SERA TR-052-22-03b

Glyphosate

Human Health and Ecological Risk Assessment

FINAL REPORT

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ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
AChE	acetylcholinesterase
ADD	attention-deficit disorder
ADHD	attention-deficit hyperactivity disorder
AEL	adverse-effect level
a.e.	acid equivalent
a.i.	active ingredient
ae/form	acid equivalent per formulation (used only in equations)
ae/surf	acid equivalent per surfactant (used only in equations)
ai/form	active ingredient per formulation (used only in equations)
ai/surf	active ingredient per surfactant (used only in equations)
ALP	alkaline phosphatase
ALT	alanine aminotransferase
Am	ammonium (salt)
AMPA	amino methyl phosphonic acid
APHIS	Animal and Plant Health Inspection Service
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
BNMN	binucleated cells with micronuclei
bw	body weight
CBI	confidential business information
ChE	cholinesterase
cm	centimeter
CNS	central nervous system
DAA	days after application
DAT	days after treatment
DER	data evaluation record
d.f.	degrees of freedom
DMA	dimethyl amine (salt)
EC_x	concentration causing X% inhibition of a process
EC_{25}	concentration causing 25% inhibition of a process
EC_{50}	concentration causing 50% inhibition of a process
EFED	Environmental Fate and Effects Division (U.S. EPA/OPP)
ExToxNet	Extension Toxicology Network
F	female
FH	Forest Health
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FQPA	Food Quality Protection Act
g	gram
GLP	Good Laboratory Practices
ha	hectare
HED	Health Effects Division (U.S. EPA/OPP)
HI	hazard index
HQ	hazard quotient

IARC	International Agency for Research on Cancer
IC_{50}	concentration causing 50% inhibition
IPA	isopropyl amine (salt)
IRIS	Integrated Risk Information System
K	potassium (salt)
k_a	absorption coefficient
k_e	elimination coefficient
kg	kilogram
$K_{o/c}$	organic carbon partition coefficient
$K_{o/w}$	octanol-water partition coefficient
K_p	skin permeability coefficient
L	liter
lb	pound
LC_{50}	lethal concentration, 50% kill
LD_{50}	lethal dose, 50% kill
LOAEL	lowest-observed-adverse-effect level
LOC	level of concern
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
mM	millimole
mPa	millipascal, (0.001 Pa)
MOS	margin of safety
MRID	Master Record Identification Number
MSDS	material safety data sheet
MSMA	monosodium methanearsonate
MW	molecular weight
NAWQA	USGS National Water Quality Assessment
NCI	National Cancer Institute
NCOD	National Drinking Water Contaminant Occurrence Database
NHL	non-Hodgkin lymphoma
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NOS	not otherwise specified
NRC	National Research Council
NTP	National Toxicology Program
OM	organic matter
OPP	Office of Pesticide Programs
OPPTS	Office of Pesticide Planning and Toxic Substances
OSHA	Occupational Safety and Health Administration
Pa	Pascal
PBPK	physiologically-based pharmacokinetic
POEA	polyoxyethyleneamine (surfactant)

ppm	parts per million
RBC	red blood cells
RED	re-registration eligibility decision
RfD	reference dose
S.A.	South American
SERA	Syracuse Environmental Research Associates
TEP	typical end-use product
T.G.I.A.	Technical grade active ingredient
TIPA	Triisopropanolamine
TRED	Tolerance Reassessment Eligibility Decision
UF	uncertainty factor
U.S.	United States
USDA	U.S. Department of Agriculture
U.S. EPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
WHO	World Health Organization

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 mercury	760
centigrade	Fahrenheit	1.8°C+32
centimeters	inches	0.3937
cubic meters (m^3)	liters (L)	1,000
Fahrenheit	centigrade	0.556°F-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)	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
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 acre (lb/acre)	mg/square meter (mg/m^2)	112.1
pounds per acre (lb/acre)	µg/square centimeter ($\mu g/cm^2$)	11.21
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

General Considerations

Glyphosate is a herbicide used in Forest Service programs primarily in conifer release, site preparation, and noxious weed control. The Forest Service identified more than 50 formulations which are explicitly considered in the current risk assessment. This risk assessment on glyphosate is dominated by three considerations: the extensive literature available on glyphosate, the availability of numerous glyphosate formulations, and the use of surfactants either as components in glyphosate formulations or as adjuvants added to glyphosate formulations prior to application. There are obvious, and in many cases substantial, differences among the toxicities of technical grade glyphosate, glyphosate formulations that do not contain a surfactant, and some glyphosate formulations that contain polyoxyethyleneamine (POEA) surfactants. While the available information does not permit formulation-specific toxicity values, an attempt is made to discriminate between less toxic and more toxic formulations, when possible. A general classification of formulations is given in Table 5 of this risk assessment. Formulations identified as *Low Toxicity* in Table 5 can be regarded as *less toxic formulations*. Other formulations should be regarded as *more toxic formulations,* unless data on the formulation are available to justify a different classification. Additional formulations may become available subsequent to the release of this risk assessment, which may require the use of judgment to classify new formulations as more or less toxic. In general, it would be prudent to classify any formulation that contains a POEA surfactant as more toxic, except when there is a compelling reason to do otherwise. If the presence and/or toxicity of the surfactants in the formulation cannot be determined, it would be prudent to classify the formulation as more toxic.

Human Health

The toxicity data on technical grade glyphosate are extensive, including both a standard set of toxicity studies submitted to the U.S. EPA/OPP in support of the registration of glyphosate as well as a robust open literature consisting of numerous and diverse *in vivo* and *in vitro* studies. As with any complex collection of studies, the studies on technical grade glyphosate may be subject to differing interpretations. The preponderance of the available data, however, clearly indicates that the mammalian toxicity of glyphosate is low, and very few specific hazards can be identified. Doses of technical grade glyphosate that exceed around 300 mg/kg bw may cause signs of toxicity, including decreased body weight gain, changes in certain biochemical parameters in blood as well as tissues, and inhibition of some enzymes (i.e., P450) involved in the metabolism of both endogenous and exogenous compounds. At doses from about 1000 to 5000 mg/kg bw, glyphosate can cause death. The most sensitive endpoint for glyphosate—i.e., the adverse effect occurring at the lowest dose—involves developmental effects; accordingly, the EPA-derived RfDs for glyphosate are based on developmental effects. These adverse effects relate primarily to delayed development which occurs only at doses causing signs of maternal toxicity. There is no indication that technical grade glyphosate causes birth defects.

The hazard identification for glyphosate formulations is much less clear. In most Forest Service pesticide risk assessments, the active ingredient is the agent of primary concern, and consideration of other ingredients in the formulations is limited to a brief discussion in Section 3.1.14 (Adjuvants and Other Ingredients). In the current Forest Service risk assessment, however, the way in which the formulation ingredients other than glyphosate are handled is

much different. Many glyphosate formulations include surfactants, and the toxicity of these surfactants is of equal or greater concern to the risk assessment than is the toxicity of technical grade glyphosate. Consequently, as justified by the available data, the hazard identification is subdivided into sections that address the toxicity of technical grade glyphosate, the toxicity of glyphosate formulations, and/or the toxicity of the surfactants.

Because surfactants appear to be agents of concern, a central issue in the current Forest Service risk assessment involves differences in surfactants among the glyphosate formulations used by the Forest Service (Table 2) as well as glyphosate formulations for which toxicity data are available in the open literature. As detailed in Section 3.1.14, the term *POEA* (an acronym for polyoxyethyleneamine) is commonly used to designate surfactants used in some glyphosate formulations. POEA, however, is not a single surfactant. In addition, because the constituents in the surfactants are considered propriety (trade secrets or Confidential Business Information), detailed information about the constituents is not publically available. The surfactants in many glyphosate formulations used by the Forest Service appear to consist primarily of polyethoxylated tallow amines. Nonetheless, each surfactant can be characterized as a complex mixture. In addition, the POEA surfactant used in one glyphosate formulation may be different from the POEA surfactant used in other glyphosate formulations, even among formulations provided by the same manufacturer. Thus, it is not clear whether the toxicity studies conducted on one POEA surfactant are applicable to all or any of the other glyphosate formulations currently in use.

The difference or potential difference in the composition of surfactants used in various glyphosate formulations has a practical impact on the hazard identification for the current Forest Service risk assessment. Several studies conducted outside of the United States on glyphosate formulations which are not used domestically report adverse effects of concern, including potential effects on endocrine function in rats and signs of genotoxicity in humans. In the absence of comparable studies on glyphosate formulations manufactured and used in the United States, the extent to which this information is relevant to U.S. formulations of glyphosate is unclear.

Two studies conducted in South America (Bolognesi et al. 2009; Paz-y-Mino et al. 2007) suggest that applications of glyphosate formulations may be associated with signs of chromosomal damage in human populations (Section 3.1.10.1.2). The study by Paz-y-Mino et al. (2007) has several limitations; nonetheless, the more detailed study by Bolognesi et al. (2009) suggests a temporal association between glyphosate exposure and chromosomal damage. Both of these studies involved application rates which, when expressed in units of glyphosate, are comparable to those used in Forest Service programs—i.e., about 1-4 lb a.e./acre. Neither study, however, involved glyphosate formulations used in the United States and the relevance of these studies to U.S. formulations of glyphosate is questionable.

Developmental toxicity, endocrine function, and genotoxicity are endpoints of obvious concern in any risk assessment. Based on the studies using formulations from outside the United States, there is concern that glyphosate formulations may have an impact on these endpoints and that some of these effects could be seen under typical application conditions in the United States. In the absence of comparable studies on U.S. formulations, however, is it not clear whether the

studies on glyphosate formulations used outside the United States are applicable to risks posed by U.S. formulations of glyphosate.

The quantitative risk characterization for both human health and ecological effects is expressed as the hazard quotient (HQ). For both general and accidental exposures of humans, the HQ is calculated as the estimated dose in units of mg/kg bw for acute exposures or units of mg/kg bw/day for longer-term exposures divided by the RfD of 2 mg/kg/day (U.S. EPA/OPP 1993a,b). As discussed in Section 3.3.2, the RfD is derived from a developmental study and applied to both acute and longer-term exposures. The exposure assessments on which the HQs are based are discussed in Section 3.2.2, with details provided in the EXCEL workbooks that accompany this risk assessment—i.e., Attachment 1a for backpack foliar applications, Attachment 1b for ground broadcast foliar applications, Attachment 1c for aerial foliar applications, and Attachment 2 for aquatic applications.

For both workers and members of the general public, the RfD of 2 mg a.e./kg bw/day is used to characterize risks associated with acute and longer-term exposure levels. As discussed in the exposure assessment (Section 3.2.2), all exposure assessments are based on the unit application rate of 1 lb a.e./acre. A quantitative summary of the risk characterization for workers is presented in Table 19. Quantitative summaries of risks to members of the general public are presented in Table 20 for terrestrial applications and Table 21 for aquatic applications. Because the HQs are based on the RfD, an HQ of 1 or less suggests that exposures are below the level of concern. HQs greater than 1 indicate that the exposure exceeds the level of concern.

Based on the HQ method, concern for workers is minimal. At the highest labeled application rate for terrestrial applications, about 8 lbs a.e./acre, the highest HQ is 0.6, the upper bound of the HQ for workers involved in ground broadcast applications.

For members of the general public, the only non-accidental exposure scenario of concern is for acute exposure involving the consumption of contaminated vegetation shortly after glyphosate is applied. For this exposure scenario, the HQ reaches a level of concern (HQ=1) at an application rate of about 1.4 lbs a.e./acre. At the maximum labeled application rate of about 8 lbs a.e./acre, the resulting HQ value would be about 5.6 with a corresponding dose of about 10.8 mg/kg bw.

Apart from the standard HQ method, there are additional concerns, including a report of systemic toxicity in California workers involved in glyphosate applications. In addition and as also noted above, two studies indicate a potential for chromosomal damage in South American populations exposed to glyphosate formulations containing surfactants applied aerially at rates within the range of those used in Forest Service programs. While these studies are not used quantitatively in the current Forest Service risk assessment and the studies suggest a potential for health effects that are not identified or confirmed using the standard HQ method.

Ecological Effects

The toxicity of technical grade glyphosate is relatively well characterized for both terrestrial and aquatic species. In addition, the toxicity of the original Roundup formulation as well as Rodeo is relatively well characterized. It is more difficult, however, to clearly define the hazards and assess risks associated with other glyphosate formulations.

As is the case with most Forest Service pesticide risk assessments, the data used to assess the risk to mammalian wildlife as well as human exposure to glyphosate and glyphosate formulations is largely the same. Thus, Section 4.1.2.1 focuses primarily on studies useful for assessing differences in pesticide sensitivity among various species of mammalian wildlife. The dose-response assessment for mammalian wildlife (Section 4.3.2.1) presents a fuller discussion of concerns for reproductive toxicity raised by the recent Dallegrave et al. (2007) study conducted with a South American formulation of Roundup. In some respects, however, it is some early, detailed field studies on mammalian wildlife which have a substantial impact on the hazard identification for human health and mammalian wildlife. These early studies do not report adverse reproductive effects in populations of small mammals following applications of U.S. formulations of Roundup (Ritchie et al. 1987; Sullivan 1990).

The hazard identification subsections for other groups of ecological receptors is structured in a manner similar to the hazard identification for human health effects in that distinctions between technical grade glyphosate and glyphosate formulations are maintained as clearly as possible. For birds, terrestrial-phase amphibians, and terrestrial invertebrates, relatively complete sets of studies are available on both technical grade glyphosate and some U.S. formulations. Some studies using formulations from South America suggest adverse effects on reproduction in birds, amphibians, and terrestrial invertebrates. The types of studies conducted on the South American formulations have not been conducted on formulations that will be used in Forest Service programs. Consequently, the applicability of the data on South American formulations to the current Forest Service risk assessment is difficult to assess because of the proprietary nature of the data on the surfactants used in different formulations of glyphosate.

Glyphosate is an effective herbicide, and the toxicity of glyphosate and glyphosate formulations to terrestrial plants is well characterized. In addition, there is a relatively detailed literature regarding the effects of glyphosate and glyphosate formulations to terrestrial microorganisms. While the mechanism of action of glyphosate in plants is also relevant to microorganisms, there is very little indication that terrestrial microorganisms will be adversely affected by glyphosate.

A large and detailed body of literature is available on the effects of glyphosate and some glyphosate formulations to aquatic organisms. Summaries of the available studies are provided in the following tables: Table 22 (fish), Table 25 (aquatic-phase amphibians, Table 26 (aquatic invertebrates), Table 27 (algae) and Table 28 (aquatic macrophytes). The discussions of each of these groups of aquatic organisms in the hazard identification are preceded by an overview of the available literature. The toxicity of the original Roundup and similar formulations containing POEA surfactants is far greater than the toxicity of technical grade glyphosate, Rodeo, or other formulations that do not contain surfactants. Among the formulations with surfactants, several non-U.S. formulations appear to be less toxic than some U.S. formulations of Roundup and Roundup-like formulations. Although data suggest that certain U.S. formulations of glyphosate that contain surfactants may be less toxic than others, these toxicity-related differences are not clearly documented in the EPA risk assessment on glyphosate (U.S. EPA/OPP 2008a) or in the open literature. As discussed in Section 2, data from Material Safety Data Sheets (MSDS) are neither well documented nor sufficiently clear to be used directly in this risk assessment.

Fish, amphibians, and most aquatic invertebrates appear to be about equally sensitive to the toxicity of technical grade glyphosate and glyphosate formulations, and any differences in response to exposure are more likely attributable to experimental conditions, particularly pH, than to species differences. The sensitivity of algae to glyphosate and glyphosate formulations varies among species; however, the data regarding differences among species of aquatic macrophytes are less complete. Nonetheless, there is evidence that *Lemna* species are much more sensitive than eelgrass to glyphosate acid, which suggests that there may be substantial species differences in the sensitivity of macrophytes to glyphosate formulations. Most studies on aquatic microorganisms seem consistent with studies on terrestrial microorganisms, indicating that aquatic microorganisms are not very sensitive to glyphosate. Some recent studies using changes in the composition of ribosomal RNA and DNA suggest that effects on aquatic microorganisms may occur at very low concentrations. While this may be the case, the functional significance of these effects is not apparent.

Terrestrial plants comprise the only group of nontarget species for which no distinction is made between more and less toxic formulations. Glyphosate is an effective postemergence herbicide. Foliar applications of glyphosate with an effective surfactant (POEA or otherwise) may pose a risk to terrestrial plants. The direct spray of a nontarget plant at an effective application rate is likely to kill or seriously injure most plants. Nonetheless, substantial differences in sensitivity to glyphosate are apparent among different species of plants. For sensitive species, offsite drift of glyphosate can pose a risk. The nature of the risk will depend on the application rate, application method, and site-specific conditions that can impact the extent of drift.

For groups of organisms other than terrestrial plants, risks associated with the use of more and less toxic formulations differ. Based on pesticide use reports from the Forest Service, typical application rates for glyphosate in Forest Service programs are in the range of 0.5 to 4 lbs a.e./acre. Applications of more toxic formulations of glyphosate at rates of up to 2.5-3 lb a.e./acre do not appear to present any apparent risks to terrestrial animals, based on upper bound estimates of exposures. At application rates above 2.5 lb a.e./acre, risks to mammals cannot be ruled out based on upper bound estimates of exposure, but no risks are apparent based on central estimates of exposure. At application rates above about 3.3 lb a.e./acre, the HQs for birds modestly exceed the level of concern, but there is no basis for asserting that overt toxic effects in birds are likely. Risks to terrestrial insects are a greater concern in dietary exposures than direct spray. Based on upper bound estimates of dietary exposure at the maximum application rate of 8 lb a.e./acre, the HQs for terrestrial insects can reach a value of 10. Concern for terrestrial invertebrates is enhanced by two toxicity studies using South American formulations of glyphosate which noted adverse effects on reproduction and development. While most field studies suggest that effects on terrestrial invertebrates are due to secondary effects on vegetation, the field studies do not directly contradict the South American toxicity studies or the HQs. The less toxic formulations of glyphosate do not appear to present any risks to terrestrial organisms other than terrestrial plants.

For the more toxic formulations, the risk characterization for aquatic organisms suggests that amphibians are the group at greatest risk both in terms of sensitivity and severity of effects. At an application rate of 1 lb a.e./acre, the upper bound HQ for sensitive species of amphibians is 2. The corresponding HQs for sensitive species in other groups of aquatic organisms are 1.7 for

fish, 1.1 for invertebrates, 1.0 for algae and aquatic macrophytes. Concern for amphibians is enhanced by the study by Howe et al. (2004) which indicates that two formulations of Roundup as well as the POEA surfactant used in some of the more toxic formulations of glyphosate are associated with the development of intersex gonads. The HQs for aquatic species will increase linearly with application rate. Because the upper bound HQs for most groups of aquatic organisms exceeds or reaches the level of concern at the relatively low application rate of 1 lb a.e./acre, care should be exercised when applying more toxic formulations of glyphosate near surface water.

Unlike the case with more toxic formulations, risks to amphibians and aquatic invertebrates appear to be insubstantial for the less toxic formulations. Algae appear to be the group of nontarget aquatic organisms that are most sensitive to the less toxic formulations. At an application rate of 1 lb a.e./acre, the upper bound of the HQ for sensitive species of algae is 0.8. At the maximum aquatic application rate of 3.75 lb a.e./acre, the corresponding HQ is 3. At this upper bound HQ, some inhibition of growth might be observed, but the extent of inhibition could be minor. Risks to fish cannot be ruled out based on standard and conservative assumptions and methods for applications of less toxic formulations of glyphosate at rates in excess of about 2.5 lb a.e./acre (acute effects). It seems most likely, however, that adverse effects would be observed in stressed populations of fish and less likely that effects would be noted in otherwise healthy populations of fish.

The label directions for the less toxic formulations of glyphosate state that a surfactant should be added to the formulations prior to application. Some surfactants are virtually nontoxic and are not likely to impact the toxicity of glyphosate. The use of a nontoxic surfactant would have no substantial impact on the risk characterization. Based on the available toxicity data in fish and aquatic invertebrates, however, some other surfactants which might be used with the less toxic formulations of glyphosate could pose a much greater risk than the glyphosate formulation itself. An approach to assessing risks associated with toxic surfactants is illustrated for fish (Section 4.4.3.1.3) and aquatic invertebrates (Section 4.4.3.3.3). For a fixed concentration of the surfactant in a field solution, reducing the application volume will diminish the impact of the surfactant.

1. INTRODUCTION

1.1. Chemical Specific Considerations

This document provides risk assessments for human health effects and ecological effects to support an assessment of the environmental consequences of using glyphosate in Forest Service vegetation management programs. This risk assessment is an update to previous USDA Forest Service risk assessments of glyphosate (SERA 1996, 2003).

The development of this updated risk assessment on glyphosate is dominated by three considerations: the extensive literature available on glyphosate, the availability of numerous glyphosate formulations, and the use of surfactants either as components in glyphosate formulations or as adjuvants which are added to glyphosate formulations prior to application.

There are numerous unpublished studies which registrants submitted to U.S. EPA/OPP in support of the registration of glyphosate. Many of the older registrant-submitted studies are summarized by U.S. EPA/OPP (U.S. EPA/OPP 1993a,b,c) in the initial re-registration of glyphosate as well as in a more recent risk assessment for the California Red-legged frog (U.S. EPA/OPP 2008a). The U.S. EPA has initiated a registration review of glyphosate which involves a complete review of all existing submissions as well as the completion of new registrant studies required by U.S. EPA (U.S. EPA/OPP 2009a). Since the EPA registration review of glyphosate is not scheduled to be completed until 2015, it is unlikely that any new registrant studies on glyphosate will be available for the conduct of the current Forest Service risk assessment.

In the previous Forest Service risk assessment (SERA 2003), 5829 submissions on glyphosate and glyphosate formulations were identified, and 185 submissions – i.e., full copies of the studies submitted to the U.S. EPA – were obtained from the U.S. EPA/OPP. These studies are generally classified as Confidential Business Information (CBI).

The U.S. EPA/OPP no longer releases registrant-submitted studies for non-EPA reviews and risk assessments. Certain studies that were available in the preparation of the 2003 Forest Service risk assessment are used as summarized in SERA (2003). These studies are identified in the reference list for the current Forest Service risk assessment (Section 5) as MRID03. In the preparation of this risk assessment, an updated bibliography of all the registrant-submitted studies on glyphosate was obtained from the U.S. EPA through a Freedom of Information Act (FOIA) request, HQ-FOI-00787-10. A listing of these studies is included as Supplement1 to the current Forest Service risk assessment. This listing is used in several places in the current risk assessment to clarify information on various studies submitted to the U.S. EPA.

The Forest Service is aware of and sensitive to concerns with risk assessments that are based substantially on studies submitted to the U.S. EPA in support of product registration. The general concern can be expressed as follows:

If the study is paid for and/or conducted by the registrant, the study may be designed and/or conducted and/or reported in a manner that will obscure any adverse effects that the compound may have.

This type of concern is largely without foundation. While any study (published or unpublished) can be falsified, concerns with the design, conduct and reporting of studies that are submitted to the U.S. EPA for pesticide registration are minor. The design of studies that are submitted for pesticide registration is based on strict guidelines for both the conduct and reporting of studies. These guidelines are developed by the U.S. EPA and not by the registrants. Full copies of the guidelines for these studies are available at http://www.epa.gov/opptsfrs/home/guidelin.htm. All studies are conducted under Good Laboratory Practices (GLPs). GLPs are an elaborate set of procedures that involve documentation and independent quality control and quality assurance that substantially exceed the levels typically seen in open literature publications. Lastly, each study that is submitted to the U.S. EPA is reviewed by the U.S. EPA for adherence to the relevant study guidelines. These reviews most often take the form of Data Evaluation Records (DERs). While the nature and complexity of DERs will vary with the nature and complexity of the differing studies, each DER involves an independent assessment of the study to ensure that the EPA Guidelines are followed. In addition, each DER undergoes internal review (and sometimes several layers of review).

There are real and legitimate concerns with risk assessments that based solely on registrant submitted studies but data quality and data integrity are not substantial concerns. The major limitation of risk assessments that are based solely on registrant submitted studies involve the nature and diversity of the available studies. The studies required by the U.S. EPA are based on a relatively narrow set of studies in a relatively small subset of species following standardized protocols.

For some pesticides, including glyphosate, a very large base of published studies are available, many of which are generated by academics who have a fundamental interest in understanding both the toxicology of a compound as well as underlying biological principles (e.g., physiology, biochemistry, ecology, etc.). Such studies tend to be non-standard but highly creative and can substantially contribute to or even form the basis of a risk assessment.

As discussed in the previous Forest Service risk assessment (SERA 2003), the published literature on glyphosate is substantial and complex. In the course of conducting this updated risk assessment, standard literature searches on TOXLINE and AGRICOLA were used to identify newly published literature, which includes more than 1500 references for the period from 2002 to 2010. As with the previous Forest Service risk assessment of glyphosate, no attempt is made to consider all of the new literature; instead, the focus of this updated risk assessment is the literature which specifically addresses the potential risks of glyphosate to humans and nontarget species. For the most part, literature dealing with the efficacy of glyphosate is not addressed. Other sources of relevant literature were identified through recent reviews and risk assessments in the open literature (e.g., Atkinson 1985; Bradberry et al. 2004; Brain and Solomon 2009; Burgat et al. 1998; Chen et al. 2009; Cox 1998a,b; Dost 2008; Duke and Powles 2008; FAO/WHO 1986; Giesy et al. 2000; Kegley et al. 2008; McLaren/Hart 1995; Neary et al. 1993; Pan et al. 2003; Relyea et al. 2005; Schuette 1998; Siemering et al. 2008; Smith and Oehme

1992; Solomon and Thompson 2003; Solomon et al. 2005, 2007, 2009; Vereecken 2005; Watts 2010; WHO 1994; Williams et al. 2000). Generally, these reviews are used only to identify published studies to ensure adequate coverage of the literature.

Some of the reviews and related documents, however, had access to unpublished and very relevant literature. This is particularly true for the analysis by McLaren/Hart (1995) and the review by Williams et al. (2000). The analysis by McLaren/Hart (1995) is a document submitted to New York State on aquatic uses of glyphosate and this document was funded jointly by Monsanto and Dow AgroSciences. These companies provided the document authors with many unpublished studies. Similarly, the review by Williams et al. (2000) and Giesy et al. (2000) includes summaries of a several unpublished studies from Monsanto. In these cases, information was taken from the summary documents and used in the current Forest Service risk assessment. Particularly for important studies, the use of this secondary information is noted in the text for the sake of transparency. Studies cited in the text for which information was obtained from a secondary source are explicitly identified in the bibliography (Section 5) by a Sec (for secondary source) designation enclosed in braces at the end of the reference.

Of the very large number of commercial formulations of glyphosate that are available, only those formulations specifically identified by the Forest Service are covered in Section 2.2 of this risk assessment. It should be noted that several of the formulations discussed in Section 2.2 are no longer commercially available. These formulations are included in Section 2 simply because these formulations were identified by at least one Forest Service region as being in use. This may reflect the use of existing stocks of herbicides at Forest Service facilities.

The nature of glyphosate formulations is dynamic in that new formulations are developed and other formulations are renamed or discontinued. To the extent possible, the current risk assessment attempts to structure the analysis so that this risk assessment can be used to assess any formulation which might be used in Forest Service programs.

Issues associated with the various formulations of glyphosate are closely related to the use of surfactants. Surfactants are a class of chemicals, typically chemical mixtures, which reduce the surface tension of liquids. In very general terms, soap may be considered a common type of surfactant (e.g., Kosswig 1994). As detailed further in Section 2.2, many glyphosate formulations contain surfactants and most other glyphosate formulations require the use of surfactants. In the past, surfactants were referred to as *inerts*, which is to say they are not considered to be direct acting herbicides. For glyphosate, surfactants are typically regarded as adjuvants, in that the surfactant enhances the efficacy of glyphosate. In the context of the current Forest Service risk assessment, surfactants are important because they may be toxic, at least at high concentrations; moreover, surfactants may also increase the toxicity of glyphosate to both target plants as well as nontarget species. Surfactants are addressed generally in Section 3.1.14 of the current Forest Service risk assessment. As discussed further in Section 4.3, surfactants are particularly important in terms of the toxicity of glyphosate to aquatic organisms.

1.2. General Considerations

This document has four chapters, including 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, including an

identification of the hazards, an assessment of potential exposure to this compound, an assessment of the dose-response relationships, and a characterization of the risks associated with plausible levels of exposure.

This is a technical support document and it addresses some specialized technical areas. Nevertheless an effort was 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, methods, and terms common to all parts of the risk assessment are described in plain language in a separate document (SERA 2007a). The human health and ecological risk assessments presented in this document are not, and are not intended to be, comprehensive summaries of all of the available information. The information presented in the appendices and the discussions in chapters 2, 3, and 4 of the risk assessment are intended to be detailed enough to support a review of the risk analyses.

As discussed in Section 1.1, the current Forest Service risk assessment is an update to previous risk assessments on glyphosate (SERA 1996, 2003). At some point in the future, the Forest Service will update this risk assessment again and welcomes input from the general public on the selection of studies included in the risk assessment. This input is helpful, however, only if recommendations for including additional studies specify why and/or how the new or not previously included information would be likely to alter the conclusions reached in the risk assessments.

As with all Forest Service risk assessments, almost no risk estimates presented in this document are given as single numbers. Usually, risk is expressed as a central estimate and a range, which is sometimes quite large. Because of the need to encompass many different types of exposure as well as the need to express the uncertainties in the assessment, this risk assessment involves numerous calculations, most of which are relatively simple. They are included in the body of the document.

Some of the calculations, however, are cumbersome. For those calculations, EXCEL workbooks (sets of EXCEL worksheets) are included as attachments to this risk assessment. The worksheets provide the detail for the estimates cited in the body of the document. Documentation for the use of these workbooks is presented in SERA (2009a).

The EXCEL workbooks are an integral part of the risk assessment. The worksheets contained in these workbooks are designed to isolate the large number of calculations from the risk assessment narrative. In general, all calculations of exposure scenarios and quantitative risk characterizations (i.e., hazard quotients) are derived and contained in the worksheets. The rationale for the calculations as well as the interpretation of the hazard quotients are contained in this risk assessment document.

2. PROGRAMS DESCRIPTION

2.1. Overview

Glyphosate is an herbicide used in Forest Service programs primarily in conifer release, site preparation, and noxious weed control. The Forest Service identified more than 50 formulations which are explicitly considered in the current risk assessment. This risk assessment, however, is structured to consider any current or future glyphosate formulation registered for applications used in Forest Service programs.

The formulations of glyphosate identified by the Forest Service contain the ammonium, dimethylamine, isopropylamine, or potassium salts of glyphosate. Some formulations contain only one of these salts of glyphosate as an aqueous solution. Other formulations contain surfactants. The product labels for many formulations of glyphosate that do not contain a surfactant indicate that a surfactant must be added to the field solution prior to application. Some formulations that contain a surfactant indicate that other nonionic surfactants may be added to the field solution prior to application. In addition to surfactants, other additives to field solutions of glyphosate include ammonium sulfate, dyes, and drift reducing agents.

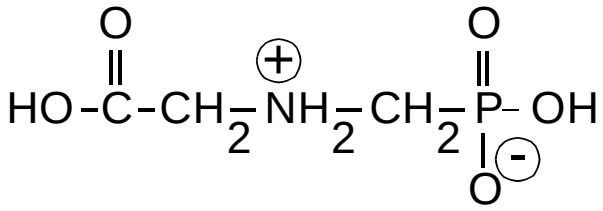
The most common application method for glyphosate in Forest Service programs is backpack-applied directed foliar sprays. Other application methods used occasionally include broadcast foliar ground applications, cut stem applications, and direct application to emergent aquatic vegetation. Some glyphosate formulations are registered for aerial application. The Forest Service avoids aerial applications when possible; nonetheless, this application method is considered in the current risk assessment.

Based on the most recent Forest Service use reports, the typical glyphosate application rate is about 2 lb a.e./acre, with most terrestrial applications using rates ranging from 0.5 to 4 lbs a.e./acre. The agricultural use of glyphosate in the United States is greater than Forest Service use by a factor of over 2900. Thus, there is no reason to believe that Forest Service programs will contribute substantially to general concentrations of glyphosate nationally.

2.2. Chemical Description and Commercial Formulations

2.2.1. Chemical Description

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



Selected chemical and physical properties of glyphosate are summarized in Table 1.

At ambient temperatures, glyphosate is a white crystalline substance. 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 *zwitterions*. In aqueous solutions, the hydrogen atoms of the carboxylic acid (**COOH**) and phosphonate (C-$\mathbf{PO_2H_2}$) groups may be associated (e.g., **-COOH**) or dissociated (e.g., $\mathbf{-COO^-}$ + $\mathbf{H^+}$) depending on the pH of the solution.

Glyphosate is a broad-spectrum, non-selective, post-emergence systemic herbicide developed by Monsanto (Franz 1985; Franz et al. 1997). As discussed further in Section 4.1.2.5, gyphosate inhibits the shikimic acid pathway in plants, which is involved in the production of essential aromatic amino acids. 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. Glyphosate is used in Forest Service programs primarily in conifer release, noxious weed control, and site preparation.

2.2.2. Commercial Formulations

Glyphosate was originally registered by the U.S. EPA in June of 1986 to Monsanto (U.S. EPA/OPP 1993a). Because of patent restrictions, all of the commercial formulations of glyphosate were produced only by Monsanto and included Accord, Rodeo, Roundup, and Roundup Pro (SERA 1996). By 2003, the year of the last Forest Service risk assessment (SERA 2003), glyphosate was no longer protected by patent, and 35 commercial formulations of glyphosate were registered for forestry applications, all of which contained the isopropylamine salt of glyphosate. Since 2003, the number of commercial formulations has increased substantially. Currently, 46 commercial formulations are listed by Greenbook (www.Greenbook.net), and the PAN pesticide database (http://www.pesticideinfo.org) lists more than 700 active formulations of glyphosate.

The commercial formulations of glyphosate identified by the Forest Service are summarized in Table 2. This list contains 52 formulations of glyphosate formulations that the Forest Service designated as formulations which should be considered in the current Forest Service risk assessment.

The issue of specifically designating formulations is complicated by different designation codes for glyphosate formulations. For example, as indicated in Table 2, Roundup Pro has an EPA Registration Number of 524-475. This formulation, however, may be marketed with any of the following names: Roundup Ultra Herbicide; Roundup Ultra RT Herbicide; Roundup Pro Herbicide; Roundup Original II CA; MON 77360 Herbicide; Roundup W Herbicide; Gly 41 Herbicide. Similarly, EPA Reg. No. 62719-517 includes Accord XRT, Durango, and Glyphomax XRT and EPA Reg. No. 62719-556 is a formulation code for Accord XRT II, Duramax, and Durango DMA (Fonseca 2010a,b).

In addition to registration numbers assigned by the U.S. EPA, product codes may be designated by the companies that provide the different formulations. These internal company codes, rather than formulation names or EPA registration numbers, may be used in study titles that are submitted to the U.S. EPA. Examples of these internal codes are included in the appendices that

accompany the current Forest Service risk assessment. It is not always possible to associate internal product codes with the corresponding formulations and no single compendium of the product codes for glyphosate formulations has been encountered. A summary of the internal product codes for glyphosate formulations that have been identified to date are included in Table 3 which gives the company, internal code, U.S. EPA registration number, and formulations. As noted by Fonseca (2010b), formulation codes may be misleading because *… codes can change to reflect even minor changes of the formulation. In other words, the formulation of the product originally registered could have now a different formulation code*. Nonetheless, the use of product codes are sometimes the only method of associating a specific study submitted to the U.S. EPA with a specific formulation or group of formulations.

As with the 2003 risk assessment of glyphosate, most of the commercial formulations contain the isopropylamine salt of glyphosate. Some formulations, however, contain the ammonium salt, dimethylamine salt, or potassium salt of glyphosate, and one formulation, Nufarm Credit Extra, contains a mixture of the ammonium and potassium salts of glyphosate. For brevity, the following abbreviations for the salts of glyphosate are used in the current Forest Service risk assessment:

Am:	ammonium salt
DMA:	dimethylamine salt
IPA:	isopropylamine salt
K:	potassium salt

As discussed further in Section 3.1.4.2 (Other Ingredients), the uses of various salts in glyphosate formulations do not have a substantial impact on the risk assessment.

A more important distinction among the various formulations of glyphosate concerns surfactants. U.S. EPA/OPPTS (2003, p. 5-2) encourages but does not require expanded inert statements on product labels which specifically identify the inert ingredients in the product. For the most part, however, use of surfactants is not clearly designated in the product labels for glyphosate.

As summarized in Table 2 and discussed further in Section 2.2.3, several liquid formulations of glyphosate consist primarily of only a glyphosate salt in water (e.g., Accord, AquaNeat, and Rodeo). Other liquid formulations of glyphosate contain a surfactant (e.g., Credit Systemic Extra, Pronto, Glyfos X-TRA, Honcho, and various Roundup formulations). What is more, the product labels and MSDSs for some glyphosate formulations do not indicate whether or not surfactants are present in the formulation. As in the previous Forest Service risk assessments, surfactants are a major issue in the risk assessment of glyphosate. As discussed in Section 3.1.14.1 (Adjuvants), surfactants not only enhance the efficacy of glyphosate but also may enhance the toxicity of glyphosate. Surfactants are also a concern in the ecological risk assessment, particularly for aquatic species (Section 4.1.3).

Relative to the large number of studies on the toxicity of glyphosate, little information is available in the open literature on the identity or toxicity of the surfactants in glyphosate formulations. In an early publication, Wan et al. (1989) indicated that the original Roundup formulation from Monsanto contained 15% MON 0818, the Monsanto code for a 75% tallow

amine surfactant. As summarized in Table 2, Dow AgroSciences has indicated the presence of surfactants in some formulations (Fonseca 2010b). In addition, Nufarm discloses the concentration of surfactants in the Material Safety Data Sheets (MSDSs) for Razor (8%) and Razor Pro (14%), and Monsanto discloses the concentration of surfactants in Roundup Pro Concentrate (13%) and Roundup UltraDry (25%).

The specific identity of the surfactants, other inerts, contaminants, and impurities has been disclosed to the U.S. EPA as part of the registration process. This information includes information on the manufacturing process, identity and quantity of the inerts/impurities in the formulations as well as additional information on the composition of some inerts, many of which are complex mixtures. This information is not disclosed publically because it is classified as *trade secret* under Sections 10(f) and 12(a)(2)(D) of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA).

Even though information on surfactants is disclosed to the U.S. EPA, uncertainties in the identity of surfactants are apparent in EPA analyses of glyphosate. For example, the U.S. EPA/OPP risk assessment on the California red-legged frog notes:

> *Also stated previously, the form of glyphosate (acid or salt) and the surfactants present in each of the formulations tested are either ambiguously reported or not reported at all. However, the Roundup® formulations generally have the IPA salt, a surfactant and water (Geisy, 2000). The formulations of Roundup® that have been tested often contain the POEA surfactant.*
>
> U.S. EPA/OPP 2008a, p.81

This ambiguity is reflected in Table 2 of the current Forest Service risk assessment. In several instances, the presence of a surfactant in a formulation is inferred from a brief note in the Material Safety Data Sheet (MSDS) for the formulation characterizing a toxic component in the formulation as *surfactant*, with no other information provided.

The issue of surfactants in the use of glyphosate is further complicated by addition of surfactants to glyphosate formulations prior to application, as illustrated in Table 4, which provides an overview of the product labels for most of the glyphosate formulations listed in Table 2. As indicated in Table 4, all of the glyphosate formulations that do not appear to contain a surfactant indicate that a nonionic surfactant should be added to the formulation prior to application. The amount and nature of the surfactant to be added is not designated precisely. In addition, some formulations which appear to contain a surfactant indicate that an additional nonionic surfactant may be used.

The ambiguities and vagaries in the use of surfactants with glyphosate formulations complicate and impair the assessment of risks associated with glyphosate applications in Forest Service programs. The most recent risk assessment by the U.S. EPA/OPP (2008a) takes the following general approach:

This document only assesses a surfactant when it is included as part of the formulated product; it does not assess a surfactant that may be included in the tank mix.

U.S. EPA/OPP 2008a, p.81

Given the abundance of new information on glyphosate to be considered and the poorly defined use of other surfactants which may be added to glyphosate formulations, the current Forest Service risk assessment adopts a similar approach. As discussed further in Section 3.1.14.1 and 4.1.3, information about other surfactants is taken into consideration; however, risks associated with the addition of surfactants to glyphosate formulations are addressed only qualitatively.

As summarized by U.S. EPA/OPP (2008a, Table 2.1, p. 21), some commercial formulations of glyphosate also contain other pesticides including 2,4-D, dicamba, diquat, imazethapyr, and metolachlor. The product labels for many of the glyphosate formulations listed in Table 2 indicate additional pesticides which may be used with glyphosate. As with the previous Forest Service risk assessments (SERA 1996, 2003) and the glyphosate risk assessments conducted by the U.S. EPA/OPP (1996a, 2008a), the current Forest Service risk assessment does not consider formulations with multiple active ingredients.

The U.S. EPA/OPP (2008a) has designated one formulation of glyphosate, identified with U.S. EPA Registration Number 524-424, as particularly hazardous. Based on information at the U.S. EPA label site (http://oaspub.epa.gov/pestlabl/ppls.home), this registration number refers to Monsanto's MON-14420 herbicide. This is a water soluble granular formulation of glyphosate that is labeled for roadside and rights-of-way applications. While rights-of-way applications are relevant to Forest Service uses of glyphosate, MON-14420 is not used by the Forest Service and is not otherwise considered in the current Forest Service risk assessment.

2.2.3. Classification of Formulations

The Forest Service prefers to quantitatively consider the toxicity of formulations. Consequently, the EXCEL utility used by the Forest Service for developing project-specific assessments (SERA 2009a) is formulation-specific. In using this utility, an active ingredient is selected and then a specific formulation is selected. In order to meaningfully implement a formulation-specific approach, sufficient information must be available on the formulation to determine if the inert ingredients in the formulation contribute substantially to the toxicity of the formulation. This analysis is typically detailed in Section 3.1.14 of the human health risk assessment as well as in appropriate subsections of the ecological risk assessment (Section 4.0) by comparing information on the toxicity of the active ingredient (a.i.) to the toxicity of formulations of the active ingredient. Information on the toxicity of the formulations is typically obtained from acute toxicity studies submitted to the U.S. EPA in support of the registration of the formulations.

For glyphosate, a formulation-specific assessment is problematic. While the U.S. EPA/OPP generally requires at least acute toxicity data on pesticide formulations, the Agency will sometimes allow toxicity studies on one formulation to support the registration of another formulation. This general approach is sometimes referred to as *bridging*. If the two formulations are identical – i.e., the same formulation is marketed under different names – data bridging is obviously sensible. If the two formulations are substantially different, however,

bridging is not permitted and formulation-specific data required. For glyphosate, a specific discussion of formulation bridging has not been encountered.

Another issue related to the large number of glyphosate formulations under consideration involves the designation of formulations in studies that submitted to the U.S. EPA/OPP. As discussed in Section 3 (Human Health) and Section 4 (Ecological Effects) and as detailed in the appendices to the current Forest Service risk assessment, studies submitted to the U.S. EPA/OPP will most often designate the formulation using an internal product code rather than the commercial name of the formulation. Thus, while a large number of toxicity studies on glyphosate formulations have been submitted to the U.S. EPA/OPP, many of these studies cannot be associated directly with the specific formulations identified by the Forest Service (Tables 2 and 4).

Material Safety Data Sheets (MSDSs) are potential sources of formulation-specific toxicity data. While MSDSs are not highly standardized, they will typically contain information of both mammalian toxicity and toxicity to some nontarget species. Mammalian toxicity data will typically consist of the acute oral and dermal LD_{50}s, 4-hour inhalation LC_{50}s, and qualitative descriptions of skin and eye irritancy. MSDSs will typically give LD_{50} or LC_{50} values for some nontarget species but the specific species that are identified are highly variable.

In an attempt to identify and meaningfully compare toxicity data for the formulations of glyphosate identified by the Forest Service, MSDSs were obtained for all of the formulations specified in Table 2. The mammalian toxicity data for these formulations are summarized in Appendix 1, Table 1. This information includes the acute oral and dermal LD_{50}s, inhalation LC50s, and qualitative descriptions of skin and eye irritancy. Ecological toxicity data from the MSDSs is summarized in Appendix 1, Table 2. This table includes the reported LC_{50} values for bluegills, rainbow trout, and *Daphnia*. These three species were selected because they are the species that are most commonly given in the MSDSs and thus form the most reasonable basis for comparing formulations. An additional column, labeled *Most Sensitive Species*, is given to accommodate a few MSDSs that do not specify individual species but simply indicate a range of toxicity values for the most sensitive aquatic species. The comparison of formulations focuses on aquatic species because, as detailed further in Section 4.3, differences in toxicities to aquatic species are the most substantial differences among the various glyphosate formulations.

Appendix 1, Table 2 also includes two additional columns with notes on the MSDS and notes on the aquatic toxicity data. The last column with notes on the aquatic toxicity data attempts to associate the toxicity values given on the MSDSs with specific toxicity studies. The attempt to associate the aquatic toxicity values on the MSDSs with specific studies is necessary in order to clearly document the units of the toxicity values – i.e., mg formulation, mg a.i., or mg a.e. The units in which the toxicity values are reported must be identified if meaningful comparisons among the formulation are to be made. While some MSDSs report the units, most do not. In addition and as discussed further below, some of the MSDSs appear to report units incorrectly.

Based on the information in Appendix 1, a classification of formulations is given in Table 5. This table classifies the formulations in terms of apparent toxicity and confidence in the classification of apparent toxicity.

Toxicity is classified as low, medium, or high. Low toxicity is exemplified by Accord and Rodeo. As discussed further below, these two formulations contain only glyphosate, water, and a dye. High toxicity is exemplified by Honcho and Roundup Original. These two formulations have the same EPA Registration number (524-445) and appear to be identical. Both formulations contain a POEA surfactant that is toxic to aquatic species. Thus, in terms of mg a.e./L values, Honcho and Roundup Original are substantially more toxic than Accord and Rodeo. As discussed further in Section 4.3 (Hazard Identification for Aquatic Organisms), some surfactants used with glyphosate are less toxic than the POEA surfactant and some formulations of glyphosate contain surfactants that are less toxic than Roundup Original. Thus, the intermediate toxicity category is used.

Confidence in the classification of the formulations is also expressed as low, medium, or high. This classification is based on ability to relate the toxicity values reported on the MSDS to a specific study as well as the toxicity data that is available on some formulations. The classification of *High* confidence is used when the toxicity values on the MSDS can be associated with a specific study on the formulation and the units of the toxicity values – i.e., formulation, a.i., or a.e – can be determined. Confidence is classified as *Medium* if some of the toxicity values on the MSDS can be associated with specific studies and the units for the MSDS can be reasonably inferred. Confidence is classified as *Low* if the toxicity data cannot be associated clearly with specific studies or the units of the toxicity values on the MSDS cannot be reasonably inferred.

2.2.3.1. Low Toxicity/High Confidence

This group consists of Rodeo (a 53.8% IPA formulation), Accord (a 41.5% IPA formulation), and other 41.5% or 53.8% IPA formulations which do not appear to have a surfactant – i.e., they are essentially equivalent to either Rodeo or Accord. Both Rodeo and Accord are known to consist primarily of the IPA salt of glyphosate and water. NCAP (2010) notes that Rodeo and Accord also contain FD&C Blue No. 1 (CAS #3844-45-9), an approved Food Additive (Clydesdale 1997). Dow AgroSciences, however, has indicated that FD&C Blue No. 1 is not used in Rodeo (Fonseca 2010a). In addition, ample toxicity and some field data are available on both Rodeo and Accord. Of the glyphosate formulations, the formulations in this group are the least toxic and have been extensively studied.

2.2.3.2. Low Toxicity/Medium Confidence

Diamondback is the only formulation in this category. Diamondback is labeled for tree injection. The MSDS for this formulation gives toxicity values that are consistent with those for Accord and Rodeo – i.e., the IPA salt of glyphosate. Diamondback is a more concentrated IPA formulation (83.5%). This, in a sense, reduces uncertainty because the composition of the bulk of the formulation is known – i.e., the IPA salt. Nonetheless, no toxicity studies on this formulation have been encountered. The ranking for slight eye irritation (Appendix 1, Table 1) does not suggest that Diamondback contains a toxic surfactant.

2.2.3.3. Low Toxicity/Low Confidence

Aqua Star is the only formulation in this category. Based on information from the MSDS (Appendix 1, Tables 1 and 2), Aqua Star could be classified as a more toxic formulation. As discussed in Section 4.1.3.3.2.1, however, the published study by Bringolf et al. (2007, p. 2095)

explicitly states that Aqua Star does not contain a POEA surfactant. In addition, the study by Bringolf et al. (2007) indicates that Aqua Star has a very low toxicity at least to a species of freshwater mussel. It appears that the information on MSDS for Aqua Star does not involve toxicity data on the Aqua Star formulation itself.

2.2.3.4. Medium Toxicity/Medium Confidence

Four of the formulations in this group are 41% IPA formulations that appear to contain a surfactant – i.e., Buccaneer Plus, Cornerstone Plus, Honcho Plus, and Gly-4 Plus. The other two formulations, Accord SP and Glyphosate Plus, are 59% IPA formulations that contain surfactants. Confidence is only *Medium* because the toxicity data on the MSDSs cannot be clearly associated with toxicity studies. The MSDS for Gly-4-Plus appears to give toxicity values for technical grade glyphosate. Gly-4-Plus is included in this group of formulations because the registrant, Universal Crop Protection Alliance, has indicated that Gly-4-Plus is simply a repackaging of Honcho Plus (Donald 2010).

