1.7 kg a.e./ha, in summer of 1985 using a spray system mounted on a crawler-tractor. Site Description: Central Georgia, herbaceous and woody species. 0.6-0.8 ha. Woody plants removed prior to treatment. Loblolly pine seedlings planted in 1982.	Observations made in 1992-1993. No significant differences in species richness for any plant groups [Arborescents, nonarborescents, legume and nonlegume forbs, grasses, and woody vines]. No effect on plant species diversity. The only effect compared to controls was a reduction in nonarborescent species *Vaccinium stamineum* and all *Vaccinium* species combined.	Boyd et al. 1995
Roundup	ED50 of 0.7-93 μg/plant for 14 non-target plant species. Dispersion model indicated that glyphosate could damage non-target plant species when aerially applied at concentrations of 6.4 g/L.	Breeze et al. 1992

Appendix 2-2. Summary of field or field simulation studies on glyphosate formulations

Application	Observations	Reference
Glyphosate (NOS)	In laboratory toxicity tests using adult carabids, no signs of toxicity at exposures equivalent to an application rate of 1.57 kg/ha. No repellant effects under laboratory conditions. In field studies, no toxic or repellent effects. Decreased numbers of carabids in field plots were secondary to effects on vegetation.	Brust 1990
Glyphosate (NOS), 3.4 kg a.i/ha.	Effects on soil invertebrates were secondary to effects on alfalfa density.	Byers and Bierlein 1984
Roundup, 2 lbs/acre by tractor mounted pump and hand-held sprayer in pine release.	Significant increase (38%) in mortality of pine seedlings after 1 year. Increased mortality also apparent after 5 years. There was, however, an increase in the number of free-to-grow survivors after 5 years.	Cain 1991
Roundup, 1.4 kg a.i./ha by hand held controlled drop band applicators in a six year old spruce plantation (North Wales)	An initial decrease in Calluna and increased amount of bare ground. After 2 years, no difference in the abundance of *Vaccinium* and *Empetrum* species. Black grouse evidenced a preference for treated areas, probably because of increased accessibility or fruiting quality.	Cayford 1988
Roundup, 0.54-3.23 kg a.i./ha	At 0.54 kg/ha, a decrease in soil fungi and bacterial populations after 2 months. No effect after 6 months. At 3.23 kg/ha, no effect on soil fungi and bacteria after 10-14 months.	Chakravarty and Chatarpaul 1990
Roundup, 2.25 kg/ha applied aerially to field to suppress angiosperms competing with conifer regeneration.	Herbicide treatment had no effect on captures of most small mammal species over a one year observation period [Masked shrew, deer mouse, pygmy shrew, short-tailed shrew, southern bog lemming, or meadow jumping mouse]. Southern Red-backed voles were more numerous in control than in treated sites. This effect was attributed to defoliation of overhead cover.	D'Anieri et al. 1987
Roundup, 6 L/ha (about 2.1 kg/ha)	Assays for the degradation of leaf litter by isopods. There was an increased decomposition of birch and a decreased decomposition of black cherry. Possible signs of toxicity but not statistically significant.	Eijsackers 1992

Appendix 2-2. Summary of field or field simulation studies on glyphosate formulations

Application	Observations	Reference
Roundup	Inhibition of growth in three species of ectomycorrhizal fungi in laboratory cultures at concentrations of over 10 mg/L.	Estok et al. 1989
Glyphosate (NOS), 2.2-3 kg/ha aerial over pine forest.	Glyphosate applications had a greater impact on stream water quality than clearcutting. Effects were evident over a five year period. Changes in water quality would not impact the suitability of the water for human consumption.	Feller 1989
Glyphosate (NOS) applied to litter.	Concentrations of 5,000 to 10,000 ppm in litter caused a significant decrease in decomposition.	Fletcher and Freedman 1986
Glyphosate, 2 lb/acre, broadcast ground application		Haywood 1994
Glyphosate (NOS), 1 kg/ha in clearcut area.	Substantial decrease utilization by mountain hare one year after spraying. A lesser decrease, not statistically significant, after 2 years.	Hjeljord et al. 1988
Roundup, 4 L/ha (1.4 kg/ha), pre-harvest treatment of pasture.	No significant effects on the consumption of treated hay by sheep.	Jones and Forbes 1984
Roundup, 2.52 kg/ha on pasture	Cattle preferred grazing on treated pasture over first 5-7 days post-treatment. There was an aversion to the treated area 15-21 days post treatment. Reasons for the preference and aversion were not apparent.	Kisseberth et al. 1986
Rodeo, 5.8 kg a.i./ha with a surfactant and drift retardant over a wetland areas.	An increase or no significant change in the usage of treated wetlands by black terns over a two year observation period. The increased usage was associated with an increase in open water and newly formed mats of dead emergent vegetation.	Linz et al. 1994
Glyphosate (NOS), 0.75-1.0 kg/ha.	Reduction of plant coverage by brush species by about 60%. Vegetation recovered after 3 years. No effect on plant species diversity. A substantial increase in the number of Norway spruce over 50 cm in height on treated vs untreated plots.	Lund-Hoie and Gronvold 1987

Appendix 2-2. Summary of field or field simulation studies on glyphosate formulations

Application	Observations	Reference
Glyphosate (NOS), 1 kg/ha by portable mist blower.	Glyphosate used for comparison to imazapyr.	Lund-Hoie and Rognstad 1990
Glyphosate (NOS), 2.3 kg/ha aerial over clearcut.	Heavy defoliation of ferns, birch, raspberry, maple, and other taxa. No difference in abundance of breeding birds in first-post spray season. A decrease in abundance of breeding birds was noted in the second post-spray season. Changes in bird density were associated with changes in vegetation.	MacKinnon and Freedman 1993
Glyphosate (NOS), 2.2 kg/ha. Tractor-mounted team sprayer.	Bioassay of drift using five species of plants in pots. Plants were placed in greenhouse after spraying. Most species evidenced no effect when placed 4 meters downwind and no plants exposed to glyphosate drift evidenced a decrease in yield at the end of the season.	Marrs et al. 1991
see Powers 1995	No effect on soil arthropods.	Moldenke 1992
Glyphosate (NOS), 2.6 kg/ha.	Initial glyphosate residues of 17 ppm in loam and 3.8 ppm in silt. No effect on soil nitrification or denitrification.	Mueller et al. 1981
Glyphosate (NOS), 0.8-3.0 kg/ha,	Three dose levels assayed at five different application times during the year to 13 species of wood ornamentals. The most sensitive species, damaged at all times and exposure levels, were ajuga, azalea, and variegated liriope. Other species, such as juniper, evidenced only minor and transient damage.	Neal and Skroch 1985
Glyphosate (NOS), 3.3 kg/ha.	Levels in wildlife monitored over a 55 day period. No residues exceeded 2 mg/kg in viscera and 0.5 mg/kg in whole body [shrews, deermice, woodrats, squirrel, voles, and chipmunks]. Body residues were consistently less than residues on vegetation.	Newton et al. 1984
Roundup, 1.7 and 3.3 kg/ha.	Vegetative hardwood and shrub cover over 1.5 meters in height virtually eliminated. Differences in height and cover were apparent at 9 years after application.	Newton et al. 1992a [NJAF 9:126]

Appendix 2-2. Summary of field or field simulation studies on glyphosate formulations

Application	Observations	Reference
Roundup, 1.7 and 3.3 kg/ha.	Conifers dominated over hardwoods. Some injury to conifers at the higher application rate.	Newton et al. 1992b [NJAF, 9:130]
Glyphosate (NOS), 0.1 g/m^2 in lysimeters (30 cm x 45 cm). [1 kg/ha]	Death of vegetation in lysimeters associated with increased leaching of nitrates and cations from soil. Reestablishment of vegetation over 28 month observation period retarded leaching.	Ogner 1987a,b
Glyphosate (NOS), 0.72 kg/ha, mechanical ground application in forest	Increase in nitrogen levels in streams, consistent with lysimeter studies. Increases were small and did not significantly affect water quality. Similar effects were observed after manual clearing and were judged to be secondary to changes in vegetation.	Ogner 1987c,d
Glyphosate (NOS), 1.2 kg/ha aerial or 1.1 kg/ha manual, 54 ha clearcut and surrounding old growth forest.	No effect on body size and apparent reproductive capacity [assayed as number of placental scars and foeti] of deer mice. Deer mice were more abundant in untreated clearcut probably due to changes in food abundance and quality secondary to changes in vegetation.	Ritchie et al. 1987
Roundup, aerial application at 4.7 L a.i./42.1 L water/ha. [≈1.7 kg/ha?] on 4-5 year old clearcuts in North Maine.	Decrease in available browse plants on 2-year post-treatment clearcuts. Moose used treated areas less than untreated areas.	Santillo 1994
Roundup, aerial application at 4.7 L a.i./42.1 L water/ha. [≈1.7 kg/ha?] on 4-5 year old clearcuts in North Maine.	Total shrub, forb, and grass cover was diminished 1-3 years post treatment. Decrease in species richness of shrubs and forbs on treated clearcuts. Decrease in numbers of invertebrates. Fewer small herbivorous mammals at 1-3 years post-treatment. No effect on carnivorous mammals. Effects attributable to changes in cover, food resources, and microclimate.	Santillo et al. 1989a,b
Rodeo, 2.8 L/ha [1.3 kg/ha] in wetlands to control cattails.	Effective control of cattails. Breeding ducks and over-water duck nest densities greater on treated areas because of increase wetland opening. Decrease in aquatic invertebrates in treated areas. Could not determine if this was due to toxicity or habitat changes.	Solberg and Higgins 1993

Appendix 2-2. Summary of field or field simulation studies on glyphosate formulations

Application	Observations	Reference
Glyphosate (NOS), 0.7, 1.4, and 2.8 g/ha, sprayed twice weekly on to culture dishes.	Earthworms evidenced decreased growth over 100 day exposure period with an uneven dose-effect relationship. Mortality observed in some worms after about 80 days. Co-exposure to Captan appeared to reduce the response. Co-exposure to azinphos-methyl and Captan had no more effect than exposure to glyphosate alone.	Springett and Gray 1992
Roundup, aerial application to conifer forest at 1.7 kg a.i./ha.	No significant impact on numbers of bacteria, fungi, and actinomycetes in litter or soil. In laboratory bioassays, no effects are rates up to 100 times field application rates.	Stratton and Stewart 1992.
Roundup, aerial application to 2-year clearcut at 3.0 kg/ha.	Little difference in recruitment of voles between control and treated areas. Decline in deer mice during first post-spray summer and winter only. Population of deer mice increased in subsequent years. Significantly ($p<0.05$) better survival of female voles on treated sites.	Sullivan 1990
Roundup, aerial application at 4 kg/ha on farmland planted for hay in previous 5 years.	No effect on any microbial soil variables tested: biomass, substrate-induced respiration, basal respiration, bacterial:fungal ratio.	Wardle and Parkinson 1991
Glyphosate (NOS), 5 kg/ha directly incorporated into soil of barley or weed plots.	No direct effect on basal soil respiration, microbial activity, or microbial biomass. Transient decrease in biomass on some plots secondary to toxic effects on weeds.	Wardle and Parkinson 1992
Glyphosate (NOS), 1.1 and 6.7 kg/ha, on cotton leaves.	Bioassay using Western bigeyed bug, *Geocoris pallens*. Females exposed to glyphosate laid slightly more viable eggs than matched controls. A slight dose/response related improvement in survival is also apparent over a 192 day observation period.	Yokoyama and Pritchard 1984