Nonetheless, the toxicity values given on the MSDSs are typical of the less toxic glyphosate/surfactant formulations – i.e., bluegill 24 mg/L; trout 42-109 mg/L; daphnids 105 to 160 mg/L. In other words, these are more toxic than aqueous glyphosate formulations but not as toxic as Roundup Original (with the POEA surfactant). NCAP (2010) notes that the formulation with EPA Reg. No. 524-454 (referenced by NCAP as Roundup RT Herbicide) contains a polyoxyethylene alkylamine (CAS #61791-26-2), FD&C Blue No. 1, sodium benzoate, and phosphoric acid. The toxicity of these and other non-herbicide compounds in glyphosate formulations is discussed in Section 3.1.14 (Adjuvants and Other Ingredients).

2.2.3.5. Medium Toxicity/Low Confidence

These formulations include Accord XRT, Durango (GF-1279), and Mirage. Accord XRT and GF-1279 are both 53.6% IPA formulations with no explicit information on the use of surfactants in the formulations. No aquatic toxicity values are given in the MSDSs. There are aquatic toxicity studies on other *GF* formulations but none on GF-1279 have been located. No submissions to the U.S. EPA/OPP on aquatic toxicity have been located for Accord-XRT. Some submissions, however, may have used an internal product code.

Mirage is a 41% IPA formulation and a surfactant in the formulation is inferred. All of the toxicity values given on the MSDS are different for those on other similar formulations, suggesting that the toxicity values on the MSDS are specific to Mirage. Also, the MSDS give an exceptionally detailed summary of toxicity values in many aquatic species other than the three considered in this comparison of formulation. The specific studies associated with the toxicity values for bluegill, trout, and daphnids, however, cannot be identified and the units for the toxicity values (formulation, a.i., or a.e.) cannot be determined. Nonetheless, the toxicity values cited in the MSDS for bluegills, trout, and daphnids are generally typical of the less toxic glyphosate/surfactant formulations but there is some overlap with the more toxic formulations – i.e., the 8.2 mg/L toxicity value for *Daphnia*.

2.2.3.6. High Toxicity/High Confidence

Some of the formulations in this group appear to be identical to Roundup Original – i.e., Honcho, Gly Star Plus, and Cornerstone. The other formulations have very similar toxicity values listed on the MSDS. While some of the toxicity values cannot be identified with a specific study – i.e.,

the trout LC_{50} of 5.4 mg/L – the toxicity values on the MSDSs are very close to those given for Roundup Original.

The MSDS for Gly Star Plus states that the units for the aquatic toxicity values are in a.e. This is incorrect. The toxicity values given on the MSDS can be identified – i.e., Forbis et al. (1982a) for bluegills and (Forbis et al. 1982b) and the toxicity values are in units of mg formulation/liter. Since the correct units for the toxicity values can be identified, the misstatement on the MSDS does not reduce the confidence for information on this formulation.

Except for Roundup ProMax (48.7% K formulation) and Roundup ProDry (a 71.4% ammonium formulation), all of the formulations in this group contain the IPA salt. Most of these formulations contain 41% IPA. The exceptions are Roundup Pro Concentrate and Roundup UltraMax (52.2% IPA) as well as Aqua Star (53.8%). NCAP (2010) has identified inerts in Roundup Ultra as a phosphate ester neutralized polyethoxylated tallow amine mixture (no CAS number given), a silicone emulsion (no CAS number given), and FD&C Blue No. 1. NCAP (2010) has also identified a polyoxyethylene alkylamine (CAS #61791-26-2) and FD&C Blue No. 1 as inerts in Roundup Original Herbicide. The toxicity of these and other non-herbicide compounds in glyphosate formulations are discussed in Section 3.1.14 (Adjuvants and Other Ingredients).

A noted above, this group includes Roundup ProDry, a 71.4% monoammonium formulation. The toxicity values for trout and *Daphnia* on the MSDS for this formulation can be identified with reasonable certainty – i.e., the toxicity values appear to be in mg a.e./L from formulation studies and the differences between the values on the MSDS and the MRIDs summarized by U.S. EPA/OPP (2008a) are insubstantial.

2.2.3.7. High Toxicity/Medium Confidence

This group consists of only three formulations, Glyphogan (IPA 41%), Roundup Original Max (K, 48.7%), and Glyphos X-TRA (IPA, 41%). For Roundup Original Max, the toxicity values cannot be associated with specific studies on K formulations but the toxicity values are consistent with those for other Roundup formulations. For Glyphogan, the toxicity value for *Daphnia*, 12.9 mg/L, cannot be associated with a specific study but is in the range of Roundup formulations assuming that the value is in units of mg a.e./L. For Glyphos X-TRA, only the toxicity value for bluegills can be reasonably associated with a specific study.

2.2.3.8. High Toxicity/Low Confidence

This group includes four formulations of 50.2% DMA, all from DowAgro Sciences – i.e., Accord XRT II, DuraMax, Durango DMA (GF-1280), and RapidFire. These formulations may be identical. The only aquatic toxicity information on the MSDSs is that the LC_{50} for the most sensitive species (NOS) is 0.1 mg/L. The units for the LC_{50}s (formulation, a.i., or a.e.) are not specified. No aquatic toxicity information for these formulations has been identified. There is a rat LD_{50} for GF-1280 (>2005 mg a.e./kg bw, MRID 46775603). The MSDSs for these formulations give a rat oral LD_{50} of >5000 mg/kg bw. Assuming that the LD_{50} given on the MSDS is in units of mg formulation/kg bw, the MSDS is consistent with MRID 46775603 [>2005 mg a.e./kg bw ÷ (0.502 x 0.79) = >5055 mg formulation/kg bw]. The presence of surfactants in these formulations is inferred from the eye irritation information on the MSDSs.

Note that MSDSs for both DuraMax and Durango DMA note corneal damage, suggesting a toxic/corrosive surfactant.

This group of formulations also includes Helosate Plus (IPA, 41%). The MSDS for Helosate Plus indicates that the aquatic toxicity data are in units of a.e. and this statement is consistent with aquatic toxicity values for the more toxic glyphosate formulations. The MSDS for Helosate Plus is somewhat unusual in that it designates the formulation as a **Severe** eye irritant. This terminology is not used on other MSDS for glyphosate formulations in this group. An eye irritation study for this formulation has not been identified.

Lastly, this group of formulations includes two 48.8% K formulations, Roundup WeatherMax and RT 3. The MSDSs for the two K formulations do not specify units for the toxicity values. Assuming that the units are for the formulation – i.e., mg formulation/L – the daphnid toxicity value (8 mg/L) is consistent with an LC_{50} of 3.2 mg/L for a potassium formulation of glyphosate.

2.2.3.9. Formulations Not Classified

Some formulations cannot be classified in terms of their toxicity relative to other formulations of glyphosate. A listing of these formulations with the rationale for not classifying them is presented in Table 6.

It must be emphasized that the failure to classify these formulations does not imply that the formulations listed in Table 6 are highly toxic or that the use of these formulations should be avoided. The lack of a classification simply indicates that the toxicity of the formulations relative to other formulations cannot be determined based on the information that is available. Nonetheless, all of these formulations are registered by the U.S. EPA/OPP for uses that are relevant to Forest Service programs and any of these formulations could be used in Forest Service programs.

No toxicity studies have been identified on any of the formulations listed in Table 6. Several of the formulations listed in Table 6 are probably identical, or nearly so, to other formulations that are listed in Table 5 – i.e., the formulations that are classified. Several formulations listed in Table 6 do contain or appear to contain surfactants but the aquatic toxicity values on the MSDSs can be associated with specific toxicity studies on unformulated glyphosate. These formulations include Credit Extra, Credit Systemic Extra, Razor, and Razor Pro. While it seems likely that these formulations are no more toxic than some of the formulations listed in Table 5, it is not possible to classify the toxicity of these formulations. The MSDS for Roundup UltraDry specifically notes that no environmental toxicity studies have been conducted for this formulation.

2.3. Application Methods

2.3.1. Foliar Applications

Glyphosate formulations may be applied by directed foliar, ground broadcast foliar, or aerial methods. In Forest Service Programs, the most common method of applying glyphosate is by backpack-applied directed foliar sprays.

In directed 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, which means that chemical contact with the arms, hands, or face is plausible. To reduce the likelihood of significant exposure, application crews are directed not to apply pesticide to vegetation above shoulder height, and not to walk through treated vegetation. Usually, a worker treats approximately 0.5 acres/hour with a plausible range of 0.25-1.0 acre/hour.

Broadcast foliar ground applications, which may be conducted occasionally, involve the use of a two to six nozzle boom mounted on a tractor or other heavy duty vehicle. With this equipment, workers typically treat 11-21 acres/hour, with the low end of this range representative of a four-wheel drive vehicle in tall grass and the upper end of the range representative of a large bulldozer (USDA/FS 1989, p 2-9 to 2-10).

In addition, some glyphosate formulations are labeled for aerial applications. 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. These operations involve burning a treated area 45-180 days after treatment with the herbicide. The potential risks associated with brown-and-burn operations are discussed further in Section 3.1.13.2.

2.3.2. Other Ground Applications

Glyphosate may also be applied in hack and squirt applications, 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. Other application methods may include cut-stump or wicking. These and other application methods involving the treatment of noncontiguous areas are not covered explicitly in the current risk assessment because standard estimates of the amount of material that a worker might handle as well as worker exposure rates are not available. In addition, these types of applications to noncontiguous areas do not readily lend themselves to the methods used for estimating exposures standard broadcast applications.

One formulation of glyphosate used by the Forest Service, EZ-Ject Diamondback, is labeled only for tree injection. Tree injections are made with special equipment such as the Arborjet Tree Injection Delivery Systems (http://www.arborjet.com/products/devices.htm)

2.3.3. Aquatic Applications

The other method of application is an aquatic application for aquatic noxious weeds. As summarized in Table 2, several formulations of glyphosate are labeled for aquatic applications. Glyphosate formulations are applied only to emergent vegetation—i.e., vegetation that is above the surface of the water. Glyphosate is not used to control subsurface aquatic weeds.

2.4. Mixing and Application Rates

2.4.1. Foliar Applications

Foliar applications account for most of the use of glyphosate in Forest Service programs. As discussed further in Section 2.5 (Use Statistics), use statistics from the Forest Service are available up to 2004, and these statistics include uses defined by Forest Service region and by management objective. The uses defined by management objective over the 5-year period from 2000 to 2004 are summarized in Table 7. As indicated in Table 7, the major uses of glyphosate in Forest Service programs involve conifer release (58.5%), site preparation (19.7 %), noxious weed control (9.9%), and hardwood release or release programs not otherwise specified (3.6%). All of these management objectives, which account for about 92% of the use of glyphosate in Forest Service programs, would primarily involve foliar applications.

The maximum application rate for glyphosate cited in the RED as well as the more recent U.S. EPA risk assessment for the California red-legged frog is 7.95 lb a.e./acre (U.S. EPA/OPP 1993a, 2008a). On some glyphosate labels, this value is rounded to 8 lb a.e./acre (e.g., Roundup Original Max Herbicide). This very minor difference appears to reflect a simple rounding of the maximum application rate. As indicated in Table 7, the Forest Service typically uses much lower application rates—i.e., an average of about 2 lbs a.e./acre.

Foliar applications may involve the use of formulations containing or not containing surfactants. The product labels for most of the formulations which do not contain a surfactant recommend adding a surfactant to the formulation prior to application; furthermore, some of the formulations containing surfactants indicate that additional nonionic surfactants may be added prior to application. Formulations containing surfactants generally recommend adding a surfactant at a concentration of 0.25-0.5%; while formulations which do not contain a surfactant generally recommend adding a surfactant at a concentration of about 10%. Depending on the specific type of application, some formulations simply note that surfactants may be added without specifying the surfactant concentration.

As summarized in Table 4, the product labels for most glyphosate formulations also note that ammonium sulfate may be added as an adjuvant to water at rates of 8.5 to 17 pounds/acre prior to adding glyphosate. Ammonium sulfate is used to increase the efficacy of glyphosate (e.g., Belles et al. 2006; O'Sullivan et al. 1981). Other materials that may be added to the field solution include colorants or dyes as well as drift reducing agents.

The final field solution may be applied at various application volumes expressed as gallons of field solution/acre. For a given application rate expressed in units of lb a.e./acre, lower application volumes will result in higher concentrations of glyphosate in the field solution. This detail is important to the current risk assessment as well as to the assessment of any site-specific application of glyphosate because the extent to which a formulation of glyphosate is diluted prior to application primarily influences dermal and direct spray scenarios, both of which are dependent on the concentration of glyphosate in the applied spray. In all cases, the higher the concentration of glyphosate - equivalent to the lower dilution of glyphosate - the greater the risk. For this risk assessment, the lowest dilution is taken as 5 gallons/acre. The highest dilution is based on 25 gallons of water per acre. A typical dilution rate is taken as 10 gallons/acre. Details regarding the calculation of field dilution rates from application volumes are given in Worksheet A01 of the EXCEL workbooks that accompany this risk assessment.

2.4.2. Aquatic Applications

As summarized in Table 2, several glyphosate formulations are labeled for *aquatic* applications. The term *aquatic*, however, refers only to emergent aquatic vegetation; none of the glyphosate formulations is labeled for the control of submerged aquatic vegetation.

Except for the application rate, aquatic applications of glyphosate are essentially identical to broadcast applications to control terrestrial vegetation. The maximum application rate for aquatic applications is 3.75 lb a.e./acre. Note that Table 7 indicates that the average application rate used in Forest Service programs is 6.23 lbs/acre. These types of apparent inconsistencies are occasionally noted in Forest Service use reports and may due be due to the use report using units of formulation rather than acid equivalents (a.e.).

The use of surfactants with aquatic applications is restricted. As discussed further in Section 3.1.14.1 (Adjuvants) and 4.3 (Hazard Identification for Aquatic Species), some formulations that are intended for terrestrial applications contain a surfactant that consists of a mixture of polyethoxylated tallow amines (POEA). The POEA surfactant is toxic to aquatic organisms and this surfactant is not permitted in formulations that are designed for aquatic applications (U.S. EPA/OPP 2008a, p. 9). POEA surfactants are not added to glyphosate field solutions that are applied to aquatic sites.

2.5. Use Statistics

Most Forest Service risk assessments attempt to characterize the use of an herbicide or other pesticide in Forest Service programs relative to the use of the herbicide or other pesticide in agricultural applications. The information on Forest Service use is typically taken from Forest Service pesticide use reports (http://www.fs.fed.us/ foresthealth/pesticide/reports.shtml), and information on agricultural use is typically taken from use statistics compiled by the U.S. Geological Survey (http://water.usgs.gov/nawqa/pnsp/usage/maps/) and/or detailed pesticide use statistics compiled by the state of California (http://www.calepa.ca.gov/).

The use of glyphosate by the Forest Service over the period from 2000 to 2004 is summarized in Table 7 and illustrated in Figure 1. As illustrated in Figure 1, the Forest Service classification divides the United States into nine regions designated from Region 1 (Northern) to Region 10 (Alaska). [Note: There is no *Region 7* in the Forest Service system.] The heaviest use of glyphosate occurs in Region 5 (Pacific Southwest) in terms of the number of acres treated

(42.4%), the number of pounds used (78.2%), and the average application rate (3.8 lbs/acre). Based on total pounds applied, glyphosate use is also substantial in Region 8 (Southern, 11.1%) and Region 9 (Eastern 5.2%) with moderate use in Region 6 (Pacific Northwest, 2.7%). Glyphosate use by the Forest Service in other regions is insubstantial—i.e., less than 3% of total. As summarized in Table 7, the total use of glyphosate in Forest Service programs over the 5-year period from 2000 to 2004 was about 173,000 pounds or approximately 35,000 pounds/year.

Many glyphosate formulations are used extensively in agriculture. A summary of the agricultural use of glyphosate is illustrated in Figure 2 (USGS 2003a). These use statistics are for 2002, the most recent year for which data are available. As indicated in this figure, over 100,000,000 lbs of glyphosate were applied to crops annually during 2002. This is about a factor of about 2900 greater than the average annual use of glyphosate in Forest Service programs. Thus, while the use of glyphosate by the Forest Service is not trivial, Forest Service use is much less than agricultural use.

More recent use statistics are available for California for the year 2007 (CDPR 2008). According to CDPR (2008), the isopropylamine salt of glyphosate is most commonly used in forestry applications (Table 1). For this salt, a total of 4,299,462 lbs was applied in California during 2007 (CDPR 2008, p. 190). Of this amount, the uses most clearly related to forestry applications are 81,657 lbs applied to timberland—i.e., about 1.9% of the total use of isopropylamine salt of glyphosate. CDPR (2008, p. 185) also reports that a total of 564,466 lbs of glyphosate (salt not specified) was applied in California during 2007. Of this amount, 38,822 lbs (≈ 6.9%) was applied in rights-of-way management. As indicated in Table 6, the use of glyphosate by the Forest Service in Region 5 between 2000 and 2005 was about 136,000 lbs or 27,200 lbs/year.

3. HUMAN HEALTH

3.1. HAZARD IDENTIFICATION

3.1.1. Overview

The toxicity data on technical grade glyphosate are extensive, including both a standard set of toxicity studies submitted to the U.S. EPA/OPP in support of the registration of glyphosate as well as a robust open literature consisting of numerous diverse *in vivo* and *in vitro* studies, including some studies in humans. As with any complex collection of studies, the studies on technical grade glyphosate may be subject to differing interpretations. The preponderance of the available data, however, clearly indicates that the mammalian toxicity of glyphosate is low, and very few specific hazards can be identified. Oral doses that exceed around 300 mg/kg bw, glyphosate may cause signs of toxicity, including decreased body weight, changes in certain biochemical parameters in blood as well as tissues, and inhibition of some enzymes (i.e., P450) involved in the metabolism of both endogenous and exogenous compounds. At doses from about 1000 to 5000 mg/kg bw, glyphosate can cause death. The most sensitive endpoint for glyphosate—i.e., the adverse effect occurring at the lowest dose—involves developmental effects; accordingly, the EPA-derived RfDs for glyphosate are based on developmental effects. These adverse developmental effects, which consist primarily of delayed development, occur only at doses causing signs of maternal toxicity. There is no indication that glyphosate causes birth defects.

The hazard identification for glyphosate formulations is much less clear. In most Forest Service pesticide risk assessments, the active ingredient is the agent of primary concern, and consideration for ingredients in the formulations is limited to a brief discussion in Section 3.1.14 (Adjuvants and Other Ingredients). In the current Forest Service risk assessment, however, the way in which the formulation ingredients other than glyphosate are handled is much different. Many glyphosate formulations include surfactants, and the toxicity of these surfactants is of equal or greater concern to the risk assessment than is the toxicity of technical grade glyphosate. Consequently, as justified by the available data, the hazard identification is subdivided into sections that address the toxicity of glyphosate, the toxicity of glyphosate formulations, and/or the toxicity of the surfactants.

Because surfactants appear to be agents of concern, a central issue in the current Forest Service risk assessment involves differences in surfactants among the glyphosate formulations used by the Forest Service (Table 2) as well as glyphosate formulations for which toxicity data are available in the open literature. As detailed in Section 3.1.14, the term *POEA* (an acronym for polyoxyethyleneamine) is commonly used to designate surfactants used in some glyphosate formulations. POEA, however, is not a single surfactant. POEA surfactants are mixtures. Because the constituents in the surfactants are considered proprietary (trade secrets or Confidential Business Information), detailed information about the constituents is not publically available. The POEA surfactant used in one glyphosate formulation may be different from the POEA surfactant used in other glyphosate formulations, even among formulations provided by the same manufacturer. Thus, it is not clear whether the toxicity studies conducted on POEA surfactants are applicable to all or any of the glyphosate formulations currently in use.

The difference or potential difference in the composition of surfactants used in various formulations of glyphosate has a practical impact on the hazard identification for the current Forest Service risk assessment. Several studies conducted outside of the United States on glyphosate formulations which are not used domestically report adverse effects of concern, including potential effects on endocrine function in rats and signs of genotoxicity in humans. In the absence of comparable studies on glyphosate formulations manufactured and used in the United States, the extent to which this information is relevant to U.S. formulations of glyphosate is unclear.

3.1.2. Mechanism of Action

Glyphosate's mechanism of action as an herbicide is well characterized. As discussed in Section 4.1.2.5.3, the herbicidal activity of glyphosate is due primarily to the inhibition of the shikimate pathway which is involved in the synthesis of aromatic amino acids in plants and microorganisms (Section 4.1). This metabolic pathway does not occur in humans or other animals; accordingly, this mechanism of action is not directly relevant to the human health risk assessment. Nonetheless, shikimate pathway inhibitors are considered antimicrobial agents for the control of pathogens (Roberts et al. 1998; Roberts et al. 2002; Schonbrunn et al. 2001), and glyphosate has been shown to be effective in prolonging survival in mice infected with a pathogen, *Cryptococcus neoformans* (Nosanchuk et al. 2001).

A mechanism by which glyphosate exerts toxic effects in humans or experimental mammals is not clear. As discussed below, two biochemical mechanisms of action are discussed in the literature on glyphosate: uncoupling of oxidative phosphorylation and inhibition of hepatic mixed function oxidases. In addition, both glyphosate and the POEA surfactant used in Roundup will damage mucosal tissue, although the mechanism of this damage is likely to differ for these two agents.

Oxidative phosphorylation is a fundamental metabolic process in which metabolic energy derived from the oxidation of nutrients is transferred to and stored in high-energy phosphate bonds. The uncoupling of this process results in energy loss in the organism and leads to death. Symptoms of uncouplers of oxidative phosphorylation include increased heart rate (tachycardia), increased respiratory rate, labored breathing, profuse sweating, fever, metabolic acidosis, and weight loss (ATSDR 2001). Based on a series of experiments using rat liver mitochondria exposed to the isopropanolamine salt of glyphosate, an uncoupling of oxidative phosphorylation has been reported in several studies (Bababunmi et al. 1979, Olorunsogo 1982, Olorunsogo and Bababunmi 1980, Olorunsogo et al. 1977, Olorunsogo et al. 1979a,b). This effect was observed after intraperitoneal doses as low as 15 mg/kg (Olorunsogo et al. 1979a).

Some of the observations on whole animals and isolated mitochondria are consistent with an uncoupling of oxidative phosphorylation, including decreased body weight gain, decreased food conversion efficiency, and increased body temperature (Section 3.1.3). It is less clear, however, that uncoupling of oxidative phosphorylation is a significant factor in acute *in vivo* exposures to glyphosate. Of the 97 patients covered in the Tominack et al. (1991) report, only seven individuals had mild elevations in body temperature (>99.5°F). In addition, acute gavage doses of 50, 100, or 200 mg glyphosate a.e./kg in rats were associated with hypothermia (a decrease in body temperature) rather than hyperthermia (Horner 1996a).

The other specific mechanism of action that may account for some the effects of glyphosate involves the inhibition of mixed-function oxidases. This is a class of enzymes comprised of various isozymes of cytochrome P450 which is involved in the metabolism of various endogenous compounds as well as xenobiotics. Decreases in hepatic mixed function oxidase activity *in vivo* were noted after doses of 500 mg/kg/day of glyphosate (as Roundup 360 g/L) for 4 days followed by doses of 300 mg/kg/day for 6 days (Hietanen et al. 1983). This decrease in mixed function oxidase activity is only suggestive of cytochrome P450 inhibition, since a general decrease in mixed function oxidase activity could also be caused by direct liver damage. *In vitro* studies, however, have demonstrated the inhibition of P450 activity in both mammalian cells (Richard et al. 2005) and plant cells (Lamb et al. 1998).

Many of the effects of acute oral exposure to high doses of glyphosate or Roundup are consistent with corrosive effects on the mucosa. In case studies of the suicidal ingestion of the original Roundup formulation, corrosive effects on the gastric mucosa as well as other tissue have been noted (Chang et al. 1999; Hung et al. 1997). While somewhat speculative, it is likely that the mechanisms for this effect differ between glyphosate and the POEA surfactant. As indicated in Section 2, glyphosate is a zwitterion that will have a net negative charge and can be expected to act as an acid at physiological pH. Thus, the effects of glyphosate on mucosal tissue may be due to the acidic action of glyphosate, similar to the effects of high concentrations of hydrochloric acid in dog (Talbot et al. 1991). As detailed in Section 3.1.11, the POEA surfactant appears to have a different mechanism of action, behaving essentially like a soap to dissolve cell membranes.

Glyphosate has been assays in a large number of *in vitro* studies. These studies are reviewed in some detail by Williams et al. (2000), and the most significant *in vitro* studies are summarized in the current Forest Service risk assessment in Appendix 2 (Table 7 for studies relating to endocrine function and Table 8 for other *in vitro* studies). Except as noted specifically in the following subsections, the *in vitro* studies do not contribute substantially to the hazard identification because of the large number of relevant *in vivo* studies on glyphosate. In addition, *in vitro* studies may be useful in attempts to characterize mechanisms of action but *in vitro* exposures may be of limited use in identifying the potential or likelihood of effects in whole organisms.

3.1.3. Pharmacokinetics and Metabolism

3.1.3.1. General Considerations

Pharmacokinetics involves the quantitative study of the absorption, distribution, and excretion of a compound. Pharmacokinetics is particularly important to this risk assessment on glyphosate for two reasons. First, many of the most plausible and quantitatively most significant exposure assessments (Section 3.2) involve dermal exposure, although most of the dose-response assessments (Section 3.3) used to interpret the consequences of dermal exposure involve oral exposure levels. Accordingly, it is necessary to understand the kinetics of both oral and dermal absorption so that dermal exposure assessments can be appropriately compared with oral dose-response assessments. Second, the *in vitro* studies on glyphosate are conducted over a wide range of concentrations, some of which are beyond concentrations that may occur *in vivo*. Thus, an understanding of likely concentrations of glyphosate following *in vivo* exposures in mammals can be useful in interpreting the available data from *in vitro* studies.

The general characteristics of the pharmacokinetics of glyphosate have been reviewed in a number of sources (e.g., Bradberry et al. 2004; Burgat et al. 1998; FAO/WHO 1986; Smith and Oehme 1992; Solomon et al. 2005, 2007, 2009; U.S. EPA/OPP 1993b; WHO 1994; Williams et al. 2000) . At physiological pH, glyphosate has a net negative charge (Franz 1985). Charged molecules do not readily cross normal and intact biological membranes. Consequently, as discussed further in Section 3.1.3.2, glyphosate is not readily absorbed by humans or other mammals.

As discussed further in Section 3.1.3.2, glyphosate is not rapidly absorbed by the dermal route although absorption across abraded skin is much more rapid than absorption across intact skin. After oral administration, most glyphosate remains in the gastrointestinal tract (e.g., Brewster et al. 1991). The oral bioavailability of glyphosate was recently estimated to be about 23% (Anadon et al. 2009). Vasiluk et al. (2005) report that at high concentrations—i.e., greater than 10,000 mg/L or 1% w/v—glyphosate can damage intestinal cells, which could contribute to more rapid absorption after oral exposures. As discussed further in Section 3.1.4, there is ample evidence from poisoning incidents in humans that damage to the gastrointestinal tract is common.

Glyphosate is not extensively metabolized, and more than 95% of administered glyphosate is excreted unchanged (e.g., U.S. EPA/OPP 1993b; WHO 1994; Williams et al. 2000). Of the small proportion of glyphosate that is metabolized, the most commonly noted metabolite is amino methyl phosphonic acid (AMPA), which is the only metabolite quantified in pharmacokinetic studies (e.g., Anadon et al. 2009). Differences in metabolic pathways can be an important consideration regarding differences in species sensitivity to some chemical agents. There is no indication, however, that this is an important consideration for glyphosate. Because glyphosate is not extensively metabolized, differences in metabolic pathways are not likely to be an important consideration in extrapolations from animal toxicity data to potential risks in humans.

Most toxicity studies on glyphosate involve experiments with laboratory mammals. Understanding the differences between animals and humans with respect to the absorption, distribution, and excretion of glyphosate, helps to interpret better the consequences of glyphosate exposure for both workers and members of the general public. As discussed below, very little information is available on the pharmacokinetics in humans and a physiologically-based pharmacokinetic (PBPK) model for glyphosate has not been developed. While interspecies differences in the metabolism of glyphosate do not appear to be a major concern, the lack of a PBPK model limits the use of information on tissue levels from *in vivo* studies in the interpretation of some of the *in vitro* studies.

Three pharmacokinetic studies on glyphosate are available that could form the basis of a PBPK model for glyphosate (Anadon et al. 2009; Brewster et al. 1991; NTP 1992). The study by Brewster et al. 1991 is a relatively standard pharmacokinetic study in male rats which were administered a single dose of radiolabelled glyphosate at 10 mg/kg bw. Radioactivity was then monitored in various tissues over 7 days (168 hours). As discussed further in Section 3.1.8

(Endocrine Disruption), information on the concentrations of glyphosate in different tissues is most relevant to the current Forest Service risk assessment.

Brewster et al. (1991) do not provide explicit concentrations of glyphosate in tissues, but tissue concentrations can be reasonably approximated from the data given in the publication. Table 4 in the Brewster et al. (1991) paper gives the tissue to blood ratios ($\boldsymbol{P_T}$) for 12 tissues at periods from 2 to 168 hours after dosing. Thus, if the concentration in blood ($\boldsymbol{C_B}$) is known, the concentration in tissues ($\boldsymbol{C_T}$) can be calculated: $\boldsymbol{C_T = P_T}$ x $\boldsymbol{C_B}$.

Table 3 in the Brewster et al. (1991) paper gives the concentration of glyphosate in blood as a percent of the administered dose—i.e., 10 mg/kg bw. The paper also gives the body weights of the rats as ranging from 115 to 125 grams. Taking the average body weight as 0.12 kg, each rat was administered an average of 1.2 mg glyphosate. The percent of the administered dose in blood is given as 0.38, 0.33, and 0.06% at 2, 6.3, and 28 hours, respectively, after dosing. By comparison, NTP (1992) reports that after rats were dosed with 5.6 mg/kg bw, the blood concentrations of glyphosate were 0.28, 0.18, 0.31, and 0.03% of the administered dose at 3, 6,12, and 24 hours, respectively, after dosing.

For the Brewster et al. (1991) study, the amount of glyphosate in the blood can be estimated from these percentages and the total dose of 1.2 mg—i.e., 0.00456, 0.00396, and 0.00072 mg at 2, 6.3, and 28 hours, respectively, after dosing. In a footnote to Table 3, Brewster et al. (1991) also note that the blood was estimated at 8% of the body weight—i.e., 0.08 x 120 g = 9.6 g. Taking the density of blood as approximately 1 mL/g, the total blood volume would be 9.6 mL. Thus, the blood concentration of glyphosate can be estimated as 0.475, 0.4125, and 0.075 mg/L at 2, 6.3, and 28 hours, respectively, after dosing.

Using the above estimates of the concentration of glyphosate in whole blood and the tissue to blood ratios given in Table 4 of Brewster et al. (1991), the concentrations of glyphosate in various tissues are summarized in Table 10 and illustrated in Figure 3 of the current Forest Service risk assessment. Figure 3 consists of four graphs. The upper left graph plots all of the data from Table 10.

The upper right graph plots the tissue concentrations for the gastrointestinal tract—i.e., stomach, small intestine, and colon. The highest concentrations of glyphosate are found in the small intestine and colon. The relatively low concentrations of glyphosate in the stomach reflect rapid transit time in the stomach. As summarized in Durkin et al. (2004), the approximate transit time for the rat stomach is about 32 minutes, while the first observation period in the study by Brewster et al. (1991) is 2 hours after dosing. The pattern of high tissue concentrations of glyphosate in the gastrointestinal tract reflects the slow absorption of glyphosate after oral exposure, as noted above.

The lower left graph in Figure 3 illustrates the concentration of glyphosate in bone, kidney, liver, spleen, blood plasma, and red blood cells. Bone is the only tissue in which glyphosate concentrations increase over the initial 24 hour period after oral dosing. As discussed by Brewster et al. (1991), the glyphosate concentration in bone is probably associated with the formation of ionic bonds between glyphosate and bone calcium. As noted above, glyphosate has

a net negative charge at physiological pH (Franz 1985), and the suggestion by Brewster et al. (1991) that glyphosate would bind to Ca++ ions in the bone matrix seems reasonable.

The lower right graph in Figure 3 illustrates the concentration of glyphosate in testes, testicular fat, blood plasma, and red blood cells. As discussed further in Section 3.1.9.3 (Reproductive Effects, Target Organ Toxicity), some reports indicate that testes are a target organ for glyphosate. As illustrated in Figure 3, glyphosate does not accumulate in testes tissue or testicular fat, relative to blood plasma or red blood cells, respectively.

In terms of toxicologically significant exposure, the dose of 10 mg/kg bw used in the pharmacokinetic study by Brewster et al. (1991) is relatively low. As discussed further in Section 3.3, the RfD derived by U.S. EPA/OPP (2002) is based on a NOAEL in rabbits of 175 mg/kg bw from a teratology study, and the RfD derived by the U.S. EPA/ORD (1990) is based on a rat NOAEL of 10 mg/kg bw/day from a 3-generation reproduction study. Thus, the single 10 mg/kg bw dose used by Brewster et al. (1991) would clearly be considered a nontoxic exposure.

The pharmacokinetic study by Anadon et al. (2009) used a single oral dose of 400 mg/kg bw and noted a peak plasma concentration of about 10 mg/L at about 4 hours after dosing (see Figure 2 in the Anadon paper). At about 6 hours after dosing, comparable to the 6.3 hour observation from the study by Brewster et al. 1991, the concentration in plasma was about 8 mg/L. As indicated in Table 10 of the current Forest Service risk assessment, the concentration in plasma from the Brewster et al. (1991) study at 6.3 hours is about 0.83 mg/L. Assuming linear pharmacokinetics, the expected concentration at a 40-fold higher dose (i.e., 400 mg/L) would be about 33.2 mg/L [0.83 mg/L x 40], a factor of about 4 less than the peak concentration observed by Anadon et al. (2009) [33.2 mg/L ÷ 8 mg/L = 4.15]. At 24 hours after dosing, the concentration in plasma noted by Anadon et al. (2009) is about 1 mg/L. As indicated in Table 10, the concentration of glyphosate in plasma at 28 hours after an oral dose of 10 mg/kg bw is 0.08 mg/L. Correcting for the 40-fold lower dose and assuming linear kinetics, the expected concentration of glyphosate in plasma following a 400 mg/kg bw dose would be 3.2 mg/L. This expected concentration is about 3 times greater than the concentration observed by Anadon et al. (2009) [3.2 mg/L ÷ 1 mg/L = 3.2].

The above comparisons are limited. Anadon et al. (2009) does not provide error estimates on the observed plasma concentrations. In the Brewster et al. (1991) study, error estimates of the plasma concentration cannot be made for the 6.3-hour observation period because the standard error of the mean (SEM) for blood concentration is reported as 0.00. For the 28-hour observation period, Brewster et al. (2009) report the mean as 0.06 and the SEM as 0.03.

While the statistics are limited, the overall pattern suggests that the pharmacokinetic parameters for glyphosate may not scale linearly with dose. While somewhat speculative, the lower than expected peak plasma concentration at the 400 mg/kg bw dose at about 6 hours after dosing suggests lower rates of absorption at higher doses. The higher than expected concentrations at the 400 mg/kg dose at about 24 hours after dosing suggests lower rates of elimination; nevertheless, the mechanism for the lower rate—e.g., impaired excretion, sequestering in bone, etc.—cannot be identified.

A similar pattern is noted in NTP (1992) in which a dose of 56 mg/kg bw resulted in peak blood concentrations that are 30 times greater than those following a dose of 5.6 mg/kg bw. In addition, the higher dose resulted in a longer period to peak blood concentrations—i.e., 1 hour at 5.6 mg/kg bw and 2 hours at 56 mg/kg bw. Based on drinking water studies of both glyphosate and Roundup (glyphosate with POEA), NTP (1992) notes that the surfactant in Roundup does not affect the rapid elimination rate of glyphosate.

3.1.3.2. Dermal Absorption

Most of the occupational exposure scenarios and many of the exposure scenarios for the general public involve the dermal route of exposure. For these exposure scenarios, dermal absorption is estimated and compared to an estimated acceptable level of oral exposure based on subchronic or chronic toxicity studies in animals. Hence, it is necessary to assess the consequences of dermal exposure relative to oral exposure and the extent to which glyphosate is likely to be absorbed from the skin surface.

Two types of dermal exposure scenarios are considered: immersion and accidental spills. As detailed in SERA (2007), the calculation of absorbed dose for dermal exposure scenarios involving immersion or prolonged contact with chemical solutions uses Fick's first law and requires an estimate of the zero-order permeability coefficient (K_p) expressed in cm/hour. In exposure scenarios like direct sprays or accidental spills involving deposition of the compound onto the skin's surface, first-order dermal absorption rates (k_a), expressed as a proportion of the deposited dose that is absorbed per unit time, are used in the exposure assessment—e.g., $hour^{-1}$. Experimental estimates are available for both first-order and zero-order dermal absorption rates of glyphosate.

3.1.3.2.1. First-Order Dermal Absorption

Wester et al. (1991) assayed the first-order dermal absorption rate of ^{14}C-labeled glyphosate in a Roundup formulation in both an *in vitro* system using skin from human cadavers and in an *in vivo* study in monkeys. *In vitro* skin preparations were exposed to undiluted Roundup formulations for up to 8 hours, and 1:20 and 1:32 dilutions of Roundup were treated similarly for up to 16 hours (Wester et al. 1991, Table 1, p. 728). Based on the 16-hour exposures to the dilute solutions, first-order dermal absorption rates ranged from 1.3×10^{-4} to 1.0×10^{-3} $hour^{-1}$ with an average value of 4.1×10^{-4} $hour^{-1}$. Based on the 8-hour exposures to the concentrated Roundup, first-order dermal absorption rates ranged from 7.5×10^{-5} to 5.0×10^{-4} $hour^{-1}$. Thus, glyphosate in undiluted Roundup—i.e., containing the POEA surfactant—does not appear to be more rapidly absorbed than glyphosate in a more dilute solution of the surfactant. The *in vivo* studies in monkeys indicate that about 1.5% of the glyphosate was absorbed in 12 hours, corresponding to a first-order dermal absorption rate of 1.3×10^{-3} $hour^{-1}$ [k_a = ln(1-proportion absorbed)/duration].

These experimental measurements of dermal absorption are consistent with the standard methods used to estimate first-order dermal absorption rates (SERA 2001a). The details of the method specified in SERA (2001a) for estimating the first-order dermal absorption coefficient based on the molecular weight and octanol-water partition coefficient are given in worksheet B06. The application of this method to glyphosate is detailed in worksheet B03. Based on a molecular

weight of 169.07 and K_{ow} of 0.00032 from Schuette (1998), the estimated k_a for glyphosate is about 5.4×10^{-4} hour^{-1} with a range of 8.6×10^{-5} to 3.3×10^{-3} hour^{-1}.

Given the similarities between the estimated values of the first-order dermal absorption rates in worksheet B03 and the experimental values calculated from the study by Wester et al. (1991), the use of either set in this risk assessment makes relatively little difference. Nonetheless, the experimental values for human skin preparations from Wester et al. (1991) are used in all exposure assessments requiring first-order dermal absorption rates, as specified in worksheet B05— i.e., 4.1×10^{-4} (1.3×10^{-4} to 1.0×10^{-3}) hour^{-1}.

3.1.3.2.2. Zero-Order Dermal Absorption

Three studies are available on the zero-order dermal absorption of glyphosate, all of which involve the use of *in vitro* human skin preparations. Wester et al. (1996) examined five test systems, one involving a 1% glyphosate solution and the others involving exposure of the skin preparation to glyphosate on cotton cloth treated for 0-2 days prior to use. The latter series of tests were conducted to determine whether glyphosate binds to skin with increasing affinity over time. The most rapid K_p, expressed as mean plus or minus the standard error of the mean, was $4.59\pm1.56\times10^{-4}$ cm/hour with a lag time of 10.48 hours, and this value was from the assay involving the 1% glyphosate solution. Two K_p values using intact human skin preparations are reported by Nielsen and coworkers, as $5.9\text{x}10^{-5}$ cm/hour with a lag time of 8 hours (Nielsen et al. 2007) and $4\text{x}10^{-5}$ cm/hour with no detectable lag time (Nielsen et al. 2009).

Using an abraded skin preparation, Nielsen et al. (2007) reported a much higher K_p of $9.7\text{x}10^{-4}$ cm/h with a lag time of 8.7 hours. The lack of a detectable lag time with intact skin versus an 8.7 hour lag time with abraded skin may not be intuitive. Nielsen et al. (2007) attributed the failure to detect a lag time with intact skin to the low rate of absorption. In other words, a time lag between application and absorption probably occurred; however, the lag time could not be quantified.

As with the first-order dermal absorption rates, Forest Service risk assessments rely on quantitative structure activity relationships in the absence of experimental data and use the algorithm recommended by U.S. EPA/ORD (1992) to estimate K_p values. As detailed in Worksheet B05, the estimated K_p values for glyphosate are about 1.5×10^{-6} (3.7×10^{-7} to 6.2×10^{-6}) cm/hour. These rates are substantially less than the experimental estimates of K_p values as summarized above.

In selecting the K_p values to use in the current Forest Service risk assessment, the issue of lag time is important. The zero-order exposure scenarios used in Forest Service risk assessments (Section 3.2) assume that the individual comes into contact with the pesticide for a relatively brief period of time (minutes to several hours) and that the pesticide is effectively removed after the exposure period. This exposure scenario is supported by the Wester et al. (1994) study which indicates that about 90% of glyphosate applied to the skin can be effectively removed by washing with soap and water. Thus, while the absorption rates based on lag times are higher than those based on the U.S. EPA/ORD (1992) model (which does not consider a lag time), using a lag time in Forest Service risk assessments would result in essentially no absorption over exposure periods of fewer than 8 hours. Thus, the K_p values in the current Forest Service risk

assessment are based on the U.S. EPA/ORD (1992) model—i.e., 1.5 x 10^{-6} (3.7 x 10^{-7} to 6.2 x 10^{-6}) cm/hour.

The uncertainties associated with the higher dermal absorption rate for abraded versus intact skin are difficult to consider quantitatively. As discussed above, the study by Nielsen et al. (2007) demonstrates that the K_p for abraded skin (9.7x10^{-4} cm/h) is higher than the K_p for intact skin (5.9x10^{-5} cm/h) by a factor of about 16. Despite the lack of specific studies on first-order dermal absorption rates in abraded skin, it is likely that relative to intact skin, abraded skin is far more permeable to any pesticide, based on either zero-order or first-order absorption kinetics. The impact of abraded skin on potential risk is addressed semi-quantitatively in Section 3.4 (Risk Characterization).

3.1.3.3. Excretion

Although excretion rates are not used directly in either the dose-response assessment or risk characterization, excretion half-lives can be used to infer the effect of longer-term exposures on body burden, based on the *plateau principle* (e.g., Goldstein et al. 1974). The concentration of the chemical in the body after a series of doses (X_{Inf}) over an infinite period of time can be estimated based on the body burden immediately after a single dose, X_0, by the relationship:

$$\frac{X_{Inf}}{X_0} = \frac{1}{1 - e^{-kt^*}}$$

where ***t**** is the interval between dosing and k is the first-order excretion rate.

The elimination of glyphosate from plasma is extremely rapid. For example, the recent study by Anadon et al. (2009) reports terminal plasma half-lives of about 10 hours after intravenous administration and 14 hours after oral administration. In terms of applying the plateau principle to estimating body burden, however, the most relevant half-life involves total body burden. Reported whole body half-lives for glyphosate are about 52 hours or about 2.2 days (Brewster et al. 1991). A half-life of 2.2 days corresponds to a whole body elimination rate of about 0.3 day^{-1} [$k = \ln(2)/t_{½}$]. Substituting this value into the above equation for the plateau principal, the estimated plateau in the body burden after daily doses over a prolonged period of time would be about 4 [$1 \div (1 - e^{-0.3}) \approx 3.86$].

3.1.4. Acute Oral Toxicity

3.1.4.1. Technical Grade Glyphosate

One very basic type of acute toxicity information involves time-specific LD_{50} or LC_{50} values (i.e., doses or concentrations of a toxicant that result in or are estimated to result in 50% mortality of the test species during a specified exposure or observation period). These values can be viewed as an index of acute lethal potency. Studies that are useful in estimating the LD_{50} involve testing at a number of different dose levels which result in mortality rates that bracket 50% of the treated animals. These data are then used to estimate the oral LD_{50} value. In the registration process, however, the U.S. EPA will accept *limit tests* in which the compound is tested at only a single high dose, typically 2000 mg/kg bw or 5000 mg/kg bw. If the compound does not cause mortality rates of 50% or more, the requirement for a full study to determine the

LD_{50} value may be waived. In these instances, LD_{50} values are expressed as greater than the limit dose—e.g., >2000 mg/kg bw or >5000 mg/kg bw.

Consistent with the terminology used in U.S. EPA/OPP (2008a), LD_{50} values expressed as greater than a particular value are referred to as *non-definitive* LD_{50} values, and LD_{50} values expressed as a specific value (with or without confidence intervals) are referred to as *definitive* LD_{50} values. This convention is also applied to inhalation LC_{50} values discussed in Section 3.1.13 as well as LC_{50} values for aquatic species discussed in Section 4.1.3. While *non-definitive* LD_{50} values are often associated with limit tests, occasionally, standard multi-dose acute toxicity studies result in mortalities which are substantially below 50% and the dose-response relationship may be such that the LD_{50} or other comparable value cannot be estimated. In these instances, a non-definitive LD_{50} is reported in which the *greater than* value is the highest dose or concentration tested.

U.S. EPA/OPP (2008a) summarizes a number of acute oral toxicity studies in rats, which are summarized in Appendix 2, Table 1 of the current Forest Service risk assessment. All of the LD_{50} values reported by U.S. EPA/OPP (2008a) are non-definitive and range from >1920 to >4860 mg a.e./kg bw. No mortality was observed in any of the studies. Thus, the "range" of values merely reflects the range of doses used in the individual toxicity studies, and these ranges do not imply any differences or uncertainties in the acute toxicity of technical grade glyphosate.

The U.S. EPA/OPP uses a general system to categorize acute toxicity from Category I (the most toxic) to Category IV (the least toxic). Details of this system are summarized in U.S. EPA/OPPTS (2003, Table 1, p. 7-2). For acute oral toxicity, all of available acute LD_{50} values for technical grade glyphosate place this pesticide in Category III (LD_{50} >500 mg/kg to 5000 mg/kg). The only less toxic category is Category IV which applies to compounds with acute LD_{50} values >5000 mg/kg bw. The actual acute toxicity of technical grade glyphosate might warrant a Category IV classification, except that the available toxicity studies did not use doses greater than 5000 mg/kg bw. In other words, the classification of technical grade glyphosate as Category III rather than Category IV may be purely an artifact of the doses used in the acute oral toxicity studies reviewed by the U.S. EPA/OPP (2008a).

Other available LD_{50} values for technical grade glyphosate are summarized in Appendix 2, Table 1 of the current Forest Service risk assessment. Most of the LD_{50} values are from reviews (Smith and Oehme 1992; WHO 1994) which summarize unpublished toxicity values, most of which appear to be early studies conducted by Monsanto as well as a goat study conducted by the USDA.

For rats, some of the early LD_{50} studies yielded non-definitive LD_{50} values >5000 mg/kg bw. Other definitive LD_{50} values range from 1568 mg a.e./kg bw for mice (Babaunmi et al. 1978) to 5957 mg a.i./kg bw for rats, using the isopropylamine salt of glyphosate (Baba et al. 1989). Converting a.i. to a.e., the LD_{50} from Baba et al. (1989) corresponds to about 4400 mg a.e./kg bw [5957 mg a.i./kg bw x 0.74 a.e./a.i. = 4408.18 mg a.e./kg bw]. Baba et al. (1989) do not report confidence intervals for the LD_{50} but do provide dose-response data, as summarized in Appendix 2, Table 1. A reanalysis of these data, using probit analysis, yielded an LD_{50} of 5960 (5305-6719) mg a.i./kg bw or about 4410 (3926-4972) mg a.e./kg. The minor difference in the LD_{50} of

5957 mg a.i./kg bw reported by Baba et al. (1989) and the LD_{50} of 5960 mg a.i./kg bw is inconsequential and is associated with the older method used in the Baba paper to calculate the LD_{50}—i.e., Litchfield and Wilcoxon 1949.

Based on the definitive LD_{50} values in mice, rats, rabbits, and goats, there is no apparent relationship between body weight and sensitivity to glyphosate. The study by Baba et al. (1989) is from the Japanese literature. As discussed further in Section 3.1.4.3 (Surfactants) the study by Baba et al. (1989) is the only oral LD_{50} study in mammals that tested glyphosate, a glyphosate/surfactant formulation, and a surfactant alone.

3.1.4.2. Glyphosate Formulations

As summarized in Table 2, the Forest Service has identified 53 formulations of glyphosate that have been used in Forest Service programs. While LC_{50} values are not used as the basis for dose-response assessments in Forest Service risk assessments, acute oral LD_{50} values are useful for comparing relative toxic potency among formulations. Thus, it would be useful to have LD_{50} values for each formulation.

For glyphosate, as well as many other pesticides, this type of information is not available because the U.S. EPA does not require LD_{50} values or other standard acute toxicity studies for every formulation. This approach is taken because some formulations are either identical to or at least very similar to other formulations. For example,as noted in Section 2, Gly-4 Plus (distributed by Universal Crop Protection Alliance) is simply a repackaging of Honcho Plus (distributed by Monsanto). Thus, it would not be sensible to require separate LD_{50} studies for these two formulations, which are identical. Consequently, the U.S. EPA will allow studies on one formulation to be used in support of the registration of other formulations so long as the formulations are identical or at least reasonably similar. This process is sometimes referred to as *data bridging*, in which data on one formulation can be used to support another formulation.

For glyphosate formulations, two sources of acute toxicity information are available: data from the material safety data sheets (MSDSs) and data from studies submitted to the U.S. EPA or studies published in the open literature. MSDS typically report oral LD_{50} values in rats. For the formulations considered in the current Forest Service risk assessment, the oral LD_{50} values for rats from the MSDS are summarized in Appendix 1, Table 1. The acute oral LD_{50} values in rats available from studies submitted to the U.S. EPA or studies published in the open literature are summarized in Appendix 2, Table 2.