Appendix 2-3. Toxicity of glyphosate and glyphosate formulations to fish

Formulation	Species	Nature of Exposure	Exposure Time	Effects	Comments[a]	Reference
Roundup Pro	rainbow trout	NS	96 hours	LC_{50} = 8.3 ppm		Matura 1996a
Roundup Pro	bluegill sunfish	NS	96 hours	LC_{50} = 6.5 ppm		Matura 1996a
Roundup	rainbow trout	static bioassay	24 hours 96 hours	LC_{50} = 8.3 ppm LC_{50} = 8.3 ppm	(7.0-9.9 ppm) 12°C (54°F) (7.0-9.9 ppm) 12°C (54°F)	Folmar et al. 1979
Roundup	fathead minnow	static bioassay	24 hours 96 hours	LC_{50} = 2.4 ppm LC_{50} = 2.3 ppm	(2.0-2.9 ppm) 22°C (72°F) (1.9-2.8 ppm) 22°C (72°F)	Folmar et al. 1979
Roundup	channel catfish	static bioassay	24 hours 96 hours	LC_{50} = 13 ppm LC_{50} = 13 ppm	(11-16 ppm) 22°C (72°F) (11-16 ppm) 22°C (72°F)	Folmar et al. 1979
Roundup	bluegill	static bioassay	24 hours 96 hours	LC_{50} = 6.4 ppm LC_{50} = 5.0 ppm	(4.8-8.6 ppm) 22°C (72°F) (3.8-6.6 ppm) 22°C (72°F)	Folmar et al. 1979
Roundup	rainbow trout eyed eggs	static bioassay	24 hours 96 hours	LC_{50} = 46 ppm LC_{50} = 16 ppm	(35-61 ppm) (13-19 ppm)	Folmar et al. 1979
Roundup	rainbow trout sac fry	static bioassay	24 hours 96 hours	LC_{50} = 11 ppm LC_{50} = 3.4 ppm	(8.8-13 ppm) (2.2-5.3 ppm)	Folmar et al. 1979
Roundup	rainbow trout swim-up fry	static bioassay	24 hours 96 hours	LC_{50} = 2.4 ppm LC_{50} = 2.4 ppm	(2.0-2.9 ppm) (2.0-2.9 ppm)	Folmar et al. 1979
Roundup	rainbow trout fingerling (1.0 g)	static bioassay	24 hours 96 hours	LC_{50} = 2.2 ppm LC_{50} = 1.3 ppm	(0.93-5.2 ppm) (1.1-1.6 ppm)	Folmar et al. 1979
Roundup	rainbow trout fingerling (2.0 g)	static bioassay	24 hours 96 hours	LC_{50} = 8.3 ppm LC_{50} = 8.3 ppm	(7.0-9.9 ppm) (7.0-9.9 ppm)	Folmar et al. 1979
Roundup	channel catfish eyed eggs	static bioassay	24 hours 96 hours	LC_{50} = 43 ppm LC_{50} = ND	(36-51 ppm) ND	Folmar et al. 1979
Roundup	channel catfish sac fry	static bioassay	24 hours 96 hours	LC_{50} = 4.3 ppm LC_{50} = 4.3 ppm	(3.6-5.1 ppm) (3.6-5.1 ppm)	Folmar et al. 1979
Roundup	channel catfish swim-up fry	static bioassay	24 hours 96 hours	LC_{50} = 3.7 ppm LC_{50} = 3.3 ppm	(3.4-4.1 ppm) (2.8-3.9 ppm)	Folmar et al. 1979
Roundup	channel catfish fingerling (2.2 g)	static bioassay	24 hours 96 hours	LC_{50} = 13 ppm LC_{50} = 13 ppm	(11-16 ppm) (11-16 ppm)	Folmar et al. 1979
Roundup	rainbow trout fingerling (1.4 g)	static bioassay (laboratory)	96 hours	LC_{50} = 54.8 ppm	(50-60 ppm)	Hildebrand et al. 1982

Appendix 2-3. Toxicity of glyphosate and glyphosate formulations to fish

Formulation	Species	Nature of Exposure	Exposure Time	Effects	Comments[a]	Reference
Roundup	rainbow trout fingerling (1.6 g)	static bioassay (field)	96 hours	LC_{50} = 52 ppm	not reported	Hildebrand et al. 1982
Roundup	rainbow trout fingerling (2.1 g)	manual application	1 hour	100% survival; short period (15 minutes) of increased swimming activity during and shortly after application; no acute manifestations of physical discomfort such as coughing or loss of equilibrium	indigenous cutthroat trout and caddis fly larae in pools along the stream course did not show signs of stress during the period of spraying	Hildebrand et al. 1982
Roundup	rainbow trout fingerling (2.3 g)	aerial application	NS	100% surival; no obvious signs of physical stress or discomfort from the time of spraying to conclusion of study (17 days)	no indication of stressful behavior by fish after first rainfall	Hildebrand et al. 1982
Roundup	rainbow trout	static bioassay	96 hours	LC_{50} = 26 ppm	(12-38 ppm) 11°C	Mitchell et al. 1987a
Roundup	chinook salmon	static bioassay	96 hours	LC_{50} = 20 ppm	(17-27 ppm) 11°C	Mitchell et al. 1987a
Roundup	coho salmon	static bioassay	96 hours	LC_{50} = 22 ppm	(12-38 ppm) 11°C	Mitchell et al. 1987a
Roundup	bluegill	not reported	96 hours	TL_{50} = 14 ppm	none	Monsanto Co. 1982b
Roundup	carp	not reported	96 hours	TL_{50} = 3.9 ppm	none	Monsanto Co. 1982b
Roundup	trout	not reported	96 hours	TL_{50} = 11 ppm	none	Monsanto Co. 1982b
Roundup	catfish	not reported	96 hours	LC_{50} = 16 ppm	none	Monsanto Co. 1982b
Roundup	fathead minnow	not reported	96 hours	LC_{50} = 9.4 ppm	none	Monsanto Co. 1982b
Roundup	rainbow trout	not reported	96 hours	TL_{50} = 48 ppm	none	USDA 1981

Appendix 2-3. Toxicity of glyphosate and glyphosate formulations to fish

Formulation	Species	Nature of Exposure	Exposure Time	Effects	Comments[a]	Reference
Roundup	bluegill	not reported	96 hours	TL_{50} = 24 ppm	none	USDA 1981
Roundup	rainbow trout	static bioassay	24 hours 96 hours	LC_{50} = 8.3 mg/L LC_{50} = 8.3 mg/L	none	Folmar et al. 1979
Roundup	channel catfish	static bioassay	24 hours 96 hours	LC_{50} = 13 mg/L LC_{50} = 13 mg/L	none	Folmar et al. 1979
Roundup	bluegill	static bioassay	24 hours 96 hours	LC_{50} = 6.4 mg/L LC_{50} = 5.0 mg/L	none	Folmar et al. 1979
Roundup	bleak	static bioassay	96 hours	LC_{50} = 16 ppm	(15-18 ppm)	Linden et al. 1979
Roundup	harpacticoid	static bioassay	96 hours	LC_{50} = 22 ppm	(17-29 ppm)	Linden et al. 1979
Roundup	coho salmon smolts	sublethal exposure	10 days	no affect on seawater adaptation: plasma sodium values not significantly different from control	there was no effect on growth and several sublethal parameters at exposure concentrations up to 2.78 ppm	Mitchell et al. 1987b
Roundup	grass carp	intermittent dosing	24 hours	LC_{50} = 26 ppm	(22-30 ppm) 18-21°C; pH 8.1; hardness 270 mg/L	Tooby et al. 1980
			48 hours	LC_{50} = 24 ppm	(21-28 ppm) 18-21°C; pH 8.1; hardness 270 mg/L	
			96 hours	LC_{50} = 15 ppm	(13-18 ppm) 18-21°C; pH 8.1; hardness 270 mg/L	
Roundup	sockeye (fingerling)	static bioassay	96 hours	LC_{50} = 26.7 ppm	4.2°C; pH 7.95; average weight 3.8 g	Servizi et al. 1987
Roundup	sockeye (fingerling)	static bioassay	96 hours	LC_{50} = 27.7 ppm	4.2°C; pH 8.0; average weight 3.7 g	Servizi et al. 1987
Roundup	sockeye (fry)	static bioassay	96 hours	LC_{50} = 28.8 ppm	4.5°C; pH 7.7; average weight 0.25 g	Servizi et al. 1987
Roundup	rainbow trout (fry)	static bioassay	96 hours	LC_{50} = 28.0 ppm	15°C; pH <6.3; average weight 0.33 g	Servizi et al. 1987
Roundup	rainbow trout (fry)	static bioassay	96 hours	LC_{50} = 25.5 ppm	14.5°C; pH <6.3; average weight 0.60 g	Servizi et al. 1987