Most of the LD_{50} values reported on the MSDS are non-definitive and indicate that the LD_{50} values for most formulations are >5000 mg/kg bw. Based on the categorization system used by the U.S. EPA, as discussed in the previous subsection, these formulations would be classified as Category IV, the least toxic category in the EPA classification system. As discussed in Section 2.2.2, some liquid formulations of glyphosate consist primarily of only a glyphosate salt in water (e.g., Accord and Rodeo). For these formulations, the oral LD_{50} values on the MSDS are given as >5000 mg/kg bw, which is consistent with LD_{50} values for technical grade glyphosate, as discussed in previous subsection. Interest in the oral LD_{50} values for other glyphosate formulations is focused on an attempt to identify formulations that may contain other ingredients, particularly surfactants, which may be of concern in the risk assessment of glyphosate.

LD_{50} values reported on the MSDS should be, and most probably are, related to specific studies submitted to the U.S. EPA—i.e., those studies summarized in Appendix 2, Table 2. These relationships, however, are not always clear. Most MSDS do not provide references to the specific studies used to derive the toxicity values. In addition, the MSDS are specific to a formulation. Most of the toxicity studies submitted to U.S. EPA/OPP do not specify a formulation name; moreover, many of those studies do not specify a product code in their title. Thus, although full studies in some way include the identity of the formulation, that information most often is not evident in the publically available information on a pesticide. Thus, most of the indefinite LD_{50} values given in Appendix 1, Table 1 cannot be directly related to the toxicity values on the MSDS.

In some instances, however, the identity of the studies can be linked directly to the toxicity values given on the MSDS. For example, as noted in Section 2.2.3.7, DowAgro Sciences has identified the GF-1280 formulation code as applying to Accord XRT II, DuraMax, Durango DMA, and RapidFire. These are all 50.2% glyphosate DMA formulations. The MSDS for these formulations indicate a rat oral LD_{50} of >5000 mg/kg bw. In the summary of mammalian toxicity studies, U.S. EPA/OPP (2008a, Appendix J, Table J-26) indicates that the acute oral LD_{50} of GF-1280 is >2005 mg a.e./kg bw and that this value is from MRID 46775603. Back calculating for the percent a.i. (50.2%) and the conversion factor for a.i. to a.e. (0.74), the LD_{50} of >2005 mg a.e./kg bw corresponds to >5397 mg formulation/kg bw [>2005 mg a.e./kg bw ÷ (0.502 x 0.74)], which is consistent with the value of >5000 mg/kg bw on the MSDS, once it is understood that the MSDS value is reported in mg formulation/kg bw.

Most MSDS, however, do not clearly specify the units for the reported LD_{50} values as mg formulation/kg bw, mg a.i./kg bw, or mg a.e./kg bw; furthermore, the units in which the toxicity values are expressed are not consistent. As discussed above, the MSDS for the GF-1280 formulations report the toxicity value for the oral LD_{50} only in units of mg formulation/kg bw. As further discussed below, the MSDS for Roundup UltraDry reports the rat oral LD_{50} as: *5827 mg/kg bw, slightly toxic, FIFRA Category III (LD50 female rats – 3700 mg/kg bw).* In discussing definitive LD_{50} values, U.S. EPA/OPP (2008a, Table 5.5) identifies a rat oral LD_{50} of 5827 mg formulation/kg bw with U.S. EPA registration number 524-504, which corresponds to Roundup UltraDry, and associates this LD_{50} with MRID 44615502. Elsewhere in the EPA's assessment (U.S. EPA/OPP 2008a, Table 4.32), MRID 44615502 is associated with MON 77063 (presumably Monsanto's formulation code for Roundup UltraDry) and this study is described as yielding a rat oral LD_{50} of 5827 mg formulation/kg bw, which is equated to an LD_{50} of 2599 mg a.e./kg bw. The source of the mg a.e. dose given by U.S. EPA/OPP (2008a) is not clear. As summarized in Table 2, Roundup UltraDry is a 71.4% formulation of the monoammonium salt of glyphosate. Thus, a dose of 5827 mg formulation/kg bw would correspond to a dose of about [5827 mg formulation/kg bw x 0.714 (a.i./formulation) x 0.77 (a.i. to a.e.) = 3204 mg a.e./kg]. While the information from (U.S. EPA/OPP 2008a) permits a connection between the value reported on the MSDS to a specific toxicity study, the value of 3700 mg/kg bw given on the MSDS remains unclear. It is possible that the second LD_{50} value of 3700 mg/kg bw is a typographical error for the conversion to 3200 mg a.e./kg bw.

Because LD_{50} values are not used directly in Forest Service risk assessments, the above discussion may seem excessively detailed. Nonetheless, an attempt is made to clearly identify

differences in toxicity among the glyphosate formulations that contain surfactants as well as differences in the toxicity of the surfactants. It is desirable in doing so to identify reported toxicity values with specific studies submitted to the U.S. EPA and to clearly understand the units in which the toxicity values are reported. Because of the nature of the available data on glyphosate formulations as well as proprietary concerns among its suppliers the degree of clarity that can be achieved is limited.

While most of the oral LD_{50} values for the glyphosate formulations designated by the Forest Service (Table 2) are non-definitive and reported as >5000 mg/kg bw, four of the formulations do report definitive oral LD_{50} values on the MSDS. As summarized above, the MSDS for Roundup UltraDry reports an oral LD_{50} of 5827 mg/kg bw, which presumably is given in units of mg formulation/kg bw. Roundup ProDry, which is another 71.4% monoammonium salt of glyphosate, gives an oral LD_{50} of 3794 mg/kg bw. The units for this LD_{50} value are not clear. Ranger Pro and Roundup Pro, both of which are 41% IPA formulations from Monsanto, specify an oral LD_{50} of 5108 mg/kg bw. Another 41% IPA formulation, Helosate Plus from Helm Agro U.S., reports a very similar oral LD_{50} of 5000 mg/kg bw.

All of these oral LD_{50} values are similar to the oral LD_{50} of 5338 mg formulation/kg bw for Roundup reported in the open literature by Baba et al. (1989). The formulation tested by Baba et al. (1989) appears to have been what is now called Roundup Original. Baba et al. (1989) identified the formulation as consisting of 41% glyphosate IPA and 15% surfactant. As with the results for glyphosate IPA (Section 3.1.5.1), Baba et al. (1989) do not provide confidence intervals on the LD_{50} for Roundup but do provided the dose-response data, which is summarized in Appendix 2, Table 2. A reanalysis of these data using probit analysis (Stephan 1976) yielded an LD_{50} of 5046 (4446-5738) mg formulation/kg bw. As noted above, the differences in the LD_{50} values are associated with the use of the Litchfield and Wilcoxon (1949) method by Baba et al. (1989).

U.S. EPA/OPP (2008a, Table 5.5) identifies other definitive LD_{50} values for glyphosate formulations. While the U.S. EPA does not specifically identify the formulations, the EPA registration numbers are identified. As noted above, these EPA registration numbers can be linked to specific formulations using the information from the U.S. EPA label system (http://www.epa.gov/pesticides/pestlabels/index.htm). The definitive formulation LD_{50} values given by U.S. EPA/OPP (2008a) along with the definitive LD_{50} values discussed above are summarized in Table 9 of the current Forest Service risk assessment. When expressed in units of mg a.e./kg bw, the LD_{50} values in Table 9 vary by a factor of about10, ranging from 357 mg a.e./kg bw (HM-2028) to 3204 mg a.e./kg bw (Roundup UltraDry). This range discounts the LD_{50} of 3794 mg/kg because the units (mg formulation, a.i., or a.e.) for this LD_{50} are not clear.

U.S. EPA/OPP (2008a) reports some unit conversions that are not consistent with LD_{50} values derived in this Forest Service risk assessment. As discussed above, the LD_{50} value of 5827 mg formulation/kg bw given on the MSDS for Roundup UltraDry is converted to 3204 mg a.e./kg bw based on the percent a.i. in the formulation (71.4%) and the conversion factor for the monoammonium salt to a.e. (0.77 as specified in Table 1). As indicated in Table 9, U.S. EPA/OPP (2008a, Appendix J, Table J-26) reports the LD_{50} value for this MRID as 2599 mg a.e./kg bw, identifying the formulation as MON 77063. These types of discrepancies are

common in dealing with toxicity data on glyphosate formulations. From a practical perspective, however, the important point for the current Forest Service risk assessment is that none of the more highly toxic formulations summarized in Table 9 are designated by the Forest Service as products that might be used in Forest Service programs.

3.1.4.3. Surfactants

3.1.4.3.1. Acute Oral Toxicity

The information on surfactants which are or may be used in glyphosate formulations is discussed generally in Section 3.1.14. As noted in Section 3.1.1.1, the current risk assessment on glyphosate is somewhat atypical with respect to other Forest Service risk assessments in that available information on surfactants is included in each subsection of this risk assessment in order to distinguish, as clearly as possible, the differences between technical grade glyphosate or glyphosate salts, the surfactants which may be included in certain glyphosate formulations, as well as the formulations themselves.

In terms of acute oral LD_{50} values, relatively little information on surfactants used with glyphosate formulations is available in mammals (Appendix 2, Table 5). Williams et al. (2000) cite an unpublished study by Birch (1977) which reports an acute oral LD_{50} of 1200 mg/kg bw for the POEA surfactant used in the original Roundup formulation. The study by Baba et al. (1989) reports a 72-hour oral LD_{50} of 661 mg/kg bw for the surfactant used in the original formulation of Roundup – i.e., MON 0818 which consists of 75% POEA. As discussed in previous subsections, Baba et al. (1989) also reports LD_{50} values in rats for glyphosate IPA as well as the Roundup mixture. Consequently, an assessment of the joint action of glyphosate with the MON 0818 surfactant can be made, as detailed in the following subsection.

3.1.4.3.2. Joint Action of Glyphosate and Surfactant

The term *joint action* is used as a general designation for both non-interaction – i.e., none of the components in the mixture impact the toxicity of other components in the mixture – as well as interaction – one or more of the components in the mixture impact the toxicity of other components in the mixture. In the current risk assessment, most of the data on joint action involves mixtures of glyphosate with the MON 0818 surfactant used in the original Monsanto formulation of Roundup. While relatively little information is available on the joint action of glyphosate and MON 0818 in mammals, several studies are available on the joint action of glyphosate and MON 0818 in fish (Section 4.1.3.1.2.4), amphibians (Section 4.1.3.2.2.4), and aquatic invertebrates (Section 4.1.3.3.2.4). Consequently, the general approach to the analysis of joint action is given in some detail below and this discussion is referenced in the analyses presented in the ecological risk assessment.

A common model for assessing joint action is dose addition (U.S. EPA 2000). Dose addition is based on the concept of simple similar action as defined by Finney (1971). This form of non-interactive joint action assumes that the components in the mixture behave as if they were concentrations or dilutions of each other differing only in relative potency (***ρ***), which is defined as the ratio of equitoxic doses, such as LD_{50} values. For example, taking ζ_1 and ζ_2 to designate the LD_{50} values for two chemicals, the relative potency is defined as:

$$\rho = \frac{\zeta_1}{\zeta_2}$$

Equation 1

Under the assumption of dose addition, the LD_{50} for a mixture of two chemicals (ζ_M) can be estimated from the LD_{50} values for the two components in the mixture (ζ_1 and ζ_2) and the proportions of the two chemicals in the mixture (designated as π_1 and π_2):

$$\zeta_M = \frac{\zeta_1}{\pi_1 + \rho\pi_2}$$

Equation 2

Equation 2 above is identical to Equation 11.8 in Finney (1971, p. 233). The lower case Greek letter zeta (ζ) is used to designate equally toxic doses, such as LD_{50} values, following the terminology used by Finney (1971). In the addition, the more general designation of ζ is appropriate because ζ can refer to any equitoxic exposure. As discussed further in Section 4.1 (the Hazard Identification for the ecological risk assessment), the concept of dose addition can be applied to LC_{50} values. When applied to LC_{50} values, dose addition is sometime referred to as concentration-addition. While the latter designation may be viewed strictly as more appropriate when applied to LC_{50} values, the current risk assessment uses the term dose addition for both types of applications.

While simple similar action and dose addition are mathematically identical, a subtle but important distinction is maintained in the current risk assessment. The concept of simple similar action, as defined by Finney (1971), has mechanistic implications in that compounds that display simple similar action are assumed to have the same or at least a very similar mechanism of action. Deviations from simple similar action are may be classified with terms such as *antagonism* or *synergism* and both other these terms also have mechanistic implications.

Glyphosate and the surfactants that may be used with glyphosate are very different substances that may cause damage in unrelated ways. Thus, in the current risk assessment, the LD_{50} or LC_{50} of a mixture of glyphosate and a surfactant will be calculated using Equation 2 – i.e., the assumption of dose addition – and compared to the observed LD_{50} or LC_{50}. The comparison will be based on the ratio of the LD_{50} or LC_{50} predicted from Equation 2 to the observed LD_{50} or LC_{50}. These ratios may be referred to interaction ratios (IR). Interaction ratios of approximately one are consistent with the assumption of additivity. Ratios less than one suggest a less than additive joint action and ratios greater than one suggest a greater than additive joint action. While less than additive joint action (IR<1) may sometimes be referred to as antagonism and greater than additive joint action (IR >1) may sometimes be referred to as synergism, the terms *antagonism* and *synergism* are avoided in the current risk assessment to avoid the appearance of mechanistic implications that cannot be supported by the available data on glyphosate, the surfactants used with glyphosate, and formulations of glyphosate that contain surfactants.

The Baba et al. (1989) study was conducted on the original Roundup formulation which consisted of about 41%$_{w/w}$ glyphosate IPA (equivalent to about 30.% $_{w/w}$ glyphosate a.e.) and 15%$_{w/w}$ MON 0818 which contained a POAE surfactant at a concentration of 75%$_{w/w}$. The LD_{50} values reported in Baba et al. (1989) are listed below:

Glyphosate IPA	= 5957 mg a.i./kg bw
Surfactant	= 661 mg surfactant/kg bw
Roundup	= 5338 mg formulation/kg bw.

Using glyphosate IPA as chemical 1 (i.e., ζ_1 in Equation 1), the potency of the surfactant relative to glyphosate IPA is about 9:

$$\rho = \frac{5957 \text{ mg a. i./kg bw}}{661 \text{ mg surfactant/kg bw}} = 9.0121\ a.i./surfactant$$

Equation 3

In other words, based on the LD_{50} values, the surfactant is about 9 times more toxic than glyphosate IPA.

For the Roundup formulation tested by Baba et al. (1989), the proportion of glyphosate IPA (π_1) in the mixture is 0.41 (41%) and the proportion of the surfactant in the Roundup formulation (π_2) is 0.15 (15%). Thus, under the assumption of dose addition (Equation 2), the expected LD_{50} of the Roundup formulation would be:

$$\zeta_{Roundup} = \frac{5957 \text{ mg a. i./kg bw}}{0.41_{ai/form} + (9_{ai/surf} \times 0.15_{surf/form})} \cong 3385\ mg\ formulation/kg\ bw$$

Equation 4

In the denominator of the above equation, the subscripts explicitly note units. In Equation 4, the subscripts are abbreviated *ai/form* (for active ingredients/formulation), *ai/surf* (for active ingredient/surfactant), and *surf/form* (for surfactant/formulation). These somewhat unconventional abbreviations and other similar abbreviations – e.g., *ae/form* for acid equivalents/formulation) – are used above and in other similar equations in the current risk assessment for the sake of brevity.

While the proportion of the a.i. in the formulation as well as the proportion of surfactant in the formulation may often be regarded as unitless, this is not actually the case. For example, if the formulation contains the surfactant at a concentration of 15% w/w, the proportion of the surfactant in the formulation is actually in units of mg surfactant/mg formulation or surfactant/formulation. The use of explicit units in Equation 4 is intended to clearly indicate the estimated LD_{50} is in units of mg formulation/kg bw rather than mg a.i./kg bw. In other words, the numerator of Equation 4 has units of mg a.i./kg bw and the denominator has units of a.i./formulation. Thus, the resulting calculation has units of mg formulation/kg bw.

The observed LD_{50} of the Roundup formulation is reported in Baba et al. (1989) as 5338 mg formulation/kg bw, which is to say, the observed LD_{50} is higher than the expected LD_{50}. For the data reported by the Babe et al. (1989), the interaction ratio is about 0.6 [3385 mg formulation/kg

bw ÷ 5337 mg formulation/kg bw ≈ 0.6343], indicating that the joint action of glyphosate and POEA is less than additive.

While the mathematics of dose addition (i.e., Equation 2) are not particularly complicated, there are several different ways in which the assumption of dose addition can be formulated and these differences can lead to errors in the calculation of the predicted value, ζ, for the mixture. These errors can be minor, due to rounding, or substantial if the units used in the calculations are not properly formulated.

For example, Equation 4 could be modified to calculate the expected LD_{50} for Roundup in terms of acid equivalents and level of POEA in the Roundup surfactant. In this modification, the LD_{50} of 5957 mg a.i./kg bw for glyphosate IPA would be adjusted to 4408 mg a.e./kg bw [5957 mg a.i./kg bw x 0.74 a.e./a.i. = 4408 mg a.e./kg bw]. The MON 0818 surfactant used in the original Roundup formulation consists of 75% POAE. Thus, the LD_{50} of the surfactant, 661 mg surfactant/kg bw, would be adjusted to about 496 mg POEA/kg bw [661 mg surfactant/kg bw x $0.75_{POEA/surfactant}$ = 495.75 mg POEA/kg bw]. Note that if this approach is taken, the proportions of the components in the mixture must also be adjusted. In this example, the proportion of glyphosate acid in the formulation must be adjusted to $0.3034_{a.e./form}$ [$0.41_{a.i./form}$ x $0.74_{a.e./a.e.}$] and the proportion of POEA in the formulation must be adjusted to $0.1125_{POEA/form}$ [$0.15_{MON\ 0818/form}$ x $0.75_{POEA/MON\ 0818}$]. Lastly, the relative potency must be redefined as the ratio of the LD_{50} expressed in acid equivalents to the LD_{50} of the surfactant expressed as POEA:

$$\rho = \frac{4408\ \text{mg a. e./kg bw}}{496\ \text{mg POEA/kg bw}} = 8.89194\ a.e./POEA$$

Equation 5

Taking the relative potency as $8.9_{a.e./POEA}$, the toxicity of the formulation may then be calculated as about 3379 mg/kg bw:

$$\zeta_{Roundup} = \frac{4408\ \text{mg a. e./kg bw}}{0.3034_{ae/form} + (8.9_{ae/POAE} \times 0.1125_{POEA/form})} \cong 3379\ mg\ formulation/kg\ bw$$

Equation 6

Note that the predicted LD_{50} for the formulation using the a.i. and surfactant units in Equation 4 is about 3385 mg formulation/kg bw whereas the result given in Equation 6 is 3379 mg formulation/kg bw, lower than the result of Equation 4 by about 0.2%. This difference is due entirely to rounding errors in calculating values of relative potencies. If the values of relative potency are not rounded, both Equation 4 and Equation 6 yield same estimate of the LD_{50}, about 3381.17 mg formulation/kg bw.

Rounding errors are typically trivial although these errors can be a source of confusion. The errors in the adjustments that must be made if different methods are used in the application of Equation 2, however, can lead to errors that are substantial. While the discussion of units in the application of dose addition as well as the discussion of rounding may seem and perhaps is somewhat pedantic, errors in the application of Equation 2 were noted in the previous Forest Service risk assessment of glyphosate (SERA 2003). In the preparation of the current Forest Service risk assessment, additional errors were noted in the peer review draft.

In order to more clearly document the application of dose addition and reduce the potential for errors, all applications of Equation 2 in the current risk assessment clearly specify the units for proportions (i.e., π_1 and π_2 in Equation 2) as well as the units for relative potency and the LD_{50} or LC_{50} used in the numerator of Equation 2). In addition, all calculations presented in the text are based on the rounded values presented in the text. While this may lead to very minor rounding errors, as illustrated above, this approach will facilitate the independent verification of the values presented in the risk assessment. In other words, an individual checking the calculations should be able to reproduce all of the calculations based on Equation 2 if the specific examples (e.g., Equation 4 and Equation 6) are checked with the numbers given in the text.

Lastly and as an additional check of the application of dose addition, all calculations presented in the text of this risk assessment are also included in Attachment 3, an EXCEL workbook. This workbook consists of a series of worksheets for each application of dose addition discussed in this risk assessment. Each worksheet designates the specific study covered by the worksheet as well as the section in the risk assessment in which the study is discussed. For example, the worksheet named "Baba et al. 1989" duplicates the calculations of the Baba et al. (1989) study given in Equation 4. The rounding conventions used in these worksheets are identical to the rounding conventions used in the text of the risk assessment. The worksheets are structured, however, in a manner that allows the user to change the rounding used in the calculations. Thus, if values in column labeled "Rounding" in worksheet "Baba et al. 1989" are increased to a large value such as 10, the predicted LD_{50} is about 3381.17 mg formulation/kg bw. As discussed above with respect to Equation 6, a dose of 3381.17 mg formulation/kg bw is the predicted value (without rounding errors) of the LD_{50} for Roundup based on the study by Baba et al. (1989).

3.1.4.4. Suicides and Suicide Attempts Involving Glyphosate Formulations

Formulations of glyphosate with a POEA surfactant have been used in many suicides and attempted suicides. The published literature on human poisonings is summarized in Appendix 2, Table 6. These publications include individual case reports (Chang and Chang 2009; Hsiao et al. 2008; Moon et al. 2006; Pushnoy et al. 1998; Sampogna and Cunard 2007; Stella and Ryan 2004; Temple and Smith 1992;) as well as the analyses of poisoning incidents (Chen et al. 2009; Lee et al. 2008; Nagami et al. 2005; Sawada et al. 1988; Talbot et al. 1991; Tominack et al. 1991; Yang et al. 1997; Weng et al. 2008; Wu et al. 2006). Most, but not all, of the analyses of poisoning incidents also involve suicidal ingestion. The largest number of incidents is reported from the orient (i.e., China, Japan, South Korea, and Taiwan). Only one suicide attempt has been reported in the United States (Sampogna and Cunard 2007). Although most incidents involve ingestion , there are two reports of suicide attempts by injection, one involving intramuscular injection (Weng et al. 2008) and the other involving intravenous injection (Wu et al. 2006).

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, have been observed in human cases of glyphosate/surfactant exposure. As detailed in Section 3.1.11, the POEA surfactants used in glyphosate formulations (e.g., various formulations of Roundup) are a factor, and probably the dominant factor, in some of the effects seen in humans in cases of suicidal ingestion of glyphosate formulations. Surfactants, including the POEA surfactants used in

Roundup and other glyphosate formulations will break down and essentially dissolve biological membranes. Thus, ingestion of a large quantity of a surfactant damages the integrity of the gastrointestinal tract.

Although suicide attempts are not directly germane to the current Forest Service risk assessment, they are useful for assessing the relative sensitivity of humans to the toxicity of glyphosate formulations. Most of the reports of suicide incidents involving glyphosate formulations do not involve reliable estimates of exposure. Uncertainties regarding dose are common issues in the assessment of suicide attempts with pesticides.

Some of the case studies summarized in Appendix 2 (Table 6), however, do provide relatively detailed estimates of exposure, and these studies are summarized in Table 11. While these studies all provide estimates of the amount of the glyphosate formulations that were consumed, none of the studies provides information on the body weights of the individuals. As a crude approximation, standard body weights of 60 kg for females and 70 kg for males are used. The amount of formulation consumed is specified in these studies in units of mL. To estimate the doses in units of mg formulation/kg bw, a density of 1.2 g/mL is used. This density is reasonably close to the density of many glyphosate formulations.

Table 11 summarizes eight case reports of suicidal ingestions of glyphosate formulations, four of which resulted in mortality with estimated doses ranging from 4500 to about 17,000 mg formulation/kg bw. In the other four cases, the individuals survived doses estimated to range from about 1700 to 5000 mg formulation/kg bw. The geometric mean of all doses from Table 11 is 5337 mg formulation/kg bw, which is identical to the LD_{50} for Roundup in rats (Baba et al. 1998). While this exact correspondence is most certainly coincidental, the mortality data from individual case reports suggest that lethal doses in humans are similar to lethal doses in rats.

The summaries of case reports are also useful in estimating the acute lethal toxicity of glyphosate/surfactant formulations. In the analysis of poisoning incidents associated with suicides or attempted suicides in Taiwan (Lee et al. 2000), fatalities were associated with doses of glyphosate/surfactant formulations in the range of 330±42 mL; whereas, survival of poisoning incidents was associated with doses of 122±12 mL. Again using a formulation density of 1.2 g/mL but assuming an average body weight of 65 kg (i.e., males and females combined), the estimated average dose from Lee et al. (2000) is about 2252 mg formulation/kg bw for nonfatal exposures and 6092 mg/kg bw for fatal exposures, and the average of these two values is about 4200 mg formulation/kg bw. Again, the value of 4200 mg formulation/kg bw cannot be regarded as a human LD_{50}; nevertheless, the Lee et al. (2000) data are consistent with the assertion that the acute lethal potency of glyphosate/surfactant formulations is comparable in humans and rats.

The concordance between rats and humans is important to the current risk assessment because the toxicity values used in the current Forest Service risk assessment are based on toxicity studies conducted with rats. As discussed further in Section 3.3 (Dose-Response Assessment), the toxicity values used directly to characterize risks to humans involve uncertainty factors based on the assumption that humans are more sensitive than experimental mammals. For many pesticides, this assumption cannot be evaluated very well, due to the limited amount of

quantitative data regarding human sensitivity to pesticides. Despite the limitations of the available information involving suicide attempts with glyphosate formulations, the information does indicate that humans and rats are essentially equally sensitive to the acute lethal effects of glyphosate formulations.

3.1.5. Subchronic or Chronic Systemic Toxic Effects

Systemic toxicity encompasses virtually all effects of chemical absorption. Certain types of effects, however, are of particular concern and are considered below as they relate to the nervous system (Section 3.1.6), immune system (Section 3.1.7), development or reproduction (Section 3.1.8), and carcinogenicity or mutagenicity (Section 3.1.9). This section encompasses the remaining signs of general and non-specific toxicity.

3.1.5.1. Technical Grade Glyphosate

Studies on the subchronic and chronic toxicity of glyphosate are summarized in Appendix 2, Table 4. Most of the subchronic and chronic toxicity studies on technical grade glyphosate are unpublished studies submitted to U.S. EPA/OPP in support of the registration of glyphosate. As indicated in Appendix 2, Table 4, summaries of these studies are taken from U.S. EPA/OPP (1993b), the U.S. EPA/OPP Science Chapter prepared in support of the Reregistration Eligibility Decision (RED) document for glyphosate (U.S. EPA/OPP 1993a). In Appendix 2, the registrant-submitted studies are cited by MRID number, and full references to these studies are included in the RED (U.S. EPA/OPP 1993a). These studies are also discussed in Williams et al. (2000).

One of the more consistent signs of subchronic or chronic exposure to glyphosate is decreased body weight gain. This effect has been observed in mice (MRIDs 40559401, 00036803, 00130406 and 00150564; NTP 1992), and rats (MRID 41643801; NTP 1992). As summarized in Appendix 2, Table 3, decreases in body weight gain are also reported in several reproduction studies, including, Daruich et al. (2001), Farmer et al. (2000a,b), Beuret et al. (2005) as well as a subchronic oral toxicity studies in male rabbits (Yousef et al. 1995). The reproduction studies are discussed further in Section 3.1.8.

Decreased body weight gain 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. Decreased body weight gain, particularly in studies using dietary exposure, can also be secondary to decreased food consumption. In the NTP bioassay conducted with 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 observed weight loss was not associated with a decrease in food consumption (Yousef 1995). In the NTP study conducted with 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.

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 (MRID 41643801; NTP 1992). Signs of kidney toxicity, which might be expected based on observations from human suicide attempts (Appendix 2, Table 6), have not been reported consistently and are

not severe (e.g., MRIDs 00130406 and 00150564; NTP 1992). As summarized by NTP (1992), various hematological changes have been observed in rats and mice at high doses; however, these effects are attributed to mild dehydration and are not associated with overt signs of toxicity.

3.1.5.2. Glyphosate Formulations

Subchronic and chronic toxicity studies on formulations are not required for pesticide registration, and no such registrant-submitted studies have been identified in the glyphosate literature. One subchronic toxicity study on a glyphosate formulation is published in the open literature (Benedetti et al. 2004). This study involved a Brazilian formulation, Glyphosate-Biocarb. A product label and MSDS for this formulation have not been located. In the Benedetti et al. (2004) publication, the formulation is described as containing glyphosate IPA at a concentration of 480 g a.i./L (360 g a.e./L) with 18% (w/v) of a polyoxyethyleneamine surfactant. Note that the concentration of 480 g glyphosate IPA/L corresponds to many 41% w/w formulations included in Table 2, including, Accord SP, Glyp-4 Plus, Honcho, Razor, Razor Pro, and Ranger Pro. All of the 41% w/w formulations in Table 2 indicate glyphosate acid equivalent concentrations of 356 g a.e./L. The report of 360 g a.e./L by Benedetti et al. (2004) has been noted on several South American labels and some European studies (Benachour et al.2007b). The different reports of acid equivalents may reflect a simple difference in rounding conventions.

As summarized in Appendix 2, Table 4, Wistar rats were dosed at 4.87, 48.7, or 487 mg /kg bw every other day for 75 days. The doses appear to be expressed in units of formulation. The Benedetti et al. (2004) publication focuses on signs of liver toxicity. Based on biochemical indices of toxicity—increased serum ALT—effects were noted at all doses, although the differences between the lowest and highest doses were not remarkable. Liver pathology was observed only at the highest dose.

Benedetti et al. (2004) do not provide information on body or organ weights, food consumption, or signs of toxicity. Thus, it is difficult to compare the results of this study with the results of subchronic studies on glyphosate acid. Assuming that Benedetti et al. (2004) used a 41% w/w IPA formulation, the conversion factor for formulation dose to an a.e. dose would be about 0.3 [0.41 x 0.74 ≈ 0.3034]. Thus, the doses in the Benedetti et al. (2004) study correspond to about 3.6, 36, and 360 mg a.e./kg bw/day. It seems reasonable to assume that Benedetti et al. (2004) would have reported overt toxic effects if any had been noted. The lack of reported overt toxic effects at doses up to 360 mg a.e./kg bw/day is consistent with the NOAEL of 500 mg a.e./kg bw/day from the 90-day study in mice (MRID 00036803). The biochemical changes noted at low doses are consistent with the 90-day feeding study in rats (MRID 40559401) in which effects were noted at doses of 63 mg a.e./kg bw/day. Both of these MRID studies are summarized in Appendix 2, Table 4.

3.1.5.3. POEA Surfactant

As summarized in Appendix 2, Table 5, two subchronic toxicity studies have been conducted in rats and one subchronic toxicity study has been conducted in dogs. None of these studies, briefly summarized in the review by Williams et al. (2000) and apparently conducted by or for Monsanto, is published in the open literature.

Both studies in rats as well as the study in dogs note gastrointestinal irritation as a prominent effect. In rats, this effect was noted at a dietary concentration of 1500 ppm (mg POEA/kg diet). Based on food consumption rates provided by Williams et al. (2000) for rats dosed at 500 ppm, gastrointestinal irritation occurred at a dose of about 100 mg/kg bw. As discussed in Section 3.1.4.4, gastrointestinal irritation is commonly noted in cases of suicidal ingestion of glyphosate formulations, and damage to the gastrointestinal tract is generally attributed to the POEA surfactant. The NOAEL for POEA in rats appears to be 500 ppm, corresponding to a dose of about 36 mg/kg bw (Williams et al. 2000).

In the dog study summarized by Williams et al. (2000), irritation to the gastrointestinal tract was noted over doses which may have been lower than 30 mg/kg bw, but the review does not specify the doses used early in the study. A dose of 90 mg/kg bw/day used over the last 10 weeks of the dog study is associated with decreased body weight gain. The magnitude of the decrease, however, is not specified in the review. Williams et al. (2000) also note that a *slight* (NOS) decrease in body weight gain was observed in female dogs at doses of 30 and 60 mg/kg bw but that the decreases were… *not always dose related*.

While the summary of the subchronic toxicity data by Williams et al. (2000) is not very detailed, this summary is consistent with the acute toxicity data suggesting that POEA surfactants are more toxic than technical grade glyphosate. Quantitative comparisons between technical grade glyphosate and POEA surfactants, however, are difficult both because of the limited details available on the POEA studies and the differences regarding the experimental designs of the studies on glyphosate and POEA. For example, the NTP (1992) study and MRID 40559401appear to be comparable to the subchronic dietary studies on POEA—all are subchronic feeding studies. As noted in Appendix 2, Table 4, however, neither subchronic study with technical grade glyphosate establishes a clear NOAEL. Specifically, MRID 40559401 (U.S. EPA/OPP 1993b, pp. 4) notes changes in serum biochemistry at a dose of 63 mg a.e./kg bw/day, which is not remarkably different from the LOAEL for POEA in rats of about 100 mg/kg bw.

For dogs, however, the quantitative differences between technical grade glyphosate and POEA are clear. As summarized in Appendix 2, Table 4, no adverse effects were seen in dogs administered glyphosate in capsules for 1 year at a dose of 500 mg/kg bw/day (MRID 00153374, U.S. EPA/OPP 1993b, p. 6). The subchronic dog study with POEA, as summarized by Williams et al. (2000), notes clear adverse effects at 90 mg/kg bw/day and equivocal adverse effects at doses as low as 30 mg/kg bw/day over a much shorter period of exposure. Based on this comparison, POEA appears to be about 10 times more toxic than technical grade glyphosate to dogs, which is remarkably similar to the relative potency of a POEA surfactant to glyphosate IPA, based on acute oral LD_{50} values in which the POEA surfactant is 9 times more toxic than glyphosate IPA (Section 3.1.4.3).

3.1.6. Effects on Nervous System

In severely poisoned animals, virtually any chemical may cause gross signs of toxicity which can be attributed to neurotoxicity—e.g., incoordination or convulsions. A direct neurotoxicant, however, is defined as a chemical that interferes with the function of nerves, either by interacting with nerves directly or by interacting with supporting cells in the nervous system. This definition of a direct neurotoxicant distinguishes agents that act directly on the nervous system

(direct neurotoxicants) from those agents that might produce neurological effects secondary to other forms of toxicity (indirect neurotoxicants). U.S. EPA has developed a battery of assays to test for neurotoxicity (U.S. EPA/OCSPP 2010), and U.S. EPA/OPP requires neurotoxicity studies for pesticides when standard toxicity studies or other considerations such as chemical structure suggest that concerns for effects on the nervous system are credible.

3.1.6.1. Technical Grade Glyphosate

Glyphosate is sometimes referred to as an organophosphate (e.g., Boutin et al. 2004). The term *organophosphate*, however, is more commonly used to designate a group of neurotoxic insecticides. As illustrated in Figure 7, the structure of glyphosate and organophosphate insecticides is only superficially similar. Structurally, glyphosate can be viewed as a substituted phosphorous acid. Organophosphate insecticides can be viewed as substituted phosphoric acids, and the nature of the substitution is somewhat specific—i.e., either methyl or ethyl groups along with a leaving group. The leaving group is important in terms of the mechanism of neurotoxicity because loss of the leaving group allows for covalent binding to (i.e., phosphorylation of) enzymes such as AChE which are important to normal neurological function (Anthony et al. 1996; NPIC 2010a).

In the Reregistration Eligibility Decision (RED) document for glyphosate (U.S. EPA/OPP 1993a), the U.S. EPA notes that standard toxicity studies of glyphosate do not suggest that this pesticide is neurotoxic and that specific toxicity tests for neurotoxicity are not necessary:

> *The acute and 90-day neurotoxicity screening battery in the rat (guidelines 81-8-SS, 82-7) is not being required since there was no evidence of neurotoxicity seen in any of the existing studies at very high doses and this chemical lacks a leaving group; therefore, it would not seem likely to inhibit esterases (the presumptive neurotoxic mechanism of concern for all organophosphates).*
>
> U.S. EPA/OPP 1993a, p. 18

As noted above, the reference to a *leaving group* in the above quotation refers to the lack of structural element on the phosphorus atom in glyphosate which would be indicative of a neurotoxic agents, such as a halide, sulfur, or thiocyanate group.

Subsequent to the RED, standard neurotoxicity studies on glyphosate were conducted, including an acute and subchronic neurotoxicity studies in rats (Horner 1996a,b) and a delayed neurotoxicity study in hens (Johnson 1997). In the acute study by Horner (1996a), 10 male and 10 female rats were given doses of 50, 100, or 200 mg glyphosate a.e./kg and observed for 2 weeks. Initially—i.e., 6 hours after dosing —the animals exhibited decreased activity, subdued behavior, and hypothermia. There were, however, no effects on landing foot splay, sensory perception, muscle strength, or locomotor activity and no abnormal histological changes in the central or peripheral nervous system tissue. In the subchronic study (Horner, 1996b), groups of 12 male and 12 female rats were exposed to dietary concentrations of 2000, 8000, or 20,000 ppm glyphosate for 13 weeks. Although effects were noted on growth and food consumption, there were no neurological effects, based on locomotor activity, no changes in brain weight or dimensions, and no evidence of damage to nerve tissue (peripheral or central).

In hens (n=20) given a single dose (gavage) of glyphosate at 2000 mg/kg, a slight decrease in brain acetylcholinesterase (AChE) activity was observed, but there were no signs of delayed locomotor ataxia and no signs of neuropathology (Johnson 1997). The lack of AChE inhibition has also been confirmed in studies on ducks with a granular glyphosate formulation used in Mexico (Osten et al. 2005) and mollusks exposed to technical grade glyphosate (Da Silva et al. 2003). As noted above, glyphosate is not be expected to inhibit AChE.

A study by El-Demerdash et al. (2001) does report an IC_{50} for the *in vitro* inhibition of human *serum AChE* of 714.3 mM. The term *serum AChE* is italicized because serum does not contain acetylcholinesterase (AChE). In blood, acetylcholinesterase is in red blood cells and pseudocholinesterase is in plasma. It is not clear whether this study was conducted with technical grade glyphosate or a formulation. Since the IC_{50} is reported in molar units, it is reasonable to conclude that the IC_{50} is reported as the a.e. The 714.3 mM corresponds to a concentration of about 120,700 mg a.e./L—i.e., about a 12% solution of glyphosate, which is a factor of about 140,000 higher than would be found in plasma after a nontoxic dose of glyphosate—i.e., 0.86 mg a.e./L, as summarized in Table 10. As also noted in El-Demerdash et al. (2001, Figure 1, p. 33), concentrations of glyphosate up to 2000 mM (≈338,000 mg/L) result in only about 60% inhibition of *ChE*. Thus, the *in vitro* concentrations used by El-Demerdash et al. (2001) are implausibly high, and this study does not contradict the assessment by U.S. EPA/OPP (1993a) regarding the neurotoxicity of glyphosate.

In the subchronic studies in mice and rats (NTP 1992), morphological examinations were conducted on brain tissue (including basal ganglia, a site of injury in Parkinsonism); however, it is unclear from the report whether or not spinal cord and sciatic nerve tissues were examined. Nonetheless, NTP (1992) does not report abnormal findings in these tissues; moreover, it does not report clinical signs of neurotoxicity. In the NTP (1992) study, histological changes in salivary glands were observed in both rats and mice. These changes were less severe in animals that received glyphosate in combination with a dose of propranolol, an antagonist of β-adrenergic neurotransmitters. Propranolol also completely prevented similar changes produced by isoproterenol, a β-adrenergic agonist. NTP (1992) concludes from these results that glyphosate may have produced the salivary gland changes by acting through an adrenergic mechanism. This conclusion has been challenged as being difficult to reconcile with the absence of β-adrenergic effects (e.g., on heart rate and blood pressure) when glyphosate was administered intravenously to dogs or rabbits (Williams et al., 2000). Nevertheless, it is possible that rather than acting by a direct adrenergic mechanism, glyphosate could have produced an adrenergic-mediated stimulation of the salivary glands through some indirect mechanism exerted during prolonged repeated dosing.

Schiffman et al. (1995) studied the effects of glyphosate on taste response in gerbils. This study appears to be the only reported investigation of the effects of glyphosate on sensory mechanisms in mammals. Glyphosate (1 and 10 mM, equivalent to 169-1690 mg/L) applied to the tongue of anesthetized gerbils decreased taste receptor response to table salt, sugars, and acids. These tests on glyphosate involved exposure periods of 1 minute and were conducted along with tests on 10 other pesticides, with 1 minute rinses between each agent. The mechanism that caused the taste response has not been investigated, and its implications for dietary preferences in the field cannot be assessed. The effect could have been produced by a general biochemical alteration in the

epithelial cells of the tongue, including the specialized cells that detect taste, by chemical injury to the tongue, or by a direct neurotoxic effect on the sensory nerve endings. Thus, effects reported in Schiffman et al. (1995) cannot be classified clearly as a glyphosate-induced neurological effect.

3.1.6.2. Glyphosate Formulations

The only mammalian study regarding the neurotoxicity of a glyphosate formulation is an unpublished study by Monsanto, which is summarized in the Williams et al. (2000) review and cited as *Naylor (1988)*. This study is not cited in any EPA documents on glyphosate and is not listed in the compendia of registrant-submitted studies from U.S. EPA/OPP (Supplement 1). The summary by Williams et al. (2000) indicates that dogs were given a single oral dose of 59 or 366 mg/kg of Roundup. According to Williams et al. (2000):

> *"A detailed examination consisting of 12 different measurements of spinal, postural, supporting, and consensual reflexes was performed before treatment, during the post administration observation period, and again on the following day. Reflexes appeared normal, and there were no clinical signs indicative of neuromuscular abnormalities."*

As discussed in Section 3.1.4.4, many human suicide attempts involving glyphosate formulations are documented, and most appear to involve formulations of Roundup or other formulations of glyphosate which contain surfactants. In the hundreds of reported cases, neurological symptoms unrelated to respiratory tract distress and shock (confusion, drowsiness, collapse, coma) associated with severe acute toxicity cannot be identified. In a review of 92 cases, only 11 individuals were reported as having an abnormal mental state prior to the onset of severe respiratory and/or cardiovascular complications. In most of these cases, the individuals received atropine or pralidoxime, neurotoxicants used as antidotes for certain organophosphate insecticides that inhibit acetylcholinesterase (in these cases, organophosphate intoxication and cholinesterase inhibition was suspected, although glyphosate is not a potent cholinesterase inhibitor) (Tominack et al., 1991). In a review of 93 cases, 12 individuals were reported as having neurological symptoms (confusion, coma), two of which occurred after cardiovascular resuscitation. The causes of symptoms in 10 other cases were not distinguished from secondary respiratory tract and/or cardiovascular distress (Talbot et al., 1991). Thus, the weight of evidence suggests that neurological signs and symptoms associated with the suicidal ingestion of glyphosate-surfactant formulations were secondary to other toxic effects.

Reports of non-suicidal human exposures to glyphosate formulations also do not provide any compelling indication that glyphosate formulations are neurotoxic. Garry et al. (2002) conducted a self-reporting survey of individuals exposed to herbicides and other pesticides, including glyphosate. This study reports that 6 of 14 children of parents who used phosphonamino herbicides had parent-reported attention-deficit disorder (ADD) or attention-deficit hyperactivity disorder (ADHD). Garry et al. (2002) indicates that the odds ratio for the association between glyphosate exposure and attention deficit disorder is statistically significant (3.6 with 95% confidence intervals of 1.35-9.65).

Note that *odds ratio* is a term for the chance of an event occurring in one group divided by the chance of the event occurring in another group. In the case of the Garry et al. (2002) study as

well as other odds ratios cited in this risk assessment, the numerator for the odds ratio is associated with a group exposed to glyphosate and the denominator is associated with a group not exposed to glyphosate. Thus, if the odds ratio is greater than 1, an association is suggested. If the lower bound of the confidence interval is greater than 1, then the association may be considered statistically significant.

While the reported association by Garry et al. (2002) is statistically significant, it should be appreciated that the use of lay diagnosed disease and self-reported exposure histories diminishes the ability of the study to demonstrate a causal association between glyphosate exposure and attention deficit disorder. While Garry et al. (2002) notes that the parent-reported diagnoses were reviewed by a physician, it is not clear that the diagnoses were clinically confirmed. Finally, as noted by Acquavella et al. (2006a), self-reported exposures are not highly correlated with levels of exposure that can be verified by biomonitoring. Garry et al. (2002) offer a reasonably conservative assessment of their results: … *our present study shows a tentative associationbetween ADD/ADHD and use of this herbicide* (Garry et al. 2002, p. 447). Since the time of this publication in 2002, no additional studies further clarifying this tentative association between ADD and glyphosate exposure were found in the glyphosate literature.

A recent publication (Bouchard et al. 2010) notes an association between ADHD and levels of urinary metabolites of organophosphate pesticides. This association is based on generic metabolites of organophosphates (i.e., diethyl and dimethyl phosphates, thiophosphates, dithiophosphates). As illustrated in Figure 7 and discussed further in a National Pesticide Information Center monograph (NPIC 2010a), these metabolites would be associated with exposures to organophosphate insecticides. These metabolites, however, would not be associated with exposure to glyphosate.

Ptok (2009) reports an unusual incident in which an individual used an unspecified glyphosate formulation and subsequently developed difficulty speaking, which lasted for approximately 6 weeks. In discussing this case, Ptok (2009) notes that:

> *Glyphosate neurotoxicity has been discussed in the literature therefore, the dysphonia observed here may have been due to an intermittent neuropraxia of the laryngeal nerve.*

While it is true that glyphosate neurotoxicity is *discussed in the literature*, the discussion in the literature does not suggest that glyphosate is a neurotoxin. Thus, to suggest that glyphosate exposure caused the impairment of speech seems highly speculative. As noted further by Ptok (2009), no other similar cases of speech impairment associated with glyphosate exposure have been reported. Given the large number of survivors in glyphosate suicide attempts with no subsequent reports of speech impairment (Appendix 2, Table 6), the association suggested by Ptok (2009) does not seem credible.

Similarly, Barbosa et al. (2001) report a case of Parkinsonism in an adult male exposed to glyphosate. Parkinsonism is a degenerative disease of the central nervous system which impairs movement. The subject of the Barbosa et al. (2001) report is a 54-year old male who experienced an extensive dermal exposure to the herbicide while spraying a garden. The acute

and transient symptoms included eye irritation (*conjunctival hyperemia*) and skin rash which progressed to blisters. One month after the exposure, the individual developed hand tremors and was diagnosed with Parkinsonism, based on the results of a neurological examination and brain imaging. Parkinsonism is a chronic degenerative disorder which may have been present in the patient prior to the exposure.

While the case reported by Barbosa et al. (2001) may have involved gross over-exposure to glyphosate, this over-exposure, in itself, is not dismissive of a possible neurological risk. As noted above, extreme and sometimes fatal over-exposures to glyphosate are not generally associated with neurologic effects. In addition, there is, at least, a tenuous biological basis for suggesting a potential association. Glyphosate is a structural analog of glycine, a physiological agent that serves as an inhibitory neurotransmitter in the CNS. Glycine, which is also a naturally occurring amino acid and is essential for normal growth and development, has been implicated as an excitotoxin when present at high concentrations in brain tissue (Johnson and Ascher, 1987; Newell et al., 1997). Excitotoxicity has been hypothesized as a possible mechanism of Parkinsonism induced by the neurotoxicant MPTA (1-methyl-4-phenyl–2-3-6-tetrahydropyridine) and N-methylamino-L-alanine (Kanthasamy et al., 1997; Karcz et al., 1999; Spencer et al., 1987).

At this point, there is no evidence to conclude that glyphosate can produce or exacerbate Parkinsonism; indeed, the Barbosa et al. (2001) observation stands in contrast to the abundant case literature which suggests that glyphosate is not a neurotoxicant in humans. The possible connection between the onset of Parkinsonism and the exposure to glyphosate cannot be established from the single case reported by Barbosa et al. (2001), as the apparent concurrence of the two effects could be coincidental. A coincidental association is suggested by the fact no other cases of glyphosate-related Parkinsonism have been reported in the literature in the nearly 10-year period since the Barbosa et al. (2001) publication. Thus, as with the report by Ptok (2009) on speech disorder, the report by Barbosa et al. (2001) is essentially anecdotal and does not demonstrate a causal relationship between glyphosate and the development of Parkinsonism.

3.1.7. Effects on Immune System

There are various methods for assessing the effects of chemical exposure on immune responses, including assays of antibody-antigen reactions, changes in the activity of specific types of lymphoid cells, and assessments of changes in the susceptibility of exposed animals to resist infection from pathogens or proliferation of tumor cells. Typical subchronic or chronic animal bioassays conduct morphological assessments of the major lymphoid tissues, including bone marrow, major lymph nodes, spleen and thymus (organ weights are sometimes measured as well), and blood leukocyte counts. These assessments can detect signs of inflammation or injury indicative of a direct toxic effect of the chemical on the lymphoid tissue. Changes in morphology of lymphoid tissue and blood, indicative of a possible immune system stimulation or suppression, can also be detected.

3.1.7.1. Technical Grade Glyphosate

With the exception of skin sensitization studies, specific studies regarding the effects of pesticides on immune function are not required for pesticide registration. Thus, no registrant-submitted studies —i.e., those with MRID numbers —are available on technical grade glyphosate, other than skin sensitization studies. As discussed further in Section 3.1.11.2,

glyphosate and glyphosate formulations do not cause skin sensitization under standard test protocols designed by the U.S. EPA. As noted in the previous discussions of subchronic and chronic toxicity studies (Section 3.1.5), none of the studies conducted on technical grade glyphosate report morphological abnormalities in tissues indicative of an effect on the immune system. In an *in vitro* study using human natural killer cells or cytotoxic T cells (Flaherty et al 1991), technical grade glyphosate had no adverse effect on the function of these immune cells at concentrations ranging from 0.01 to 10 µM (i.e.,≈1.7 µg/L or 1.7 mg/L).

3.1.7.2. Glyphosate Formulations

The potential for Roundup to impact immune function has been assayed in one *in vivo* study (Blakley 1997) and one *in vitro* study (Flaherty et al. 1991). In the *in vivo* study by Blakley (1997), mice were exposed for 26 days to Roundup in drinking water (0, 0.35, 0.70, or 1.05 %), and humoral (antibody) immune response was assessed using sheep red blood cell challenge. The response in exposed mice was not different than that of control (unexposed) mice. In the *in vitro* study by Flaherty et al. (1991), the assay using Roundup had the same result as the assay with technical grade glyphosate—i.e., no effect on either human natural killer cells or cytotoxic T cells at concentrations ranging from 0.01 to 10 µM (i.e.,≈1.7 µg/L or 1.7 mg/L).

An additional *in vivo* study by Rank et al. (1993) used bone marrow cells to assess the genotoxicity of both glyphosate IPA and Roundup in mice exposed to doses of up to 200 a.i. mg/kg bw (≈148 mg a.e./kg bw). An increased incidence of damage to spleen cells is noted only for Roundup and only at the highest dose tested. While the spleen is a relevant target organ for the assessment of immunotoxic agents, the endpoint observed in this study (an increase in polychromatic erythrocytes) is relevant to the assessment of genotoxicity (Section 3.1.10) rather than immunotoxicity.

Experimental, clinical, and field studies have evaluated the ability of glyphosate formulations to induce allergic responses in humans. Maibach (1986) exposed volunteers to Roundup and found that direct dermal application did not produce allergic or photoallergic responses. Williams et al. (2000) describe an unpublished study in which dermal exposure to Roundup (approximately 0.9% or 4.1% glyphosate as the isopropylamine salt) did not produce skin sensitization in human volunteers (Shelanski et al., 1973). A study of five forest workers who participated in mixing and spraying operations does not report changes in blood leukocyte counts or symptoms of allergy (e.g., skin rash, respiratory symptoms) (Jauhiainen et al., 1991). Although there are reported cases of skin rashes following dermal exposures to glyphosate formulations (Barbosa et al., 2001), these effects are thought to derive primarily from irritation rather than allergy, based on observations of Maibach (1986). Hindson and Diffey (1984a) report that Tumbleweed, a glyphosate formulation used in the United Kingdom, may cause photosensitization. Subsequently, however, the effect was 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 Maibach (1986) study conducted with volunteers, there is no evidence that glyphosate itself causes photoirritation or photosensitization.

Reported cases of suicide attempts (Section 3.1.4.4) comprise the only other data regarding human exposure to glyphosate, and the only observation potentially relevant to the effects of glyphosate on immune function is the reported increase in leukocytes counts observed in about

70% of the 131 suicide attempts covered in the Lee et al. (2000) analysis. This effect, however, was observed in severely poisoned individuals, and the increase in leukocytes may have been associated with secondary effects including damage to the gastrointestinal tract. While somewhat speculative, damage to the gastrointestinal tract may have led to infections that stimulated leukocyte production. This speculation is consistent with the development of fevers in about 40% of the individuals who ingested glyphosate formulations.

As discussed further in Section 4.3.1 (hazard identification for fish), there are reports of immune suppression in fish (El-Gendy et al. 1998; Terech-Majewska et al. 2004). As detailed in Section 4.1.31, the fish studies involve extremely high exposure levels and are of limited use in assessing risks to fish and not directly useful in the hazard identification for humans.

Gagnaire et al. (2007) observed immune suppression in mussels exposed to glyphosate along with seven other pesticides (atrazine, alachlor, metolachlor, fosetyl-alumimium, terbuthylazine, diuron and carbaryl). This appears to have been a well conducted study and measured a highly relevant endpoint for immune suppression—response to pathogens. Because individual compounds were not assayed, however, this study cannot be used to suggest that glyphosate was a causative agent in the immune suppression.

3.1.8. Endocrine Effects

Assessment of the direct effects of chemicals on endocrine function are most often based on mechanistic studies on estrogen, androgen, or thyroid hormone systems (i.e., assessments on hormone synthesis, hormone receptor binding, or post-receptor processing). The U.S. EPA/OPP has developed a battery of screening assays for endocrine disruption (i.e., http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series890.htm) and glyphosate has been selected as one of the pesticides for which the screening assays are being required (U.S. EPA/OPP 2009b). No results of the screening assays were located in a search of the EPA web site.

In addition, inferences concerning the potential for endocrine disruption can sometimes be made from responses seen in standard toxicity tests—i.e., changes in the structure of major endocrine glands (i.e., the adrenal, hypothalamus, pancreas, parathyroid, pituitary, thyroid, ovary, and testis) or changes in growth rates. As with effects on the nervous system and immune function, however, effects on organs associated with endocrine function may be secondary to other toxic effects. Thus, in the absence of information on specific endocrine mechanisms, pathological changes in endocrine tissues do not necessarily indicate a direct effect on endocrine function.

In terms of functional effects that have important public health implications, effects on endocrine function would be expressed as diminished reproductive performance or abnormal development. This issue is addressed specifically in the following section (Section 3.1.9), while this section is limited to mechanistic assays that can be used to assess potential direct action on the endocrine system.

Most of the *in vitro* studies discussed in this section assayed both glyphosate as well as glyphosate formulations, and most of the studies clearly indicate that the biological activity of glyphosate is less than that of glyphosate formulations. In order to more clearly compare the differences between glyphosate and glyphosate formulations noted in the various studies, the

discussion in the current subsection is organized by study rather than by presenting the information on glyphosate and glyphosate formulations in different subsections.

A summary of the available *in vitro* studies relevant to the assessment of glyphosate or glyphosate formulations on endocrine function is given in Appendix 2, Table 7. Several early *in vitro* assays suggest that glyphosate as well as Roundup have a low or equivocal potential for endocrine disruption (Lin and Garry, 2000; Petit et al., 1997; Walsh et al., 2000), but some more recent studies raise concern that glyphosate and some glyphosate formulations may be able to impact endocrine function through the inhibition of hormone synthesis (Richard et al. 2005; Benachour et al.2007a,b), binding to hormone receptors (Gasnier et al. 2009), or the alteration of gene expression (Hokanson et al. 2007).

Both glyphosate and Roundup were inactive as estrogen receptor agonists (i.e., the substances did not exhibit *estrogenic activity*) in MCF-7 human breast cancer cells (Lin and Garry, 2000). Similarly, glyphosate did not evidence binding to estrogen receptors from trout (Petit et al., 1997). The study by Petit et al. (1997) is a survey of the activity of several different pesticides The publication does not clearly identify the form of glyphosate tested and there is no indication in the publication that a glyphosate formulation was tested.

Walsh et al. (2000) assayed the ability of glyphosate and Roundup to interfere with steroidogenic acute regulatory (StAR) protein. StAR protein is important to the synthesis of all steroid hormones because this protein is involved in the transport of cholesterol (a hormone precursor) into mitochondria. Within the mitochondria, cholesterol is metabolized by P450 enzymes to generate steroid hormones. At concentrations of up to 100 mg a.e./L, glyphosate itself did not inhibit progesterone synthesis in MA-10 mouse Leydig tumor cells by disrupting StAR protein. A 180 mg a.e./L Roundup formulation, however, did inhibit steroid synthesis with an IC_{50} of 24.4 mg formulation/L.

Levine et al. (2007) conducted a follow-up study in progesterone production in MA-10 mouse Leydig cells using the same Roundup formulation as that used by Walsh et al. (2000). Levine et al. (2007) describe the Roundup formulation as containing 12.2% (w/w) glyphosate acid and 6.1% MON 0818. Levine et al. (2007) also assayed the formulation blank – i.e., the same components as the formulation but without glyphosate – as well as several other surfactants –i.e., benzalkonium chloride, an alcohol ethoxylate, a linear alkylbenzenesulfonate, and sodium lauryl sulfate. All test compounds inhibited progesterone production with IC_{50} values over a relatively narrow range of about 1 to 6 mg/mL with similar slopes ranging from 1.4 to 3.6. Levine et al. (2007) suggest that these similarities indicate that the effect on progesterone production in this *in vitro* assay system is attributable to nonspecific effects of surfactants on cell membranes.

As noted in Section 3.1.2 (Mechanism of Action), glyphosate and glyphosate formulations can inhibit the activity of mixed-function oxidases, a class of enzymes comprised of various isozymes of cytochrome P450. One of these enzymes, referred to generically as a *aromatase*, is involved in the synthesis of sex hormones from cholesterol, specifically the conversion of male hormones (i.e., androgens such as androstenedione and testosterone) to female hormones (i.e., estrogens such as estrone and estradiol) (e.g., Bulun et al. 2003). There are two studies

(Benachour et al.2007b ; Richard et al. 2005) that indicate that glyphosate and glyphosate formulations may alter the activity of aromatase.

As detailed in Appendix 2, Table 7, Benachour et al. (2007b) assayed glyphosate and a 480 g glyphosate IPA/L Roundup formulation in human embryonic cells and human placental microsomes. Glyphosate causes a slight stimulation of activity at concentrations less than 1000 mg/L and about 50% inhibition at concentrations of 8000 mg/L. The Roundup formulation is somewhat more active, causing a 50% inhibition of aromatase at about 1800 mg a.e./L in human placental microsomes. As discussed Section 3.1.3.1, these concentrations of glyphosate are far higher than credible *in vivo* concentrations.

Richard et al. (2005) assayed the effect of glyphosate and a formulation of Roundup (360 mg a.e./L from Monsanto, Belgium) on aromatase in a human placental cell preparation. In these assays, glyphosate caused no significant inhibition of aromatase and no significant changes in messenger RNA (mRNA) associated with the synthesis of aromatase. In an 18-hour assay, the Roundup formulation caused a concentration-related inhibition in aromatase activity (from about a 15 to 55% decrease) over a concentration range of about 0.01to 0.04% formulation (i.e., 100-400 ppm formulation or about 36-144 mg a.e./L). Higher concentrations of up to about 800 ppm formulation (≈288 mg a.e./L) did not result in a greater inhibition of aromatase activity. In a 1-hour assay, formulation concentrations of 0.01-0.2% (100-2000 ppm formulation or about 36-720 mg a.e./L) resulted in a significant but not a concentration-related increase in aromatase activity to about 140% of normal activity. Similar to the study by Benachour et al. (2007b), the concentrations used in the Richard et al. (2005) assays are higher than typical *in vivo* concentrations (Section 3.1.3.1).

As noted above, the study by Petit et al. (1997) found no indication of significant binding of glyphosate to trout estrogen receptors (Petit et al., 1997). More recently, Gasnier et al. (2009) examined the binding of glyphosate and several glyphosate formulations to an estrogen receptor using a human hepatoma cell line (HepG2) culture. As with the study by Petit et al. (1997), glyphosate did not bind to estrogen receptors. Glyphosate, however, did inhibit androgen receptor binding over a concentration range from about 500 to 3000 mg a.e./L but the inhibition was not concentration related.

Gasnier et al. (2009) also assayed four glyphosate formulations, referenced as Roundup formulations purchased from Monsanto, Anvers, Belgium. The specific formulations are referenced as Roundup Express (7.2 g/L), Bioforce (360 g/L), Grands Travaux (400 g/L), and Grands Travaux Plus (450 g/L). Details of these formulations are not given in the Gasnier publication and have not been identified elsewhere. By analogy to the formulations identified in Table 4 of the current Forest Service risk assessment, the concentrations for the formulations appear to be expressed in units of g a.e./L. As detailed in Appendix 2, Table 7, all four formulations bound to the estrogenic receptors and androgenic receptor; what is more, for each formulation the IC_{50} values for binding were lowest for the androgenic receptor, relative to the estrogenic receptors. The inhibitory potencies, however, are not related to the concentrations of glyphosate in the formulations. The 400 g/L formulation displayed the lowest IC_{50}, 0.36 mg a.e./L, for the androgen receptor. The next lowest IC_{50}, 5.55 mg/L, was noted for the 7.2 g/L formulation. The intermediate formulation with a glyphosate concentration of 360 g/L displayed

a much higher IC_{50}, 112 mg/L. Similar to the discussion by Levine et al. (2007) on progesterone production in MA-10 mouse Leydig cells, Gasnier et al. (2009) note that the inhibition of hormone binding the estrogen and androgen receptors does not appear to be attributable directly to glyphosate but appears to be more closely related to other ingredients, presumably surfactants, in the formulations.

While not detailed in Appendix 2, Table 7, Gasnier et al. (2009) also assayed glyphosate and the glyphosate formulations for the inhibition of aromatase activity as well as levels of aromatase mRNA. Gasnier et al. (2009) do not provide detailed data on these assays but a graphical summary is presented in Figure 4 of the publication. As with the study by Richard et al. (2005), glyphosate had no substantial or significant effect on either aromatase activity or mRNA. The four formulations did appear to generally inhibit aromatase activity and increase levels of mRNA, but the concentration-response curves are not consistent among the formulations, and there is an absence of concentration-dependent patterns.

Changes in levels of mRNA imply changes in gene (DNA) regulation—i.e., mRNA is synthesized by DNA. In the glyphosate literature, there is only one study that specifically addresses the potential effect of glyphosate on estrogen-regulated genes (Hokanson et al. 2007). The Hokanson et al. (2007) study involves two types of assays with a human cell line (MCF-7), a preliminary screening assay of numerous genes using a commercial microarray and a more refined assay (quantitative real time polymerase chain reaction or qrtPCR) to confirm the activities noted in the screening assay.

In the screening assay, MCF-7 cells were exposed to glyphosate with and without 17 β-estradiol as well as concentrations of a glyphosate formulation at concentrations ranging from 0.0001 to 0.1% (i.e., from1to 1000 ppm dilutions of the formulation) for 18 hours. It is not clear that a 0% control was used. Furthermore, the formulation is specified only as a *…15% home use formulation…purchased from a small retail supply*. Changes in DNA regulation were assayed using a commercial microarray chip for 1550 genes. Hokanson et al. (2007) do not provide detailed concentration response information. Table 2 of the Hokanson publication indicates that changes (either up or down regulation) were observed in ≈44% of the genes. Using changes of more than a factor of 2 as an index of biological significance, up-regulation was observed in about 1.4% of the genes (21/1550) and down-regulation was observed in about 0.5% of the genes (8/1550).

The more refined qrtPCR assays were also conducted on 7 of the 29 genes evidencing positive activity in the microarray assay. Altered regulation in 3 of the 7 genes was not confirmed with qrtPCR. A fourth gene, INPP1 (the gene associated with the inositol polyphosphate 1-phosphatase) was significantly up-regulated by a factor of about 2.7, but only at a glyphosate concentration of 0.023% —i.e., ≈ 230 mg a.e./L. This result was discounted by the authors as being *…a concentration that is likely not reasonable for cellular exposure*. As discussed in Section 3.1.3.1, this assessment appears to be correct. The three remaining genes were significantly dysregulated at a concentration of 0.00023% or 2.3 mg a.e./L, based on the discussion given by the authors. The genes impacted included HIF1 (hypoxia-inducible factor 1), CXCL12 (chemokine ligand 12), and EGR1 (early growth response 1). As discussed in 3.1.3.1 and illustrated in Figure 3, the concentration of 2.3 mg/L is greater than expected

concentrations in most internal tissue (except for the bone and kidney) of rats at a nontoxic oral dose of 10 mg/kg bw.

In discussing the above concentrations that caused changes in genetic expression, Hokanson et al. (2007) reference Figures 1 through 3 in their publication. The legend to these figures appears to indicate that a 1% (0.01) dilution of the 15% glyphosate (150,000 mg a.e./L) formulation was used in the qrtPCR assays, which is equivalent to a glyphosate concentration of 150 mg a.e./L. The corresponding author, David Busbee (dbusbee@cvm.tamu.edu) was queried on this apparent discrepancy on May 30, 2010. No response has been received to date.

3.1.9. Reproductive and Developmental Effects

An overview of the reproduction and developmental studies on glyphosate, glyphosate formulations, and surfactants is given in Table 12. Technical grade glyphosate has been assayed in developmental studies for its ability to cause birth defects and in multi-generation reproduction studies to measure its overall effects on reproductive capacity. Developmental and reproduction studies on technical grade glyphosate are the bases for RfDs proposed by different offices within the U.S. EPA. Developmental studies also have been published on a Roundup formulation from Brazil as well as the POEA surfactant used in Roundup. The study on the Brazilian formulation of Roundup raises concern for impacts on male offspring. This concern, however, is not supported by the developmental and reproduction studies on glyphosate and the POEA surfactant used in some glyphosate formulations, the available epidemiology studies on workers applying glyphosate formulations, and field studies on mammalian wildlife. Finally, some studies are available on the effect of glyphosate or glyphosate formulations on testes. Each of these types of studies is discussed in the following subsections. A discussion of the impact on these studies on the quantitative risk assessment is given in Section 3.3 (Dose-Response Assessment).

3.1.9.1. Developmental Studies

As discussed in Section 3.1.3, glyphosate is negatively charged at physiologic pH, and anions do not readily transport across biological membranes. Consistent with this characteristic, *in vitro* studies by Poulsen et al. (2009) suggest that glyphosate has a low potential for transport across the placenta. Using human placental preparations, Mose et al. (2008) notes that glyphosate is not readily transported across the placenta but that as much as 15% of glyphosate in maternal circulation might reach the developing fetus.

The potential for glyphosate to disrupt normal fetal development can be directly assessed from several developmental studies. These studies entail gavage administration to pregnant rats or rabbits on specific days of gestation. Developmental assays as well as studies on reproductive function (Section 3.1.9.2) are generally required for the registration of pesticides. Very specific protocols for developmental studies are established by U.S. EPA/OPPTS and are available at http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized. The developmental studies on glyphosate and glyphosate formulations are summarized in Table 12, and additional details of these studies are given in Appendix 2, Table 3.

3.1.9.1.1. Glyphosate

Two sets of standard developmental toxicity studies were submitted to U.S. EPA/OPP in support of the registration of glyphosate. An early set of studies in rats (Rodwell et al. 1980a) and

rabbits (Rodwell et al. 1980b) is reviewed in the RED for glyphosate (U.S. EPA/OPP 1993a,b). Subsequent to the RED, another set of studies were submitted in rats (Moxon 1996a) and rabbits (Moxon 1996b). The studies by Moxon (1996a,b) were obtained for the preparation of the previous Forest Service risk assessment of glyphosate (SERA 2003).

The developmental studies submitted to the EPA clearly indicate that rabbits are more sensitive than rats. This is not an unusual pattern in developmental studies. The NOAELs for rats are 1000 mg/kg bw/day for both maternal toxicity and fetal toxicity. For rabbits, NOAELs for maternal toxicity range from 100 to 175 mg/kg bw/day, and the NOAELs for fetal toxicity range from 175 to 350 mg/kg bw/day. Thus, based on these studies, the developing fetus appears to be less sensitive than dams to glyphosate.

The developmental studies by Beuret et al. (2005) and Daruich et al. (2001) are from the open literature. These studies are not standard developmental studies but are focused on specific biochemical endpoints following exposure to glyphosate in drinking water. The study by Daruich et al. (2001) is generally consistent with the standard developmental studies in that only a decrease in maternal body weight gain along with changes in some biochemical parameters were noted at a dose of about 455 mg/kg bw/day. The study by Beuret et al. (2005) is also reasonably consistent with the standard gavage studies, with a dose of about 1000 mg/kg bw causing increases in maternal liver peroxidation (but no overt signs of maternal toxicity) and no effects in offspring.

3.1.9.1.2. Glyphosate Formulations

The developmental toxicity of a Brazilian glyphosate formulation has been assayed by Dallegrave et al. (2003, 2007). As summarized in Table 12 and detailed in Appendix 2 (Table 3), the initial study by Dallegrave et al. (2003) is a relatively standard developmental study similar to those conducted on technical grade glyphosate and submitted to the U.S. EPA. This initial study used relatively high doses (0, 500, 750, or 1000 mg/kg bw/day) and noted skeletal malformations, suggestive of delayed development, at all doses and severe maternal toxicity at the highest dose. The NOAELs and LOAELs in this study—750 and 1000 mg/kg bw/day—are only somewhat lower than the corresponding NOAELs and LOAELs reported for rats exposed to technical grade glyphosate—1000 and 3500 mg/kg bw/day.

The second study (Dallegrave et al. 2007) used lower doses of 0,50, 150 or 450 mg/kg/day, but continued exposures for 21 days into the lactation period. In addition, Dallegrave et al. (2007) assayed endpoints in male and female offspring at 65 days after birth (i.e., the time at which young rats typically reach puberty) and 140 days after birth (young adult rats). These types of observations are not typically reported in standard developmental studies submitted to the U.S. EPA. In 65-day-old offspring, Dallegrave et al. (2007) observed a decrease in serum testosterone. For the doses of 0, 50, 150 or 450 mg/kg/day, the mean concentrations of serum testosterone were 5.2, 4.0, 3.2, and 1.5 ng/mL. No effect on testosterone levels was noted in the 140-day-old rats.

Based on statistically significant differences relative to control animals for the 65-day-old rats, the NOAEL and LOAEL values for decreased testosterone were 150 and 450 mg/kg bw/day, respectively. As summarized in Table 12, these NOAEL and LOAEL values for decreased testosterone are not substantially different for the NOAEL and LOAEL values for rabbits

exposed to technical grade glyphosate, but they are substantially lower than the NOAEL and LOAEL values reported in developmental studies in which rats were exposed to technical grade glyphosate.

Dallegrave et al. (2007) do not conduct a statistical analysis on the dose-response relationship; however, there is an apparent dose-response relationship for testosterone in the 65-day-old rats. As an exploratory effort, the exponential model (i.e., log transformation of the mean testosterone levels in serum) was used to fit the dose-response relationship for serum testosterone. As illustrated in Figure 4, the data fit the exponential model. The squared correlation coefficient is 0.99 with a *p*-value for the model of 0.0053.

Two other studies, one in rats (Romano et al. 2010) and the other in mallard ducks (Oliveira et al. 2007), have also reported decreases in testosterone in animals exposed to glyphosate formulations that contain surfactants. Figure 4 illustrates the data from the study by Romano et al. (2010). This study is not a developmental study and is discussed further in Section 3.1.9.3. The study by Oliveira et al. (2007) on the effect of glyphosate formulations on testosterone in mallards is discussed further in Section 4.1.2.2.2.

As noted by Dallegrave et al. (2007, Table 4), various other endpoints resulted in statistically significant differences relative to the controls—e.g., a significant decrease in sperm production in 140-day-old rats at 50 and 450 mg/kg bw. This and other differences, however, do not demonstrate a dose-response relationship. In the case of sperm production, 140-day-old rats in the 150 mg/kg/day dose group had daily sperm production comparable to that of control rats.

Other endpoints from the Dallegrave et al. (2007) study are suggestive of a dose-response relationship, specifically the sex ratios (Table 2 in the Dallegrave paper which shows a general increase in the number of males with increasing dose) and percentage of tubules with spermatogenesis (Table 4 in the Dallegrave paper which shows a general decrease with increasing dose in both 85- and 140-day-old rats). Exploratory analyses of these data indicate no statistically significant dose-response relationships with or without log transformation of the responses (*p*-values >0.15).

As noted by Dallegrave et al. (2007, p. 670): *The best male reproductive outcomes to be evaluated in toxicity studies are the testis relative weight, testis histology, sperm number and morphology*. The only quantitative endpoint in this list is relative testis weight, which was not affected in either 65- or 140-day-old rats (Dallegrave et al. 2007). While Dallegrave et al. (2007) note pathological changes in the testes, including elongated spermatid vacuolization and tubular degeneration, there is no indication that these changes are dose related or statistically significant except at the highest dose tested. Additional studies suggesting that glyphosate or glyphosate formulations may cause damage to sperm are discussed in Section 3.1.9.3.

The only other consistent and potentially significant adverse effect noted by Dallegrave et al. (2007) was a delay in the opening of the vaginal canal in female offspring. This delay was statistically significant, relative to the control group, at all doses: increases of about 7.7% at 50 mg/kg bw/day, 5.7% at 150 mg/kg bw/day, and 5.1% at 450 mg/kg bw/day. The magnitude of the increases, however, is not substantial, and the increases are not dose related. As discussed by

Dallegrave et al. (2007, p. 669): *These differences were statistically significant but did not show biologic significance*.

Because the study by Dallegrave et al. (2007) did not concurrently test glyphosate without a surfactant and the surfactant alone, it is not clear if the effects on serum testosterone are attributable to glyphosate, the surfactant, the combined exposures to the two agents, or other unidentified inerts in the formulation.

A final consideration in assessing the significance of the Dallegrave et al. (2003, 2007) studies involves the formulation that was tested. In both publications, the formulation is designated as Roundup purchased from Monsanto of Brazil and is specified as containing 360 g a.e./L and 18% w/v of a polyoxyethyleneamine surfactant. In the preparation of the current Forest Service risk assessment, a product label and MSDS for a Brazilian formulation of Roundup was obtained from the Brazilian web site for Monsanto: http://www.monsanto.com/who_we_are/locations/brazil.asp. This formulation consists of the IPA salt of glyphosate at a concentration of 460 g a.i./L. The formulation density is specified as 1.163 g/mL (1,163 g/L). Thus, the formulation appears to be a 41% (w/w) a.i. formulation, similar to many of the formulations specified in Table 2 [460 g a.i./L ÷ 1,163 g/L = 0.4127 a.i. w/w/ ≈ 41% a.i. w/w.]

As summarized in Table 2 and discussed in Section 2.2.2, the amount of surfactant in many U.S. formulations of glyphosate is not disclosed. Based on information from Nufarm and Dow AgroSciences, some liquid formulations of glyphosate contain surfactants at concentrations of 8-14%, and some granular formulations contain surfactants at concentrations of 13-25%. Monsanto's original Roundup formulation contained a POEA surfactant, MON 0818, at a concentration of 15% and this concentration appears to apply to many current Roundup formulations. While the Roundup formulation used by Dallegrave et al. (2003, 2007) contained a surfactant at a concentration similar to those in some U.S. formulations, the specific composition of the surfactants is considered proprietary and there is no way of knowing if the surfactant in the Roundup formulation used by Dallegrave et al. (2003, 2007) is identical or reasonably similar to any or all of the surfactants used in U.S. formulations of glyphosate.

3.1.9.1.3. Surfactants

The publication by Farmer et al. (2000b) summarizes two developmental toxicity studies, one on the POEA surfactant used in some glyphosate formulations (doses of 0, 15, 100, or 300 mg/kg bw/day) and another on a phosphate ester neutralized POEA (doses of 0, 15, 50, or 150 mg/kg bw/day). Details of these studies are given in Appendix 2, Table 5. As noted in Section 3.1.9.1.1, the publication by Farmer et al. (2000b) is an abstract from Monsanto. Full publications of the data presented in Farmer et al. (2000b) are not to be found in the glyphosate literature.

In terms of developmental toxicity, such as effects on the developing fetus or offspring, neither study summarized by Farmer et al. (2000b) reports adverse effects at doses of up to 300 mg/kg bw/day for the POEA surfactant (highest dose tested) and 150 mg/kg bw/day for the neutralized POEA surfactant (also the highest dose tested). Nevertheless, maternal toxicity was observed at these doses. This outcome is consistent with the studies on both glyphosate and Roundup, in which none of the agents is toxic to the developing fetus at doses that are nontoxic to dams. For

the POEA surfactant, no adverse effects on offspring are apparent, even at doses that cause signs of maternal toxicity.

Farmer et al. (2000b) do not comment on testosterone. Testosterone levels are not typically assayed in developmental studies submitted to the U.S. EPA. Thus, the studies summarized by Farmer et al. (2000b) do not impact the assessment of the effects on testosterone observed in the Roundup study by Dallegrave et al. (2007).

3.1.9.2. Reproduction Studies

Reproduction studies involve exposing one or more generations of the test animal to a chemical compound. Generally, the experimental method involves dosing the parental (P or F_0) generation (i.e., the male and female animals used at the start of the study) to the test substance prior to mating, during mating, after mating, and through weaning of the offspring (F_1). In a 2–generation reproduction study, this procedure is repeated with male and female offspring from the F_1 generation to produce another set of offspring (F_2). During these types of studies, standard observations for gross signs of toxicity are made. Additional observations often include the length of the estrous cycle, assays on sperm and other reproductive tissue, and number, viability, and growth of the offspring.

As discussed in Section 3.1.9.2.1, three multi-generation studies are available on technical grade glyphosate. Apparently, multi-generation studies have not been conducted on glyphosate formulations or any of the surfactants used in glyphosate. As discussed further in Section 3.3 (Dose-Response Assessment), the lack of multi-generation studies on glyphosate formulations and surfactants is a concern. Notwithstanding this concern, several epidemiology studies involving the use and application of glyphosate formulations are available (Section 3.1.9.2.2).

3.1.9.2.1. Laboratory Studies on Glyphosate

As summarized in Table 12 and detailed further in Appendix 2, Table 3, there are available three multi-generation reproductive studies on glyphosate. Two of these studies (Reyna 1985; Schroeder and Hogan 1981) were submitted to and reviewed by the U.S. EPA (U.S. EPA/OPP 1993b; EPA/ORD 1990). The third study is published only as an abstract (Farmer et al. 2000a). All studies were conducted with glyphosate acid.

The initial 3-generation reproduction study conducted by Schroeder and Hogan (1981) used very low doses: 0, 3, 10, or 30 mg/kg bw/day. 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 discussing this effect, Schroeder and Hogan (1981) noted that the historical control indices for tubular lesions varied markedly in male weanling rats. Schroeder and Hogan (1981) concluded that the highest dose tested (30 mg/kg/day) had no adverse reproductive effects.

As discussed further in Section 3.3, U.S. EPA/ORD (1990) elected to use the dose of 30 mg/kg/day as a systemic LOAEL. This approach may be viewed as reasonable in that the incidence of tubular dilation at 30 mg/kg bw/day was 7/10 and the incidence of this effect in the matched control group was 2/10. Using the Fisher Exact test, the increased incidence in the 30 mg/kg bw/day dose group is statistically significant (p=0.0349). Thus, U.S. EPA/ORD (1990) identifies 10 mg/kg bw/day as the systemic NOAEL. In terms of reproductive effects, however, the reproduction NOAEL for this study is clearly 30 mg/kg bw/day.

The later study by Reyna (1985) and the study summarized in the abstract by Farmer et al. (2000a) both involved much higher doses ranging from 100 mg/kg bw/day (the low dose in the study by Reyna 1985) to greater than 2000 mg/kg bw/day (based on the high dose reported by Farmer et al. 2000a). The study by Reyna (1985) noted no effects on reproductive capacity with a NOAEL of 500 mg/kg bw/day and a LOAEL of 1500 mg/kg bw/day, based on systemic toxicity manifested as soft stool and decreased food consumption and body weight gain in both adults and offspring. The study summarized by Farmer et al. (2000a) notes very similar results, a NOAEL of 740 mg/kg bw/day and a LOAEL of 2268 mg/kg bw/day based on reduced body weight gain and reduced litter size. This LOAEL of 2268 mg/kg bw/day is the only dose in the multi-generation studies which suggests a frank effect on reproduction—i.e., reductions in litter sizes. The summary by Farmer et al. (2000a) does not provide details on the magnitude of the reductions in litter sizes.

As discussed further in Section 3.3 (Dose Response Assessment), U.S. EPA/OPP (1993b) uses the NOAEL of 175 mg/kg bw/day as the basis for an RfD for glyphosate. This dose is about 6 times greater than the 30 mg/kg bw/day LOAEL from the study by Schroeder and Hogan (1981). The rationale for this approach is provided in U.S. EPA/OPP (1993b, p. 6) as follows:

> *Since the focal tubular dilation of the kidneys was not observed at the 1500 mg/kg/day level (HOT) in the 2-generation rat reproduction study* [Reyna 1985] *but was observed at the 30 mg/kg/day level in the 3-generation rat reproduction study* [Schroeder and Hogan 1981]*, the Office of Pesticide Programs (OPP) Developmental Peer Review Committee concluded that the latter was a spurious rather than glyphosate-related effect.*
>
> U.S. EPA/OPP 1993b, p. 6.

This judgment made by the EPA is independent of but consistent with the judgment of the study authors, based on the variability of tubular kidney lesions in the historical controls for male rats (Schroeder and Hogan (1981).

3.1.9.2.2. MON 0818 Surfactant

The U.S. EPA/OPP (2009c) provides a relatively detailed synopsis of a two generation reproduction study in rats on MON 0818, designating the study only as MRID 47097401. Based on the list of MRID studies included in Supplement 1 to the current risk assessment, MRID 47097401 corresponds to the study by Knapp (2006). A brief summary of this study is also included in Appendix J (Table J-28) of U.S. EPA/OPP (2008a).

As summarized in Appendix 2 (Table 5) of the current Forest Service risk assessment, the two generation reproduction study by Knapp (2006) involved exposures to MON 0818 at dietary concentrations of 0, 100, 300, and 1000 ppm (mg MON 0818/kg diet). The test material is designated as MON 0818 containing "69-73% a.i.". While the test material is not discussed in detail by U.S. EPA/OPP (2009c), the designation of "69-73% a.i." may refer to the concentration of POEA in the sample of MON 0818 that was tested. As discussed in Section 2.2.2 and Section 3.1.4.3, MON 0818 typically contains POAE at a concentration of about 75%. The dietary

exposures corresponded to doses of about 5 to 7 mg/kg bw in the 100 ppm groups, 15 to 20 mg/kg bw in the 300 ppm groups, and 50 to 65 mg/kg bw in the 1000 ppm groups.

No adverse effects were noted in any parental rats. The 1000 ppm exposure level was classified as a LOAEL based on decreases in live litters sizes, increases in the number of unaccounted for implantation sites (presumably resorptions), and small litter sizes in some F_0 dams, and decreases in post-natal survival in F_1 offspring. No effects in any reproductive parameters were noted in the 100 ppm or 300 ppm exposure groups.

In addition to standard measures of reproductive performance, testosterone was assayed in one F_1 male from each liter. Sperm motility and sperm morphology were also assayed in all F_1 males. No effects on testosterone or sperm were noted at any dose level. In addition, no effects were noted on estrous cycles or thyroid hormone levels in the F_1 generation.

3.1.9.2.3. Human Experience with Formulations

Numerous epidemiological studies have examined relationships between pesticide exposures or assumed pesticide exposures in agricultural workers and reproductive outcomes. Very few studies, however, have attempted to characterize exposures, either qualitatively or quantitatively, to specific pesticides (e.g., Arbuckle and Sever, 1998; Driscoll et al. 1998). Of those studies that specifically address potential risks from glyphosate exposures, none has demonstrated a statistically significant association between exposure and adverse reproductive effects.

The Ontario Farm Health Study collected information on pregnancy outcomes and pesticide use among Ontario farm couples. Three retrospective cohort studies of this group (Arbuckle et al. 2001; Curtis et al. 1999; Savitz et al. 1997) examined relationships between exposures to glyphosate formulations (defined as self-reported participation in mixing and/or spraying operations) and reproductive outcomes. One study analyzed self-reported spontaneous miscarriages of 3984 pregnancies among 1898 couples who self-reported exposures to glyphosate formulations within a period beginning 2 months before pregnancy and ending the month of conception (Savitz et al., 1997). Risk of miscarriage was unrelated to self-reported exposure to glyphosate formulations. A second study of spontaneous abortions among 2110 women and 3936 pregnancies disaggregated the herbicide exposures into pre- and post-conception and spontaneous abortions into early- (< 12 wk) and late-term (12-19 wk) abortions (Arbuckle et al., 2001). Spontaneous abortions were not associated with post-conception glyphosate formulation exposure; however, the odds ratio for abortions and post-conception exposure was 1.4 (1.0-2.1), and for late-term abortions was 1.7 (1.0-2.9). The latter odds ratios were not adjusted for maternal age which is a risk factor for spontaneous abortion. When maternal age was considered in a regression tree analysis, spontaneous abortions were found to be unrelated to glyphosate formulation use. Curtis et al. (1999) examined fecundity among 1048 farm couples who self-reported exposures to glyphosate formulations within a period beginning 2 months prior to trying to conceive (to account for time of spermatogenesis) and ending at pregnancy. Fecundity was unrelated to glyphosate exposure.

Larsen et al. (1998a) examined relationships between pesticide use and semen quality among farmers in Denmark. Participants in the study included 161 farmers who self-reported crop spraying with a variety of pesticides, which included Roundup (7% prevalence of use) and 87 farmers who did not use pesticides. Semen samples were collected at the start of the spraying

season and 12-18 weeks after the first spraying. Evaluations included sperm count, morphology, chromatin structure and motility; and serum concentrations of reproductive hormones (testosterone, LH, FSH). Semen quality and reproductive hormone levels were unrelated to pesticide use. In a related study, fecundity was compared among farmers who did or did not participate in pesticide spraying operations. Fecundity was determined from the number of self-reported menstrual cycles or months between discontinuation of birth control and pregnancy (Larsen et al., 1998b). Participants included 450 traditional farmers who reported that they sprayed pesticides, 72 traditional farmers who did not participate in spraying operations, and 94 organic farmers who reported not using pesticides on their crops. Fecundity was unrelated to pesticide use or participation in pesticide spray operations.

Based on California Pesticide Use Reports, Rull et al. (2004) published an abstract of an analysis of potential exposures to 59 pesticides to the incidence of neural tube defects in residential populations. The only association involving glyphosate was an odds ratio of 1.55 with a 95% confidence interval of 0.85 to 2.85 for anencephaly. While Rull et al. (2004) identify this odds ratio as an association, the association is not statistically significant—i.e., the lower bound is not greater than one.

Sanin et al. (2009) examined differences in time-to-pregnancy in women living in five regions in Columbia. In three of these regions, glyphosate was applied to either sugar cane (one region) or to illicit crops. No statistically significant effects were observed.

3.1.9.2.4. Field Studies in Mammals

In addition to the epidemiology studies on human populations discussed in the previous subsection, field studies in mammalian wildlife have failed to note adverse effects on reproduction. While field studies on mammalian wildlife are not typically considered in the human health risk assessment, the mammalian field studies on glyphosate add another measure for assessing the concern with the potential effect of glyphosate formulations on reproductive function. As discussed in the ecological risk assessment (Section 4.1.2.1), the studies by Ritchie et al. (1987) and Sullivan (1990) have failed to note reproductive effects on populations of small mammals following aerial applications of glyphosate. The study by Sullivan (1990) is particularly compelling in that it involved surveys of mice and voles one year prior to and three years following an application of Roundup at a rate of 2.7 lb a.e./acre. Based on typical exposure assumptions for small mammals, this application rate could have resulted in doses in the range of about 40 (1.6 to 120) mg a.e./kg bw (Attachment 1a-c, Worksheet F03b). As discussed in Section 3.1.9.1.2, a dose-response relationship noted in the study by the Dallegrave et al. (2007) suggests a NOAEL of about 20 mg a.e./kg bw/day for the effects on testosterone. In the study by Sullivan (1990), exposures could have exceeded the dose of 20 mg a.e./kg bw by factors of up to 6. In addition, based on the observations from the Dallegrave et al. (2007) study, doses over the range of 40 to 120 mg a.e./kg bw would be expected to result in decreased testosterone levels that could have an adverse effect on reproductive capacity. On the other hand, the NOAEL of 175 mg a.e./kg bw that is used as the basis for the RfD would lead to the assessment that the exposures in the study by Sullivan (1990) should not have resulted in any adverse reproductive effects. Thus, the failure of Sullivan (1990) to note adverse reproductive effects in mammalian wildlife in exposures that may have approached 175 mg a.e./kg bw/day is consistent with the assumption that the U.S. EPA/OPP RfD is sufficiently protective.

3.1.9.3. Effects on Testes and Testosterone

As discussed further in the dose-response assessment (Section 3.3), the current RfD for glyphosate is based on a developmental study and this RfD is well-supported by multigeneration reproduction studies on both glyphosate and a surfactant used in glyphosate. Nonetheless, several publications in the open literature have suggested that glyphosate or some glyphosate formulations may have adverse effects on the testes and may lead to a reduction in testosterone. While these studies may impact the perception of risk, they do not have a substantial impact on the hazard identification because concerns for reproductive function are adequately encompassed by the current RfD for glyphosate.

Yousef et al. (1995) observed substantial decreases in libido, ejaculate volume, sperm concentrations, semen initial fructose and semen osmolality as well as increases in abnormal and dead sperm in rabbits after acute exposures to glyphosate. The authors report that all of the effects were statistically significant at $p<0.05$. A serious limitation of this study is that the authors report the doses as proportions of 0.01 and 0.1 of the LD_{50} but do not specify the actual doses. In addition, Yousef et al. (1995) do not specify the type of glyphosate tested—i.e., acid, salt, or formulation. In the absence of information on dose as well as the test material (i.e., glyphosate or a formulation), the Yousef et al. (1995) study does not contribute substantially to the hazard identification.

As discussed by Williams et al. (2000) and Dost (2008), the Yousef et al. (1995) study can be criticized for a number of other reporting and experimental design limitations or deficiencies. In addition, it should be noted that the rabbits in the Yousef et al. (1995) study were dosed by gelatin capsules. The use of gelatin capsules is a reasonable mode of administration; however, like gavage exposures, it results in a high spike in body burden which is not typical or particularly relevant to potential human exposures, except in the case of attempted suicides. Dietary exposure, on the other hand, results in more gradual and steady exposure levels over the course of the day, which is more comparable and relevant to potential human exposures.

In a subsequent *in vitro* study, Yousef et al. (1996) report that glyphosate may reduce sperm motility in the range of from 116 to about 300 µM in protein free media and from 500 to about 740 µM in a media with protein. Again, however, Yousef et al. (1996) do not specify the form of glyphosate tested. The concentrations, however, do appear to be expressed in units of a.e. The lowest reported effect concentration, 116 µM, corresponds to a concentration of about 19.6 mg a.e./L [116 µMoles/L × 169.07 µg/µMole = 19,612 µg/L]. As summarized in Table 10, the peak concentration in rats testes following a dose of 10 mg a.e./L glyphosate is about 0.16 mg/L. The concentration used in the Yousef et al. (1996) study is greater than the concentration of glyphosate in testes at a dose of 10 mg/kg bw (Table 10) by about a factor of 120 [19.6 mg a.e./L ÷ 0.16 mg/L = 120.625]. Assuming a linear relation between dose and concentration in testes tissue, a concentration of 19.6 mg a.e./L corresponds to a dose of about 1200 mg a.e./kg bw.

A statistically significant decrease (20%) in sperm count was observed in male rats exposed to 25,000 or 50,000 ppm (NTP 1992). As indicated in Appendix 2, Table 4, these dietary concentrations correspond to doses of 1678 and 3383 mg/kg bw/day. NTP (1992) concluded that there was no evidence of adverse effects on the reproductive system of rats or mice, and summarized the findings as follows:

> *Measures of sperm density, or the number of sperm/g caudal epididymal tissue, were reduced somewhat in male rats in the 2 highest dose groups (25,000, 50,000 ppm); other spermatozoal measurements were not different from controls in rats or mice. There was a slight lengthening of the estrous cycle in high dose female rats (50,000 ppm), but the biologic significance of these findings, if any, is not known.*
>
> NTP 1992, p. 35

As discussed in Section 3.1.9.1.2 and illustrated in Figure 4, Dallegrave et al. (2007) noted a decrease in testosterone in 65 day old male rats which had been exposed to a Brazilian formulation of Roundup during gestation and lactation at maternal doses of 50, 150, and 450 mg a.e./kg bw/day. Another Brazilian study, Romano et al. (2010), has recently reported decreases in testosterone in young male rats following a different dosing regimen with a different formulation. The assay by Romano et al. (2010) involved Roundup Transorb. The source of the formulation is not clearly specified other than a parenthetical reference to … *Monsanto Co., St. Louis, MO; Monsanto of Brazil Ltda, São Paulo, Brazil*. It is not clear that Roundup Transorb from Brazil is identical to Roundup Transorb from the U.S. The male rats were dosed at 0, 5, 50, or 250 mg/kg bw/day from post-natal Days 23 to 53. While not explicitly specified in the publication, the doses appear to be expressed as glyphosate acid equivalents. As in the study by Dallegrave et al. (2007), Romano et al. (2010) noted decreases in serum testosterone.

As illustrated in Figure 4, a dose-response relationship is apparent in the decrease in testosterone in the study by Romano et al. (2010). Unlike the case with the data from Dallegrave et al. (2007), however, the dose-response relationship does not fit a simple exponential model ($p \approx 0.19$). Nonetheless and as detailed in Appendix 2, Table 3, the decreases in testosterone are statistically significant ($p < 0.001$) with respect to controls at all dose levels. Other effects noted by Romano et al. (2010, Table 2, p. 313) include significant decreases in the height of seminiferous epithelium and significant increases in the luminal diameter of the seminiferous tubules. In addition, Romano et al. (2010, Table 1, p. 312) note significant increases in testicular weight and adrenal weight in the 250 mg/kg bw/day dose group. Lastly, Romano et al. (2010, Table 1, p. 312) report a significant and dose-related delay in preputial separation – i.e., the normal separation of skin from the penis after birth.

As discussed above, the studies by Dallegrave et al. (2007) and Romano et al. (2010) involve different exposure regimes as well as different glyphosate formulations. While these studies both observed decreases in testosterone, the results of the two studies are not consistent with respect to other endpoints. For example, Romano et al. (2010) note a delay in preputial separation as well as an increase in testes weight. Dallegrave et al. (2007), however, assayed but noted no effect on testes weight and either no effect or a very slight acceleration in preputial separation. Thus, while a decrease in testosterone is clearly an adverse effect, the observations in the studies by Dallegrave et al. (2007) and Romano et al. (2010) are difficult to interpret in terms of a coherent and consistent set of effects on male reproductive function.

While studies comparable to those of Dallegrave et al. (2007) and Romano et al. (2010) are not available on U.S. formulations of glyphosate, acceptable reproduction and developmental studies are available on both glyphosate and MON 0818. Specifically, the multigeneration reproduction study with MON 0810 failed to note any effect on testosterone (Knapp 2006). Concern for the results reported by Dallegrave et al. (2007) and Romano et al. (2010) are further reduced by the available epidemiology studies on glyphosate formulations (Section 3.1.9.2.3) as well as field studies on mammalian wildlife following applications of glyphosate formulations (Section 3.1.9.2.4).

In the absence of confirming studies demonstrating a decrease in testosterone in mammals following exposures to U.S. formulations of glyphosate, the reports by Dallegrave et al. (2007) and Romano et al. (2010) do not have a material impact on the hazard identification for potential effects on male reproductive function.

3.1.10. Carcinogenicity and Mutagenicity

Mutagenicity and carcinogenicity are considered together in most risk assessments. Demonstrating that a compound is mutagenic raises concern that a compound may have carcinogenic potential. This is different, however, from demonstrating that a compound is carcinogenic. The Risk Assessment Forum of the U.S. EPA has established guidelines for classifying a compound as a carcinogen and using cancer data in quantitative risk assessments (U.S. EPA/RAF 2005). Mutagenicity data can be used to evaluate the mechanism by which a potential carcinogen may operate; however, quantitative risk assessments for carcinogenicity are based on either *in vivo* cancer bioassays in experimental mammals or epidemiology studies that provide adequate measures of both exposure and risk.

Based on the available information on glyphosate, the U.S. EPA/OPP (1993b) has concluded that glyphosate should be classified as Group E (evidence of non-carcinogenicity for humans). As with any well-studied, well-tested pesticide, there is always some equivocal evidence of carcinogenic or mutagenic potential, which may remain a cause of concern, at least in terms of risk perception (e.g., Cox 1998a, 2004; Watts 2010). While these concerns are understandable, there is no compelling basis for challenging the position taken by the U.S. EPA/OPP; accordingly, no quantitative risk assessment for cancer is conducted as part of the current Forest Service risk assessment.

3.1.10.1. Mutagenicity (and Genotoxicity)

3.1.10.1.1. Laboratory Studies

There is no doubt that glyphosate and glyphosate formulations can cause damage to cells, including the chromosomes/genetic material that are in cells. This literature is the subject of numerous reviews (e.g., Cox 1998a, 2004; Watts 2010; Williams 2000). Furthermore, numerous studies demonstrating that glyphosate and glyphosate formulations can damage genetic material are summarized in Appendix 2 (Table 8) of the current Forest Service risk assessment.

Based on the studies that EPA requires for pesticide registration, the agency has concluded that glyphosate …*is neither mutagenic or Clastogenic* (U.S. EPA/OPP 2002, p. 60935). The distinction between mutagenic and clastogenic is important. Mutagenicity refers to a change in chromosomes, which can be hereditary, like a hereditable mutation, while clastogenic effects

involve chromosome breakage. More generally, the term genotoxicity can refer to any type of DNA damage. The U.S. EPA/OPP requires a number of standard bioassays in bacteria and other systems used to assay the ability of a chemical to cause hereditable mutations. As reviewed by U.S. EPA/OPP (1993b) and reasserted in U.S. EPA/OPP (2002), all assays of glyphosate for hereditable mutations have been negative.

There is only one study in the open literature on glyphosate which suggests that technical grade glyphosate may have mutagenic activity (Kaya et al. 2000). This study, which is briefly summarized in Appendix 2, Table 8, is discussed here in greater detail. In the study by Kaya et al. (2000), fruit flies were exposed from egg stage through pupation to glyphosate concentrations ranging from 16.9 to 1690 mg a.e./L. Mutagenicity was assayed as the development of visual changes (wing spots) indicative of various types of mutations. Of the 12 specific types of mutations assayed, glyphosate demonstrated a significant concentration-related increase in one type of mutation. Based on significant differences from the untreated controls, the threshold for the mutation was 0.5 mM, equivalent to about 84.5 mg/L (see Table III in Kaya et al. 2000). As illustrated in Figure 3 and summarized in Table 10 of the current Forest Service risk assessment, this concentration is about 100 times greater than glyphosate concentrations in the plasma of rats exposed to a nontoxic dose (10 mg/kg bw) of glyphosate [84.5 mg/L ÷ 0.86 mg/L ≈ 98.3].