Appendix 2-3. Toxicity of glyphosate and glyphosate formulations to fish

Formulation	Species	Nature of Exposure	Exposure Time	Effects	Comments[a]	Reference
Roundup	coho salmon (fry)	static bioassay	96 hours	LC_{50} = 42.0 ppm	15°C; pH <6.3; average weight 0.30 g	Servizi et al. 1987
Roundup	coho salmon (juvenile)	static bioassay	96 hours	LC_{50} = 31 ppm	14°C; intermediate pH	Wan et al. 1991
Roundup	pink salmon (juvenile)	static bioassay	96 hours	LC_{50} = 10 ppm	14°C; intermediate pH	Wan et al. 1991
Roundup	rainbow trout (juvenile)	static bioassay	96 hours	LC_{50} = 31 ppm	14°C; intermediate pH	Wan et al. 1991
Rodeo	carp	not reported	96 hours	TL_{50} >10,000	none	Monsanto Co. 1982d
Rodeo	trout	not reported	96 hours	TL_{50} >1000	none	Monsanto Co. 1982d
Rodeo	bluegill	not reported	96 hours	TL_{50} >1000	none	Monsanto Co. 1982d
Rodeo	plains minnow	renewal	96 hours	NOAEC = 1000 mg/L	none	Beyers 1995
Rodeo	fathead minnow	renewal	96 hours	NOAEC = 1000 mg/L	none	Beyers 1995
Rodeo	rainbow trout (0.52 g)	static bioassay	96 hours	LC_{50} = 1100	(850-1300 ppm) 11°C; pH 6.0; hardness 5.0 mg/L	Mitchell et al. 1987a
Rodeo/X-77[b])	rainbow trout (0.52 g)	static bioassay	96 hours	LC_{50} = 680 ppm	(600-820 ppm) 11°C; pH 6.0; hardness 5.0 mg/L	Mitchell et al. 1987a
Rodeo/X-77[b])	rainbow trout (0.21 g)	static bioassay	96 hours	LC_{50} = 1070 ppm	(600-1920 ppm) 11°C; pH 7.8; hardness 75 mg/L	Mitchell et al. 1987a
Rodeo/X-77[b])	chinook salmon (4.2 g)	static bioassay	96 hours	LC_{50} = 750 ppm	(600-1100 ppm) 11°C; pH 5.8; hardness 5.0 mg/L	Mitchell et al. 1987a
Rodeo/X-77[b])	chinook salmon (5.9 g)	static bioassay	96 hours	LC_{50} = 1440 ppm	(1070-1920 ppm) 11°C; pH 7.4; hardness 77 mg/L	Mitchell et al. 1987a
Rodeo/X-77[b])	coho salmon (17.9 g)	static bioassay	96 hours	LC_{50} = 1000 ppm	(600-1900 ppm) 11°C; pH 5.8; hardness 5.0 mg/L	Mitchell et al. 1987a
Rodeo/X-77[b])	coho salmon (11.8 g)	static bioassay	96 hours	LC_{50} = 600 ppm	(340-1100 ppm) 11°C; pH 6.2; hardness 4.5 mg/L	Mitchell et al. 1987a

Appendix 2-3. Toxicity of glyphosate and glyphosate formulations to fish

Formulation	Species	Nature of Exposure	Exposure Time	Effects	Comments[a]	Reference
Glyphosate (IPA salt in Rodeo)	rainbow trout (0.52 g)	static bioassay	96 hours	LC_{50} = 580	(460-730 ppm) 11°C; pH 6.0; hardness 5.0 mg/L	Mitchell et al. 1987a
Glyphosate (IPA salt in Roundup)	rainbow trout (0.37 g)	static bioassay	96 hours	LC_{50} = 12 ppm	(5.7-18 ppm) 11°C; pH 6.1; hardness 4.5 mg/L	Mitchell et al. 1987a
Glyphosate (IPA salt in Roundup)	rainbow trout (0.37 g)	static bioassay	96 hours	LC_{50} = 11 ppm	(5.7-18 ppm) 11°C; pH 7.6; hardness 85 mg/L	Mitchell et al. 1987a
Glyphosate (IPA salt in Roundup)	rainbow trout (0.37 g)	static bioassay	96 hours	LC_{50} = 7.4 ppm	(5.7-10 ppm) 11°C; pH 7.7; hardness 81 mg/L	Mitchell et al. 1987a
Glyphosate (IPA salt in Roundup)	chinook salmon (4.6 g)	static bioassay	96 hours	LC_{50} = 9.6 ppm	(7.9-13 ppm) 11°C; pH 6.1; hardness 4.5 mg/L	Mitchell et al. 1987a
Glyphosate (IPA salt in Roundup)	coho salmon (11.8 g)	static bioassay	96 hours	LC_{50} = 11 ppm	(5.7-18 ppm) 11°C; pH 6.2; hardness 4.5 mg/L	Mitchell et al. 1987a
Glyphosate (IPA salt in Rodeo/X-77[b])	rainbow trout (0.52 g)	static bioassay	96 hours	LC_{50} = 130 ppm	(120-160 ppm) 11°C; pH 6.0; hardness 5.0 mg/L	Mitchell et al. 1987a
Glyphosate (IPA salt in Rodeo/X-77[b])	rainbow trout (0.21 g)	static bioassay	96 hours	LC_{50} = 210 ppm	(120-380 ppm) 11°C; pH 7.8; hardness 75 mg/L	Mitchell et al. 1987a
Glyphosate (IPA salt in Rodeo/X-77[b])	chinook salmon (4.2 g)	static bioassay	96 hours	LC_{50} = 140 ppm	(120-220 ppm) 11°C; pH 5.8; hardness 5.0 mg/L	Mitchell et al. 1987a
Glyphosate (IPA salt in Rodeo/X-77[b])	chinook salmon (5.9 g)	static bioassay	96 hours	LC_{50} = 290 ppm	(210-380 ppm) 11°C; pH 7.4; hardness 77 mg/L	Mitchell et al. 1987a
Glyphosate (IPA salt in Rodeo/X-77[b])	coho salmon (17.9 g)	static bioassay	96 hours	LC_{50} = 200 ppm	(120-370 ppm) 11°C; pH 5.8; hardness 5.0 mg/L	Mitchell et al. 1987a
Glyphosate (IPA salt in Rodeo/X-77[b])	coho salmon (11.8 g)	static bioassay	96 hours	LC_{50} = 120 ppm	(68-220 ppm) 11°C; pH 6.2; hardness 4.5 mg/L	Mitchell et al. 1987a
Glyphosate	sockeye (fingerling)	static bioassay	96 hours	LC_{50} = 8.1 ppm	4.2°C; pH 7.95; average weight 3.8 g	Servizi et al. 1987

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Formulation	Species	Nature of Exposure	Exposure Time	Effects	Comments[a]	Reference
Glyphosate	sockeye (fingerling)	static bioassay	96 hours	LC_{50} = 8.4 ppm	4.2°C; pH 8.0; average weight 3.7 g	Servizi et al. 1987
Glyphosate	sockeye (fry)	static bioassay	96 hours	LC_{50} = 8.7 ppm	4.5°C; pH 7.7; average weight 0.25 g	Servizi et al. 1987
Glyphosate	rainbow trout (fry)	static bioassay	96 hours	LC_{50} = 8.5 ppm	15°C; pH <6.3; average weight 0.33 g	Servizi et al. 1987
Glyphosate	rainbow trout (fry)	static bioassay	96 hours	LC_{50} = 7.8 ppm	14.5°C; pH <6.3; average weight 0.60 g	Servizi et al. 1987
Glyphosate	coho salmon (fry)	static bioassay	96 hours	LC_{50} = 12.8 ppm	15°C; pH <6.3; average weight 0.30 g	Servizi et al. 1987
Glyphosate	rainbow trout	static bioassay	96 hours	LC_{50} = 10.42 ppm	(9.37-11.67) 12°C; pH 6.01; hardness 9.6 mg/L	Morgan and Kiceniuk 1992
Glyphosate	rainbow trout	static bioassay	24 hours 96 hours	LC_{50} = 140 ppm LC_{50} = 140 ppm	(120-170 ppm) 12°C (54°F) (120-170 ppm) 12°C (54°F)	Folmar et al. 1979
Glyphosate	fathead minnow	static bioassay	24 hours 96 hours	LC_{50} = 97 ppm LC_{50} = 97 ppm	(79-120 ppm) 22°C (72°F) (79-120 ppm) 22°C (72°F)	Folmar et al. 1979
Glyphosate	channel catfish	static bioassay	24 hours 96 hours	LC_{50} = 130 ppm LC_{50} = 130 ppm	(110-160 ppm) 22°C (72°F) (110-160 ppm) 22°C (72°F)	Folmar et al. 1979
Glyphosate	bluegill	static bioassay	24 hours 96 hours	LC_{50} = 150 ppm LC_{50} = 140 ppm	(120-190 ppm) 22°C (72°F) (120-190 ppm) 22°C (72°F)	Folmar et al. 1979
Glyphosate	rainbow trout fry	not reported	96 hours	LC_{50} = 50 ppm	3 lbs a.e./gallon	Folmar 1976
Glyphosate	bleak	not reported	96 hours	LC_{50} = 16 ppm	(15-18 ppm) 10°C (50°F)	Linden et al. 1979
Glyphosate (95% pure)	flagfish	pulse exposure	96 hours	LC_{20} = 29.6 ppm	fed 8-day-old flagfish	Holdway and Dixon 1988
Glyphosate, technical	rainbow trout	not reported	96 hours	TL_{50} = 38 ppm	none	USDA 1981
Glyphosate, technical	bluegill	not reported	96 hours	TL_{50} = 78 ppm	none	USDA 1981
Glyphosate, technical	bluegill	dynamic test	96 hours	TL_{50} = 24 ppm	none	USDA 1981

Appendix 2-3. Toxicity of glyphosate and glyphosate formulations to fish

Formulation	Species	Nature of Exposure	Exposure Time	Effects	Comments[a]	Reference
Glyphosate, technical	bluegill	not reported	96 hours	LC_{50} = 120 ppm	none	Monsanto Co. 1982a
Glyphosate, technical	trout	not reported	96 hours	LC_{50} = 86 ppm	none	Monsanto Co. 1982a
Glyphosate, technical	carp	not reported	96 hours	LC_{50} = 115 ppm	none	Monsanto Co. 1982a
Glyphosate, technical	harlequin fish	not reported	96 hours	LC_{50} = 168 ppm	none	Monsanto Co. 1982a
Glyphosate, technical	carp	static bioassay	48 hours	TL_{50} =119 ppm TL_1 =146 ppm TL_{99} =96.7 ppm	none	USDA 1981
Glyphosate, technical	carp	static bioassay	96 hours	TL_{50} =115 ppm TL_1 =125 ppm TL_{99} =105 ppm	none	USDA 1981
Glyphosate, technical	rainbow trout	static bioassay	96 hours	LC_{50} = 25,605 mg/L NOEC = 8,000 mg/L	36% active ingredient	Anton et al. 1994
Glyphosate, technical	rainbow trout	static bioassay	96 hours	LC_{50} = 25,657 mg/L NOEC = NR	38% active ingredient	Anton et al. 1994
Glyphosate, technical	rainbow trout	static bioassay	96 hours	LC_{50} = 7,620 mg/L NOEC = 6,250 mg/L	54.9% active ingredient	Anton et al. 1994
Glyphosate, technical	goldfish	static bioassay	96 hours	LC_{50} = 7,816 mg/L NOEC = 1,500 mg/L	54.9% active ingredient	Anton et al. 1994
Vision-10% surfactant	coho salmon	closed system respirometer	4 hours	hematocrit significantly increased over controls at lowest(3.75 and 60 ppm) concentrations ($p<0.05$) but expected to decrease as a result of stress; no significant increases in plasma lactate or plasma glucose	data suggest that a staress threshold was not reached for Vision-10% surfactant at concentrations up to 80% of the 96-hour LC_{50}	Janz et al. 1991