There are two studies in the open literature on glyphosate which suggest that glyphosate formulations may be mutagenic, as opposed to clastogenic or simply genotoxic: Kale et al. (1995) and Rank et al. (1993) As summarized in Appendix 2 (Table 8), Rank et al. (1993) assayed two strains of *Salmonella typhimurium*, TA98 and TA100, using Roundup. These are standard strains used by the U.S. EPA to assay for reverse mutations (U.S. EPA/OPPTS 1998a). Rank et al. (1993) observed a significant number of revertants (i.e., mutations) in strain TA98 without metabolic activation at an exposure of 360 µg/plate and in strain TA100 with metabolic activation at 720 µg/plate. Rank et al. (1993) also note that these exposure levels are near to those that cause cell death. The study does not provide information regarding the volume of the test solution in each plate. Assuming that Rank et al. (1993) used standard methods, the volume of the test solution in this type of assay is approximately 2-3 mL (U.S. EPA/OPPTS 1998a). Based on the upper range of 3 mL (0.003 L), the estimated concentrations in the test solutions are about 120- 240 mg/L [0.360 mg to 0.72 mg/plate ÷ 0.003 L/plate]. These concentrations would be plausible in the gastrointestinal tract following acute oral exposure to a nontoxic dose of glyphosate (Table 10) but are from about 140 to 280-fold greater than peak plausible concentrations in plasma (≈0.86 mg/L).

As with the study by Kaya et al. (2000) on technical grade glyphosate, the study by Kale et al. (1995) used fruit flies but assayed for a sex-linked recessive mutation. Two formulations of glyphosate were tested, Roundup and Pondmaster, with exposures starting with larvae and extending to pupation. Given the date of the Kale publication, the Roundup formulation tested was probably the 41% IPA formulation currently called Roundup Original. Kale et al. (1995) do not provide details about the Pondmaster formulation. Current labels for formulations identified as *Pondmaster* are for an algicidal solution of copper. The 1995 analysis by aquatic formulations of glyphosate by McLaren-Hart (1995) does not identify a glyphosate formulation designated as Pondmaster. In any event, the two formulations were each tested at only a single concentration,

0.1 mg/L for Pondmaster and 1 mg/L for Roundup. Kale et al. (1995) notes that these exposure levels approximate the LC_{50} values for the two formulations.

In the first three broods, characterized as spermatocyte broods by Kale et al. (1995), the incidence of recessive lethal mutations was 13 of 4945 (0.26%) for Roundup and 12/4892 (0.24%) for Pondmaster. These effects are both characterized as statistically significant ($p<0.001$) increases, relative to control responses of 33/49,467 (or 0.06%) using a Chi-square test. In the conduct of the current Forest Service risk assessment, the statistical significance of the results reported by Kale et al. (1995) were confirmed using the Fischer Exact test which yields *p*-values of 0.000163 for Roundup and 0.000474 for Pondmaster. Note that the magnitude of increases is a factor of about 4.3 for Roundup and about 4 for Pondmaster.

In a review of the Kale et al. (1995) study, Williams et al. (2000) notes several deficiencies with this study including … *the authors' lack of experience with the assay, absence of negative controls*, but do not elaborate on this statement. The current Forest Service risk assessment cannot comment on the experience of the nine authors of the Kale et al. (1995) publication. The journal (Environmental and Molecular Mutagenesis) as well as the publisher (Wiley InterScience) are reputable. To some extent, deference must be given to the editorial and peer review process. The comment by Williams et al. (2000) concerning the lack of *negative controls* appears to refer to the fact that all of the agents tested by Kale et al. (1995) were classified as having a positive mutagenic effect. It is correct to note that an assay which yields only positive results is of limited use. It is also worth noting that Kale et al. (1995) used standard untreated controls but that the reported incidence of mutations in the untreated controls appears to be a combination of more than one control experiment. As noted by the Kale et al. (1995, p. 149), *control experiments were not always performed simultaneously with each treatment*. Williams et al. (2000) also criticize the Kale et al. (1995) study because the exposures are close to the LC_{50} values, as noted by the study authors. Nonetheless, a 1 mg/L concentration of Roundup formulation corresponds to a glyphosate concentration of about 0.3 mg a.e./L, which is 3 times lower than plausible peak concentrations in plasma (≈0.86 mg/L, Table 10) after a nontoxic dose of glyphosate.

Roundup was also shown to increase chromosomal aberrations in a plant (*Allium sp.*), associated with cell abnormalities in spindle fiber (Rank et al. 1993), DNA adduct formation in mice (Reluso et al. 1998), and single strand breaks in mice (Bolognesi et al. 1997). 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 but is capable of causing chromosomal damage. While many of the *in vitro* studies are conducted at relatively high concentrations of glyphosate or glyphosate formulations, some studies note effects at relatively low concentrations which are in the range of plausible plasma levels of glyphosate—i.e., ≈ 1 mg/L (e.g., Kale et al. 1995; Potte and Sehgal 2005; Vigfusson and Vyse 1980).

Some *in vitro* assays do involve *in vivo* exposures. In other words, the whole animal is dosed with the chemical but the assay for toxicity involves an *in vitro* culturing of cells taken from the animal. Most assays of chromosomal damage using *in vivo* exposures with mammalian species have no reported mutagenic activity or chromosomal damage (i.e., Grisolia 2002; NTP 1992;

Rank et al. 1993). Two laboratory studies involving *in vivo* exposures to a mammalian species note DNA damage. Prasad et al. (2009) reports an increase in the incidence of chromosomal breaks and micronuclei in mice following intraperitoneal dosing at 25 and 50 mg/kg bw. Manas et al. (2009a), however, report a significant increase in micronuclei in mice after intraperitoneal doses of 200 mg/kg bw administered on 2 consecutive days, but no significant increases at doses of 50 or 100 mg/kg bw.

3.1.10.1.2. Human Populations

Two studies, both of which involve the applications of glyphosate in South America to control illicit crops, assayed chromosomal damage in populations following glyphosate-formulation sprays (Paz-y-Mino et al. 2007; Bolognesi et al. 2009).

A summary of the glyphosate exposures in the study by Paz-y-Mino et al. (2007) is given in Section 3.1.12.2. Briefly, the study involves a group of 24 individuals who were exposed to a glyphosate-surfactant formulation. The application rate is not specified in Paz-y-Mino et al. (2007). By analogy to other similar applications for the control of illegal crops, the application rate was probably between about 1.2 and 5 lb a.e./acre. In addition to uncertainties in the application rate, the number of applications is unclear. Paz-y-Mino et al. (2007) state that: *spraying with a glyphosate-based herbicide had occurred continuously during three days between December 2000 and March 2001, sporadic aerial spraying continuing for three weeks following continuous spraying*. The proximity of the exposed group to the application sites also appears to be variable: …*with half of the individuals in this group having received spraying directly over their houses and the other half living within 200m to 3 km* [≈2 miles] *from the sprayed areas*.

In the Paz-y-Mino et al. (2007) study, blood samples were taken from the exposed group and an unmatched control group of 21 individuals living at least 80 km [≈50 miles] from the treated site. Other than the glyphosate spray, Paz-y-Mino et al. (2007) state that none of the individuals in the exposed or control groups… *had been exposed to pesticides during the course of their normal daily lives*. This statement is not elaborated on or otherwise documented. Differences in DNA damage between the control and exposed groups were measured based on a standard comet assay using peripheral lymphocytes. Based on DNA migration (mean ±SD), DNA damage in the exposed group (35.5 ±6.4 μm) was significantly greater than in the unexposed group (25.94 ±0.6 μm). Both Paz-y-Mino et al. (2007) and the review by Watts (2010) consider the results of the study evidence for genetic damage in humans associated with a typical use of glyphosate.

The review by Solomon et al. (2009) notes limitations in the Paz-y-Mino et al. (2007) based on the small sample size and unmatched controls. For the type of analysis used in Paz-y-Mino et al. (2007)— i.e., a standard t-test—small sample size is a limitation for studies that fail to detect a difference—i.e., low statistical power —but is not a concern for studies that detect a significant difference.

More serious limitations in the Paz-y-Mino et al. (2007) study involve the failure to demonstrate either temporal or spatial associations between exposure and effect. Paz-y-Mino et al. (2007) simply tested two groups of individuals after a spray and noted a difference. If the individuals at both sites had been tested before and after spraying, a temporal association could have been detected. Similarly, Paz-y-Mino et al. (2007) note that the exposed group consisted of

individuals who lived at the spray site as well as individuals who lived as many as 3 miles away from the spray site. If Paz-y-Mino et al. (2007) had assessed responses based on proximity to the spray and noted some positive correlation in the responses, confidence in their assertion that the glyphosate spray caused the observed effect would be enhanced. In the absence of these types of analyses, the assertion that the differences (i.e., chromosomal damage) between the two populations are due to glyphosate exposure is weak.

The study by Bolognesi et al. (2009) is much more extensive and does address temporal relationships between exposure and effect. The study by Bolognesi et al. (2009) is part of a larger effort to address the potential human health effects of a glyphosate spray program for illicit crop eradication in Columbia (i.e., Sanin et al. 2009; Solomon et al. 2005, 2007, 2009). Bolognesi et al. (2009) monitored human populations for chromosomal damage in lymphocytes, micronuclei and binucleated cells with micronuclei (BNMN), before and after glyphosate applications. The study population consisted of 274 individuals living in five different areas of Columbia, three of which were involved in the glyphosate spray program, including Valle del Cauca, Narino, and Putumayo. The other two regions were used as control areas. In one region (Santa Marta), no pesticides were used widely. In another region (Boyaca), agricultural pesticides were used, but glyphosate was not sprayed to control illicit crops.

In the Bolognesi et al. (2009) study, gyphosate exposures were associated with aerial applications of surfactant-containing glyphosate formulations at a rates of 1 kg a.e./ha (≈1.1 lb a.e./acre) in one region in which glyphosate is applied to sugar cane (Valle del Cauca) and 3.69 kg a.e./ha (≈4.1 lb a.e./acre) in two other regions where glyphosate is applied to illicit crops (Narino and Putumayo). In the regions sprayed with glyphosate, blood samples were taken prior to spraying as well as after spraying.

In the three regions sprayed with glyphosate, a statistically significant increase in binucleated cells with micronuclei was noted 5 days after spraying; however, the magnitudes of the increases were not correlated with differences in application rates among the three regions. As summarized in Figure 1 and Table 2 of the Bolognesi et al. (2009) study, the area with the lowest application rate (Valle del Cauca) evidenced the highest increase in BMNM at 5 days after spray. At 4 months after spraying, a significant decrease in micronuclei was noted only in one of the three regions, Narino, in which an application rate of 4.1 lb a.e./acre had been used.

In discussing the significance of their findings, Bolognesi et al. (2009) suggest the following:

> *Overall, these results suggest that genotoxic damage associated with glyphosate spraying, as evidenced by the MN test, is small and appears to be transient. … A greater increase in frequency of BNMN was observed in Valle del Cauca, but it cannot be attributed only to the glyphosate exposure, because the application rate of the herbicide in this area was one-third compared with that in Narino and Putumayo. … Evidence indicates that the genotoxic risk potentially associated with exposure to glyphosate in the areas where the herbicide is applied for eradication of coca and poppy is of low biological relevance*
>
> Bolognesi et al. 2009, pp. 995-996

In the final publication for the project associated with the study by Bolognesi et al. (2009), a similar conclusion is offered:

> *In those regions where spraying of glyphosate was being carried out for agricultural and eradication purposes, frequency of MN rose after spraying but these increases were not related to the rate of application or to self-reported exposures to the spray. In some regions the frequency decreased after spraying but in one, it did not. These observations do not fulfill all of the criteria for causality, suggesting that if glyphosate spraying has any influence on MN, this is small and not of biological significance.*
>
> Solomon et al. 2007, p. 919

The above interpretations have been challenged by Watts (2010) who notes that: *Despite the authors attempts to dismiss the results because they were not consistent, this study provides further evidence that exposure to glyphosate may cause DNA damage* (Watts 2010, p. 21).

A more substantial and specific concern for the interpretations given by Bolognesi et al. (2009) and Solomon et al. (2009) involves the reliance on the lack of an application rate and the dependency on the magnitude of the response as a basis for questioning the biological significance of their results. Bolognesi et al. (2009) are correct in asserting that a dose-response relationship enhances and is typically a prerequisite in concluding that a chemical causes an effect. As discussed in Section 3.1.9.1.2, the lack of dose-response relationships for several endpoints in the study by Dallegrave et al. (2007) raises doubt concerning the effects of Roundup on several reproductive endpoints. In general, if the doses or exposures can be reliably estimated, the lack of an exposure-response relationship is a reasonable basis for doubting that a chemical induces a particular effect.

In the case of the Bolognesi et al. (2009) study, however, the exposures are not well characterized. The application rate used at one site was lower than the application rate at the other two sites; however, this does not mean that the exposure levels were necessarily less for the individuals at the site with the lower application rate. The actual exposure levels would depend on the locations of the individuals in the general area, relative to the fields that were sprayed, and any number of other factors (e.g., foliar interception) that might have reduced exposures. In addition, the self-reporting data presented by Bolognesi et al. (2009, Table 4) are not amenable to a simple interpretation. As noted in the discussion in this paper, *the mean BNMN in Narino and Putumayo was greater in respondents who self-reported exposure, but differences were not statistically significant* (Bolognesi et al. (2009, p. 992). While Bolognesi et al. (2009) may be correct in their supposition that exposures to pesticides other than glyphosate may have been involved in the observed responses, this speculation cannot be demonstrated.

Another consideration in looking at the differences in the magnitudes of the responses between the Valle del Cauca site (i.e., the site with the highest response but lowest application rate) and the Narino and Putumayo sites (i.e., the sites with lower responses but a higher application rate) involves differences among glyphosate formulations. The Valle del Cauca site involved

applications of Roundup 747. This formulation is marketed in South America but has a U.S. EPA Registration Number: 524-424 (http://www.ecuaquimica.com/index.php?option=com_content&task=view&id=124lang=). The most recent EPA label for this Registration Number, according to the EPA website (http://oaspub.epa.gov/pestlabl/ppls.home), is December 12, 1988, more than 20 years old, suggesting that this formulation is no longer active in the United States. The EPA label identifies the product designation only as MON 14420, a water soluble granular formulation consisting of the mono ammonium salt of glyphosate at a concentration 74.7%. As discussed in Section 2.2.2, this formulation is identified by the U.S. EPA/OPP (2008a) as the most toxic of all glyphosate formulations; moreover, this formulation is not used in Forest Service programs.

The two sites with the high application rate but lower responses involved applications of Glyphos (NOS) with Cosmo-Flux 411F surfactant. As noted in Section 3.1.12.2, this surfactant is an adjuvant developed in South America consisting of a mixture of linear and aryl polyethoxylates at a concentration of 17% (w/v) and isoparaffins at a concentration of 83% (Solomon et al. 2005, p. 24). Given the apparent importance of surfactants to the genotoxicity of glyphosate formulations (Section 3.1.10.1.1), the comparisons by Bolognesi et al. (2009) on applications expressed in units of lb glyphosate/acre may be missing the point. It seems equally credible that the differences in the magnitude of the responses are attributable to differences in the formulations themselves and/or their surfactants.

A more direct interpretation of the Bolognesi et al. (2009) study is that glyphosate was applied at three sites over a standard range of application rates and that significant increases in chromosomal damage were observed after 5 days at each of the three sites. Even if viewed as totally random events with probabilities of 0.5 for increased or decreased damage, the probability of all three events all indicating damage is 0.125—i.e., 0.5^3. While this probability is not significant at the standard *p*-value of 0.05, these observations of chromosomal damage on three exposed populations would be of concern. These observations at these three sites, however, should not be viewed as random events because the temporal differences—i.e., before and 5 days after spraying—were statistically significant at each of the three sites.

The reason(s) for the persistence of the effects at 4 months after treatment cannot be identified. Nonetheless, while the study by Bolognesi et al. (2009) raises concern that applications of some glyphosate formulations ranging from about 1 to 4 lbs a.e./acre could result in an increased incidence of chromosomal damage humans, this does not demonstrate that glyphosate is mutagenic in humans—i.e., causes hereditable mutations—or that increased risks of cancer or any overt signs of toxicity would be expected. Instead, the study by Bolognesi et al. (2009) is consistent with *in vitro* assays demonstrating that glyphosate and glyphosate formulations can cause chromosomal damage.

In terms of a practical impact on hazard identification for the current Forest Service risk assessment, the central issue in the Bolognesi et al. (2009) study is not whether an effect was noted but whether the formulation and adjuvants used in the application are relevant to formulations that might be used by the Forest Service. As discussed above, neither the Paz-y-Mino et al. (2007) nor the Bolognesi et al. (2009) studies used formulations identified for use by the Forest Service (Table 2). As discussed further in Section 3.1.14 (Adjuvants and Other Ingredients), the composition of the surfactants used in Forest Service programs are not

identified because they are considered as trade secrets. In this respect, the two South American studies could be viewed as relevant to the current Forest Service risk assessment as a *worst case* assumption.

Conversely and as noted above, it is clear that the Roundup 747 formulation used in the Bolognesi et al. (2009) is more toxic than the formulations identified for use by the Forest Service. In addition, the available information on the Cosmo-Flux 411F surfactant indicates that the composition of this surfactant is different from that of the POEA surfactants used in the formulations identified for use by the Forest Service (Section 3.1.14). In the absence of studies comparable to Bolognesi et al. (2009) but based on formulations directly relevant to the current Forest Service risk assessment, the study by Bolognesi et al. (2009) raises concern. Nonetheless, the study by Bolognesi et al. (2009) is not directly applicable to the hazard assessment for the current Forest Service risk assessment.

3.1.10.2. Carcinogenicity

Information regarding the carcinogenicity of glyphosate is reviewed in detail by the EPA (U.S. EPA/OPP 1993a,b,c, 2002), the World Health Organization (WHO 1994), and in the open literature (Cox 1998a, 2004; Smith and Oehme 1992; Solomon et al. 2005, 2007, 2007; Watts 2010; Williams et al. 2000). The different reviewers have asserted very different conclusions. Based on standard *in vivo* bioassays, most reviews, including those by U.S. EPA and WHO conclude that there is no substantial evidence that glyphosate is carcinogenic. Other reviewers (Cox 1998a, 2004; Watts 2010) emphasize data from *in vitro* bioassays (discussed in Section 3.1.10) as well as some epidemiological studies on non-Hodgkin lymphoma (NHL) and suggest that glyphosate may be carcinogenic.

Based on standard animal bioassays for carcinogenic activity *in vivo* (Appendix 2, Table 4), there is no basis for asserting that glyphosate is likely to pose a substantial risk of causing cancer. In support of the preparation of the U.S. EPA Re-registration Eligibility Decision document, the Health Effects Division (HED) of U.S. EPA/OPP reviewed the available *in vivo* studies and reached the following conclusion:

> *On June 26, 1991, the HED Carcinogenicity Peer Review Committee classified glyphosate in Group E (evidence of non-carcinogenicity for humans), based on a lack of convincing evidence of carcinogenicity in adequate studies with two animal species, rat and mouse.*
>
> U.S. EPA/OPP 1993b, p. 5

This conclusion is reflected in the Re-registration Eligibility Decision document on glyphosate (U.S. EPA/OPP 1993a). This classification is also indicated in U.S. EPA's publication of tolerances for glyphosate (U.S. EPA/OPP 2002, 2007a). Finally, this conclusion is also consistent with the assessments by WHO (1994), WHO/FAO (2004) and several reviews in the open literature (Smith and Oehme 1992; Solomon et al. 2005, 2007, 2007; Williams et al. 2000).

However, the study by Stout and Ruecker (1990) does indicate 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 in males and females), the effects were not dose related. Gold et

al. (1997) report cancer potency estimates of 5.9×10^{-5} to 4.8×10^{-4} $(mg/kg/day)^{-1}$ for glyphosate, based, however, on experimental data in which there were no statistically significant increases in tumor rates at any dose level.

Studies on the potential carcinogenicity of glyphosate in humans is based primarily on studies involving self-reports of exposure to glyphosate by individuals with cancer. Hardell and Erikson (1999a) reported an increased cancer risk of non-Hodgkin lymphoma (NHL) in individuals in Sweden who reported a history of exposure to glyphosate. The increased risk, however, was not statistically significant. Acquavella et al. (1999a) have criticized the methodology used by Hardell and Erikson (1999a). As part of the response to this criticism, Hardell and Erikson (1999b) reported that an additional analysis of their data pooled with data from another study demonstrated a statistically significant increase in non-Hodgkin lymphoma associated with exposures to glyphosate. This pooled analysis is published in Hardell et al. (2002). Based on eight cases of non-Hodgkin lymphoma with eight case controls, Hardell et al. (2002) report an odds ratio of 3.04 (1.08-8.52) for glyphosate. While based on a small number of cases, this univariate odds ratio is marginally significant. Hardell et al. (2002) do not provide a multivariate odds ratio—i.e., an odds ratio considering co-exposures to other agents associated with non-Hodgkin lymphoma . Hardell et al. (2002), however, do provide a multivariate odds ratio for all herbicides combined, and this odds ratio, 1.39 (0.96-2.02), is not statistically significant.

McDuffie et al. (2001) conducted another case-control study on the associations between non-Hodgkin lymphoma and pesticide exposure in Canada. Odds ratios for glyphosate were calculated based on two different stratifications of the data, and neither odds ratio was statistically significant. However, when the analyses were stratified based on reported frequency of exposure—i.e., ≤ 2 days/year versus >2 days per year—the univariate odds ratio for the subgroup (n=23) reporting use frequencies of >2 days per year was 2.12 (1.20-3.73), which is statistically significant. Based on a multivariate analysis considering exposures to glyphosate as well as other agents associated with non-Hodgkin lymphoma , the odds ratio for glyphosate is 1.85 (0.55-6.2), which is not statistically significant.

De Roos et al. (2003) suggest several pesticides, including glyphosate, may be associated with an increased risk of non-Hodgkin lymphoma . In a subsequent analysis of the glyphosate data, however, De Roos et al. (2005a) indicate that the association could not be confirmed as statistically significant—i.e., an odds ratio of 2.6 with a 95% confidence interval of 0.7 to 9.4.

Based on a self-reporting study of individuals presumably exposed to pesticides, Eriksson et al. (2008) report odds ratios of 2.02 (1.10–3.71) for non-Hodgkin lymphoma associated with glyphosate exposure. For individuals reporting exposures to glyphosate for more than 10 years, the odds ratio was 2.26 (1.16–4.40). Both of these odds ratios are statistically significant; however, they both involve comparisons only between the control group and the subgroup of individuals with non-Hodgkin lymphoma who reported some exposure to glyphosate—i.e., a univariate analysis. Eriksson et al. (2008) also provide a multivariate analysis which considers exposures to glyphosate along with exposures to other pesticides. Based on the multivariate analysis, the odds ratio for glyphosate was not statistically significant—i.e., 1.51 (0.77–2.94) from Table VII in Eriksson et al. (2008).

The nature of the available epidemiology data on glyphosate is addressed in the U.S. EPA/OPP (2002) assessment:

> *This type of epidemiologic evaluation does not establish a definitive link to cancer. Furthermore, this information has limitations because it is based solely on unverified recollection of exposure to glyphosate-based herbicides.*

Based on an evaluation of the available animal studies as well as epidemiology studies, U.S. EPA/OPP (2002, p. 60943) classifies the carcinogenic potential of glyphosate as *Group E, No Evidence of Carcinogenicity*. Given the marginal mutagenic activity of glyphosate (Section 3.1.10.1), the failure of several chronic feeding studies to demonstrate a dose-response relationship for carcinogenicity, and the limitations in the available epidemiology studies on glyphosate, the Group E classification in U.S. EPA/OPP (1993a, 2002) appears to be reasonable.

3.1.11. Irritation and Sensitization (Effects on the Skin and Eyes)

3.1.11.1. Skin Irritation

Technical grade glyphosate causes only slight skin irritation and is classified as Category IV (the least hazardous category) for this endpoint (U.S. EPA/OPP 1993b). The U.S. EPA/OPP requires assays for skin irritation for both active ingredients and formulations. As discussed in Section 2.2.2, however, tests on every formulation may not be required because of data bridging.

As an example of the impact of data bridging, information on required assays of glyphosate formulations was solicited from the registrants for the glyphosate formulations considered in the current Forest Service risk assessment. Nufarm kindly provided copies of full studies as well as Data Evaluation Records (DERs) for Foresters' Non Selective (Nufarm product code NUP3a99), including a study relating to dermal irritation (Ehresman 2010a). DERs for other products, however, were not available due to bridging provisions.

Most pesticide manufacturers provide information on the dermal irritancy of pesticide formulations through the material safety data sheets (MSDS). Information on the dermal irritation of the glyphosate formulations identified by the Forest Service are taken from the MSDS and summarized in Appendix 1, Table 1. As reviewed by U.S. EPA/OPP (1993b) some glyphosate formulations are classified as Toxicity Category I (the most hazardous) or Category II (the second most hazardous) for skin irritation. Based on the information from the MSDS, none of the glyphosate formulations identified for use by the Forest Service appear to fall into these categories. The formulations that contain primarily glyphosate and water with no surfactants as well as most formulations with surfactants are classified as either non-irritating or only slightly irritating to skin. Some of the formulations which do contain surfactants, like Roundup Original Max, Roundup WeatherMax, and RT 3, are moderately irritating to the skin. The MSDS for one formulation, Hi-yield Killzall, indicates that the formulation causes skin irritation, but does not specify the severity of the skin irritation.

As reviewed by U.S. EPA/OPP (1993b), records from California indicate that exposures to glyphosate formulations (not specifically identified) have been associated with skin irritation. This summary by U.S. EPA/OPP (1993b) is similar to a later published report by Goldstein et al. (2002) covering calls reported to the California EPA Pesticide Illness Surveillance Program. Out

of a total of 815 calls involving glyphosate, about 30% (250) involved reports of skin irritation of which 54 were classified as skin irritation that was definitely associated with exposure to glyphosate formulations. A case of skin irritation and possibly skin sensitization associated with the domestic use of a glyphosate formulation is reported in the Italian literature (Amerio et al. 2004). The formulation is not identified specifically but is characterized as containing 41% glyphosate and 15% POEA surfactant. The 41% glyphosate is not specifically identified as the IPA salt, but this may be an oversight in the publication. Glyphosate formulations have also been associated with skin irritation in Japan; however, specific formulations are not identified (Horiuchi et al. 2008).

It is likely that the irritant effects of some glyphosate formulations to the skin are due to the surfactants in the formulations. As discussed in Section 3.1.14, POEA and other surfactants used in glyphosate formulations may be severely irritating to the eyes, skin, and other mucosal surfaces such as the gastrointestinal tract and lungs.

3.1.11.2. Skin Sensitization

Skin sensitization in guinea pigs is a standard test required for pesticide registration. Both glyphosate and Roundup were assayed for skin sensitization, and no skin sensitization was noted (U.S. EPA/OPP 1993b). As summarized in Supplement 1, numerous skin sensitization assays have been conducted on various glyphosate formulations (Guideline 81-6, Dermal sensitization, Supplement 1, pp. 155-162). While not specifically included in Appendix 1, Table 1), none of the MSDS for the formulations identified for use by the Forest Service indicates that the formulation tested positively for skin sensitization. Further, U.S. EPA/OPP (1993a,b) does not note any positive assays for skin sensitization for either glyphosate or glyphosate formulations.

As noted in the previous section, Amerio et al. (2004) report a case of skin irritation with potential sensitization after the use of an Italian glyphosate-surfactant formulation. Heras-Mendaza et al. (2008) report a rather unusual incident in Spain in which an individual experienced severe skin irritation at a site other than the contact site after using Touchdown Premium , a Spanish formulation containing 35% glyphosate ammonium salt, at 1.6% dilution. It does not seem likely that this effect represented skin sensitization, since the individual had a negative patch test after recovery. Finally, Penagos et al. (2004) report skin sensitization to glyphosate (based on patch test results) in 2/60 workers involved in mixed pesticide exposures at a banana plantation in Panama. The publication does not specify whether the patch tests were conducted with glyphosate, a glyphosate salt, or with a glyphosate formulation. In addition, the glyphosate formulation used by the workers is not specified.

3.1.11.3. Ocular Effects

Based on several eye irritation studies submitted to the U.S. EPA as part of the registration process, U.S. EPA/OPP (1993c) classifies glyphosate as mildly irritating to the eyes (Category III). As with skin irritation, however, some formulations of glyphosate are classified by the U.S. EPA/OPP as corrosive (Category I – corneal opacity not reversible within 7 days) or severe eye irritants (Category II – corneal opacity reversible within 7 days or other eye irritation persisting for 7 days or more).

Also as with skin irritation (Section 3.1.11.1), the MSDS are the source of most of the available formulation-specific information on eye irritation for the formulations identified for use by the

Forest Service, and this information is summarized in Appendix 1, Table 1. Only one formulation, Helosate Plus, has the potential to cause severe eye injury. This formulation is provided by Helm Agro US, Inc and has an EPA Registration Number of 74530-4. As noted in Table 2 of this Forest Service risk assessment, Helosate Plus is a 41% liquid formulation of the IPA salt of glyphosate. No specific information is available on the surfactant in this formation, and the presence of a surfactant in the formulation is inferred. The classification of severe eye irritation is one of the bases for this inference. It seems likely that Helosate Plus is a formulation that would be classified as a Category I eye irritant.

Four formulations specifically note the potential for corneal damage, including DuraMax, Durango DMA, RapidFire, and Accord XRT II. As noted in Table 3, all of these formulations have the same Dow AgroSciences product code of GF-1280, which means they are identical to one another. As noted on the MSDS for these formulations, however, the corneal damage is characterized as *slight*. Several formulations listed in Appendix 1, Table 1, indicate the potential for *moderate* eye irritation, and two formulations indicate simply that the formulation *may cause* eye irritation. For these formulations, the MSDS are not sufficiently detailed to determine if corneal damage was documented in eye irritation studies.

As with dermal irritation (Section 3.1.11.1) and as discussed further in Section 3.1.14 (Adjuvants and Other Ingredients), surfactants are probably the cause for the irritation to or corrosive effects on eyes associated with some glyphosate formulations. Notwithstanding this assertion, the MSDS for some formulations that contain a POEA surfactant are noted as causing only *slight* eye irritation. For example, Dow AgroSciences has confirmed that formulations designated as Dow AgroSciences product code GF-1279 (i.e., Accord XRT; Durango; Glyphomax XRT) are identical and contain a POEA surfactant. The MSDS for these formulations all indicate the following: "*May cause slight eye irritation. Corneal injury is unlikely*,".

For the Dow AgroSciences formulations, the nature and concentrations of the surfactants in the GF-1279 and GF-1280 products are not disclosed. As summarized in Table 2, Nufarm does disclose that both Razor and Razor Pro contain a POEA surfactant at concentrations of 8% and 14%, respectively. The MSDS for both of these formulations indicate moderate eye irritation, which might suggest that the concentration of the surfactant in these formulations is not a controlling factor in eye irritation. Nonetheless, of the 118 eye irritation studies submitted to the U.S. EPA/OPP (in Supplement 1 to the current Forest Service risk assessment), only one study is identified with Nufarm, MRID: 45605406. Thus, it may be that bridging was used and only one eye irritation study was required for the two Nufarm formulations.

In addition to the information on the MSDS for the different formulations, two published studies are available which indicate human experience with ocular irritation during the use of glyphosate formulations (Acquavella et al. 1999b; Goldstein et al. 2002). The study by Acquavella et al. (1999b) covers calls to U.S. poison control centers from 1993 to 1997. A total of 1513 calls involved ocular effects associated with the use of Roundup. Of these calls, 21% were associated with no injury, 70% with transient minor injury, and 2% with some temporary injury. One case was classified as a major effect which took more than 2 weeks to resolve. This case, however, involved an individual exposed to a dilute solution of Roundup while wearing extended wear contact lenses. In addition, symptoms were apparent in both the exposed and unexposed eye.

Thus, it is unclear if the ocular signs observed in this individual were attributable to the Roundup exposure. For all patients, the most frequently noted symptoms included blurred vision, a stinging or burning sensation, and lacrimation. No cases of permanent eye damage were reported. (Acquavella et al. 1999b).

The study by Goldstein et al. (2002) noted similar results in an analysis of 815 calls involving glyphosate exposure reported to the California EPA Pesticide Illness Surveillance Program between 1982 and 1997. About half of the calls (399 or 48.9%) involved reports of eye irritation. Of these, slightly more than half were classified as eye irritation definitely associated with exposure to glyphosate formulations (223 of 399 or about 56%). The most severe cases of eye irritation appear to involve accidental exposures in which the glyphosate formulation was sprayed into eyes under pressure.

3.1.12. Systemic Toxic Effects from Dermal Exposure

3.1.12.1. Experimental Studies

As discussed in section 3.1.3, glyphosate is poorly absorbed from the skin; accordingly, systemic toxicity is likely to be less from dermal exposure than from oral exposure. On the other hand, in terms of acute exposure levels, there is relatively little difference in the oral and dermal toxicity of glyphosate, because glyphosate is relatively non-toxic by either route. For example, the acute oral toxicity of glyphosate expressed as the LD_{50} in rats is listed by U.S. EPA/OPP (1993c) as >4320 mg/kg. Based on this LD_{50}, glyphosate is classified as Category III for oral toxicity. Similarly, the acute dermal toxicity of glyphosate expressed as the LD_{50} in rabbits is listed by U.S. EPA/OPP (1993c) as >2000 mg/kg and is also classified as Category III. As discussed in Section 3.1.4.2, these are indefinite LD_{50} values in which the "greater than" designation (>) indicates that fewer than 50% of the animals died at the maximum dose tested, in this example 4320 mg/kg for oral exposure and 2000 mg/kg for dermal exposure. The difference in these doses is an artifact of the highest doses used in the toxicity studies and does not indicate that glyphosate is more toxic by the dermal route than by the oral route of exposure.

As summarized in Appendix 1, Table 1, virtually all of the dermal LD_{50} values from the MSDS for the glyphosate formulations identified for use by the Forest Service are indefinite LD_{50} values ranging from >2000 to >5000 mg/kg bw. None of these differences should be construed to mean that one formulation is more or less toxic than the other. The only exception is Helosate Plus which indicates a definite dermal LD_{50} of 5000 mg/kg bw. As discussed in the Section on eye irritation, Helosate Plus appears to be more damaging to the eyes, relative to other glyphosate formulations. Nonetheless, the dermal LD_{50} of 5000 mg/kg bw for Helosate Plus would classify this formulation as Category III for dermal toxicity, the same classification for all of the other glyphosate formulations.

A more meaningful assessment of the dermal toxicity of glyphosate can be made from repeated dose 21-day studies. The U.S. EPA RED for glyphosate (U.S. EPA/OPP 1993c) cites a 1982 study (MRID 00098460) in which glyphosate was applied to the intact or abraded skin of rabbits at doses of 10, 1000 or 5000 mg/kg/day, 5 days per week, for 3 weeks. The only treatment-related effects included slight irritation of the abraded skin (a local and not a systemic effect), decreased food consumption, and decreased serum lactic dehydrogenase activity at 5000 mg/kg/day. In a more recent but similarly designed study in rats (Pinto 1996), dermal doses of

250, 500, or 1000 mg/kg/day caused no effects on body weight, food consumption, hematology, clinical chemistry, or organ weights, and there were no signs of dermal irritation or pathological changes in any tissue.

3.1.12.2. Human Studies

Two very different types of studies suggest an association between exposures to glyphosate formulations and systemic toxicity in humans (Goldstein et al. 2002; Paz-y-Mino et al. 2007). The study by Goldstein et al. (2002) involves poison control center calls to the California EPA Pesticide Illness Surveillance Program between 1982 and 1997. The paper by Paz-y-Mino et al. (2007) involves reports of illnesses in a small population in Ecuador following an aerial application of glyphosate. While multiple routes of exposure may have been involved in both populations considered by these papers, the primary route of exposure was probably dermal, and for that reason these papers are considered in this subsection.

Goldstein et al. (2002) analyzed 815 calls involving glyphosate exposures. Of these, 22 calls (≈2.7%) were classified as reports that could definitely be associated with systemic effects. As discussed by Goldstein et al. (2002), the primary route of exposure in these cases was dermal. The specific symptoms reported included … *nausea, vomiting, tiredness, diarrhea, dizziness, lightheadedness, headache, fever, shakes and chills, blurred vision and double vision with unilateral scotomata* [visual impairment in one eye]*, and lethargy*.

The reports by Goldstein et al. (2002) of systemic toxicity associated with dermal exposure are difficult to interpret. The effects on vision are consistent with the effects of glyphosate following direct ocular exposures (Section 3.1.11.3) but not with systemic toxicity. Most of the symptoms would be consistent with oral intoxication. Goldstein et al. (2002), however, specifically note that significant oral exposures in these cases can be ruled out. As also noted by Goldstein et al. (2002) …the *available literature on glyphosate product toxicity would not support the occurrence of systemic symptoms following the types of exposures reported*. Goldstein et al. (2002) go on to suggest that these atypical reports may reflect reporting deficiencies in the California Pesticide Illness Surveillance Program. While the reports of systemic toxicity following dermal exposures discussed by Goldstein et al. (2002) could be an artifact of the reporting system, this speculation cannot be confirmed.

The paper by Paz-y-Mino et al. (2007) involves a small group (N=24) of individuals who appear to have been exposed to an application of Roundup Ultra associated with the eradication of illicit crops. The primary focus of the Paz-y-Mino et al. (2007) publication involves DNA damage, and this aspect of the paper is discussed further in Section 3.1.10.1. The individuals were exposed to applications of Roundup as well as Cosmoflux 411F. As discussed by Solomon et al. (2005, p. 24), Cosmoflux 411F is an adjuvant developed in South America to be used in drug control programs. The adjuvant consists of a mixture of linear and aryl polyethoxylates at a concentration of 17% (w/v) and isoparaffins at a concentration of 83%. The individuals in the Paz-y-Mino et al. (2007) were exposed to a glyphosate-surfactant aerial spray at a rate of 23.4 liters ha^{-1}. The exact application rate cannot be calculated from the data in the Paz-y-Mino et al. (2007) paper. As noted by Solomon et al. (2005, p. 30), the application rates typically used in South American drug enforcement applications of glyphosate are 1.2 lb a.e./acre for poppy control and about 5 lb a.e./acre for coca control. Signs of systemic toxicity reported in the group of exposed individuals included: … *intestinal pain and vomiting, diarrhea, fever, heart*

palpitations, headaches, dizziness, numbness, insomnia, sadness, burning of eyes or skin, blurred vision, difficulty in breathing and blisters or rash. The report of the symptoms in the Paz-y-Mino et al. (2007) paper is very brief, and the association between the symptoms and actual levels of glyphosate exposure to the individuals is not clear. Some of the symptoms (e.g., sadness) cannot be interpreted. Most of the symptoms, however, are generally consistent with the reported symptoms in the paper by Goldstein et al. (2002). As with the commentary offered by Goldstein et al. (2002), the symptoms reported in the paper by Paz-y-Mino et al. (2007) would be consistent with signs of gross over-exposure to glyphosate but would not be expected under normal circumstances.

While the above reports are difficult to interpret clearly, the potential risks of systemic effects from dermal exposures is a potential hazard considered in all Forest Service risk assessments. The plausibility of these risks from the use of glyphosate in Forest Service programs is discussed further in Section 3.4 (Risk Characterization).

3.1.13. Inhalation Exposure

3.1.13.1. Glyphosate and Formulations

Glyphosate has a very low vapor pressure—i.e., 1.31×10^{-2} mPa at 25°C (Tomlin 2004)—and will not tend to volatilize. As discussed further in Section 3.2.2. (exposure assessment for workers), the low volatility of glyphosate is reflected in biomonitoring studies in workers, which indicate that inhalation exposure levels for workers applying glyphosate are low relative to the dermal exposure levels (Jauhiainen et al. 1991; Johnson et al. 2005). Specifically, Jauhiainen et al. (1991) monitored air samples in the breathing zone of forestry applicators who participated in mixing and spraying (brush saw applications) operations. The highest monitored air concentration was 15.7 µg/m^3, equivalent to 1.57×10^{-5} mg/L [m^3=1000 L]. Much higher concentrations in air are reported in the more recent study by Johnson et al. (2005), which assayed air samples in the breathing zone of workers applying glyphosate using all-terrain vehicles and backpack sprayers. Concentrations of glyphosate in air ranged from 7 to 37 mg/m^3 for all-terrain vehicles and from 0.2 to 0.61 mg/m^3 for backpack applications.

The U.S. EPA waived the requirement of an acute inhalation study for technical grade glyphosate (U.S. EPA 1993b, p. 10) because adequate inhalation toxicity data are available on glyphosate formulations. As with acute oral and acute inhalation studies, the U.S. EPA/OPP does require acute inhalation studies on pesticide formulations. These studies follow a standard protocol in which the inhalation LC_{50} is determined in rats over a 4-hour exposure period. The currently available data for glyphosate formulations are taken from the MSDS. As indicated in Appendix 1 (Table 1) and illustrated in Figure 5, the inhalation data fall into three categories: acute reported LC_{50} determinations, limit tests in which the LC_{50} is reported as a *greater than* (>) value, and limit tests in which no numeric value is reported. The availability of acute LC_{50} determinations indicates that a concentration-response relationship was observed and the LC_{50} could be estimated. Limit tests indicate that the compound was tested at the highest feasible concentration—i.e., the $>LC_{50}$ that is reported—and that fewer than 50% of the animals died. These are indefinite LC_{50} values, analogous to the indefinite oral LD_{50} values discussed in Section 3.1.4. The limit tests in which no LC_{50} value is given reflect difference in reporting methods on the MSDS. The lack of a reported value indicates that fewer than 50% of the animals died but that the limit concentration is not reported on the MSDS.

Some of the concentrations reported in the MSDS can be associated with specific studies submitted to the U.S. EPA/OPP. The limit value of 6.37 mg/L for three 58.8 % formulations of the IPA salt of glyphosate appears to be from the inhalation study by (McGuirk 1999a). The LC_{50} of >2.6 mg/L for Roundup ProDry appears to reference the acute inhalation study by Dudek and Cortner (1998) in which 20% mortality (1/5 rats of each sex) was observed after 4-hour inhalation exposures to MON 77063 at a concentration of 2.6 mg/L. The lowest reported definite LC_{50} is 1.6 mg/L—i.e., Hocho Plus, a 41% (w/w) formulation of the IPA salt of glyphosate. Thus, the LC_{50} of 1.6 mg formulation/L corresponds to about 0.66 mg a.i./L [1.6 mg formulation/L x 0.41 a.e. w/w]. Using the a.i. to a.e. conversion factor of 0.74 for the IPA salt (Table 1), this concentration is equivalent to about 0.5 mg a.e./L [0.66 mg a.i./L x 0.74 a.e/a.i. = 0.4884 mg a.e./L]. A concentration of 0.4884 mg a.e./L is above the highest detected concentration of glyphosate in air during glyphosate applications —i.e., $2.5x10^{-5}$ mg a.e./L from the study by Schneider et al. (1999)—by a factor of 20,000 [0.5 mg/L ÷ $2.5x10^{-5}$ mg/L].

A case of *"Roundup Pneumonitis"* has been reported by Pushnoy et al. (1998). This case concerned an individual with shortness of breath, respiratory irritation, and dizziness. Exposure to Roundup involved disassembling sprayer equipment used to apply Roundup. As discussed by Goldstein et al. (1999), the association between Roundup exposure and the development of these symptoms is tenuous, given that this individual may have been exposed to diesel fuel aerosols, chlorinated solvents, or welding fumes.

Jamison et al. (1986) suggested a possible health effect associated with the inhalation of dust from glyphosate-treated flax dust. In this study, 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 dust consistently caused a greater depression in respiratory function, compared with the dust from flax not treated with glyphosate. As noted by the authors, the glyphosate was applied to the flax 6 weeks prior to testing and it is likely that there was very little glyphosate residue on the flax. The authors also note that particle size distribution of the two dusts used in the study was not significantly different. Based on particle size distribution data presented in this publication (Jamison 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 (e.g., Rozman and Klaassen 1996). Thus, even though the differences in the particle size distributions may not be statistically significant, the apparent difference in biological activity may be attributed to the higher concentration of respirable particles in the glyphosate-treated flax.

The frequency of nasal irritation (rhinitis) in workers involved in pesticide applications in North Carolina was investigated by Slager et al. (2009). Although rhinitis is considered a localized effect and not a sign of broader toxicity to the respiratory tract, the endpoint is most closely related to inhalation exposure. Exposure to glyphosate alone was not associated with a significant increase in rhinitis (an odds ratio of 1.07 with a 95% confidence interval of 0.78-1.48). Nevertheless, there was a significant increase in rhinitis in workers exposed to to both glyphosate and 2,4-D (not quantitatively defined) with an odds ratio of 1.42 and 95% confidence interval of 1.14-1.77.

One study in the open literature, Marc et al. (2004b), raises concerns for inhalation exposures but these concerns are not support by the data in the study. This study involves cultures of sea urchin eggs exposed to several glyphosate formulations in which disruption of normal egg development was noted at reported concentrations of 0.1 to 10 mM (Marc et al. 2004b). While not specifically stated in the publication, the concentrations appear to be in units of acid equivalents rather than formulation, because molar concentrations are reported—i.e., it is not meaningful to report formulation (mixture) concentrations in molar units. Under this assumption, the effect level concentrations are in the range of about 16.7 mg a.e./L (0.1 M) to 1670 mg a.e./L (10 mM).

The Marc et al. (2004b) study appears to be a well conducted and is discussed further in the ecological risk assessment (Section 4.3.1). Nonetheless, the discussion of results of the study include statements concerning the hazard identification and risk characterization for human inhalation exposure levels, which must be addressed in this section of the Forest Service risk assessment. To ensure that the assertions from Marc et al. (2004b) are presented in full context, their statements are quoted in detail in the following paragraphs.

At end of the abstract to the paper, Marc et al. (2004b) make the following statement:

> *The threshold concentration for induction of cell cycle dysfunction was evaluated for each product and suggests high risk by inhalation for people in the vicinity of the pesticide handling sprayed at 500 to 4000 times higher dose than the cell-cycle adverse concentration.*
>
> Marc et al. 2004b, p. 245

In the body of the paper, these investigators note that the concentrations causing cell dysfunction are

> *… a concentration much lower than the concentration of the product in the micro-droplets sprayed for herbicide intention suggesting high risk by inhalation for people in the vicinity of spraying*
>
> (Marc et al. 2004b, p. 246).

Finally, the authors state:

> *Therefore, glyphosate-based pesticides are clearly of human health concern by inhalation in the vicinity of spraying. Our experiments detect very early a long term risk for humans since cancer may originate from a single cell several years or decades after the initial stress.*
>
> Marc et al. 2004b, p. 248

Although the above statements are found in a peer-reviewed journal from a reputable publisher and reiterated in glyphosate reviews (Watts 2010), they appear to be unjustified. Marc et al. (2004b) do not present an exposure assessment for inhalation. The allusion to a quantitative exposure in the statement concerning risks *at 500 to 4000 times higher dose* implies extremely high concentrations of glyphosate. The effect concentrations noted by Marc et al. (2004b) range from 16.7 mg a.e./L (0.1 M) to 1670 mg a.e./L. A factor of 4000 greater than the lower bound

of this range is about 66.8 g/L and a factor of 500 greater than the upper bound of this range is about 668 g/L. The concentration of glyphosate in the formulations assayed by Marc et al. (2004b) ranges from 170 to 360 g/L. These dose levels are essentially undiluted concentrations of glyphosate formulations.

An exposure assessment for pulmonary contact with an undiluted glyphosate formulation could be described as follows: An individual is in an area of glyphosate use and the individual is immersed in an undiluted formulation of glyphosate for a sufficient period of time that the individual aspirates a significant amount of the undiluted glyphosate formulation into the lungs. This highly implausible exposure scenario would be of concern, but cancer risk would not be an issue. Apart from the risk of drowning, the aspiration of an undiluted glyphosate formulation into the lungs could be fatal. As discussed in Section 3.1.4.4 and detailed in Appendix 2 (Table 6), many deaths involving the suicidal ingestion of glyphosate formulations which contain surfactants are attributed to aspiration of the formulation into the lungs (e.g., Chang et al. 1999; Hung et al. 1997; Kageura et al. 1988).

Marc et al. (2004b) do not provide an objective basis for asserting that the results of their study have any impact on the hazard identification for inhalation exposures to members of the general public. As noted above, inhalation exposures for workers involved in the application of glyphosate have been documented at glyphosate concentrations of up to 37 mg a.e./m^3, equivalent to 0.037 mg a.e./L. Even accepting the premise that the results in sea urchins are directly relevant to human inhalation exposures, the maximum documented concentration of glyphosate in the breathing zone of workers is below the minimum effect level noted by Marc et al. (2004b) by a factor of about 450 [i.e., 16.7 mg a.e./L $\div$ 0.037 mg a.e./L $\approx$ 451.4]. There are no known monitoring data associated with exposure of the general public to air concentrations of glyphosate during an aerial spray; nevertheless, it is reasonable to assume that members of the general public will be exposed to air concentrations of glyphosate that are far lower than those associated with worker exposure.

3.1.13.2. Brown-and-Burn Operations

In brown-and-burn operations, unwanted vegetation is removed by burning 30-180 days after a herbicide application. Although glyphosate is used in brown-and-burn operations, there is no information specifically regarding the amounts of glyphosate residue in treated vegetation before it is burned or the levels of glyphosate in smoke as the treated vegetation is burned. Bush et al. (1987) assayed concentrations of several herbicides, including 2,4-D, dicamba, dichlorprop, picloram, and triclopyr, in the combustion of logs and observed that relatively hot fires, 800-1000 °C, resulted in greater than 95% thermal degradation of these herbicides. Slower fires—i.e., ≈500 °C—resulted in relatively little thermal degradation.

Given that the inhalation toxicity of glyphosate is low, inhalation exposure to glyphosate during brown-and-burn operations does not appear to be a substantial concern. In addition, glyphosate will decompose at >200°C (Tomlin 2004), meaning that even in a very slow fire, little glyphosate should be released. As noted by Dost (2003), however, concern may be expressed for the thermal degradation products of herbicides for which the is little information with respect to glyphosate. Dost (2003) notes that small amounts of acetonitrile may be released in the combustion of glyphosate and that the nitrogen in glyphosate could form ammonia. Flora and Simon (1981) report that the low combustion temperature (200°C to 240°C) of glyphosate can

result in the formation of a glyphosate dimer. Whether or not and to what extent these compounds would form during brown-and-burn operations with glyphosate is not known.