Appendix 2-3. Toxicity of glyphosate and glyphosate formulations to fish

Formulation	Species	Nature of Exposure	Exposure Time	Effects	Comments[a]	Reference
Vision	rainbow trout	sublethal exposure	1 month	fish in highest concentration (45.75 µg/L) had significantly higher frequency of wigwags	little overall effect of exposure to Vision on rainbow trout	Morgan and Kiceniuk 1992
Vision	rainbow trout	sublethal exposure	2 months	fish in lowest concentration (4.25 µg/L) performed significantly fewer wigwags	little overall effect of exposure to Vision on rainbow trout; it is not clear what the implications of a change in one agonistic activity in the repetoire of aggressive behavior would be in terms of fish's ability to hold a feeding station	Morgan and Kiceniuk 1992
MONO818	sockeye (fingerling)	static bioassay	96 hours	LC_{50} = 4.0 ppm	4.2°C; pH 7.95; average weight 3.8 g	Servizi et al. 1987
MONO818	sockeye (fingerling)	static bioassay	96 hours	LC_{50} = 4.2 ppm	4.2°C; pH 8.0; average weight 3.7 g	Servizi et al. 1987
MONO818	sockeye (fry)	static bioassay	96 hours	LC_{50} = 4.3 ppm	4.5°C; pH 7.7; average weight 0.25 g	Servizi et al. 1987
MONO818	rainbow trout (fry)	static bioassay	96 hours	LC_{50} = 4.2 ppm	15°C; pH <6.3; average weight 0.33 g	Servizi et al. 1987
MONO818	rainbow trout (fry)	static bioassay	96 hours	LC_{50} = 3.8 ppm	14.5°C; pH <6.3; average weight 0.60 g	Servizi et al. 1987
MONO818	coho salmon (fry)	static bioassay	96 hours	LC_{50} = 6.3 ppm	15°C; pH <6.3; average weight 0.30 g	Servizi et al. 1987
Glyphosate	rainbow trout	static bioassay	96 hours	LC_{50} = 10.42 ppm	(9.37-11.67) 12°C; pH 6.01; hardness 9.6 mg/L	Morgan and Kiceniuk 1992
Surfactant used in Roundup	rainbow trout	static bioassay	24 hours 96 hours	LC_{50} = 2.1 ppm LC_{50} = 2.0 ppm	(1.6-2.7 ppm) 12°C (54°F) (1.5-2.7 ppm) 12°C (54°F)	Folmar et al. 1979
Surfactant used in Roundup	fathead minnow	static bioassay	24 hours 96 hours	LC_{50} = 1.4 ppm LC_{50} = 1.0 ppm	(1.2-1.7 ppm) 22°C (72°F) (1.2-1.7 ppm) 22°C (72°F)	Folmar et al. 1979
Surfactant used in Roundup	channel catfish	static bioassay	24 hours 96 hours	LC_{50} = 18 ppm LC_{50} = 13 ppm	(8.5-38 ppm) 22°C (72°F) (10-17 ppm) 22°C (72°F)	Folmar et al. 1979

Appendix 2-3. Toxicity of glyphosate and glyphosate formulations to fish

Formulation	Species	Nature of Exposure	Exposure Time	Effects	Comments[a]	Reference
Surfactant used in Roundup	bluegill	static bioassay	24 hours	LC_{50} = 3.0 ppm	(2.5-3.7 ppm) 22°C (72°F)	Folmar et al. 1979
			96 hours	LC_{50} = 3.0 ppm	(2.5-3.7 ppm) 22°C (72°F)	

[a]Values in parentheses are the 95% confidence limits.
[b]Rodeo /X-77 consists of 312 mL Rodeo mixed with 699 mL water and 4 mL X-77 surfactant.
NOEC = No-observed-effect concentration; NOAEC = No-observed-acute-effect concentration; ND = not determined

Appendix 2-4. Acute toxicity of glyphosate to aquatic invertebrates

Formulation	Species	Nature of Exposure	Exposure Time	Effects	Comments[a]	Reference
Glyphosate	midge larvae (*Chironomus plumosus*; insecta)		48 hours	EC_{50} = 55 ppm	(31-97 ppm) 22°C (72°F)	Folmar et al. 1979
Roundup surfactant	midge larvae (*Chironomus plumosus*; insecta)		48 hours	EC_{50} = 13 ppm	(7.1-24 ppm) 22°C (72°F)	Folmar et al. 1979
Glyphosate, technical	grass shrimp (crustacea)		96 hours	TL_{50} = 281 ppm NOEL at 210 ppm	(207-391 ppm)	Monsanto Co. 1982a, USDA 1981
Glyphosate, technical	fiddler crab (crustacea)		96 hours	TL_{50} = 934 ppm NOEL at 650 ppm	(555-1570 ppm)	Monsanto Co. 1982a, USDA 1981
Roundup	red swamp crawfish (*Procambarus clarkii*)		96	LC_{50} = 47.31 ppm	(41.06-51.69)	Holck and Meek 1987
Roundup	fourth instar *Anopheles quadrimaculatus* larvae	combination of techniques	24	LC_{50} = 673.43 ppm	(572.57-770.17)	Holck and Meek 1987
Roundup	fourth instar *Psurophora columbiae* larvae	combination of techniques	24	LC_{50} = 940.84 ppm	(823.08-1067.12)	Holck and Meek 1987
Roundup	fourth instar *Culex salinarius* larvae	combination of techniques	24	LC_{50} = 1563.69 ppm	(1262.00-2214.54)	Holck and Meek 1987
Roundup	cladoceran (*Daphnia magna*; crustacea)		48 hours	EC_{50} = 3.0 ppm	(2.6-3.4 ppm) 22°C (72°F)	Folmar et al. 1979
Roundup	cladoceran (*Daphnia pulex*; crustacea)	basic static test with suspended sediment	48 hours	EC_{50} = 3.2 ppm	(3.0-3.4 ppm) 22°C (72°F)	Hartman and Martin 1984
Roundup	cladoceran (*Daphnia pulex*; crustacea)	basic static test without suspended sediment	48 hours	EC_{50} = 7.9 ppm	(7.2-8.6 ppm) 22°C (72°F)	Hartman and Martin 1984
Roundup	cladoceran (*Daphnia* sp.; crustacea)		48 hours	LC_{50} = 192 ppm	(181-205 ppm)	USDA 1981
Roundup	cladoceran (*Daphnia* sp.; crustacea)		48 hours	LC_{50} = 5.3 ppm	NS	Monsanto Co. 1982b
Roundup Pro	cladoceran (*Daphnia magna*; crustacea)		48 hours	LC_{50} = 8.9 ppm		Matura 1996a