Further concern for thermal degradation products of glyphosate is raised in a case report by Fisher et al. (2008). This report involves an agricultural worker who was involved in applications of a 41% glyphosate IPA formulation over a 3-year period. In one incident, the individual disposed of used drums of this formulation by burning the drums in an open field. Neither the composition of the drums nor the method of burning the drums is specified in the publication. What is clear is 2 days after the drum burning incident, the individual developed severe skin irritation on the upper body, arms, and legs. Fisher et al. (2008) diagnosed this condition as pemphigus vulgaris caused by exposure to the fumes of burning glyphosate. As reviewed by Brenner et al. (2001, 2003), pemphigus vulgaris is an autoimmune condition associated with severe skin irritation. The patient treated by Fisher et al. (2008) evidenced as an immune response the presence of immunoglobin in the skin (epidermis). As noted by Fisher et al. (2008), the mechanism by which pesticides might induce pemphigus vulgaris is not known. As also noted by Fisher et al. (2008), formation of antibodies after exposure to an antigen (i.e., an agent that causes an immune or allergic response) typically takes1week. In the case reported by Fisher et al. (2008), the patient responded within 2 days. Fisher et al. (2008) speculate that their patient may have been predisposed to the more rapid development of pemphigus vulgaris by genetic factors as well as prior exposure to fumes.

The relevance of the case reported by Fisher et al. (2008) to brown-and-burn operations with glyphosate cannot be determined with any certainty. The publication does not specify whether the drums used in the operation were metal or plastic. The publication also does not describe either the nature of the fire—wood burning, petroleum fuel or some other source of fire—used to burn the drums or the amounts of glyphosate in the drums. Notably, however, the patient described by Fisher et al. (2008) clearly required immediate medical care for the severe effects on his skin. Brown-and-burn operations with glyphosate have been conducted as part of Forest Service and other vegetation management programs for many years, and it seems reasonable to suggest that severe effects such as those described by Fisher et al. (2008) would have been noted in workers involved in brown-and-burn operations. No such reports have been encountered.

While there is no basis for asserting that exposure to glyphosate combustion products are likely to pose a risk to humans during brown-and-burn operations, the available information on the nature and toxicity of the combustion products and the levels of exposure to these products during brown-and-burn operations prevents any standard development of a HQ. This limits confidence in the assessment of risks associated with brown-and-burn operations. Dost (1982, 2003) developed an extremely conservative approach to a rough quantitative assessment of risks based on the assumption that toxic materials formed during combustion would be directly proportional to particulates in air during combustion relative to the concentration of a pesticide residue in vegetation prior to burning.

For glyphosate, Dost (2003) assumes that the nitrogen in glyphosate is completely converted to ammonia. By comparison to an 8-hour occupational exposure limit for ammonia of 17.4 mg/m^3, Dost (2003) estimates that the concentration of ammonia in air associated with the combustion of glyphosate would be below the occupational exposure limit by a factor of 7000. The Dost

(2003) analysis is based on combustion occurring immediately after the application of glyphosate at 1 lb a.e./acre. As noted above, brown-and-burn operations conducted by the Forest Service occur 30-180 days after herbicide treatment, after which time, glyphosate residues on treated vegetation would be substantially lower than those used in the Dost (2003) analysis.

3.1.14. Adjuvants and Other Ingredients

3.1.14.1. General Considerations

U.S. EPA is responsible for regulating all ingredients in pesticide formulations. The term *inert* is used widely to designate compounds that do not have a direct toxic effect on the target species. While the term *inert* is codified in FIFRA, some inert ingredients can be toxic, and the U.S. EPA now uses the term *Other Ingredients* rather than *inerts* (http://www.epa.gov/opprd001/inerts/). The nomenclature is adopted in the current Forest Service risk assessment.

The role of other ingredients in pesticide formulations is most often assessed quantitatively by comparing the toxicity of the active ingredient in various types of bioassays with comparable data on the pesticide formulations. In most Forest Service pesticide risk assessments, the active ingredient is the agent of primary concern, and a discussion of other ingredients is limited to this subsection of the risk assessment.

The handling of other ingredients in the risk assessment of glyphosate, however, is much different. The surfactants used in many glyphosate formulations may be of equal or greater concern to the risk assessment than the toxicity of glyphosate itself. Consequently, as justified by the available data, most subsections of the current Forest Service risk assessment on glyphosate are subdivided into discussions of the toxicity of glyphosate, the toxicity of glyphosate formulations, and/or the toxicity of the surfactants.

While a number of surfactants may be used in conjunction with glyphosate, the most important class of surfactants is the POEA (polyoxyethyleneamine) group. A specific POEA surfactant, designated as MON 0818, was originally used with glyphosate in Roundup formulations at a concentration of 15% (Wan et al. 1989). The surfactant was a complex mixture consisting of a tallow amine surfactant at a concentration of 75% and other unidentified components.

The general structure of a tallowamine surfactant is relatively simple. As illustrated in Figure 6, a polyethoxylated tallow amine consists of three hydrocarbon moieties linked via a nitrogen atom (i.e., the amine). The hydrocarbon group (i.e., the CH_3-$(CH_2)_a$—structure on the left side of Figure 6) is derived from tallow. Tallow is a general term for the harder or denser fat 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). The bold subscripted ***a*** in Figure 6 indicates that the tallow moiety consists of a number of methylene (CH_2) groups. In other words, this moiety is a polymer of varying lengths in different tallow amines. The other two groups in tallow amine linked to the nitrogen atom consist of a series of ethoxy groups (i.e., CH_2-CH_2-O-). Ethoxy groups can be linked together by ether (-C-O-C-) bonds. For example, -CH_2-CH_2-O- CH_2-CH_2-O- is a di-ethoxy group The bold subscripted ***b*** and ***c*** designations indicate that number of ethoxy groups can vary between the two polyethoxy groups linked to the nitrogen in a tallowamine surfactant.

Because animal fat is a complex mixture and tallow amine is made from animal fat, tallow amines are complex mixtures. Because animal fat can be rendered in different ways and ethoxylation can be conducted under different conditions, POAE surfactants may differ substantially. As discussed by Brausch and Smith (2007), the properties of POEA surfactants vary, depending on differences in the length of the three groups attached to the nitrogen atom.

The differences among POEA surfactants are critical to the current Forest Service risk assessment. As discussed in several sections of this hazard identification for human health and as detailed in the hazard identification for the ecological risk assessment (Section 4.1), the toxicity of formulations which contain surfactants is greater than the toxicity of formulations which do not contain surfactants. Thus, a focus of the current Forest Service risk assessment is to describe differences in the toxicity of different formulations, and these differences are most likely due to differences in the surfactants. These differences among formulations are important because the toxicity data on formulations are limited, particularly for longer-term toxic effects.

For example, as discussed in further detail in Section 3.1.9.1.2, one study on a Brazilian formulation of Roundup (Dallegrave et al. 2007) indicates that a dose of 450 mg a.e./kg bw of this formulation results in a decrease in testosterone in male rats. While this effect cannot be unequivocally associated with the surfactant, the available *in vitro* studies on endocrine function suggest that the surfactant is the component in the formulation which most likely to impact endocrine function (Section 3.1.8). The sensible question in terms of the Forest Service risk assessment, which focuses on formulations manufactured in the United States, is whether the results from the Brazilian study are relevant to U.S. formulations.

Because the manufacturing processes and compositions of the surfactants are not disclosed, this question cannot be answered directly. In terms of mammalian toxicity data on POEA surfactants, the data are extremely limited. As summarized in Appendix 2 (Table 5), two mammalian LD_{50} values are available for POEA surfactants: 661 mg/kg bw from the Japanese study by Baba et al. (1989) and 1200 mg/kg bw from the unpublished study summarized in Williams et al. (2000). The difference between these two LD_{50} values is not remarkable. On the other hand, other types of toxicity information summarized in MSDS do suggest that some surfactant-containing formulations may be substantially more toxic than others. As discussed in Section 3.1.11, the MSDS for Helosate Plus indicates that this formulation is a severe eye irritant.

In order to obtain more detailed information than is available on the MSDS, suppliers of the formulations identified by the Forest Service were queried for information on the toxicity of formulations in the conduct of the current Forest Service risk assessment. As noted in Section 3.1.11.1, Nufarm kindly provided copies of full studies as well as DERs for Foresters' Non Selective (Nufarm product code NUP3a99 which does not contain a surfactant). With regard to formulations which contain surfactants, Nufarm provided the following comment:

> *For Razor & Razor Pro- these products contain proprietary surfactant blends which are in certain instances exclusive to Nufarm. If this*

information is made public, we stand to lose unique product attributes and competitive advantage.

Ehresman 2010a

This comment suggests that some Nufarm products contain surfactants which differ from other surfactants used by other suppliers. Again, however, this statement does not suggest that the surfactants from Nufarm differ in toxicity from those of other suppliers.

Given the lack of specific information about the composition of the surfactants used by the various suppliers of glyphosate formulations as well as any differences in surfactants which might be used by a single supplier for different formulations, potential differences between surfactant-containing formulations of glyphosate limit the hazard identification for some toxic effects.

In terms of acute toxicities, the uncertainties associated with differences in the composition of POEA or other surfactants used in glyphosate formulations is not a substantial concern. As discussed in the different subsections on acute toxicities, the U.S. EPA requires formulation testing and reviews the results of the tests. Although specific details about the individual studies are not available, the available information does not suggest that the potential for systemic toxicity after exposure to any of the various glyphosate formulations which contain surfactants is substantially different. Irritant effects to the eyes and skin are discussed qualitatively in the risk characterization (Section 3.4). While the bridging practices of the U.S. EPA do not require testing of every surfactant, the U.S. EPA has access to the data on the composition of the surfactants.

In terms of chronic toxicity, particularly reproductive effects, concerns with differences among formulations are more substantial. When faced with data limitations, a typical practice in risk assessment is to make *worst case* or at least very conservative assumptions. Given the paucity of data on the longer-term toxicities of glyphosate formulations and POEA surfactants, however, it is not clear that the available data would adequately encompass what might be considered a *worst case* – i.e., data on the most toxic formulation or surfactant in the most sensitive species.

3.1.14.2. Other Data

To the extent possible, data on surfactants have been reviewed along with data on glyphosate formulations in previous subsections of this hazard identification. Some studies, however, involve routes of exposure or experimental designs which do not lend themselves to integration into other subsections. These studies are discussed in the current subsection.

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, or 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 3050 mg/kg [3 mL · 356 mg a.e./mL ÷ 0.350 kg]. This value is within the range of definitive LD_{50} values for glyphosate formulations – i.e., 2300 to 5827 mg/kg bw, as summarized in Table 9.

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 (Section 3.1.4.4). 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"*.

Intratracheal instillations in rats resulted in much more toxic effects at much lower dose levels. Roundup at doses of 0.1, 0.2, or 0.4 mL/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, respectively, 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. Martinez and Brown (1991), however, did not test the pulmonary toxicity of glyphosate alone. Consequently, the assertion of potentiation seems speculative, and a simple additive response cannot be ruled out.

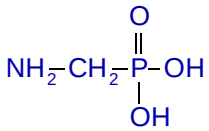
Adam et al. (1997) studied the effects of glyphosate, POEA, mixtures of glyphosate and POEA, as well as a commercial formulation of Roundup (41% glyphosate IPA and 18% POEA) in rats after gavage (oral) and intratracheal installations (i.e., directly to the lungs). Respiratory effects and pulmonary damage were more severe in the rats dosed with any of the POEA containing treatments than with glyphosate alone. Similarly, the gastrointestinal effects of the POEA containing treatments were uniformly more severe than seen in rats treated with glyphosate alone. Tai et al. (1990) report that injections of Roundup in rats led to cardiac depression caused solely by POEA and partially antagonized by glyphosate.

3.1.15. Impurities and Metabolites

One metabolite (aminomethyl phosphonate) and two impurities (N-nitrosoglyphosate and 1,4-dioxane) are specifically addressed in the following subsection. The only other relevant information encountered in the literature is the report by Brosillon et al. (2006) on the impact of water chlorination on glyphosate residues in water. During water chlorination, glyphosate will degrade rapidly with decomposition products similar to those of amino acids.

3.1.15.1. Aminomethyl phosphonate (AMPA)

The primary metabolite of glyphosate in mammals and other organisms is aminomethyl phosphonate (AMPA):



AMPA

which is formed together with glyoxylic acid (HCO-COOH).

In mammals, only very small amounts of AMPA, less than 1% of the absorbed dose, are formed (e.g., Brewster et al. 1991). In addition, AMPA is an environmental metabolite of glyphosate. This is to say that AMPA is formed in environmental media such as soil and water as a breakdown product of glyphosate (Gard et al. 1997). AMPA is also formed in the degradation of amino(trimethylenephosphonic) acid, a compound used as a scale inhibitor and additive in washing agents (Schweinsberg et al. 1999)

These two differing sources of exposure, an endogenous metabolite in mammals and an environmental metabolite, must be handled differently in this risk assessment. The approach of examining the potential importance of the metabolism of a chemical agent by a mammal is common in the risk assessment of xenobiotics, which generally involve the formation of one or more mammalian 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, risk assessments typically express dose 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. Thus, in terms of assessing direct exposures to technical grade glyphosate, the inherent exposures to AMPA as a metabolite are encompassed by the existing toxicity data on glyphosate.

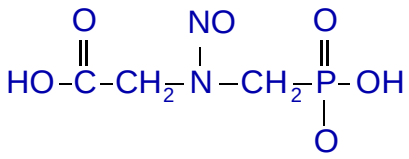
This approach does not, however, encompass concern for exposures to AMPA as an environmental metabolite. As noted above, about 20% of applied dose of glyphosate may be found in water as AMPA after about 6 months. The toxicity and environmental fate of AMPA was reviewed recently by WHO (1997), Cox (2002), and Williams et al. (2000). In addition, the U.S. EPA/OPP (2002) reviewed this information and assessed the potential consequences of exposures to AMPA as an environmental degradate. Based on this review, the U.S. EPA/OPP (2002) concludes:

> *The nature of the residue in plants and animals is adequately understood and consists of the parent, glyphosate. The Agency has decided that only glyphosate parent is to be regulated in plant and animal commodities and that the major metabolite, AMPA (aminomethylphosphonic acid) is not of toxicological concern regardless of its levels in food.* – U.S. EPA/OPP (2002, p. 17725)

While Cox (2002) has cited concerns for AMPA based on a limited subset of the literature on this compound, no formal dose-response and exposure assessment is presented which argues against the position of U.S. EPA/OPP (2002). Furthermore, the position taken in U.S. EPA/OPP (2002) is supported by the conclusions of the more extensive reviews by both WHO (1997) and Williams et al. (2002). The position taken by U.S. EPA/OPP (2002) appears to be reasonable and is well-supported. Consequently, in this risk assessment, AMPA is not quantitatively considered in the dose-response and exposure assessments.

3.1.15.2. 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. 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 (RED) 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.*

As with AMPA, a detailed dose-response and exposure assessment for NNG does not appear to be warranted.

3.1.15.3. 1,4-Dioxane

1,4-Dioxane is a contaminant in POEA. The upper limit of 1,4-dioxane in the POEA surfactant used in Roundup is about 0.03% (SERA 1997; Watts 2010). The U.S. EPA (U.S. EPA/IRIS 1992) considers dioxane to be a carcinogen, Class B2: Probable human carcinogen and 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).

The potential risks associated with dioxane can be crudely approximated. These calculations, detailed below, are included in a custom worksheet (`Dioxane`), in the EXCEL workbooks for terrestrial applications, which accompany this risk assessment. As summarized in Table 2, most liquid formulations of glyphosate which contain a POEA surfactant consist of about 30% (w/w) glyphosate acid—i.e., a proportion of 0.3. As summarized in Table 2, the information on the concentration of the POEA surfactant in glyphosate is limited but several formulations contain about 15% POEA or a proportion of about 0.15. If POEA contains 0.03% dioxane (a proportion of 0.0003), a typical liquid glyphosate formulation will contain dioxane at a proportion of

0.000045 [0.15 x 0.003]. Thus, the proportion of dioxane relative to glyphosate acid is about 0.00015 [0.000045 ÷ 0.3].

As summarized in Worksheet E03 of the EXCEL workbooks that accompany this risk assessment, the highest longer-term exposures to glyphosate are associated with the consumption of contaminated fruit. At a unit application rate of 1 lb a.e./acre, the doses of glyphosate for this exposure scenario are about 0.03 (0.002 to 0.2) mg/kg bw/day. As detailed in Worksheet D03a, these estimated doses are based on 90-day average concentrations rather than yearly average concentrations. Cancer risks are based on lifetime daily exposures. Adjusting the exposure period in Worksheet D03a to 365 days, the estimated doses of glyphosate are about 0.0064 (0.00044 to 0.053) mg/kg bw/day. Taking the proportion of 1,4-dioxane as 0.00015, the average doses of dioxane would be about $9.6x10^{-6}$ ($6.6x10^{-8}$ to $7.95x10^{-6}$) mg/kg bw/day. Multiplying these average daily doses by the cancer potency factor of 0.011 $(mg/kg/day)^{-1}$, the cancer risks would be about $1.1x10^{-8}$ ($7.3x10^{-10}$ to $8.75x10^{-8}$). These risks would be associated with a unit application rate of 1 lb a.e./acre, and the risks are linearly related to the application rate. Thus, at the maximum application rate of about 8 lb a.e./acre, the resulting risks would be $8.45x10^{-8}$ ($5.8x10^{-9}$ to $7.0x10^{-7}$). The upper bound risk is equivalent to a cancer risk of 1 in about 1.5 million.

The above assessment of the risks associated with 1,4-dioxane is similar to that of the analysis by Borrecco and Neisess (1991) which assesses the risks of 1,4-dioxane as a contaminant in Roundup and demonstrates that the upper limit of risk associated with 1,4-dioxane is extremely low—e.g., $<1 \cdot 10^{-7}$. The cancer potency factor used by Borrecco and Neisess (1991) 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).

3.1.16. Toxicological Interactions

As discussed in Section 3.1.4.3, glyphosate and a POEA surfactant used in some glyphosate formulations appear to have a less than additive toxicological interaction. As discussed further in the ecological risk assessment, additional information is available on the joint action of glyphosate and POEA surfactants. As with effects in mammals, the POEA surfactants and glyphosate formulations with POEA surfactants are more toxic than technical grade glyphosate to aquatic organisms. As with mammals, the joint action appears to be less than additive.

As noted in Section 3.1.2 and discussed further in Section 3.1.8, glyphosate and glyphosate formulations inhibit at least some cytochrome P450 isozymes—i.e., aromatase. At high concentrations or high doses, this inhibition is associated with alterations in endogenous hormones. Thus, it seems reasonable to speculate that glyphosate could have an impact on the metabolism of exogenous compounds, such as pesticides, which are also metabolized by cytochrome P450 enzymes. This inhibition of P450 enzymes could enhance or diminish the toxicity of other compounds, depending on whether metabolism increases or decreases the toxicity of the other compound (e.g., Lewis et al. 1998).

3.2. EXPOSURE ASSESSMENT

3.2.1. Overview

All exposure assessments for glyphosate are summarized in Worksheet E01 for workers and Worksheet E03 for the general public in the EXCEL workbooks that accompany this risk assessment. All exposure assessments are based on unit application rate of 1 lb a.e./acre. The consequences of varying this application rate are considered in the risk characterization (Section 3.4).

For workers applying glyphosate, three types of application methods are modeled: directed foliar (backpack), broadcast ground spray, and aerial spray. In non-accidental scenarios involving the normal application of glyphosate, central estimates of exposure for workers are approximately 0.015 mg/kg bw/day for aerial, 0.022 mg/kg bw/day for ground broadcast, and 0.013 mg/kg bw/day for directed foliar applications. Upper ranges of exposures are approximately 0.08 mg/kg bw/day for aerial, 0.15 mg/kg bw/day for ground broadcast, and 0.08 mg/kg bw/day for directed foliar applications. All of the accidental exposure scenarios for workers involve dermal exposures. Because glyphosate is not readily absorbed by the dermal route and because the accidental dermal exposure scenarios involve relatively brief periods of time, the estimated doses are much lower than those associated with general exposures over the course of a workday.

For the general public (Worksheet E03), acute levels of exposures range from minuscule (e.g., $1x10^{-10}$ mg/kg/day, the lower bound for swimming in contaminated water, to about 2 mg/kg bw. The upper bound of exposure, 2 mg/kg bw, is associated with the consumption of contaminated water by a child shortly after an accidental spill. This exposure scenario is highly arbitrary. The upper bound of the dose associated with the consumption of contaminated vegetation, a more plausible but still extreme exposure scenario, is about 1.4 mg/kg bw. The other acute exposure scenarios lead to much lower dose estimates.

The chronic or longer-term exposure levels are much lower than the estimates of corresponding acute exposures. The highest longer-term exposure levels are associated with the consumption of contaminated vegetation, and the upper bound for this scenario is about 0.2 mg/kg/day, which is followed by the scenario for the longer-term consumption of contaminated fruit with an upper bound of 0.03 mg/kg/day. As with the acute exposures, the lowest longer-term exposures are associated with the consumption of surface water.

3.2.2. Workers

Exposure assessments for workers are summarized in Worksheet E01 of the EXCEL workbook that accompanies this risk assessment (Attachment 1). This workbook contains a set of worksheets on glyphosate that detail each exposure scenario discussed in this risk assessment as well as summary worksheets for both workers and members of the general public. Documentation for these worksheets is presented in SERA (2009a). This section on workers and the following section on the general public provide a plain language description of the worksheets and discuss the glyphosate-specific data used in the worksheets.

Two types of exposure assessments are considered: general and accidental/incidental. The term *general exposure* is used to designate exposures involving absorbed dose estimates based on handling a specified amount of chemical during specific types of applications. The

accidental/incidental exposure scenarios involve specific events that may occur during any type of application. All exposure assessments (i.e., those for workers, members of the general public, and ecological receptors) are based on a unit application rate of 1 lb a.e./acre. The unit application rate is adopted as a convenience. For most exposure scenarios, exposure and consequent risk will scale linearly with the application rate, and the consequences of using lower or higher application rates are considered in the risk characterization (Section 3.4).

3.2.2.1. General Exposures

3.2.2.1.1. Terrestrial Applications

Based on analyses of several different pesticides using a variety of application methods, default exposure rates are estimated for three different types of applications: directed foliar (backpack), boom spray (hydraulic ground spray), and aerial. These exposure rates, taken from Table 3-3 in SERA 2007a, are summarized below:

Application Method	Exposure Rate (mg/kg bw per lb a.i.)
Directed foliar	0.003 (0.0003 to 0.01)
Broadcast foliar, boom spray	0.0002 (0.00001 to 0.0009)
Aerial	0.00003 (0.000001 to 0.0001)

As described in SERA (2001a), the ranges of estimated occupational exposure rates vary substantially among individuals and groups, (i.e., by a factor of 50 for backpack applicators and a factor of 100 for mechanical ground sprayers).

Sometimes, Forest Service pesticide risk assessments incorporate an adjustment to the worker exposure rates to consider the use of personal protective equipment (PPE). For glyphosate, the use of extraordinary PPE (e.g., Tyvek suits, respirators, etc.) is neither required on the product label nor specified by the Forest Service. Consequently, the worksheets for worker exposures (i.e., C01 series) use a clothing protection factor of 0 (i.e., no protection). As documented in Section 3.4.2 (Risk Characterization for Workers), all of the HQs for general worker exposure are substantially below the level of concern, and the use of extraordinary PPE does not have an impact on the risk characterization.

As detailed in Section 2, the most common method of application for glyphosate in Forest Service programs is ground based directed foliar spray (backpack). As indicated above and in SERA 2007a (Table 3-2), the default rate for this method of application is 0.003 mg/kg bw per lb applied with a range of 0.0003 to 0.01 mg/kg bw per lb applied.

For glyphosate, there are several worker exposure studies involving backpack applications which can be used to assess the quality of these values (Edmiston et al. 1995; Jauhianen et al. 1991; Johnson et al. 2005; Lavy et al. 1992; Machado-Neto et al. 2000; and Middendorf 1993). Three of these studies (Edmiston et al. 1995; Johnson et al. 2005; Machado-Neto et al. 2000) provide only deposition data and cannot be used directly to assess the use of the standard exposure rates. The other three studies (Jauhianen et al. 1991;Lavy et al. 1992; Middendorf 1993) involve backpack applications with both biomonitoring—i.e., urinary analysis—as well as deposition data as measures of exposure. Consequently, these three studies are the most relevant to the assessment of the general exposure rates used for backpack applications.

In the study by Jauhiainen et al. (1991), biological monitoring was conducted on five workers applying Roundup. Each worker handled an average of 9.8 L of an 8% solution of Roundup (360 g a.i./L or 270 g a.e/L). Thus, the amount of glyphosate acid handled each day was approximately 0.211 kg [9.8 L × 0.08 × 0.270 kg/L] (Jauhiainen et al. 1991, p. 62, column one, top of page) or about 0.5 lbs. 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 was assayed for glyphosate by gas chromatography/mass spectroscopy (GC/MS), and glyphosate was detected at a level of 0.085 ng/µL, equivalent to 0.085 µg/mL. Assuming that this urine sample was representative and using the default body weight of 70 kg and an approximate urinary output of 2000 mL/day (ICRP 1975, p. 354), the absorbed dose would be 119 µg [0.085 µg/mL × 1,400 mL] or 0.0017 mg/kg bw [0.119 mg ÷ 70 kg]. The corresponding exposure rate would be 0.0034 mg/kg bw per lb a.e. applied [0.0017 mg/kg bw ÷ 0.5 lb a.e.]. This value is quite similar to the central estimate of 0.003 mg/kg bw per lb applied generally used for directed foliar applications (SERA 2007a, Table 3-2).

As with the study by Jauhianen et al. (1991), the Lavy et al. (1992) study involves applications of Roundup. Nursery 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 weeds with Roundup. Biological monitoring consisted of 5-day complete urine collections. In a total of 355 urine samples, no glyphosate was detected (limit of detection = 0.01 µg/mL). Assuming that the concentration of glyphosate in the urine was just below the limit of detection and assuming a urinary output of 2000 mL, the total absorbed dose would be 20 µg or 0.020 mg [0.01 µg/mL x 2000 mL]. The most exposed individual in this study weighed 63.5 kg and handled, on average, 0.54 kg [1.18 lbs] of glyphosate per day. Thus, the maximum absorbed dose of 0.02 mg corresponds to 0.00031 mg/kg bw [0.02 mg ÷ 63.5 kg] and 0.00027 mg/kg bw per lb applied [0.00031 mg/kg ÷ 1.18 lbs]. This is modestly below the lower range of the value of 0.0003 mg/kg bw per lb applied is generally used for directed foliar applications (SERA 2007a, Table 3-2).

The study by Middendorf (1993) also involves backpack (directed foliar) applications of Roundup, albeit in a more dilute mixture (2.3%). Middendorf (1993) provides data (urinary excretion, lbs applied, body weight, and deposition) on 15 workers at three different application sites. The average exposure rate for all workers was approximately 0.00032 mg/kg bw per lb applied with a range of 0.00013-0.001 mg/kg bw per lb applied. The central estimate from the Middendorf (1993) study is virtually identical to the lower range of 0.0003 mg/kg bw per lb typically used for directed foliar applications. The upper range noted in the Middendorf (1993) study is somewhat below the central estimate of 0.003 mg/kg bw given in SERA (2007a, Table 3-2).

Given the concordance of the glyphosate-specific data with the general exposure rates used in most other Forest Service risk assessments (i.e., SERA 2007a, Table 3-2) justifies using the general exposure rates in the current Forest Service risk assessment on glyphosate. Other

general exposure considerations, including the number of acres treated per hour and number of hours worked per day, are standard exposure assumptions used in most Forest Service risk assessments and are based on information from the Forest Service (SERA 2007a, Section 3.2.2.1.).

Based on monitoring data from agricultural workers, it appears that forestry workers may be exposed to greater amounts of glyphosate compared with agricultural workers. Acquavella et al. (2004) estimate that farmers involved in agricultural applications of glyphosate may be exposed to systemic doses of up to 0.004 mg/kg bw but that most farmers would be exposed to a much lower dose—i.e., <0.001 mg/kg bw as illustrated in Acquavella et al. 2004, Figure 1. For backpack workers, the current Forest Service risk assessment estimates systemic doses of about 0.013 (0.0004 to 0.08) mg/kg bw/day at a unit application rate of 1 lb a.e./acre (Attachment 1a, Worksheet C01). This estimate suggests that backpack applicators involved in forestry applications are likely to be subject to greater exposure levels than are most farm workers. Given the intensive nature of backpack applications, the differences in estimated exposure levels for forestry workers and farmers seem intuitive.

3.2.2.1.2. Aquatic Applications

The literature on glyphosate does not include data regarding workers exposed to aquatic applications. There is, however, a study on worker exposure rates during aquatic applications of 2,4-D (Nigg and Stamper 1983). This study involves the application of a liquid formulation of 2,4-D by airboat handguns to control water hyacinths. The absorbed doses of 2,4–D were assayed in four workers as total urinary elimination over a 24-hour period. The estimated occupational exposure rates for the 2,4-D workers were 0.0009 (0.0004-0.002) mg/kg body weight per lb handled.

To estimate worker exposure rates for glyphosate applications, the estimated occupational exposure rates for the 2,4-D workers are used with the estimated amount of glyphosate handled. As specified in Worksheets C01 of Attachment 2, the amount handled is calculated as the product of the application rate (lbs a.e./acre) and the number of acres of surface water to be treated. For this exposure scenario, the unit application rate of 1 lb a.e./acre is used, and the worker is assumed to apply glyphosate to a 10-acre area. These inputs can be modified in Worksheet A01 of Attachment 2. The consequences of using different application rates and treating different surface areas are discussed in the risk characterization.

Using 2,4-D data to estimate worker exposures to glyphosate adds uncertainty to the risk assessment. In the absence of a worker exposure study involving aquatic applications of glyphosate, there is no alternative approach to reduce this uncertainty.

3.2.2.2. Accidental Exposures

Typical occupational exposures may involve multiple routes of exposure (i.e., oral, dermal, and inhalation); nonetheless, dermal exposure is generally the predominant route for herbicide applicators (Ecobichon 1998; van Hemmen 1992). Typical multi-route exposures are encompassed by the methods used in Section 3.2.2.1 on general exposures. Accidental exposures, on the other hand, are most likely to involve splashing a solution of herbicide into the eyes and may also involve various dermal exposure scenarios.

Quantitative exposure scenarios for ocular exposures are not developed in this or other Forest Service risk assessments. As discussed in Section 3.1.11.3 (Ocular Effects), ocular exposures to some formulations of glyphosate may cause moderate to severe eye damage. This effect is considered qualitatively in the risk characterization for workers (Section 3.4.2).

Accidental dermal exposure to glyphosate is considered quantitatively in this risk assessment. The two types of modeled dermal exposure include direct contact with a pesticide solution and accidental spills of the pesticide onto the surface of the skin. In addition, two exposure scenarios are developed for each of the two types of dermal exposure, and the estimated absorbed dose for each scenario is expressed in units of mg chemical/kg body weight. Both sets of exposure scenarios are summarized in Worksheet E01, which references other worksheets which provide detailed calculations.

Exposure scenarios involving direct contact with glyphosate solutions are characterized either by immersion of the hands in a field solution for 1 hour or wearing pesticide contaminated gloves for 1 hour. The assumption that the hands or any other part of a worker's body will be immersed in a chemical solution for a prolonged period of time may seem unreasonable; however, it is quite plausible that the gloves or other articles of clothing worn by a worker may become contaminated with a pesticide. For these exposure scenarios, the key assumption is that wearing gloves grossly contaminated with a chemical solution is equivalent to immersing the hands in the solution. In both cases, the chemical concentration in contact with the skin and the resulting dermal absorption rate are essentially constant.

For both scenarios (hand immersion and contaminated gloves), the assumption of zero-order absorption kinetics is appropriate. For these types of exposures, the rate of absorption is estimated, based on a zero-order dermal absorption rate (K_p). Details regarding the derivation of the K_p value for glyphosate are provided in 3.1.3.2.2. The amount of the pesticide absorbed per unit time depends directly on the concentration of the chemical in solution. As discussed in Section 2.4.1, the current risk assessment uses an application volume of 10 gallons/acre with a range of 5-25 gallons per acre, which encompasses the potential range of application rates to be used in ground and aerial applications.

Exposure scenarios involving chemical spills onto the skin are characterized by a spill on to the lower legs as well as a spill on to the hands and are based on the assumption that a certain amount of the chemical adheres to the skin. The absorbed dose is then calculated as the product of the amount of chemical on the surface of the skin (i.e., the amount of liquid per unit surface area multiplied by the surface area of the skin over which the spill occurs and the chemical concentration in the liquid), the first-order absorption rate, and the duration of exposure. The first-order absorption rate (k_a) is derived in Section 3.1.3.2.1.

While most Forest Service risk assessments rely solely on QSAR estimates for both zero-order and first-order dermal absorption rates, such is not the case for glyphosate, and the dermal absorption rates used in the current Forest Service risk assessment are supported by studies in humans and other primates (Nielsen et al. 2007; Wester et al. 1991, 1996).

Numerous exposure scenarios could be developed for direct contact or accidental spills by varying the amount or concentration of the chemical on, or in contact with, the skin surface, the surface area of the affected skin, and the duration of exposure. The impact of these variables on the risk assessment is discussed further in the risk characterization (Section 3.4.2).

3.2.3. General Public

3.2.3.1. General Considerations

3.2.3.1.1. Likelihood and Magnitude of Exposure

The chances that members of the general public will be exposed to glyphosate in Forest Service applications are highly unpredictable. In some Forest Service applications, glyphosate could be applied in recreational areas, including campgrounds, picnic areas, and trails. Because of the conservative exposure assumptions used in the current risk assessment, neither the probability of exposure nor the number of individuals who might be exposed has a substantial impact on the risk characterization presented in Section 3.4. As noted in Section 1 (Introduction) and detailed in SERA (2007a, Section 1.2.2.2), the exposure assessments developed in this risk assessment are based on *Extreme Values* rather than a single value. Extreme value exposure assessments, as the name implies, bracket the most plausible estimate of exposure (referred to statistically as the central or maximum likelihood estimate) with lower and upper bounds of credible exposure levels.

This Extreme Value approach is essentially an elaboration on the concept of the *Most Exposed Individual* (MEI), sometime referred to as the M*aximum Exposed Individual.* As this name implies, exposure assessments that use the MEI approach attempt to characterize the extreme but still plausible upper limit on exposure. This common approach to exposure assessment is used by U. S. EPA, other government agencies, and the International Commission on Radiological Protection (e.g., ATSDR 2002; ICRP 2005; Payne-Sturges et al. 2004). In the current risk assessment, all upper bounds on exposure are intended to encompass exposures to the MEI.

In addition to this upper bound MEI value, the Extreme Value approach used in this risk assessment provides a central estimate of exposure as well as a lower bound on exposure. Although not germane to assessing the upper bound risk, using the central estimate and especially the lower bound estimate is not intended to lessen concern. To the contrary, the central and lower estimates of exposure are used to assess the prospect of mitigation—e.g., protective measures to limit exposure. If lower bound exposure estimates exceed a level of concern (which is not the case in the current risk assessment), there is strong indication that the pesticide cannot be used in a manner that will lead to acceptable risk.

In addition to concern for the most exposed individual, there is concern for individuals who may be more sensitive than most members of the general population to glyphosate exposure. This concern is considered in the dose-response assessment (Section 3.3) which bases exposures on the most sensitive endpoint in the most sensitive species and uses an uncertainty factor for sensitive individuals. Atypical sensitivities—i.e., special conditions that might increase an individual's sensitivity to a particular agent—are also considered separately in the risk characterization (Section 3.4.4).

There is information regarding general population exposure to glyphosate applications which are not specifically related to Forest Service use. Although exposure to certain pesticides may be higher for persons involved in agriculture, relative to the general public, Curwin et al. (2005) did not detect glyphosate in dust from either farm or nonfarm homes. Similarly, Curwin et al. (2007a) report that despite higher peak concentrations of glyphosate in the urine, individuals living on farms did not have significantly different urinary concentrations of glyphosate from those of individuals who do not live on farms. Based on the peak concentrations of glyphosate in urine, the highest estimated dose to an individual is 0.00034 mg/kg bw (Curwin et al. 2007b). Based on dietary surveys in Europe, Harris and Gaston (2004) estimate substantially higher daily intakes of glyphosate—i.e., 0.0007 to 0.033 mg/kg bw/day. The upper bound of the range of doses estimated by Harris and Gaston (2004) is very similar to estimates of maximum daily dietary exposures from the U.S. EPA/OPP (1993b, p. 12)—i.e., 0.028-0.058 mg/kg bw/day.

As summarized in Worksheet E03 of the EXCEL workbook that accompanies this risk assessment, much higher non-accidental, daily doses are estimated in this Forest Service risk—i.e., up to 1.35 mg/kg bw for acute exposures and up to 0.74 mg/kg bw/day for longer-term exposures. It is usual for Forest Service risk assessments to estimate much higher doses for members of the general public than are typically estimated from dietary surveys such as those used by Harris and Gaston (2004) and U.S. EPA/OPP (1993b). The dietary surveys consider a typical mix of consumed items with anticipated residues in food from agricultural food tolerances (e.g., U.S. EPA/OPP 2002, 2007). Forest Service risk assessments, on the other hand, assume that individuals consume fruit or vegetation taken directly from a treated site either immediately (acute scenario) or following (long-term scenario) application. The impact of these extremely conservative exposure assumptions on the risk characterization is discussed further in Section 3.4.

3.2.3.1.2. Summary of Assessments

The exposure scenarios developed for the general public are summarized in Worksheet E03 of the EXCEL workbook that accompanies this risk assessment. As with the worker exposure scenarios, details about the assumptions and calculations used in these assessments are given in the detailed calculation worksheets in the EXCEL workbook (Worksheets D01–D11).

As summarized in Worksheet E03, the kinds of exposure scenarios developed for the general public include acute accidental, acute non-accidental, and longer-term or chronic exposures. The accidental exposure scenarios assume that an individual is exposed to the compound of concern either during or shortly after its application. As well, the nature of the accidental exposures is intentionally extreme. Non-accidental exposures involve dermal contact with contaminated vegetation as well as the consumption of contaminated fruit, vegetation, water, or fish. The longer-term or chronic exposure scenarios parallel the acute exposure scenarios for the consumption of contaminated fruit, water, or fish. All of the non-accidental exposure scenarios are based on levels of exposure to be expected in the routine uses of glyphosate at a unit application rate of 1 lb a.e./acre. The upper bounds of the exposure estimates for the non-accidental scenarios involve conservative assumptions intended to reflect exposure for the MEI (*Most Exposed Individual*). The impact of lower or higher application rates on the risk characterization is discussed in Section 3.4.

3.2.3.2. Direct Spray

Direct sprays involving ground applications are modeled similarly to accidental spills for workers (Section 3.2.2.2). In other words, the scenarios assume that an individual is sprayed with a chemical solution, some of which remains on the skin and is absorbed by first-order kinetics. Two direct spray scenarios are included in this risk assessment: one for a young child (D01a) and the other for a young woman (D01b).

The exposure scenario involving the young child assumes that a naked child is sprayed directly with a chemical during a ground broadcast application and is completely covered (i.e., 100% of the surface area of the body is exposed). This exposure scenario is intentionally extreme. As discussed in Section 3.2.3.1.1, the upper limits of this exposure scenario are intended to represent the *Extreme Value* upper limits of exposure for the *Most Exposed Individual* (MEI).

The exposure scenario involving the young woman (Worksheet D01b) is somewhat less extreme, but more credible. In this scenario, it is assumed that the woman is accidentally sprayed over the feet and lower legs. The preference for using a young woman rather than an adult male in many of the exposure assessments relates to concerns for both the *Most Exposed Individual* (MEI) as well as the most sensitive individual. Based on general allometric considerations, the smaller the individual, the greater will be the chemical doses per unit body weight (e.g., Boxenbaum and D'Souza. 1990). According to standard reference values used in exposure assessments (e.g., U.S. EPA/ORD. 1989), the female body size is smaller than that of males. Thus, in direct spray exposure scenarios, females are subject to somewhat higher doses than males. More significantly, reproductive effects are a major concern in all Forest Service risk assessments. Consequently, exposure levels for a young woman of reproductive age are used in order to better assess the potential for adverse effects in the population at risk from potential reproductive effects—i.e., the most exposed and the most sensitive individual.

For this exposure scenario, assumptions are made regarding the surface area of the skin and the body weight of the individual, as detailed in Worksheet A03. The rationale for and sources of the specific values used in these and other exposure scenarios is given in the documentation for the worksheets (SERA 2009a) as well as the documentation for the preparation of Forest Service risk assessments (SERA 2007a). As with the similar worker exposure scenarios, the first-order absorption dermal absorption rates are taken from the study by Wester et al. (1991).

3.2.3.3. Dermal Exposure from Contaminated Vegetation

The exposure scenario involving contaminated vegetation assumes that the herbicide is sprayed at a given application rate and that a young woman comes in contact with the sprayed vegetation or with other contaminated surfaces sometime after the spray operation (D02). This exposure scenario depends on estimates of dislodgeable residue (a measure of the amount of the chemical that could be released from the vegetation) and the availability of dermal transfer rates (i.e., the rate at which the chemical is transferred from the contaminated vegetation to the surface of the skin). Dermal transfer rates are reasonably consistent for a number of different pesticides (Durkin et al.1995). In addition, the methods and rates derived in Durkin et al. (1995) are used as defined in Worksheet D02, using default dislodgeable residue rate of 0.1 of the application rate from (Harris and Solomon 1992). This exposure scenario assumes both a contact period of 1hour and that the chemical is not effectively removed by washing for 24 hours. Other estimates used in this exposure scenario involve estimates of body weight, skin surface area, and first-order

dermal absorption rates, as discussed in the previous section. As with the direct spray scenarios, the first-order absorption dermal absorption rates are taken from the study by Wester et al. (1991).

3.2.3.4. Contaminated Water

3.2.3.4.1. Accidental Spill

The accidental spill scenario assumes that a young child consumes contaminated water shortly after an accidental spill of a field solution into a small pond. The specifics of this scenario are given in Worksheet D05. Because this scenario is based on the assumption that exposure occurs shortly after the spill, no dissipation or degradation is considered. Since this exposure scenario is based on assumptions that are somewhat arbitrary and highly variable, it may overestimate exposure. The actual chemical concentrations in the water will vary according to the amount of compound spilled, the size of the water body into which it is spilled, the time at which water consumption occurs relative to the time of the spill, and the amount of contaminated water consumption. To reflect the variability inherent in this exposure scenario, a spill volume of 100 gallons (range of 20-200 gallons) is used to reflect plausible spill events. The glyphosate concentrations in the field solution are also varied to reflect the plausible range of concentrations in field solutions—i.e., the material that might be spilled—using the same values as in the accidental exposure scenarios for workers (Section 3.2.2.2). Based on these assumptions, the estimated concentration of glyphosate in a small pond ranges from about 0.36 to about 18 mg/L, with a central estimate of about 4.5 mg/L (Worksheet D05). As discussed further in Section 3.2.3.4.3, glyphosate may be extensively bound to some types of soils. This binding is not considered in the accidental spill scenario and thus the concentrations that might be seen following a spill could be overestimated for some types of soils.

3.2.3.4.2. Accidental Direct Spray/drift for a Pond or Stream

These exposure scenarios involving drift are less severe but more plausible than the accidental spill scenario described above. If a 1 meter deep pond is directly sprayed with glyphosate at a unit application rate of 1.0 lb a.e./acre, the peak concentration in the pond would be about 0.11 mg/L, equivalent to 110 µg/L or 110 ppb (Worksheet D10a). This concentration is a factor of about 40 below the upper bound of the central estimate of the concentration in pond water after the accidental spill – i.e., of 4.5 mg/L (Section 3.2.3.4.1, Worksheets D05). Worksheet D10a also models concentrations at distances of 25-900 feet down wind based on standard values adapted from AgDrift (SERA 2008). Based on these estimates, concentrations of glyphosate in a small pond contaminated by drift would range from about 0.00004 mg/L (40 part per trillion) to 0.001 mg/L (2 part per billion) depending on the application method and the distance of the pond from the treated site.

Similar calculations can be made for the direct spray of or drift into a stream. For this scenario, the resulting water concentrations depend on the surface area of the stream and the rate of water flow in the stream. The stream modeled using GLEAMS (see below) is about 6 feet wide (1.82 meters), and it is assumed that the pesticide is applied along a 1038 foot (316.38 meters) length of the stream with a flow rate of 710,000 L/day. Using these values, the concentration in stream water after a direct spray is estimated at about 0.09 mg/L (90 parts per billion). Much lower concentrations, ranging from about 0.00003 mg/L (30 part per trillion) to 0.0008 mg/L (0.8 parts per billion) are estimated based on drift at distances of 25-900 feet (Worksheet D10b).

3.2.3.4.3. Gleams-Driver Modeling

The Forest Service developed a program, Gleams-Driver, to estimate expected peak and longer-term pesticide concentrations in surface water. Gleams-Driver serves as a preprocessor and postprocessor for GLEAMS (Knisel and Davis 2000). GLEAMS is a field scale model developed by the USDA/ARS and has been used for many years in Forest Service and other USDA risk assessments (SERA 2007b).

Gleams-Driver offers the option of conducting general exposure assessments using site-specific weather files from Cligen, a climate generator program developed and maintained by the USDA Agricultural Research Service (http://horizon.nserl.purdue .edu/Cligen). Gleams-Driver was used in the current risk assessment to model glyphosate concentrations in a small stream and small pond.

The generic site parameters used in the Gleams-Driver runs are summarized in Table 13, and additional details are available in the documentation for Gleams-Driver (SERA 2007b). For each site modeled, simulations were conducted using clay (high runoff, low leaching potential), loam (moderate runoff and leaching potential), and sand (low runoff, high leaching potential) soil textures. The locations of the generic sites selected for modeling include a total of nine sites, as summarized in Table 14. As discussed in SERA (2007b), these locations are standard sites for the application of Gleams-Driver in Forest Service risk assessments and are intended to represent combinations of precipitation (dry, average, and wet) and temperature (hot, temperate, and cool). For each site, Gleams-Driver was used to simulate 100 applications at a unit application rate of 1 lb/acre, and each of the simulations was followed for a period of more than 1½ years post application.

Table 15 summarizes the chemical-specific values used in Gleams-Driver simulations. For the most part, the chemical properties used in the Gleams-Driver simulations are taken from U.S. EPA/OPP (2007c). The EPA modeling efforts are discussed below (Section 3.2.3.4.4). In the current risk assessment, most of the model input values are based on the environmental fate studies submitted to the EPA by registrants as well as standard values for GLEAMS modeling recommended by Knisel and Davis (2000). The notes to Table 15 indicate the sources of the chemical-specific values used in the GLEAMS modeling effort.

Two of the chemical specific parameters used in Gleams-Driver modeling, soil K_{oc} and sediment K_d, are based on distributions rather than single values and this approach differs from the approach used in the modeling done by U.S. EPA/OPP (2008a). As summarized in Table 1, soil K_{oc} and sediment K_d values for glyphosate are highly variable. In general, glyphosate will bind tightly to soil and its leaching capacity is extremely low—i.e., glyphosate is relatively immobile in soil (e.g., Alex et al. 2008; Landry et al. 2005; Mamy and Burriuso et al. 2005). Thus, the K_{oc} and sediment K_d values are relatively high. Notwithstanding this consistency, the reported K_{oc} values in studies submitted to and accepted by the U.S. EPA/OPP (2008a, Table 2.4) span a factor of nearly 20, ranging from 3100 to 58,000. In other words, the binding of glyphosate to soil does not follow the simple K_{oc} model in which the K_{oc} should be relatively constant because soil binding is directly proportional to the organic carbon in the soil (e.g., Winegardner 1996). Because of the apparent lack of correlation between soil binding and organic carbon, the K_{oc} and

sediment K_d values are not specified by soil type. Instead, these values are represented by triangular distributions which are identical for each of the three soils modeled.

Details of the results for the Gleams-Driver runs are provided in Appendix 10. A summary of the results for the Gleams-Driver runs are presented in Table 16, along with a summary of other modeling efforts and monitoring data, both of which are discussed further in the following subsections.

3.2.3.4.4. Other Modeling Efforts

To estimate concentrations of a pesticide in ambient water, the U.S. EPA will typically use either Tier 1 screening models (i.e., GENEEC and SCIGROW) or PRZM/EXAMS, a more refined Tier 2 modeling system. In the U.S. EPA's most recent risk assessment on glyphosate, U.S. EPA/OPP (2008a), the Agency used two approaches, GENEEC for standard terrestrial applications of glyphosate and a simple dilution model for surface applications. The dilution model is based on the application rate, expressed in lb a.e./acre, and the assumption that glyphosate is applied to a pond with a surface area of 1 hectare and a water volume of 20,000,000 liters —i.e., a depth of 2 meters (U.S. EPA/OPP 2008a, Appendix D). As detailed in Worksheet B04a, the current Forest Service risk assessment takes a similar approach except that water depth is taken as 5 feet (the central estimate) with a range of 2-10 feet).

As summarized in Table 16, the surface water modeling by U.S. EPA/OPP (2008a) for terrestrial applications yields a peak concentration of 11 ppb (µg/L) with a longer-term concentration of 5.8 ppb. As also illustrated in Table 16, the upper bound estimates from Gleams-Driver exceed the EPA peak estimates by a factor of about 3 for the pond scenario and 8 for the stream scenario. The upper bound of the longer-term concentration for the pond scenario, 4.5 ppb, is only somewhat less than the concentration of 5.8 ppb from GENEEC. For aquatic applications, the differences in concentrations simply reflect minor differences in the underlying model assumptions. The concentration modeled by U.S. EPA/OPP (2008a) of 56 ppb is encompassed by the estimated concentrations of 74 (37-184) ppb in the EXCEL workbook (Worksheet B04a of Attachment 2) that accompanies this Forest Service risk assessment.