Appendix 2-4. Acute toxicity of glyphosate to aquatic invertebrates

Formulation	Species	Nature of Exposure	Exposure Time	Effects	Comments[a]	Reference
Rodeo	cladoceran (*Daphnia* sp.; crustacea)		48 hours	LC_{50} = 930 ppm	NS	Monsanto Co. 1982d
Rodeo	*Daphnia magna*	static bioassay	48 hours	LC_{50} = 218 ppm	(150-287 ppm)	Henry et al. 1994
Rodeo	*Hyalella azteca*	static bioassay	96 hours	LC_{50} = 720 ppm[b]	(399-1076 ppm)	Henry et al. 1994
Rodeo	*Chironomus riparius*	static bioassay	48 hours	LC_{50} = 1216 ppm[b]	(996-1566 ppm)	Henry et al. 1994
Rodeo	*Nephelopsis obscura*	static bioassay	96 hours	LC_{50} = 1177 ppm[b]	(941-1415 ppm)	Henry et al. 1994
Glyphosate, technical	cladoceran (*Daphnia* sp.; crustacea)		40 hours	LC_{50} = 780 ppm	NS	Monsanto Co. 1982a
Glyphosate, technical	larval Atlantic oysters (mollusca)		48 hours	NOEL at 10 ppm	no effect on embryonic development of larvae	Monsanto Co. 1982a, USDA 1981
Glyphosate	snails (*Pseudosuccinea columella*)	snails reared in sublethal concentrations	4 weeks	biochemical alteration	increased protein concentration of snails reared in 1.0 mg/L compared with those reared in 0.1 mg/L; exact mechanism for response not determined	Christian et al. 1993
Roundup	amphipod (*Gammarus pseudolimnaeus*; crustacea)		48 hours 96 hours	LC_{50} = 62 ppm LC_{50} = 43 ppm	(40-98 ppm) 12°C (54°F) (28-66 ppm) 12°C (54°F)	Folmar et al. 1979
Roundup	crayfish (crustacea)		96 hours	LC_{50} >1000 ppm	NS	Monsanto Co. 1982b
Roundup	Harpacticoid (*Nitocra spinipes*; crustacea)		96 hours	LC_{50} = 22 ppm	(17-29 ppm) 21.1°C (70 ± 2°F)	Linden et al. 1979

[a]Values in parentheses are the 95% confidence limits.
[b]Only 50% of the test organisms were killed in the highest concentration tested.
NS = Not specified.

Appendix 2-5: Toxicity of glyphosate and glyphosate formulations to aquatic plants

Species	Endpoint	Reference
	Glyphosate	
Selenastrum capricornutum, green algae	4 day EC_{50} = 12.5 mg/L	U.S. EPA 1993b
Navicula pelliculosa, diatom	4 day EC_{50} = 39.9 mg/L	
Skeletonema costatum,	4 day EC_{50} = 0.85 mg/L	
Anabaena flosaquae, cyanobacter	4 day EC_{50} = 11.7 mg/L	
Lemna gibba, duckweed	7 day EC_{50} = 11.7 mg/L	
Chlorella pyrenoidosa, green algae	4 day EC_{50} = 590 mg/L	Maule and Wright 1984
Chlorococcum hypnosporum, green algae	4 day EC_{50} = 68 mg/L	
Zygnema cllindricum, green algae	4 day EC_{50} = 88 mg/L	
Anabaena flosaquae, cyanobacter	4 day EC_{50} = 304 mg/L	
Cyclotella meneghiana, green algae	73% inhibition at 2.8 mg/L	Peterson et al. 1994
Nitzschia sp., green algae	77% inhibition at 2.8 mg/L	[Inhibition of carbon fixation after 24 hours. Negative values indicate stimulation.]
Scenedesmus quadricauda, green algae	3% inhibition at 2.8 mg/L	
Selenastrum capricornutum, green algae	18% inhibition at 2.8 mg/L	
Microcystis aeruginosa, cyanobacter	-41% inhibition at 2.8 mg/L	
Microcystis aeruginosa, cyanobacter	16% inhibition at 2.8 mg/L	
Oscillatoria sp., cyanobacter	-12% inhibition at 2.8 mg/L	
Pseudoanabaena sp, cyanobacter	12% inhibition at 2.8 mg/L	
Anabaena inaequalis, cyanobacter	11% inhibition at 2.8 mg/L	
Aphanizomenon flos-aquae, cyanobacter	74% inhibition at 2.8 mg/L	
Lemna minor, duckweed	no inhibition at 2.8 mg/L over 5 days	
	Roundup	
Mixed colonies of periphytic aglae	4 hour EC_{50}s = 35.4-44.4 mg/L for inhibition of photosynthesis. NOEC = 0.89 mg/L.	Goldsborough and Brown 1989