3.2.3.4.5. Monitoring Data

As summarized in Table 16, several relevant monitoring studies are useful for assessing the plausibility of the modeling effects discussed in the previous two subsections. After an aerial application of 2 kg a.i./ha (about 1.8 lb a.i./acre) Roundup over 10 km^2 in Vancouver Island, British Columbia, the maximum glyphosate concentrations in streams intentionally over-sprayed reached about 0.16 mg/L and rapidly dissipated to less than 0.04 mg/L after 10 minutes. After a storm event, peak concentrations in stream water were less than 0.15 mg/L, rapidly dissipating to less than 0.02 mg/L before the end of the storm event (Feng et al. 1990, Kreutzweiser et al. 1989). At the same application rate, another Canadian study reports maximum stream concentrations of 0.109–0.144 mg/L, occurring 7–28 hours after aerial application. Similar results were noted in a study conducted in Oregon (Newton et al. 1984). Maximum water levels in streams reached 0.27 mg/L. This concentration was associated with repeated helicopter applications (i.e., direct spray) across a small stream at an application rate of 3.3 kg/ha (equivalent to 2.9 lbs/acre). In a more recent series of studies conducted in Oregon, Michigan, and Georgia, peak concentrations in streams shortly after application of glyphosate at 4.1 kg/ha (about 3.6 lbs/acre) ranged from less than 0.1 to about 1 mg/L (Newton et al. 1994, Figure 4, p.

1799). The upper range of 1 mg/L corresponds to 0.28 mg/L per lb applied. As reviewed by Neary and Michael (1996), some applications have resulted in much lower concentrations in streams, in the range of 0.003-0.007 mg/L per lb applied (Neary and Michael 1996, Table 11, p. 253).

For most of the monitoring studies summarized in Table 16 which can be associated with a defined application rate, the results of the Gleams-Driver modeling as well as the U.S. EPA/OPP (2008a) modeling encompass the monitoring estimates. The only exception is the report by Newton et al. (1994) for which a water contamination rate (WCR) of up to 280 ppb/lb per acre can be derived. Notably, Newton et al. (1994) monitored streams that were directly sprayed during aerial applications of glyphosate, the remarkably high WCR probably reflects an application to a wide stream with a slow flow rate. The relatively high concentrations reported by Battaglin et al. (2009), Scribner et al. (2008), and Peruzzo et al. (2008) cannot be associated with application rates; accordingly, these values are not comparable directly to the Gleams-Driver or GENEEC modeling.

Detection rates for glyphosate in surface water of 29% (Scribner et al. 2003) and 39% (Scribner et al. 2007) have been reported. Some applications, however, produced no detectable concentrations in adjacent water bodies (e.g., Adams et al. 2007). The failure to detect glyphosate after an application is consistent with the Gleams-Driver modeling. As summarized in Appendix 10, Table 7, the median peak concentration of glyphosate in ponds after applications of 1 lb a.e./acre is zero (no contamination is expected) in arid areas, suggesting that with very little or no rainfall, glyphosate will not be transported to surface water.

3.2.3.4.6. Concentrations in Water Used for Risk Assessment

Table 17 summarizes the surface water concentrations of glyphosate used in this risk assessment for both terrestrial and aquatic applications. The concentrations are specified as water contamination rates (WCRs)—i.e., the concentrations in water expected at a normalized application rate of 1 lb a.e./acre, converted to units of ppm or mg/L per lb a.e./acre. In Table 16, units of exposure are expressed as ppb or µg/L, as a matter of convenience. In Table 17, however, ppb is converted to ppm because ppm (i.e., mg/L) is the unit of measure used in the EXCEL workbooks for contaminated water exposure scenarios in both the human health and ecological risk assessments. The WCR are entered in Worksheet B04 in each of the EXCEL workbooks that accompany this risk assessment. The values in Worksheet B04 are linked to the appropriate scenario-specific worksheets in the EXCEL workbooks.

Two sets of concentrations are given, one for terrestrial applications and the other for aquatic applications. The concentrations for terrestrial applications are based on a composite of the results from the Gleams-Driver modeling (Section 3.2.3.4.3) and the modeling done by U.S. EPA/OPP (3.2.3.4.4). The concentrations for aquatic applications are based on a simple dilution model as detailed in Worksheet B04a of Attachment 2, the EXCEL workbook for aquatic applications. As discussed in Section 3.2.3.4.4, the concentrations estimated by modeling are well supported and encompass the available monitoring data.

The selection of specific water contamination rates for the current Forest Service risk assessment is more judgmental than analytical. As discussed in Section 3.2.3.4.3 and detailed in Appendix 10, the concentrations of glyphosate that might be expected in surface water will vary

substantially depending on site-specific factors such as rainfall rates and soil textures. Because the current Forest Service risk assessment does not consider a specific site, the water contamination rates summarized in Table 17 are intended to reflect plausible ranges of glyphosate concentrations based on both Gleams-Driver modeling, the modeling efforts by the U.S. EPA, and the available monitoring data (Table 16). As discussed further in the risk characterization for human health effects (Section 3.4.3), the water contamination rates have a minimal impact on the human health risk assessment because the upper bound estimates of glyphosate exposure are far below the level of concern. As detailed further in Section 4.4, water contamination rates do impact the risk characterization for some groups of aquatic organisms. Consequently, in any site-specific assessment in which aquatic organisms are at potential risk, refinements to the water contamination rates given in Table 17 based on site-specific considerations are warranted.

3.2.3.5. Oral Exposure from Contaminated Fish

Many chemicals may be concentrated or partitioned from water into the tissues of aquatic animals or plants. This process is referred to as bioconcentration. Generally, bioconcentration is measured as the ratio of the concentration in the organism to the concentration in the water. For example, if the concentration in the organism is 5 mg/kg and the concentration in the water is 1 mg/L, the bioconcentration factor (BCF) is 5 L/kg [5 mg/kg ÷ 1 mg/L]. As with most absorption processes, bioconcentration depends initially on the duration of exposure but eventually reaches steady state. Details regarding the relationship of bioconcentration factor to standard pharmacokinetic principles are provided in Calabrese and Baldwin (1993).

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 appears to reflect the binding of glyphosate metabolites, including mineralized carbon, to fish tissue.

Based on the study by Forbis (1989), the U.S. EPA/OPP (1993c, p. 36) cites maximum bioconcentration factors of 0.38 for edible tissues and 0.52 for whole fish. These bioconcentration factors are consistent with a range of whole body bioconcentration factors—i.e., from 0.11 to 0.68—based on unpublished studies summarized briefly in FAO/WHO (1986, Table 21). Calabrese and Baldwin (1993) reviewed a number of different methods for estimating bioconcentration factors in fish based on chemical and physical properties. Using a log $K_{o/w}$ of -4.85 at pH 6.86 (from Chamberlain et al. 1996 as summarized in Table 1), the estimated bioconcentration factors in fish would be well below unity, consistent with the study by Forbis (1989) and the bioconcentration factors used by U.S. EPA/OPP (1993c).

For the current risk assessment, the bioconcentration factors reported by Forbis (1989) and used by EPA/OPP (1993c) are used to estimate dietary exposure to fish. These values are included in Worksheet B02 and used in all exposure assessments involving the consumption of contaminated fish. In the exposure assessment for humans, the assumption is made that the individual consumes only the edible portion of the fish. In the ecological risk assessment, the assumption is made that the predator completely consumes the fish, so the whole body bioconcentration factor is used.

3.2.3.6. Dermal Exposure from Swimming in Contaminated Water

Some geographical sites maintained by the Forest Service or Forest Service cooperators include surface water in which members of the general public might swim. To assess the potential risks associated with swimming in contaminated water, an exposure assessment is developed for a young woman swimming in surface water for 1 hour (Worksheet D11). Conceptually and computationally, this exposure scenario is virtually identical to the contaminated gloves scenario used for workers (Section 3.2.2.2)—i.e., a portion of the body is immersed in an aqueous solution of the compound at a fixed concentration for a fixed period of time.

As in the corresponding worker exposure scenario, the 1-hour period of exposure is somewhat, but not completely, arbitrary, given that longer periods of exposure are plausible. Nonetheless, the 1-hour period is intended as a unit exposure estimate. In other words, the exposure and consequently the risk will increase linearly with the duration of exposure, as indicated in Worksheet D11. Thus, a 2-hour exposure would lead to a HQ that is twice as high as that associated with an exposure period of 1 hour. In cases in which this or other similar exposures approach a level of concern, further consideration is given to the duration of exposure in the risk characterization (Section 3.4).

3.2.3.6. Oral Exposure from Contaminated Vegetation

Although none of the Forest Service applications of glyphosate will involve crop treatment, Forest Service risk assessments typically include standard exposure scenarios for the acute and longer-term consumption of contaminated vegetation. Two sets of exposure scenarios are provided: one for the consumption of contaminated fruit and the other for the consumption of contaminated vegetation. These scenarios are detailed in Worksheets D03a and D03b for acute exposure and Worksheets D04a and D04b for chronic exposure.

The concentration of the pesticide on contaminated fruit and vegetation is estimated using the empirical relationships between application rate and concentration on different types of vegetation (Fletcher et al. 1994). The rates provided by Fletcher et al. (1994) are based on a reanalysis of data originally compiled by Hoerger and Kenaga (1972) and represent estimates of pesticide concentration in different types of vegetation (mg chemical/kg vegetation) after a normalized application rate of 1 lb a.i./acre. Although the human health risk assessments conducted by the EPA do not consider this exposure scenario, the residue rates recommended by Fletcher et al. (1994) are used by U.S. EPA/OPP in their ecological risk assessment of glyphosate (U.S. EPA/OPP 1993c, p. 24).

The residue rates recommended by Fletcher et al. (1994) are given in Table 18 of the current Forest Service risk assessment. Fletcher et al. (1994) and Hoerger and Kenaga (1972) provide only central and upper bound estimates of residue rates. Accordingly, the lower bound estimates in Table 18 are made under the assumption that the ratio of the central estimate to the upper bound estimate is identical to the ratio of the lower bound estimate to the central estimate (i.e., the variability is log-symmetrical).

The residue rates from Fletcher et al. (1994) are somewhat higher than those from the study by Siltanen et al. (1981) in which glyphosate levels on cowberries and bilberries were assayed 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]. The central estimate of residues immediately after application was approximately 1.6

ppm (mg/kg) with a 95% upper limit of 4 ppm. This number corresponds to the central estimate of a residue rate of about 2.4 ppm per lb per acre [1.6 ppm ÷ 0.67 lb a.i./acre] with an upper limit of 5.9 ppm per lb per acre [4 ppm ÷ 0.67 lb a.i./acre]. The central estimate from Siltanen et al. (1981), 2.4 ppm per lb per acre, is somewhat less than the lower limit of 3.2 ppm per lb per acre derived in Table 18. The upper bound from Siltanen et al. (1981), 5.9 ppm per lb per acre, is somewhat below the central estimate of 7 ppm per lb per acre from Fletcher et al. (1994). Thus, while the study by Siltanen et al. (1981) is not inconsistent with the rates from Fletcher et al. (1994), the rates from Fletcher et al. (1994) provide somewhat more conservative (i.e., higher) estimates of exposure, which are used in the current Forest Service risk assessment.

The residue rates from Fletcher et al. (1994) are also useful in that they provide different residue rates for different types of vegetation. Residue rates on vegetation are a function of the application rate and the physical characteristics of the vegetation—i.e., surface area and volume. As noted in Table 18, plants with higher surface area to volume ratios (e.g., grasses) will tend to have higher residues rates, compared with plants which have lower surface area to volume ratios (e.g., fruits). In a survey of herbicide residues on plants important to native Americans, Segawa et al. (1997) note that glyphosate residues on some plants may exceed 10 ppm. These residue rates are clearly encompassed by the residue rates derived from Fletcher et al. (1994) which range from 3.2 to 240 ppm.

For longer-term exposures, the time-weighted average exposure is estimated using the initial pesticide concentration and its half-life on vegetation (Worksheet D04a and D04b). The U.S. EPA/OPP does not explicitly use half-lives on vegetation in exposure assessments for human health effects. As an alternative, U.S. EPA/OPP uses a market basket survey approach, as discussed in Section 3.2.3.1.1. In ecological risk assessments, however, U.S. EPA/OPP uses a field based exposure assessment for the consumption of treated vegetation by wildlife. In its most recent ecological risk assessment, U.S. EPA/OPP uses a vegetation half-life of 7 days. As noted in Table 15, the current Forest Service risk assessment uses a modestly more conservative half-life of 10 days (Feng and Thompson 1990 and Newton et al. 1984). This half-life of 10 days is also used for the exposure assessment for the longer-term consumption of contaminated vegetation.

As with all Forest Service risk assessments on herbicides, the use of the exposure scenario for the longer-term consumption of contaminated vegetation is probably not realistic and may be grossly conservative. Glyphosate is an effective herbicide which will cause visual damage to vegetation. While acute exposures to contaminated vegetation may be plausible (i.e., vegetation treated shortly prior to consumption), it is unlikely that humans would consume vegetation exposed to significant levels of glyphosate over a prolonged period because the vegetation would show obvious signs of injury.

3.3. DOSE-RESPONSE ASSESSMENT

3.3.1. Overview

The current Forest Service risk assessment adopts the RfD of 2 mg/kg bw/day which is based on a NOAEL of 175 mg/kg bw/day from a developmental study in rabbits (U.S. EPA/OPP 1993a,c, 2000). Relative to other similar criteria which are available from the U.S. EPA and WHO, the RfD derived by U.S. EPA/OPP (1993a,c, 2000) is preferable because it is based on a study that defines both a NOAEL and a LOAEL. The other available exposure criteria are based on free standing NOAELs—i.e., studies that do not define an adverse effect level.

Using an RfD derived by the EPA is standard practice in most Forest Service risk assessments. The U.S. EPA RfDs are used because they generally provide a level of analysis, review, and resources that far exceed those that are or can be conducted in the support of most Forest Service risk assessments. In addition, it is desirable for different agencies and organizations within the federal government to use concordant risk assessment values.

3.3.2. Acute RfD

U.S. EPA/OPP sometimes derives an acute RfD for 1-day pesticide exposures. These acute RfDs are usually based on developmental studies in which an adverse effect is associated with a single dose of a pesticide. The U.S. EPA has not derived an explicit acute RfD for glyphosate. As detailed in the following subsection, the current chronic RfD from U.S. EPA/OPP (1993a,b, 2000) is based on a developmental study and is basically equivalent to an acute RfD considered to be protective for longer-term exposures. Consequently, and consistent with the EPA approach (U.S. EPA/OPP 1993a,b, 2000), the current Forest Service risk assessment does not adopt an explicit acute RfD for glyphosate and uses the chronic RfD to characterize risks associated with both acute and longer-term exposures.

The Office of Drinking Water (U.S. EPA/ODW 1998) proposes a 20 mg/L 10-day health advisory for glyphosate. The 10-day health advisory is based on the NOAEL of 175 mg/kg/day from the rabbit reproduction study by Rodwell et al. (1980b) discussed in Section 3.1.9.1.1. As discussed further below (Section 3.3.3.1), this is the same study used by EPA to derive the chronic RfD (U.S. EPA/OPP 1993a,b, 2000). An uncertainty factor of 100 was applied to this NOAEL and the 10-day exposure limit was set at 1.75 mg/kg/day and rounded to 2 mg/kg bw/day, identical to the chronic RfD derived by U.S. EPA/OPP. This dose was multiplied by 10 kg, the default weight for a child used by U.S. EPA/ODW (1998) and divided by 1 L, the default amount of water consumed by a child—i.e., 2 mg/kg bw x 10 kg ÷ 1 L = 20 mg/L. Thus, the 10-day health advisory of 20 mg/L is equivalent to the chronic RfD of 2 mg/kg bw/day.

3.3.3. Chronic RfD

3.3.3.1. Existing Guidelines

Three different longer-term exposure criteria have been derived for glyphosate, including a chronic RfD derived by the U.S. EPA/ORD (1990), a chronic RfD derived by the U.S. EPA/OPP (1993a,b, 2000), and an Acceptable Daily Intake (ADI) derived by WHO (2005).

The RfD of 2 mg/kg/day was proposed originally in the RED for glyphosate (U.S. EPA/OPP 1993a,b) and was also used in glyphosate pesticide tolerances (U.S. EPA/OPP 2002). This RfD

is based on developmental study in rabbits (Rodwell et al. 1980b) in which doses of 75, 175, or 350 mg/kg/day were administered by gavage on days 6-27 of gestation. As detailed in Appendix 2 (Table 3) and discussed in Section 3.1.9.1.1, no effects were observed in offspring at any dose levels. Maternal toxicity, manifested as nasal discharge, diarrhea, altered physical appearance and death in some dams, was observed at 350 mg/kg/day. Using an uncertainty factor of 100, 10 for sensitive individuals and 10 for species-to-species extrapolation, the U.S. EPA/OPP derived the RfD of 2 mg/kg/day (U.S. EPA/OPP 1993c), rounding the value of 1.75 mg/kg/day to one significant digit.

The U.S. EPA's Office of Research and Development also derived an RfD for glyphosate. This RfD was originally derived in 1990 by the U.S. EPA Integrated Risk Information System (IRIS) workgroup and is the current (June 2010) RfD posted on IRIS. As discussed in Section 3.1.9.2.1, this RfD is based on a dietary 3-generation reproduction study (Schroeder and Hogan 1981), which is summarized also in Appendix 2 (Table 3). In this study, rats were exposed to dietary concentrations of glyphosate resulting in dose rates of 0, 3, 10, or 30 mg/kg/day. No signs of maternal toxicity were observed. The only effect in offspring was an increased incidence of unilateral renal tubular dilation in male pups from the F_{3b} mating group. Thus, the NOAEL was identified as 10 mg/kg/day and an uncertainty factor of 100 was applied to derive an RfD of 0.1 mg/kg/day.

WHO (2005) proposes an ADI of 0.3 mg/kg bw/day for glyphosate. ADIs are similar to RfDs in that they are intended to represent a dose which will not be associated with adverse effects in humans. Unlike the RfDs derived by the U.S. EPA, the ADI proposed by WHO is based on chronic systemic toxicity, specifically a chronic toxicity study in rats summarized in Appendix 2 (Table 4) as MRID 00093879. In this study, no signs of toxicity were noted in 26-month dietary exposures to glyphosate at concentrations of 30, 100, or 300 ppm. The highest concentration corresponded to daily doses of about 31 mg/kg bw/day in male rats and 34 mg/kg bw/day in female rats. WHO (2005) rounds the highest NOAEL to one significant digit, and, as with the RfDs from U.S. EPA, divides the NOAEL by an uncertainty factor of 100 to reach the ADI of 0.3 mg/kg bw/day.

3.3.3.2. Selection of RfD

The current Forest Service risk assessment adopts the RfD of 2 mg/kg bw/day from U.S. EPA/OPP (1993a,b, 2000). This approach is taken because the RfDs derived by U.S. EPA/ORD (1990) and WHO (2005) are based on what may be viewed as free standing NOELs—i.e., no adverse effect levels are defined in the studies on which the criteria are based. This is clearly the case for the ADI from WHO (2005). Because no adverse effects were noted at the highest dose tested in the chronic rat study, the ADI of 0.3 mg/kg bw/day from WHO (2005) is essentially a non-definitive value much like the *greater than* LD_{50} and LC_{50} values discussed in Section 3.1. In other words, the NOAEL from the chronic rat study should be viewed not as 30 mg/kg bw/day but as >30 mg/kg bw/day, because the study provides no information concerning doses that would cause adverse effects. Consequently, the ADI of 0.3 mg/kg bw/day should actually be expressed as >0.3 mg/kg bw/day.

The U.S. EPA/ORD RfD 0.1 mg/kg bw/day (U.S. EPA/ORD 1990) presents a somewhat different issue. As discussed in detail in Section 3.1.9.2.1, U.S. EPA/ORD (1990) bases the RfD of 0.1 mg/kg bw/day on the 3-generation study by Schroeder and Hogan (1981) in which no

adverse effects were noted at a dose of 10 mg/kg bw/day but an increase in the incidence of tubular dilation of the kidneys was noted at 30 mg/kg bw/day. While the study authors dismissed this effect based on comparisons to historical controls, the EPA judged that 30 mg/kg bw/day was a LOAEL because the incidence of tubular dilation was statistically significant, relative to matched controls (U.S. EPA/ORD 1990). Looking only at the data from the study by Schroeder and Hogan (1981), the EPA judgment is supportable. As discussed by U.S. EPA/OPP (1993b), the multi-generation study in rats by Reyna (1985) failed to note any adverse kidney effects at a dose of 500 mg/kg bw/day, which is about 17 times greater than the presumed LOAEL of 30 mg/kg bw/day in study by Schroeder and Hogan (1981). Consequently, U.S. EPA/OPP (1993b) concurred with the assessment by Schroeder and Hogan (1981) and considers the finding of kidney tubule dilation a spurious effect. As summarized in Table 12 of the current Forest Service risk assessment, this judgment is further supported by the results summarized in Farmer et al. (2000a) from a multi-generation study in which adverse effects on rats (parental or offspring) were not observed at a dose of 740 mg/kg bw/day. Thus, as with the ADI derived by WHO (2005), the RfD derived by U.S. EPA/ORD (1990) may be regarded as an indefinite toxicity value.

The EPA/OPP RfD of 2 mg/kg bw/day (U.S. EPA/OPP 1993a,b) is based on the study by Rodwell (1980b) which defines a NOAEL of 175 mg/kg bw/day and a LOAEL of 350 mg/kg bw/day. This RfD can be viewed as definitive RfD. There are, however, two concerns with this RfD. The LOAEL of 350 mg/kg bw/day can be viewed as a frank effect level because mortality was noted in some dams. Thus, there is a very narrow margin between a dose viewed as nontoxic (175 mg/kg bw/day) and a lethal dose (350 mg/kg bw/day), as discussed further in Section 3.3.4 (Dose-Severity Relationships).

A further reservation about the RfD of 2 mg/kg bw/day centers on the more recent developmental studies by Moxon (1996a,b). As summarized in Table 12, the developmental study in rats conducted by Moxon (1996a) is consistent with the developmental study in rats by Rodwell et al. (1980a) in that both studies yield a NOAEL of 1000 mg/kg bw. The Moxon (1996b) study in rabbits, however, notes adverse effects in dams at a dose of 175 mg/kg bw/day—i.e., considered a NOAEL in the study by Rodwell (1980b) and on which the U.S. EPA/OPP (1993a,b) RfD is based. U.S. EPA/OPP (1993a,b) do not cite the studies by Moxon (1996a,b), which may not have been available during the preparation of the RED (U.S. EPA/OPP 1993a). The much more recent pesticide tolerances for glyphosate (U.S. EPA/OPP 2002) includes a more detailed discussion of glyphosate toxicity. The lower NOAEL from Moxon (1996b) is not cited and the RfD of 2 mg/kg bw/day is maintained in U.S. EPA/OPP (2002). It is not clear if the EPA review of the Moxon (1996b) study noted problems with the study or if the study is simply overlooked in U.S. EPA/OPP (2002).

If the Moxon (1996b) study were acceptable, it seems likely that the EPA would have derived an RfD of 1 mg/kg bw/day. Since the difference between an RfD of 2 mg/kg bw/day and 1 mg/kg bw/day is not substantial, the current Forest Service risk assessment maintains the EPA RfD of 2 mg/kg bw/day. Additional and more substantial concerns with the current EPA RfD for glyphosate involve its applicability to exposures involving glyphosate formulations, as discussed below.

3.3.3.3. Application to Formulation Exposures

The RfD derived by U.S. EPA/OPP (1993a,b, 2000) as well as the other criteria from U.S. EPA/ORD (1990) and WHO (2005) are based on studies using technical grade glyphosate. As discussed in Section 2.4 and summarized in Table 4, glyphosate formulations used in Forest Service programs either contain surfactants or recommend adding surfactants to the formulation prior to application. As discussed in SERA (1997), the toxicology data on surfactants which may be added to glyphosate formulations that do not contain surfactants (e.g., Rodeo) are limited. Section 4.1.3 (Hazard Identification for Aquatic Organisms) discusses some of the available data on the surfactants which may be added to glyphosate formulations. These data indicate that at least some of these surfactants are relatively nontoxic, at least to aquatic organisms.

In terms of potential human health effects, however, the toxicity data in mammals as well as various *in vitro* bioassays clearly indicate that the toxicity of POEA surfactants included in some glyphosate formulations may be of equal or greater concern than glyphosate itself (Section 3.1). Consequently, the adequacy of using the U.S. EPA/OPP (1993a,b, 2000) RfD for technical grade glyphosate in the risk characterization of potential human health effects associated with the use of glyphosate formulations containing POEA surfactants may be questioned.

The *in vitro* studies on glyphosate formulations (i.e., Sections 3.1.8 and 3.1.10.1.1) suggest that glyphosate formulations as well as the POEA surfactants are more toxic than technical grade glyphosate; however, these studies are not directly useful in the dose-response assessment. The acute oral toxicity data indicate that the POEA surfactant is about 9 times more toxic than glyphosate (Section 3.1.4.3). Longer-term toxicity studies on formulations and the POEA surfactants are limited to developmental and reproduction toxicity studies (Section 3.1.9). These studies are the most relevant to determining the adequacy of the RfD for glyphosate when applied to glyphosate formulations, because the EPA RfD is based on a developmental study (U.S. EPA/OPP 1999a,c). As summarized in Table 12 and discussed in Section 3.1.9.1, the developmental NOAELs for POEA or acid neutralized POEA in rats are much lower than the developmental NOAELs for glyphosate in either rats or rabbits. This observation is consistent with the relative toxicities of POEA and glyphosate in both the *in vitro* studies and the acute LD_{50} studies.

The recent developmental study by Dallegrave et al. (2007) conducted with a Roundup formulation may seem consistent with the acute LD_{50} studies in that it reports a NOAEL of 450 mg/kg bw for fetal effects in rats—i.e., the NOAEL for effects observed at birth—which is clearly below the comparable NOAEL of 1000 mg/kg bw/day in rats exposed to glyphosate (Moxon 1996a; Rodwell et al. 1980a). In other words, based the comparison of comparable NOAELs from reproduction studies, Roundup appears to about twice as toxic as glyphosate [1000 mg/kg bw/day ÷ 450 mg/kg bw/day ≈ 2.22]. As noted above, this difference is similar to the differences in acute oral LD_{50} values—i.e., a factor of 2.88 from the study by Baba et al. (1989). Furthermore, the EPA/OPP RfD (U.S. EPA/OPP 1993a,b, 2000) is based on a NOAEL of 175 mg/kg bw/day, which is lower than the NOAEL of 450 mg/kg bw/day for fetal effects observed *post partum* in the Dallegrave et al. (2007) study. In this respect, the study by Dallegrave et al. (2007) may be viewed as having a minimal impact on concern for the use of the RfD cited in U.S. EPA/OPP (1993a,b, 2000).

As discussed in Section 3.1.9.1.2, however, the study by Dallegrave et al. (2007) is atypical in that exposures to a Brazilian formulation of Roundup were extended through lactation and observations were made on offspring at both puberty (65 days after birth) and in young adult rats (140 days after birth). Dallegrave et al. (2007) observed that at puberty, males rats in the 450 mg/kg bw/day dose group had a significant drop in testosterone and that the effect appeared to be dose related (Figure 4). The effect on testosterone is support by another Brazilian study (Romano et al. 2010) As discussed in some detail in Section 3.1.9.3, however, these two studies are not concordant with each other in observations on other measures of male reproductive capacity.

Because neither Dallegrave et al. (2007) nor Romano et al. (2010) tested glyphosate alone or the POEA surfactant alone, it is not clear if the effect on testosterone is attributable to glyphosate, the surfactant, or both. Concerns with exposures to glyphosate, however, are encompassed by the RfD for glyphosate. Concerns with the potential impact of surfactants used in U.S. formulations is reduced by the availability of the multigeneration study using MON 0818, the surfactant used in the original Roundup formulation. A discussed in Section 3.1.9.2.2, the multigeneration reproduction study by Knapp (2006) specifically assay for but noted on effect on testosterone levels.

Several other countervailing factors may be suggested to diminish concerns with the studies by Dallegrave et al. (2007) and Romano et al. (2010). As discussed in Section 3.1.4.4, information about suicides involving Roundup formulations suggest that rats and humans are equally sensitive to Roundup. Because the current RfD is based on data from rabbits and rabbits appear to be more sensitive to glyphosate than rats, the current RfD for glyphosate may be overly protective. In addition, there are no mammalian studies which confirm these results of the Brazilian studies with a U.S. formulation. Lastly, the epidemiology study by Larson et al. (1998a) does not report an association between glyphosate use and testosterone levels in pesticide workers. As discussed further in Section 4.1.2.2, Oliveira et al. (2007) report a decrease in testosterone in drakes exposed to a Roundup formulation; however, the test material in the study is the same Brazilian formulation tested by Dallegrave et al. (2007), and there is no way of knowing whether this formulation is representative of formulations used in Forest Service programs in the United States.

Given the absence of *in vivo* mammalian studies with U.S. formulations of glyphosate which corroborate the results of Dallegrave et al. (2007), there is no compelling basis for proposing an alternative and lower RfD for glyphosate.

3.3.4. Dose-Severity Relationships

As established in the previous subsections, the data on glyphosate and glyphosate formulations are complex and inconsistent. In some respects, this inconsistency and complexity are reflected in the range of available RfDs which span a factor of 20—i.e., from the 0.1 mg/kg bw/day RfD from U.S. EPA/ORD (1990) to 2 mg/kg bw/day from U.S. EPA/OPP (1996a,b). The current Forest Service risk assessment adopts the 2 mg/kg bw/day RfD from U.S. EPA/OPP (1990a,b). As discussed in Section 3.3.3.2, however, the NOAEL on which this RfD is based is 175 mg/kg bw/day from the developmental study with rabbits (Rodwell 1980b). Although this study defines a LOAEL of 350 mg/kg bw/day, because this dose caused maternal mortality, it may be viewed

as a frank effect level. From a practical perspective, any dose that exceeds the RfD should be viewed with concern, at least in terms of potentially sensitive individuals, like pregnant women.

Conversely, the relationship of the NOAEL to the LOAEL in toxicity study in rabbits (Rodwell 1980b) does not mean that a dose of 4 mg/kg bw/day is likely to cause mortality in humans, which is clearly not the case. As summarized in Table 11, individuals may well survive suicidal ingestions of more than 4000 mg/kg bw, so long as they receive prompt medical attention. The difficulty with glyphosate is in defining a clear threshold for adverse effects.

In addition and as discussed further in Section 3.4, dose-severity relationships are not central to the risk characterization for glyphosate. Exposure to doses of more than 2 mg/kg bw are unlikely. The greater concern in the risk characterization is the uncertainties associated with the adequacy of the 2 mg/kg bw/day RfD, in terms of effects that might be linked to the surfactants in some glyphosate formulations.

3.4. RISK CHARACTERIZATION

3.4.1. Overview

The quantitative risk characterization is expressed as the hazard quotient (HQ). For both general and accidental exposures, the HQ is calculated as the estimated doses in units of mg/kg bw for acute exposures or units of mg/kg bw/day for longer-term exposures divided by the RfD of 2 mg/kg/day (U.S. EPA/OPP 1993a,b). As discussed in Section 3.3.2, the RfD is derived from a developmental study and applied to both acute and longer-term exposures. The exposure assessments on which the HQs are based are discussed in Section 3.2.2, with details provided in the EXCEL workbooks that accompany this risk assessment—i.e., Attachment 1a for backpack applications, Attachment 1b for ground broadcast applications, Attachment 1c for aerial applications, and Attachment 2 for aquatic applications.

For both workers and members of the general public, the RfD of 2 mg a.e./kg bw/day is used to characterize risks associated with acute and longer-term exposure levels. As discussed in the exposure assessment (Section 3.2.2), all exposure assessments are based on the unit application rate of 1 lb a.e./acre. A quantitative summary of the risk characterization for workers is presented in Table 19. Quantitative summaries of risks to members of the general public are presented in Table 20 for terrestrial applications and Table 21 for aquatic applications. Because the HQs are based on the RfD, an HQ of 1 or less suggests that exposures are below the level of concern. HQs greater than 1 indicate that the exposure exceeds the level of concern.

Based on the HQ method, concern for workers is minimal. At the highest labeled application rate for terrestrial applications, about 8 lbs a.e./acre, the highest HQ is 0.6, the upper bound of the HQ for workers involved in ground broadcast applications.

For members of the general public, the only non-accidental exposure scenario of concern is for acute exposure involving the consumption of contaminated vegetation shortly after glyphosate is applied. For this exposure scenario, the HQ reaches a level of concern (HQ=1) at an application rate of about 1.4 lbs a.e./acre. At the maximum labeled application rate of about 8 lbs a.e./acre, the resulting HQ value would be about 5.6 with a corresponding dose of about 10.8 mg/kg bw.

Apart from the standard HQ method, there are additional concerns, including a report of systemic toxicity in California workers involved in glyphosate applications. In addition, two studies indicate a potential for chromosomal damage in South American populations exposed to glyphosate formulated with surfactants from aerial sprays at application rates in the range of those used in Forest Service programs. While these studies are not used quantitatively in the current Forest Service risk assessment, they suggest a potential for health effects that are not identified or confirmed using the standard HQ method.

3.4.2. Workers

A quantitative summary of the risk characterization for workers is presented in Table 19 for the unit application rate of 1 lbs a.e./acre. Given the very low HQs for accidental acute exposures, the risk characterization is reasonably unambiguous. None of the accidental exposure scenarios approach a level of concern. While the accidental exposure scenarios are not the most severe one might imagine (e.g., complete immersion of the worker or contamination of the entire body surface for a prolonged period of time) they represent reasonable accidental exposures. The

highest HQ for any accidental exposure scenario is 0.003, the upper bound of the HQ for a pesticide spill over the lower legs which is not effectively mitigated for1hour. This HQ is below the level of concern by a factor of greater than 300. Confidence in this assessment is reasonably high because of the availability of dermal absorption data in humans (Section 3.1.3.2). The HQ is linearly related to the application rate and the duration of exposure. Thus, to reach a level of concern (i.e., an HQ of 1) would require an application rate of 300 lbs/acre or an exposure duration of 300 hours or approximately 12 days, none of which is credible.

The HQs for general or longer-term exposures in workers are also unambiguous. Even at the upper bound of plausible exposures, all HQs are below the level of concern. For an application rate of 1 lb a.e./acre, the highest HQ is 0.08, the upper bound for workers involved in broadcast ground spray. HQs are, by convention, rounded to one significant decimal. The underlying numerical value for the HQ of 0.08 is 0.0756. Thus, to reach a level of concern or an HQ of 1, would require an application rate of about 13 lbs a.e./acre. As discussed in Section 2, the maximum application rate for glyphosate is about 8 lbs a.e./acre. At this application rate of 8 lbs a.e./acre the upper bound of the HQ value for broadcast spray workers would be 0.6.

As noted in Section 3.2.2.1.2, the exposure assessment for aquatic applications is based on a unit application rate of 1 lb a.e./acre with an application to 10 acres of surface water. The upper bound HQ based on these assumptions is 0.01, below the level of concern by a factor of 100. For this exposure scenario, the HQ is linearly related to the application rate and the number of acres that are treated. To reach a level of concern (HQ=1) at the maximum labeled rate for aquatic applications of 3.75 lb a.e./acre, would require a worker to treat more than 250 acres in a single day.

As summarized in Section 3.1.11, some glyphosate formulations may pose the risk of skin and eye irritation. Maibach (1986) notes that the original Roundup formulation is about as irritating to the skin as standard dish washing detergents, all purpose cleaners, and baby shampoos. This risk characterization, however, may not be applicable to all formulations of glyphosate that contain a surfactant. As discussed in Section 3.1.11, some surfactant containing formulations of glyphosate appear to be greater irritants to the skin and eyes compared with other nominally similar formulations. Because formulations may change over time, care should be taken to read and understand the MSDS for any formulation of glyphosate which may contain a surfactant.

The above relatively benign risk characterization for workers is based on the HQ approach considering exposures only to glyphosate. This risk characterization, however, must be tempered by two considerations, reports of adverse effects in workers using glyphosate and a consideration of the quality and stability of the RfD.

As discussed in Section 3.1.12.2, Goldstein et al. (2002) summarizes poison control center reports suggesting that occupational exposures to glyphosate may be associated with overt signs toxicity; however, as the investigators indicate, the signs of toxicity are generally consistent with grossly excessive levels of oral exposure to glyphosate, which are uncharacteristic of worker exposure. Goldstein et al. (2002) suggest that the reports may be an artifact of or reflect limitations in the reporting system. Despite its merit, the assumption that the reports are an artifact cannot be confirmed. In addition, the report by Goldstein et al. (2002) is supported by

another publication indicating signs of systemic toxicity in members of the general public following a glyphosate spray (Paz-y-Mino et al. 2007). The paper by Paz-y-Mino et al. (2007), however, is not well-documented, does not appear to have involved a control group for the report of symptoms, and some of the reported symptoms, like *sadness*, diminish confidence in the objectivity of the analysis.

As noted in Section 1, the U.S. EPA has initiated registration review of glyphosate. In addition and as noted in Section 3.1.8, the EPA is requiring additional testing of glyphosate for effects on the endocrine system. It seems very likely that the EPA will review the Dallegrave et al. (2007) and Romano et al. (2010) studies and any additional data on glyphosate which become available. Thus, the status of U.S. EPA review of glyphosate should be monitored with some care over the next several years.

3.4.3. General Public

3.4.3.1. General Considerations

A quantitative summary of the risk characterization for members of the general public is presented in Table 20 for terrestrial applications and in Table 21 for aquatic applications. Like the corresponding table for workers, Table 20 and Table 21 are based on a unit application rate of 1 lbs a.e./acre. The HQs for most scenarios are similar for terrestrial and aquatic applications. The major difference between terrestrial and aquatic applications is that the risk assessment for aquatic applications does not include some exposure scenarios, including the consumption of contaminated vegetation and contaminated fruit, and the exposure assessments for glyphosate concentrations in surface water differ for terrestrial and aquatic applications.

For an application rate of 1 lb a.e./acre, none of the HQs exceed a level of concern. The highest HQ is for the consumption of contaminated water after an accidental spill. The upper bound of the HQ for this exposure scenario reaches but does not exceed the level of concern (i.e., the HQ is equal to 1.0) for an application rate of 1 a.e./acre. The HQ for this scenario is linearly related to the application rate.

3.4.3.2. Terrestrial Applications

For terrestrial applications of glyphosate, the non-accidental exposure scenario of greatest concern involves the consumption of contaminated vegetation. For the longer-term consumption of contaminated vegetation, the upper bound of the HQ is 0.1 at an application rate of 1 lb a.e./acre. Thus, even at the maximum application rate of about 8 lbs a.e./acre, this exposure scenario would not exceed the level of concern (HQ=1).

For acute exposures, however, the consumption of contaminated vegetation yields a HQ of 0.7 at an application rate of 1 lb a.e./acre. This HQ is substantially greater than the HQ of 0.09 for contaminated fruit. As summarized in Table 18, the differences in these scenarios for fruit and contaminated vegetation are related to the substantial differences in residue rates for these two commodities from Fletcher et al. (1997). For contaminated vegetation, the application rate associated with an HQ of 1 is about 1.4 lbs a.e./acre. At the maximum labeled application rate of about 8 lbs a.e./acre, the resulting HQ value would be about 5.6 with a corresponding dose of about 10.8 mg/kg bw.

There is no basis for asserting that a dose of 10.8 mg/kg would lead to gross signs of toxicity. As discussed in Section 3.3.4, lethal doses would not be expected at this dose. Nonetheless, as also discussed in Section 3.3.4, the study by Rodwell (1980b) noted adverse effects, including mortality, in pregnant rabbits at a dose of 350 mg/kg bw—i.e., a factor of 2 above the NOAEL on which the RfD is based. Thus, an HQ of 5.6 would raise concerns for adverse health effects in pregnant women. Based on the more recent study by Moxon (1996b) which notes a LOAEL for fetotoxicity of 300 m/kg bw, an HQ in the range of 5 might raise concern for fetotoxicity.

The above discussion is not intended to suggest that these adverse effects on pregnant women and the developing fetus can be predicted directly from animal studies. RfDs are generally considered to incorporate highly conservative uncertainty factors that provide a substantial margin of safety. For example, U.S. EPA/OPP (1993a,b) use an uncertainty factor of 100 which incorporates factors of 10 for species extrapolation and 10 for sensitive subgroups. Pregnant mammals and the developing fetus appear to be a sensitive subgroup, and rabbits appear to be the most sensitive species. Based on the available toxicity data, however, rabbits appear to be more sensitive than rats in terms of reproductive effects, and rats appear to be about equally sensitive as humans in terms of the acute lethal toxicity of glyphosate. Thus, the RfD may be viewed as conservative in the application of the uncertainty factor of 10 for species extrapolation.

A separate concern for the risk characterization of glyphosate involves genotoxic effects. As discussed in Section 3.1.10.1.2, two studies from South America (Paz-y-Mino et al. 2007; Bolognesi et al. 2009) report signs of chromosomal damage in populations following broadcast aerial sprays of glyphosate formulations that contain surfactants. While the study by Paz-y-Mino et al. (2007) is not compelling, the study by Bolognesi et al. (2009) is more extensive and better designed. This study suggests that sprays of glyphosate formulations mixed with surfactants may be associated with genotoxic effects—i.e., micronuclei and binucleated cells with micronuclei. Whether or not these studies represent exposures that are relevant to applications in the United States is not clear.

3.4.3.3. Aquatic Applications

The major difference between aquatic and terrestrial applications of glyphosate is that exposure scenarios for the consumption of contaminated vegetation are not considered in aquatic applications of pesticides. As noted above, the consumption of contaminated vegetation is the only major route of exposure for the terrestrial application of glyphosate. For aquatic applications, the highest HQ is 0.01, the upper bound of the HQ for a child who consumes surface water immediately after an aquatic application of glyphosate. This upper bound HQ is below the level of concern by a factor of 100, and there is no basis for asserting plausible risk.

3.4.4. Sensitive Subgroups

3.4.4.1. Glyphosate Specific Issues

As discussed in Section 3.4.3.2, the most sensitive subgroup for exposure to glyphosate and glyphosate formulations appears to be pregnant women and the developing fetus. Since the RfD for glyphosate used in the current Forest Service risk assessment is based on a developmental study, the sensitivity of this subgroup is explicitly addressed.

3.4.4.2. Multiple Chemical Sensitivity

Some individuals report extreme sensitivities to many different types of chemical agents, including pesticides. This condition is generally referred to as Multiple Chemical Sensitivity (MSC). In general, individuals with MCS report that they experience a variety of adverse effects as a result of exposures to very low levels of environment chemicals that are tolerated by individuals who do not have MCS.

A major problem in constructively addressing MCS, however, involves the diagnosis of and remediation measures for this condition. While it is beyond the scope of the current Forest Service risk assessment to address MCS in detail, it is worth noting that there is no current consensus on the diagnosis and cause of MCS. What appears to be an emerging view in several recent publications (e.g., Bornschein et al. 2008a,b; Das-Munshi et al. 2006, 2007; Eis et al. 2008) is encapsulated in the recent review of MCS by Das-Munshi et al. (2006), who state:

> *We conclude that persons with MCS do react to chemical challenges; however, these responses occur when they can discern differences between active and sham substances, suggesting that the mechanism of action is not specific to the chemical itself and might be related to expectations and prior beliefs.*
>
> Das-Munshi et al. 2006, p. 1257

In other words, MCS is clearly a condition that exists in the human population, and individuals with MCS do experience effects. The above quotation, however, suggests that these individuals may be responding to a perception of hazard rather than to a specific chemical.

While the above quotation may be a basis for suggesting that MCS is psychosomatic, other investigators are more cautious:

> *Regarding the psychological assessment it should be kept in mind that until the etiology and pathogenesis of MCS has been clarified an organic cause of the MCS associated symptoms and symptom complexes cannot be entirely ruled out.*
>
> Lacour et al. 2005, p. 149

It is beyond the scope and authority of USDA to attempt to resolve concerns for MCS. The condition clearly exists and is the subject of serious study by the medical community. The key issue is that the cause of MCS is unclear.

3.4.5. Connected Actions

The most important connected action in the use of glyphosate involves surfactants. Some glyphosate formulations contain surfactants and other glyphosate formulations recommend adding surfactants prior to application. To the extent possible, the use of surfactants is explicitly considered in this human health risk assessment.

As summarized in Section 3.1.16, glyphosate inhibits some mixed-function oxidases, a very important system of enzymes in the metabolism of many xenobiotics. While the inhibition of hepatic mixed-function oxidases is a plausible mechanism of interaction, this conjecture does not

lead to any definite conclusions regarding the potential influence of glyphosate on the toxicity of other chemicals. In any event, this mechanism of action would probably be relevant only at very high doses, substantially above exposure levels anticipated in Forest Service programs.

3.4.6. Cumulative Effects

It is possible and even likely that some individuals will be exposed to multiple sources of glyphosate as a result of Forest Service programs. For example, an individual consuming contaminated fish might also consume contaminated water and/or vegetation. For glyphosate, these multiple sources of exposure are inconsequential. The only substantial exposure scenario involves the consumption of contaminated vegetation after terrestrial applications. All other plausible combinations of exposures would not have a substantial impact on the risk characterization.

Addressing cumulative effects, within the context of the Food Quality Protection Act, requires the assessment of chemicals with a similar mode of action. In the recent pesticide tolerance for glyphosate, the EPA states:

> *EPA does not have, at this time, available data to determine whether glyphosate has a common mechanism of toxicity with other substances or how to include this pesticide in a cumulative risk assessment.*
>
> U.S. EPA/OPP 2002, p. 60937

As detailed in Section 3.1.8, the EPA is currently requiring additional tests on glyphosate to assess the potential of glyphosate to cause endocrine effects. Depending on the results of these tests, exposure to other agents which affect endocrine function could be associated with cumulative effects.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview

In some ways, the hazard identification for ecological effects parallels the hazard identification for human health effects. The toxicity of technical grade glyphosate is relatively well characterized for both terrestrial and aquatic species. In addition, the toxicity of the original Roundup formulation as well as Rodeo is relatively well characterized. It is more difficult, however, to clearly define the hazards associated with other glyphosate formulations.

As is the case with most Forest Service pesticide risk assessments, the data used to assess the risk to mammalian wildlife as well as human exposure to glyphosate and glyphosate formulations is largely the same. Thus, Section 4.1.2.1 focuses primarily on studies useful for assessing differences in pesticide sensitivity among various species of mammalian wildlife. The dose-response assessment for mammalian wildlife (Section 4.3.2.1) presents a fuller discussion of concerns for reproductive toxicity raised by the recent study by Dallegrave et al. (2007) conducted with a South American formulation of Roundup. In some respects, however, it is some early but detailed field studies on mammalian wildlife which have a substantial impact on the hazard identification for human health and mammalian wildlife. These early studies do not report adverse reproductive effects in populations of small mammals following applications of U.S. formulations of Roundup (Ritchie et al. 1987; Sullivan 1990).

The hazard identification subsections for other groups of ecological receptors is structured in a manner similar to the hazard identification for human health effects in that distinctions between technical grade glyphosate and glyphosate formulations are maintained as clearly as possible. For birds, terrestrial-phase amphibians, and terrestrial invertebrates, relatively complete sets of studies are available on both technical grade glyphosate and some U.S. formulations. Some studies using formulations from South America suggest adverse effects on reproduction in birds, amphibians, and terrestrial invertebrates. The types of studies conducted on the South American formulations have not been conducted on formulations that will be used in Forest Service programs. Consequently, the applicability of the data on South American formulations to the current Forest Service risk assessment is difficult to assess because of the proprietary nature of the data on the surfactants used in different formulations of glyphosate.

Glyphosate is an effective herbicide, and the toxicity of glyphosate and glyphosate formulations to terrestrial plants is well characterized. In addition, there is a relatively detailed literature regarding the effects of glyphosate and glyphosate formulations to terrestrial microorganisms. While the mechanism of action of glyphosate in plants is also relevant to microorganisms, there is very little indication that terrestrial microorganisms will be adversely affected by glyphosate.

A large and detailed body of literature is available on the effects of glyphosate and some glyphosate formulations to aquatic organisms. Overviews of the available studies are provided in the following tables: Table 22 (fish), Table 25 (aquatic-phase amphibians), Table 26 (aquatic invertebrates), Table 27 (algae) and Table 28 (aquatic macrophytes). The discussions of each of these groups of aquatic organisms in the hazard identification are preceded by an overview of the available literature. The toxicity of the original Roundup and similar formulations containing

POEA surfactants is far greater than the toxicity of technical grade glyphosate, Rodeo, or other formulations that do not contain surfactants. Among the formulations with surfactants, several non-U.S. formulations appear to be more toxic than many U.S. formulations of Roundup and Roundup-like formulations. Although data suggest that certain U.S. formulations of glyphosate that contain surfactants may be less toxic than others, the differences in toxicity are not clearly documented in the EPA risk assessment on glyphosate (U.S. EPA/OPP 2008a) or the open literature. As discussed in Section 2, data from Material Safety Data Sheets (MSDS) are neither well-documented nor sufficiently clear to be used directly in this risk assessment.

Fish, amphibians, and most aquatic invertebrates appear to be about equally sensitive to the toxicity of technical grade glyphosate and glyphosate formulations. Many differences in toxicity appear to be more clearly related to experimental conditions, particularly pH, than to species differences. The sensitivity of algae to glyphosate and glyphosate formulations varies among species; however, the data regarding differences among species of aquatic macrophytes are less complete. Nonetheless, there is evidence that *Lemna* species are much more sensitive than eelgrass to glyphosate acid, which suggests that there may be substantial species differences in the sensitivity of macrophytes to glyphosate formulations. Most studies on aquatic microorganisms seem consistent with studies on terrestrial microorganisms, indicating that aquatic microorganisms are not very sensitive to glyphosate. Some recent studies using changes in the composition of ribosomal RNA and DNA suggest that effects on aquatic microorganisms may occur at very low concentrations. While this may be the case, the functional significance of these effects is not apparent.

4.1.2. Terrestrial Organisms

4.1.2.1. Mammals

As summarized in the human health risk assessment (Section 3.1), several standard toxicity studies in experimental mammals were conducted as part of the registration process for glyphosate; additionally, there is a large body of published information regarding the toxicity of glyphosate to mammals. Just as these studies are used in the human health risk assessment to identify the potential toxic hazards associated with exposures to glyphosate, they can also be used to identify potential toxic effects in mammalian wildlife.

Based on acute lethality data for glyphosate, there appear to be no remarkable differences in sensitivity among mammals. As discussed in Section 3.1.4.4, the approximate median lethal dose for Roundup in humans is remarkably similar to the LD_{50} of approximately 5400 mg/kg bw in rats (Baba et al. 1998). On the other hand, there is relatively little information regarding the toxicity of glyphosate or glyphosate formulations to mammalian wildlife or domestic animals. McComb et al. (2008) report only modest differences in the toxicity of glyphosate IPA in four species of small mammals, including deer mice, chipmunks, shrews, and voles, with intraperitoneal LD_{50} values ranging from 800 to 1370 mg/kg bw. The intraperitoneal LD_{50} for the common lab mouse reported in this study is1100 mg/kg bw.
While the differences in the acute lethal potency of glyphosate appear to be unremarkable among various species of small mammals, the limited available data suggests that larger mammals may be somewhat more sensitive than smaller mammals, based on repeated sublethal dosing. As discussed in Section 3.3, the most sensitive endpoints (i.e., the lowest NOAELs) for glyphosate and glyphosate formulations are derived from developmental studies. These studies involve

repeated sublethal dosing over a period of about 2 weeks. Based on two sets of developmental studies in rats and rabbits (Rodwell 1980b; Moxon 1996a,b), rabbits appear to be more sensitive than rats. While NOAELs and LOAELs are not good endpoints for assessing quantitative differences in species sensitivity, because they are experimental doses rather than statistical estimates, the NOAEL of 100 mg/kg bw/day for rabbits is a factor of 10 below the NOAEL of 1000 mg/kg bw/day for rats (Moxon (1996a,b).

An unpublished repeated-dose study suggests that cattle may be more sensitive than rabbits to glyphosate formulations. The WHO (1994) criteria document summarizes a study which involved dosing of Brahman-cross heifers with Roundup at 400, 500, 630 or 790 mg/kg bw per day by nasogastric intubation for 7 days. At 790 mg/kg, some animals died with labored breathing and pneumonia from the aspiration of rumen contents. This effect is consistent with the lung damage observed in experimental mammals exposed to glyphosate formulations (Section 3.1.4). Additional signs of toxicity at 500, 630 and 790 mg/kg body weight included diarrhea and decreased food intake. Again, these signs of toxicity are consistent with those seen in humans and laboratory mammals. No adverse effects were observed at 400 mg Roundup/kg bw (equivalent to 215 mg a.i./kg bw or about 160 mg a.e./kg bw).

Reduced body weight gain is commonly observed in mammals exposed to glyphosate. This effect may be associated with taste aversion, toxicity, or a combination of these factors. As summarized in Appendix 2, several standard toxicity studies note decreases in food consumption and body weight in experimental mammals exposed to high dietary concentrations of glyphosate (Reyna 1985; Schroeder and Hogan 1981; Williams et al. 2000). In addition, Evans and Batty (1986) note decreased food consumption in three species of mammalian wildlife exposed to a dietary concentration of 5000 ppm. While decreased body weight gain may be due in part to taste aversion, decreased food consumption was also observed in dermal, gavage, and drinking water toxicity studies (Beuret et al. 2005; MRID 00036803), suggesting that it is a sign of toxicity. In addition, as discussed in Section 3.1.2, inhibition of oxidative phosphorylation has been implicated as a possible mechanism by which glyphosate might impact body weight gain in experimental mammals.

Other information regarding the effect of glyphosate formulations on larger mammals is essentially anecdotal. Burgat et al. (1998) summarize information from reports of glyphosate poisonings of domestic animals in France. Although the survey does not provide dose data, it does identify signs of toxicity similar to those in reports of human poisoning with glyphosate, including pulmonary edema, metabolic acidosis. The study does not, however, specifically identify renal failure as a sign of toxicity, but since fatal poisonings are not reported in the review by Burgat et al. (1998), perhaps the exposure levels were lower than those in the human reports of suicidal ingestion (Section 3.1.4.4). The Texas Department of Agriculture (1992) investigated a report that a horse was fatally poisoned by glyphosate. Initially, the death was attributed to drift from the application of a glyphosate formulation; however, the investigators determined the horse died of natural causes.

Field studies in which populations of mammalian wildlife were observed after the application of glyphosate formulations are summarized in Appendix 2, Table 9. Most field studies provide no suggestion of adverse effects on mammalian populations, other than secondary effects which can

be attributed to changes in vegetation. Most of the field studies, however, are not specifically focused on and do not measure endpoints which might be associated with the toxicity of glyphosate or glyphosate formulations. Two notable exceptions, however, are the studies by Ritchie et al. (1987) and Sullivan (1990). Ritchie et al. (1987) assayed populations of deer mice following applications of about 1 lb a.e./acre of an unspecified glyphosate formulation. Based on body size as well as the number of placental scars and foeti in female deer mice, no effects on reproductive capacity were noted. Similarly, Sullivan (1990) monitored populations of deer mice and voles after applications of about 2.7 lb a.e./acre of Roundup. Over a 3-year period following application, no adverse effects on mammalian populations were noted, relative to a comparable untreated site. Based on the number of successful pregnancies as well as the number of juvenile voles and the number of successful pregnancies in deer mice, mammalian reproduction at the treated site was comparable to or better than the control site during the year of treatment as well as during the following 2 years (see Sullivan et al. 1990, Tables 1 and 2). Based on a number of additional parameters in the populations of these small mammals, no adverse effects in small mammals could be associated with the Roundup spray.

4.1.2.2. Birds

4.1.2.2.1. Technical Grade Glyphosate

Information on the toxicity of glyphosate to birds is summarized in Appendix 3. Three types of standard toxicity studies are required by the U.S. EPA/OPP for pesticide registration. These studies, which were conducted on glyphosate, include acute gavage toxicity (Appendix 3, Table 2), acute dietary toxicity (Appendix 3, Table 2), and reproduction studies (Appendix 3, Table 3).

Based on a gavage study using technical grade glyphosate, the LD_{50} in bobwhite quail is >2000 mg/kg bw. The EPA uses this study to classify glyphosate as practically nontoxic to birds (U.S. EPA/OPP 1993c, p. 8). The more recent EPA risk assessment of glyphosate cites additional gavage LD_{50} values ranging from about 1130 mg/kg bw for the monoammonium salt of glyphosate (MRID 45777402) to > 3190 mg/kg bw for an unspecified salt of glyphosate (MRID 108204) (U.S. EPA/OPP 2008a). All of these LD_{50} values are comparable to those reported in mammalian studies (Section 3.1.4.1).

Acute dietary studies in birds all yield non-definitive LC_{50} values (Appendix 3, Table 2). Four studies are clearly on technical grade glyphosate in which the LC_{50} values are reported as >4000 ppm a.e. In addition, the summary of these studies in U.S. EPA/OPP (2008a) indicates that no adverse effects were observed at the highest dietary concentrations used in these studies. While no comparable acute dietary studies are available in mammals, these studies are consistent with the low toxicity of technical grade glyphosate in mammals.

Finally, three reproduction studies conducted on birds were submitted to U.S. EPA/OPP (Appendix 2, Table 3). No adverse effects on reproduction in mallards and quail are associated with dietary concentrations of up to 833 ppm.

4.1.2.2.2. Glyphosate Formulations

As summarized in Appendix 3, Table 2, two acute dietary studies conducted with a 31.32% glyphosate IPA material referenced as MON65005 were submitted to U.S. EPA/OPP (MRID

44465701 and 44465702). Based on a 1995 MSDS from Monsanto (MAC-5050 dated November 1995), this product code corresponds to Roundup PRO. As indicated in Table 2, Roundup PRO contains 41% glyphosate IPA, which corresponds to about 30% glyphosate a.e, as well as a phosphate ester neutralized polyethoxylated tallowamine surfactant at a concentration of 14.5%. These acute dietary studies report NOAELs of 1760 ppm a.e.

As discussed above, the acute dietary NOAELs for technical grade glyphosate are about 4000 ppm a.e. Because none of these acute dietary studies defines adverse effect levels, they cannot be used quantitatively to compare the toxicities of technical grade glyphosate and the glyphosate-POEA formulation. Nonetheless, the acute dietary studies on the formulation do suggest that the formulation is not highly toxic to birds

The open literature on Roundup formulations includes three avian studies involving subchronic exposure (Appendix 3, Table 3). Decreased body weight was observed in the two studies involving dietary exposure (Evans and Batty 1986; Kubena et al. 1981). As noted in the Section 4.1.2.1., decreased body weight gain is a common observation in mammalian studies of glyphosate. In the Evans and Batty (1986) study, zebra finches were exposed to dietary concentrations of 2500 or 5000 ppm Roundup (NOS) for several days. The publication does not indicate whether the doses are expressed in units of formulation, glyphosate IPA, or glyphosate acid. Although adverse effects were not observed at 2500 ppm, at 5000 ppm, all birds died within 7 days with body weight losses ranging from about 20 to 60%, relative to controls. Evans and Batty (1986), who do not appear to have measured food consumption in the 5000 ppm group, suggest that the animals may have died due to starvation. In the Kubena et al. (1981) study, chickens were exposed to dietary concentrations of 0, 45, 450, or 4500 ppm a.e Roundup (NOS) for 21 days. Overt signs of toxicity are not reported in the study; however, the concentration of 4500 ppm a.e. was associated with substantial loss of body weight (i.e., about 45% of control body weight) by the end of the 21-day exposure period. The authors do not provide information on food consumption.

In the other avian subchronic study, 0, 5, or 100 mg formulation/kg bw of a Brazilian formulation of Roundup was administered by gavage to male mallards for 15 days (Oliveira et al. 2007). The doses correspond to 1.8 and 36 mg a.e./kg bw/day. No significant effect was noted on body weight. This study, however, focused on testicular effects, and while no effects were noted on testes weights, a significant and substantial (≈90%) decrease in testosterone was noted at both doses. This effect was accompanied by histological changes in the testes as well as changes in androgen receptor expression. In reviewing this study, the EPA noted following:: *Further studies would be needed to determine whether or not these observed effects would affect avian reproduction* (U.S. EPA/OPP 2008a, p. 111). As discussed in Section 3.1.9.1.2, significant and substantial decreases in plasma testosterone were observed in rats after exposure to the same Brazilian formulation of Roundup but only at a much higher dose – i.e., 450 mg a.e./kg/day (Dallegrave et al. 2007).

While there are no standard reproduction studies with Roundup formulations, two studies involving the immersion of eggs in a solution of Roundup suggest that Roundup is not likely to cause developmental effects 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 value probably corresponds to a concentration of approximately 200 g/L [(178 lbs · 0.45 kg/lb) ÷ (100 gallons · 3.785 L/gallon) = 80.1 kg ÷ 378.5 L ≈ 0.21 kg/L], which corresponds to a solution of about 20% (w/v). Clearly, the application rate of 178 lbs/acre is substantially higher than the maximum annual labeled application rate for glyphosate. The Batt et al. (1980) study involved a less severe exposure-immersion of eggs in a 5% solution of Roundup for 5 seconds. No malformations were noted in developing chicks.

4.1.2.2.3. Field Studies

Several field studies address the effect of glyphosate applications on bird populations. These studies include both terrestrial applications (Cayford 1988; Easton and Martin 1998; MacKinnon and Freedman 1993) and aquatic applications (Linz and Blixt 1997; Linz et al. 1994, 1996a,b, 1997; Solberg and Higgins 1993). All of the aquatic applications involve Rodeo. Some of the studies involving exposure to terrestrial applications do not specify a particular formulation, but it is likely that the formulations involved Roundup or similar formulations containing surfactants.

None of the field studies report adverse effects in birds. Most of the publications involving Rodeo applications note an increase in bird abundance due to increases in open water habitat. Similarly, effects on bird populations following terrestrial applications of glyphosate appear to be secondary to changes in habitat.

4.1.2.3. Reptiles and Amphibians (Terrestrial-Phase)

The U.S. EPA does not require standard toxicity studies on terrestrial-phase amphibians. As discussed further in Section 4.4.2.3 (risk characterization for terrestrial-phase amphibians), the EPA uses toxicity data on birds to assess risks to terrestrial-phase amphibians (U.S. EPA/OPP 2008a).

There is abundant information regarding the toxicity of glyphosate and glyphosate formulations to aquatic-phase amphibians, as discussed in Section 4.1.3.2. There is, however, relatively little information available on the toxicity of technical grade glyphosate to terrestrial-phase amphibians. Intraperitoneal studies suggest that differences in the toxicity of glyphosate IPA to several species of amphibians and several species of small mammals are not substantial (McComb et al. 2008). The definitive LD_{50} values in amphibians ranged from 1070 mg a.i./kg bw (≈790 mg a.e./kg bw) to 1250 mg a.i./kg bw (≈925 mg a.e./kg bw).

Relative to mammalian skin, amphibian skin is thinner and more permeable to many substances. Quaranta et al. (2009) demonstrated that the permeability of frog skin to glyphosate acid is 26 times greater than that of pig skin. Consequently, exposure to direct spray is a scenario of potential concern. Notably, the results of the two direct spray studies involving amphibian exposure (Relyea 2005c; Dinehart et al. 2009) are not consistent.

Relyea (2005c) sprayed three species of amphibians (tree frog, wood frog, and a toad) with Roundup Weed and Grass Killer at 1.6 mg a.i./m^2 (≈1.2 mg a.e./ m^2) and noted greater than 50%

mortality after 24 hours. Note that the application rate of 1.2 mg a.e./m^2 is equivalent to 0.012 kg a.e./ha [1.2 mg a.e./m^2 x 10,000 m^2/ha = 12 g/ha] or about 0.011 lb a.e./acre.

In what appears to be a similar study, Dinehart et al. (2009) applied three glyphosate formulations to two species of amphibians: the New Mexico Spadefoot toad and the Great Plains toad. One of the formulations, Roundup WeatherMax, is a formulation used by the Forest Service (Table 2). Dinehart et al. (2009) indicate that Roundup WeatherMax was applied at a rate equivalent to 44 oz/acre. This rate, in turn, is equivalent to about 0.34 gallons/acre [44 oz ÷ 128 oz/gallon]. As summarized in Table 4, Roundup WeatherMax contains 4.5 lbs a.e./gallon. Thus, the application rate used by Dinehart et al. (2009) was about 1.5 lb a.e./acre [0.34 gallons/acre x 4.5 lbs a.e./gallon]. Direct spray at this rate resulted in no significant mortality in either the New Mexico Spadefoot toad or the Great Plains toad.

Glaser (1998) conducted a laboratory bioassay in which eight newly metamorphosed frogs (*Rana sylvatica*) were sprayed with Vision (41% IPA formulation with a POEA surfactant) using a plant mister at a nominal application rate of 1.8 kg a.e./ha or about 1.6 lb a.e./acre. As in the study by Dinehart et al. (2009), no mortality was noted.

Bernal et al. (2009b) conducted a series of terrestrial and aquatic mesocosm studies using a Glyphos formulation with Cosmo-Flux. As discussed in Section 3.1.12.2, Cosmo-Flux is an adjuvant developed in South America consisting of a mixture of linear and aryl polyethoxylates at a concentration of 17% (w/v) and isoparaffins at a concentration of 83% (Solomon et al. 2005, p. 24). Terrestrial mesocosms were sprayed at rates of 1.85-29.52 kg a.e./ha or about 1.7-26 lb a.e./acre (Bernal et al. 2009b, Table 2). Responses in juvenile and adult frogs are reported as LC_1 values (lethal to 1% of the exposed individuals) as well as LC_{50} values in units of application rate. The definitive LC_{50} values ranged from 4.5 to about 22.8 kg a.e./ha. The definitive LC_1 values, which may be regarded as functional NOECs ranged from 0.32 kg a.e./ha (≈0.3 lb a.e./acre) to 7.02 kg a.e./ha (≈6.3 lb a.e./acre). The mesocosm studies by Bernal et al. (2009b) are not directly analogous to the direct spray studies by Relyea (2005c) and Dinehart et al. (2009), because frogs in the mesocosm exposures may have been protected from direct deposition, Nonetheless, the mesocosm studies are more closely related to field applications, and the results of the Bernal et al. (2009b) study suggest that substantial mortality would not be expected at application rates in the range of about 1-2 lb a.e./acre and that some species would tolerate much higher application rates. The Bernal et al. (2009b) study is consistent with the results of the direct spray study by Dinehart et al. (2009) but not with the earlier study by Relyea (2005c).

In a field study, Cole et al. (1997) report no effects on populations of six species of amphibians (based on capture rates) among clearcut sites with and without glyphosate applications. The study involved aerial applications of glyphosate at a rate of 1.3 kg/ha or about 1.2 lb/acre. The glyphosate formulation used, is not specified, and the units of the application rate—i.e., a.e., a.i., or formulation—are not clearly stated. Species included rough-skin newt, ensatina, Pacific giant salamander, Dunn's salamander, western redback salamander, and red-legged frog. Removal of red alder from the habitat, reduced amphibian populations regardless of the method used to remove the alder. This field study is consistent with the mesocosm study by Bernal et al. (2009b) as well as the direct spray study by Dinehart et al. (2009). As discussed above, the

direct spray study by Relyea (2005c) reported substantial mortality in frogs after a direct spray at a rate equivalent to 0.011 lb a.e./acre. This study, however, is not consistent with the field study by Cole et al. (1997).

The study by McComb et al. (2008) is an unusual field study in that it involved intraperitoneal dosing of newts with glyphosate IPA at a dose of 50 mg/kg bw and subsequent release. The animals were then monitored for activity with radio transmitters. The movement of the dosed animals (n=7) did not differ substantially from the movements of control animals (n=10). This study, while somewhat artificial in terms of exposure, does confirm the low toxicity of glyphosate IPA. No similar study is available on a glyphosate-surfactant formulation.

4.1.2.4. Terrestrial Invertebrates

Information on the toxicity of glyphosate and glyphosate formulations to terrestrial invertebrates is summarized in Appendix 4. This information includes relatively standard bioassays on honeybees (Section 4.1.2.4.1), other nontarget arthropods (Section 4.1.2.4.2), as well as a few studies on toxicity to non-arthropod terrestrial invertebrates (Section 4.1.2.4.3).

4.1.2.4.1. Honeybees

The honey bee is the standard test organism for assessing the potential effects of pesticides on terrestrial invertebrates, and there is a standard set of glyphosate studies on this species (Palmer and Beavers 1997; Palmer and Krueger, 2001a; Palmer and Krueger, 2001b). In addition, studies are available on a relatively wide range of other terrestrial invertebrates, including earthworms, isopods, snails, spiders, butterflies, and other terrestrial arthropods.

In standard oral and contact bioassays summarized in U.S. EPA/OPP (1993c), the LD_{50} values for bees are greater than100 µg/bee. Three more recent studies submitted to the U.S. EPA are consistent with these earlier reports. In an acute contact toxicity assay with MON 65005, no effects were seen at 100 µg/bee (Palmer and Beavers 1997). As noted in Table 3, MON 65005 appears to correspond to an older Roundup PRO formulation.

Similar results have been reported recently for a newer formulation, MON 77360, in which the NOEC based on mortality in a contact toxicity test was also 100 µg (Palmer and Krueger 2001a). As noted in Table 3, MON 77360 corresponds to several Monsanto formulations including the current Roundup PRO. The dose of 100 µg is classified as an NOEC because mortality (3/60 animals) was not significantly different from mortality in the matched solvent control (0/60, p=0.12 using the Fisher exact test). Combining the matched solvent control (0/60) with the negative control (0/60) for a combined control response of 0/120, the mortality of 3/60 animals is statistically significant (p=0.0358 using the Fisher exact test), albeit low (3/60 = 5%). No mortality (0/60) was observed at the next lower dose (50 µg/bee) or at any of the other lower doses down to 6.25 µg/bee.

In an acute dietary study (Palmer and Krueger 2001b), the 48-hour oral LD_{50} is reported as >100 µg/bee based on 11.7% mortality (7/60) at the highest dose tested. The NOEC is reported as 50 µg/bee based on 5% mortality (3/60). Again, this response rate is not significant with respect to solvent matched controls (0/60) but is significant when solvent and negative controls are combined (0/120, p=0.0358 using the Fisher exact test). Note that the high mortality rate (26/60) observed at 12.5 µg/bee dose was attributed to an unidentified failure in the test apparatus which

resulted in substantial direct contact of the bees with the test solution. While this sort of unexpected low dose response is noteworthy, the low mortality rates at higher doses (i.e., 1/60 at 25 µg/bee and 3/60 at 50 µg/bee) support the assessment of Palmer and Krueger (2001b) that the high mortality at 12.5 µg/bee was an aberration.

4.1.2.4.2. Other Arthropods

Glyphosate has been tested as an insecticide for spider mites, *Tetranychus urticae*, a pest species on apple trees (Ahn et al. 1997) as well as for toxicity to *Typhlodromus pyri*, an important predator of spider mites (Weppelman 1998b). Direct foliar spray of glyphosate IPA at 0.593-4.74 mg a.i. per leaf (kidney bean plants) had no adverse effect on the spider mite, based on mortality in eggs, larva, nymphs, and adults (Ahn et al. 1997) and was essentially ineffective as an insecticide.

Applications equivalent to 10 L/ha Roundup Ultra (glyphosate isopropylamine salt at 360 g/L or an application rate of 3.6 kg a.i./ha) applied to glass slides caused 100% mortality in predatory mites (*Typhlodromus pyri*) after 24 hours of contact and was classified as "harmful" (Weppelman 1998a) . In a similar assay using *Aphidius rhopalosiphi* (a beneficial wasp that is a parasite of the cereal aphid), the same contact exposure also resulted in 100% mortality after 24 hours. The relevance of the studies by Weppelman (1998a,b) to the assessment of potential effects under normal use is unclear. As noted in Weppelman (1998a),

> *the 5% v/v test solution of Roundup ULTRA produced a wet sticky layer on the treated glass plates that resulted in alterations of the moving behavior of the wasps to the point of sticking.*

In other words, it appears the application of the glyphosate formulation to the glass slides caused the test organism to stick to the slides, which may have contributed to the observed mortality. The studies by Weppelman (1998a,b) are included in the bibliography of studies submitted by registrants to the U.S. EPA/OPP (Supplement 1 of the current risk assessment). This bibliography indicates that the studies were prepared by the Monsanto Company. Monsanto, however, has indicated that the studies by Weppelman (1998a,b) used a formulation of Roundup Ultra that *…is not the same as the U.S. product, Roundup Ultra. In fact, the surfactant used in this formulation is not approved for use in the U.S. (Honegger 2010, p. 10).*

Haughton et al. (1999; 2001a,b) conducted a series of laboratory and field studies regarding the effects of glyphosate on the spider, *Lepthyphantes tenuis*. Direct spray laboratory bioassays at rates equivalent to 180, 360, 720, 1080, 1440, or 2160 g/ha resulted in low mortality rates which were not dose related (Haughton et al. 2001a). In the field, application rates of 360, 720, or 1440 g ae/ha resulted in decreased spider populations, which was attributed to secondary effects from changes in the vegetation (Haughton et al. 2001b). No substantial effects were observed in spider populations exposed to application rates of 90 or 180 g a.e./ha (Haughton et al. 1999).

In a more recent study, Benamu et al. (2010) exposed spiders to an Argentinean formulation of glyphosate (Glifoglex 48) by feeding the spiders for four days with prey dipped in a 192 mg a.i./L glyphosate IPA solution. While these exposure levels did not cause lethality, adverse effects were observed on a number of sublethal endpoints, including food consumption, web building, and reproductive capacity. In a similar study with the same Argentinean formulation,

Schneider et al. (2009) observed adverse effects in lacewings after dietary exposures for 48 hours to the eggs of a prey species dipped in 192 mg a.e./L formulation. The adverse effects included mortality, reduced reproductive capacity, and malformed offspring. Both of these studies provide information on the longer-term effects of short-term glyphosate exposure, indicating that glyphosate impacts reproduction and behavior in terrestrial arthropods.

Data on other arthropods are less detailed but also indicate a low potential of glyphosate to cause direct toxic effects. Some insects, such as grain beetles, may avoid foods contaminated with glyphosate (Castilla et al. 2010). Avoidance, however, was not noted in carabid beetles following field applications of 1.57 kg/ha or about 1.4 lb/acre (Brust 1990). In a laboratory study in which isopods were exposed to leaf litter at levels equivalent to an application rate of 2.1 kg/ha, the effect on litter degradation depended on the tree species. Direct toxic effects, manifested as increased mortality, could not be ruled out but were not statistically significant (Eijsackers 1992). Samsoe-Petersen (1995) reports no measurable effect on rove beetles (mortality and egg production) after spray of a substrate with 1% Roundup (3.6 g/L) at 6 $\mu L/cm^2$. Bramble et al. (1997) conducted a series of studies on the effects of using herbicides (including glyphosate) in rights-of-way maintenance, compared with mechanical maintenance and observed no significant or substantial differences in butterfly populations.

4.1.2.4.3. Other Terrestrial Invertebrates

Three available studies on glyphosate address its toxicity to earthworms. In a laboratory study, decreased growth rates and early mortality were observed on earthworm cultures treated with test concentrations equivalent to 0.7-2.8 g glyphosate/ha (Springett and Gray 1992). The direct relevance of this study is limited, however, because the exposure conditions (spraying twice weekly on culture dishes) do not closely approximate field conditions. Dalby et al. (1995) report no effects on earthworms in applications designed to mimic agricultural use. This study, however, does not report exposures either as g/ha or ppm soil and cannot be used directly in this risk assessment. The soil LC_{50} for glyphosate to *Aporrectodea caliginosa*, a worm common in Libya, is reported to be 246-177 mg glyphosate/kg soil dry weight over exposure periods of 8-37 days (Mohamed et al. 1995). Like grain beetles (Castilla et al. 2010), earthworms may avoid soil that is contaminated with glyphosate (Verrell and Van Buskirk 2004).

The toxicity of glyphosate to terrestrial snails is addressed in one available study. Diets containing 4994 ppm glyphosate did not cause mortality in the Brown garden snail, *Helix aspersa*, over a 14-day exposure period (Schuytema et al. 1994). Assuming a 30% food consumption factor for this species (APHIS 1993), the dietary concentration corresponds to a dose of about 1500 mg/kg (4994 ppm × 0.3 mg/kg bw ppm = 1498.2 mg/kg bw).

4.1.2.5. Terrestrial Plants (Macrophytes)

4.1.2.5.2. Standard Toxicity Studies

The testing requirements for the effects of herbicides on terrestrial plants are relatively rigorous since terrestrial vegetation is the typical target group for herbicides. The testing requirements of U.S. EPA involve bioassays for seedling germination and emergence (soil exposures) as well as vegetative vigor (foliar exposures) in several species of dicots and monocots. The toxicity studies on terrestrial plants include assays on vegetative vigor for both technical grade

glyphosate IPA (Appendix 5, Table 1) and glyphosate formulations (Appendix 5, Table 2) as well assays for seedling emergence using glyphosate formulations (Appendix 5, Table 3).

Foliar exposures to glyphosate, assayed as vegetative vigor studies, are much more toxic than soil exposures, as assayed by seedling emergence. The lesser toxicity of glyphosate in soil exposures is probably attributable at least in part to the tight binding of glyphosate to some types of soils (e.g., Accinelli et al. 2005; Borggaard and Gimsing 2008; Caceres-Jensen et al. 2009; Glass 1987; Mamy and Barriuso 2005). Seedling emergence studies involving three different glyphosate formulations indicate application rates in the range of 4-5 lb a.e./acre are relatively nontoxic (Bohn 1987; Everett et al. 1996a ; Willard 1996). Foliar applications, on the other hand, are much more toxic. In the assay using glyphosate IPA (Chetram and Lucash 1994), the NOAECs for monocots range from 0.7 to 0.56 lb a.e./acre. Dicots were somewhat more sensitive with NOAECs ranging from 0.035 to 0.46 lb a.e./acre. A similar pattern is apparent in studies on a wettable powder formulation of glyphosate (Appendix 5, Table 2). The NOAECs for monocots range from 0.07 to 0.45 lb a.e./acre. Dicots were again somewhat more sensitive with NOAECs ranging from 0.02 to 0.45 lb a.e./acre. Notably, the range of sensitivities is greatest for dicots, spanning a factor of over 20 [0.45 lb a.e./acre ÷ 0.02 lb a.e./acre = 22.5].

Boutin et al. (2004) conducted a series of bioassays similar to vegetative vigor studies—i.e., foliar applications—on 15 non-crop plant species native to Europe. These studies are summarized in Appendix 5, Table 4 of the current risk assessment. The plants were treated with Roundup Bio, a 360 g a.e./L formulation which appears to be marketed in Europe. Boutin et al. (2004) report EC_{50} values rather than NOECs and note a range of sensitivities from 14.26 to 64.66 g/ha. This variability is only a factor of about 4, much less than the variability in the registrant-submitted studies.

4.1.2.5.2. Other Toxicity Studies

Drift studies are relevant to the assessment of risk in that unintended drift is one of the more plausible exposure scenarios for nontarget terrestrial plant species (Section 4.2). The lowest reported effect level in drift studies is 1/33 of an application rate of 1.121 kg/ha which caused transient damage in soybeans, based on an assessment of visual injury, over a 30-day period after application but no net decrease in soybean production by the end of the season (Al-Khatib and Peterson 1999). This treatment level corresponds to 0.034 kg/ha [1.121 kg/ha ÷ 33] or about 0.03 lb/acre. A study by the same authors found that grapes were much less sensitive, evidencing damage at exposures equivalent to one-third of the application rate. A grass (*Poa annua*) and a dicot (*Brassica napus*) both exhibited substantial damage at deposition rates greater than 1000 $\mu g/m^2$ or about 1.8 lbs/acre. Fletcher et al. (1996) found that simulated drift in the range of 0.4-0.8% of an application rate of 0.43 kg/ha had no marked effect on canola, smartweed, soybean, or sunflower plants.

The study by Newmaster et al. (1999) suggests that some bryophytes and fungi may be sensitive to long-term effects of glyphosate exposure. The EC_{50} for a decrease in relative abundance 2 years after application is about 0.8 kg/ha or 0.7 lbs/acre (Newmaster et al. 1999, Figure 3, p. 1105). In addition, changes in relative abundance were apparent 6 weeks after application (Newmaster et al. 1999, Figure 7, p. 1108). The statistical analysis presented by Newmaster et al. (1999) involves the use of a non-threshold polynomial model. Although this method may be reasonable for quantifying the effects of the two herbicides investigated in the study (glyphosate

and triclopyr), it seems less appropriate for risk assessment, as discussed further in Section 4.3 (dose-response assessment). Nonetheless, this study does appear to present a plausible basis for concern that exposure to substantial glyphosate drift may have long-term impacts on bryophyte and lichen communities.

4.1.2.5.3. Other Considerations

There are numerous mechanism of action studies in the literature on glyphosate (Anthelme and Marigo 1998; Green et al. 1992; Hernandez et al. 1999; Hernandez et al. 2000; Hetherington et al. 1998; Jain et al. 2002; De Maria et al. 2006; Pline et al. 2002; Uotila et al. 1995; Singh and Shaner 1998; Schonbrunn et al. 2001). At the molecular level, glyphosate occupies the binding site of phosphoenol pyruvate, the second substrate of 5-enolpyruvylshikimate 3-phosphate synthase, mimicking an intermediate state of the ternary enzyme-substrate complex. This inhibits the shikimic acid pathway in plants, effectively blocking the synthesis of certain phenolic compounds and the synthesis of aromatic amino acids. This, in turn, leads to a variety of toxic effects in plants, including the inhibition of photosynthesis, respiration, and nucleic acid synthesis. At the level of the whole plant, inhibition of the shikimic acid pathway 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.

The efficacy of glyphosate is investigated in numerous field studies which focus primarily on vegetation management objectives (Appendix 5, Table 5). For the most part, efficacy studies are not covered in this risk assessment, with the exception of those that focus on understanding the pharmacology of glyphosate in plants. Such studies are germane to assessing potential effects in nontarget vegetation. Glyphosate is absorbed rapidly, primarily through foliage. Approximately 33% of applied glyphosate is absorbed within a few hours after application, and high humidity may serve to enhance absorption (Schonherr 2002). Because glyphosate is strongly adsorbed to soil, relatively little, if any, absorption occurs through the roots (Smith and Oehme 1992). The production of ^{14}C from plant-associated material does not appear to be correlated with soil microbial biomass (Von Wiren-Lehr et al. 1997). 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 propagule node, such as sugar cane, translocation from plant to plant can result in injury to plants not treated directly with glyphosate (Dal Piccolo et al. 1980). At least in sugar beets, the difference between tolerant and susceptible strains is related to the rate of glyphosate excretion (Geiger et al. 1999). The retention of glyphosate on foliage is affected by the use of adjuvants with a wash-off rate of about 50% with adjuvants and 64% without adjuvants (Leung 1994).

As with many herbicides, glyphosate may produce a hormetic response in some species, causing a stimulation of growth at low application rates of 0.0009-0.03 lb a.e./acre, depending on the species of plant (Schabenberger et al. 1999; Velini et al. 2008). Hormetic responses were noted also in yields of smartweed and soybeans (Fletcher et al. 1996: Figure 2, p. 1195).

Weed resistance is becoming an increasing concern with glyphosate (Busi and Powles 2009; Duke and Powles 2008; Huangfu et al. 2007; Reddy et al. 2008). As with efficacy studies, weed resistance is not a primary consideration in the current risk assessment except to note that application rates for glyphosate are being increased primarily in agricultural crops that are tolerant to glyphosate.

4.1.2.6. Terrestrial Microorganisms

As noted in Section 3.1.15.1, glyphosate is readily metabolized by soil bacteria with AMPA as a major metabolite. In addition, many species of soil microorganisms can use glyphosate as their sole carbon source (Dick and Quinn 1995a; Dick and Quinn 1995b; Dotson et al. 1996; Wardle and Parkinson 1992a). Microorganisms, like higher plants, use the shikimate pathway to produce aromatic amino acids. Since glyphosate inhibits this pathway, it is potentially toxic to microorganisms (Cox 2002; Issa 1999). Nonetheless, there is very little information suggesting that glyphosate will be harmful to soil microorganisms under field conditions and a substantial body of information indicating that glyphosate is likely to enhance or have no effect on soil microorganisms (Busse et al. 2001; Wardle and Parkinson 1990a,b; Wardle and Parkinson 1991).

On the other hand, a number of studies demonstrate adverse effects on soil microorganisms exposed to glyphosate under laboratory conditions, and the effects are consistent with the ability of glyphosate to inhibit the shikimate pathway. For example, the growth of soil algae and cyanobacteria might be inhibited by concentrations of 5 and 20 mM (about 845 and 3380 mg/L) glyphosate in an artificial culture medium (Issa 1999). Roundup was a more potent inhibitor, relative to glyphosate IPA, which, in turn, was a more potent inhibitor, relative to the free acid of glyphosate. The decreased growth was associated with shikimate accumulation and was antagonized by excess aromatic amino acids. At lower and more realistic concentrations (i.e., 2-20 ppm), however, glyphosate had no effect on fungi and only a slight inhibitory effect on some bacteria (Araujo et al. 2003; Castro et al. 2007; Forlani et al. 2008).

Wan et al. (1998) noted the inhibition of extraradical mycelial growth in *Glomus intraradices* after 14 days of exposure involving a preparation with carrot roots in a culture medium containing 0.5 ppm glyphosate. This effect, however, was attributed to an effect of glyphosate on the carrot roots rather than a direct toxic effect on the fungi. Glyphosate concentrations of 10 ppm or greater were directly toxic to soil fungi in culture media (Chakravarty and Sidhu 1987).

In another study regarding the non-target effects of glyphosate, application of 0.54 kg/ha caused a short-term (2 months) decrease in fungal and bacterial counts which recovered significantly at 6 months to population levels similar to those of untreated controls (Chakravarty and Chatarpaul 1990) . In the same study, an application rate of 3.23 kg/ha had no effect on soil fungi and bacteria after 10-14 months. A transient decrease in soil microbial activity was also noted by Wardle and Parkinson (1992b) after the application of glyphosate at 5 kg/ha. Sannino and Gianfreda (2001) report that glyphosate inhibited soil phosphatase activity at 20 mM. This inhibition, however, was attributed to competitive inhibition of p-nitrophenylphosphate, the substrate used in the phosphatase assay, by glyphosate. Thus, the inhibition of phosphatase activity was an artifact of the assay method rather than an indication of glyphosate toxicity.

Soil concentrations of 100 ppm of glyphosate or AMPA had no significant effect on soil denitrification (Pell et al. 1998). Bromilow et al. (1996) observed no effects on soil fertility in

repeated glyphosate applications of 1.4 kg/ha over the 14 years from 1980 to 1993, based on assays for microbial biomass and crop productivity. In humus soil, glyphosate did not retard microbial biomass at concentrations of up to 1000 mg/kg organic matter (Schnurer et al. 2006). Glyphosate formulations have been shown to retard the degradation of other pesticides (e.g., fluometuron and aldicarb); nonetheless, this effect appears to reflect the preferential metabolism of glyphosate rather than any adverse effect on microorganisms (Lancaster et al. 2006, 2008).

Several glyphosate field studies involving microbial activity in treated soil report either a lack of adverse effects or an increase rather than decrease in soil microorganisms or microbial activity (Biederbeck et al. 1997; Bromilow et al. 1996; Busse et al., 2001; Haney et al. 2002; Hart and Brookes 1996; Laatikainen and Heinonen-Tanski 2002; Nicholson and Hirsch 1998; Means et al. 2007; Sailaja and Satyapradad 2006; Stratton and Stewart 1992; Wardle and Parkinson 1991). Wardle and Parkinson (1992) report that an application rate of 5 kg/ha (≈4.5 lb/acre) caused a transient decrease in microbial biomass in soil but consider this effect secondary to toxic effects on vegetation. As discussed by Kremer (2002), glyphosate applications may cause transient increases in soil fungi, which may be detrimental to some plants. For example, Descalzo et al. (1996a,b) note that inoculation of soil with various pathogenic soil fungi may result in an apparent enhancement of glyphosate toxicity to a species of bean (*Phaseolus vulgaris*).

4.1.3. Aquatic Organisms

4.1.3.1. Fish

4.1.3.1.1. Overview

A substantial body of information is available on the toxicity of glyphosate, glyphosate formulations, and related surfactants to fish. Much of this information is reviewed in the ecological risk assessment by U.S. EPA/OPP (2008a), which was used as a major resource in the current Forest Service risk assessment. Most of the available studies are summarized in Appendix 6 (Tables 1 through 8). The following discussion focuses on those studies and concepts central to the hazard identification for fish.

As with the human health risk assessment (Section 3) and the hazard identification for terrestrial species (Section 4.1.2), the differences in the toxicity of glyphosate and glyphosate formulations to fish are substantial. Most of the comparative studies on glyphosate, glyphosate formulations, and surfactants used in or with glyphosate formulations involve assays of acute lethal potency—i.e., determinations of LC_{50} values.

Most studies that determine LC_{50} values for fish involve observations made at 24, 48, 72, and 96 hours of exposure. In an effort to focus the assessment on the most relevant information, this analysis focuses on 96-hour LC_{50} values. The EPA takes a similar approach in U.S. EPA/OPP (2008a). This approach is reasonable because the differences between 24-hour LC_{50} values and 96-hour LC_{50} values are modest for the test materials considered in the hazard identification. For example, in the extensive study by Wan et al. (1989, Table 4), LC_{50} values were determined for five species of fish in five different types of water. For glyphosate, the ratios of the 24-hour LC_{50} values to the 96-hour LC_{50} values ranged from 1.0 to 2.7. The corresponding ratios for a POEA surfactant (MON 0818) were 1 to 2.5, and the ratios for Roundup were 1 to 1.8. In other

words, in many of the bioassays, most of the dead fish died on Day 1, meaning that the 24- and 96-hour LC_{50} values are identical or differ only marginally.

The data on acute lethal potency are discussed separately for technical grade glyphosate (Section 4.1.3.1.2.1), various glyphosate formulations (Section 4.1.3.1.2.2), and surfactants (Section 4.1.3.1.2.3). In addition, two studies (Folmar et al. 1979; Wan et al. 1989) involve concurrent bioassays on glyphosate, Roundup, and MON 0818, the POEA surfactant used in Roundup. These studies suggest that the joint action of glyphosate and the MON 0818 is additive.

As discussed in Section 3.1.14.1, however, MON 0818 does not appear to be the only POEA surfactant used in glyphosate formulations. Specific information about the composition and toxicity of other POEA surfactants used in other formulations by other suppliers is not available. The toxicity data provided in the MSDS for other formulations of glyphosate (as summarized in Appendix 1, Table 2), do not clearly indicate the units for toxicity values. Consequently, these data are not explicitly considered in the following analysis.

POEA surfactants are important because they are used in Roundup formulations and appear to be used in many other glyphosate formulations. Nonetheless, different types of surfactants are used with Rodeo and other glyphosate formulations. As discussed in Section 4.1.3.1.2.4, some but not all of these other surfactants appear to be much less toxic than MON 0818. This is an important consideration in assessing differences between applications of Roundup and similar formulations, relative to Rodeo and similar formulations.

While LC_{50} values are the most common type of information available on glyphosate and glyphosate formulations, Forest Service risk assessments attempt to avoid using LC_{50} values quantitatively in the dose-response assessment (Section 4.3.3.1). Nonetheless, information on the sublethal toxicity of glyphosate and glyphosate formulations is extremely limited and often difficult to interpret (Section 4.1.3.1.3). While the chronic toxicity of technical grade glyphosate is well characterized, few studies assay the longer-term toxicity of glyphosate formulations to a wide range of toxic endpoints (Section 4.1.3.1.4).

4.1.3.1.2. Acute Lethality

4.1.3.1.2.1. Glyphosate Acid and Salts

Acute LC_{50} values for technical grade glyphosate and as well as the IPA salt of glyphosate are summarized in Appendix 6, Table 1. While some of the studies summarized in Appendix 6, Table 1 are unpublished registrant-submitted studies, the two most detailed comparative studies are from the open literature (Folmar et al. 1979; Wan et al 1989). Most of the studies summarized in Appendix 6, Table 1 are also summarized in the EPA's extensive review of the aquatic toxicity of glyphosate (U.S. EPA/OPP 2008a). As noted Appendix 6, Table 1, the values presented in U.S. EPA/OPP (2008a) are often different from those given in the original studies because the EPA elected to correct toxicity values for compound purity. These differences are noted in Appendix 6 as well as other appendices on aquatic organisms simply to avoid any confusion on the part of individuals using the current Forest Service risk assessment as well as the risk assessment prepared by U.S. EPA/OPP (2008a). For the most part, the correction for compound purity does not have a major impact on the risk assessment for fish or other aquatic organisms.

According to the U.S. EPA/OPP general classification scheme (see SERA 2007a, Table 4-1), the LC_{50} values for glyphosate acid and the IPA salt of glyphosate would be classified as slightly toxic (LC_{50} >10 to 100 mg/L) or practically nontoxic (LC_{50} >100 mg/L) to fish. The only exceptions are two LC_{50} values of 10 mg a.e./L reported by Wan et al. (1989) which would classify glyphosate as moderately toxic to fish (LC_{50} >1 to 10 mg/L). Based on this study conducted with five species of salmonids, pH is the most important factor regarding the toxicity of glyphosate to fish. As the pH decreases and the water becomes more acidic, the toxicity of glyphosate increases with a corresponding decrease in the LC_{50} values. This pattern is to be expected for a weak acid. As the pH decreases, glyphosate will be increasingly protonated, and the more protonated or less electrically charged ionic species will be more readily transported across biological membranes.

The impact of pH on the toxicity of glyphosate is substantial. Wan et al. (1989) conducted assays at pH values ranging from 6.3 to 8.2. The test species least sensitive to pH variance were Coho salmon, as indicated by the range of LC_{50} values (27 mg a.e./L at pH 6.3 to 174 mg a.e./L at pH 8.2) which varied by a factor of about 6 [174 mg a.e./L ÷ 27 mg a.e./L ≈ 6.44]. Rainbow trout were the test species most sensitive to pH variance, with LC_{50} values ranging from 10 mg a.e./L at pH 6.3 to 197 mg a.e./L at pH 8.2—i.e., a factor of nearly 20. The differences in sensitivity among the five test species are relatively minor at the same pH. In other words, pH appears to be a more important factor in acute lethal toxicity, relative to species differences.

As discussed further in Sections 4.1.3.1.2.2 and 4.1.3.1.2.3, the opposite pattern is apparent for glyphosate formulations that contain POEA as well as for the POEA surfactant itself. For these agents, toxicity increases with increasing pH (greater alkalinity).

Most acute toxicity studies in fish involve fasting the fish prior to and during testing. For example, the U.S. EPA/OPP requires that fish used for acute bioassays are fasted 48 hours prior to testing and that the fish are not fed during the bioassay (U.S. EPA/OPPTS 1996). Thus, in a 96-hour bioassay, the fish are without food for a total of 6 days. Holdway and Dixon (1988) conducted a series of bioassays involving 2-hour pulse exposures of flagfish (ages of 2, 4, or 8 days) that were either fed or fasted. While the exposure period involved only 2-hours, the results are reported as 96-hour LC_{50} values because mortality was determined over a 96-hour post-exposure period. The LC_{50} in 8-day-old fasted fish was 2.94 mg a.e./L and the LC_{50} in fed fish was 29.6 mg a.e./L. Thus, feeding diminished the toxicity of glyphosate by about a factor 10. It is also significant that the fasting schedule used by Holdway and Dixon (1988) was less severe than that recommended by U.S. EPA/OPP, in that all fish were fed up to the day of testing. A similar effect was noted for permethrin in both fed and fasted flagfish and white suckers.

4.1.3.1.2.2. Glyphosate Formulations

The number of acute LC_{50} studies conducted on various formulations of glyphosate is considerable, as documented in Appendix 6, Table 2. Table 2 of Appendix 6 includes all of the studies summarized in the recent EPA ecological risk assessment of glyphosate (U.S. EPA/OPP 2008a) as well as studies from the open literature.

The majority of the toxicity studies on glyphosate formulations involve Roundup. While there are currently a number of different Roundup formulations, most of the earlier studies appear to

involve the original Roundup formulation provided by Monsanto—i.e., a 41% (w/w) aqueous solution of glyphosate IPA with a POEA surfactant (MON 0818) at a concentration of 15% (e.g., Wan et al. 1989). As discussed in the previous subsection, the toxicity of technical grade glyphosate to fish increases as the pH decreases. The opposite pattern is seen with Roundup formulations. Based on the studies by Folmar et al. (1979) and Wan et al. (1989), the toxicity of Roundup increases with increasing pH (lower acidity). In the bioassays conducted by Wan et al. (1989) with five species of salmonids over a pH range from 6.3 to 8.2, the ratios of the 96-hour LC_{50}s at pH 6.3 to those at pH 8.2 ranged from about 2 to 3. In bioassays conducted by Folmar et al. (1979) with bluegills and trout over a pH range from 6.5 to 9.5, the ratios of the 96-hour LC_{50}s at pH 6.5 to those at pH 9.5 varied by a factor of 2.3 for bluegills and by a factor of 5.4 for trout. As discussed in Section 4.1.3.1.2.3, a similar pattern is seen with the effect of pH on the toxicity of POEA surfactants. Compared with the effect of pH on glyphosate where the differences in toxicity range from a factor of 6 to 20, the effect of pH on the toxicity of Roundup is modest.

The effect of pH on the toxicity of Roundup is almost certainly due to the effect of pH on the POEA surfactant. POEA surfactants are typically referred to as *non-ionic*. In other words and as illustrated in Figure 6, POEA, surfactants do not contain structures with positive or negative electrical charges. While this is the case for POEA surfactants in neat form (i.e., not in solution), Wang et al. (2005) note that in aqueous solutions, POEA surfactants are at least partially protonated and have a net positive charge (i.e., will be cationic rather than anionic). As the pH increases (i.e., the solution becomes less acidic and the concentration of protons in the solution decreases), a greater proportion of the POEA surfactant in solution will be electrically neutral and will have a greater tendency to cross biological membranes. Thus, as the pH increases, the toxicity of the glyphosate in the Roundup formulation will decrease; however, the increasing toxicity of the POEA surfactant has the greater effect on the toxicity of the Roundup formulation. The combined action of glyphosate and POEA surfactants is discussed in further detail in Section 4.1.3.1.2.4.

A selective overview of the toxicity of glyphosate formulations is given in Table 22. This overview is selective in that the focus is on formulations that are used or may be used in the United States, particularly formulations identified by the Forest Service (Table 2).

The first entry in Table 22 provides the range of reported toxicity values for Roundup formulations (NOS) which contain or appear to contain the POEA surfactant. The reported range of LC_{50} values is relatively narrow, about 1-10 mg a.e./L. Given that these bioassays were conducted at different facilities under different conditions with different populations and species of fish, this variability is relatively modest. As noted above, differences in pH alone may account for variability spanning factors of 2-6. One apparent and modest outlier not included in Table 22 is the Hildebrand et al. (1982) study in which LC_{50} values range from 15.8 to 16.6 mg a.e./L for rainbow trout. These somewhat atypically high LC_{50} values are probably due to pH, which, according to the study, dropped to as low as 4.8 during the bioassays. As noted above, this relatively acidic pH would be expected to result in higher LC_{50} values for Roundup.

The second through the fifth entries in Table 22 are the results of bioassays on specific Roundup formulations that have been identified by the Forest Service (Table 2). The LC_{50} values for these

formulations range from about 1 to 10 mg a.e./L. These formulations are based on different salts of glyphosate and various levels of surfactants. As summarized in Appendix 2, Table 2, the toxicity of a Vision formulation, which is essentially equivalent to Roundup, varies by nearly a factor of 4 at surfactant concentrations or loadings ranging from 7.5 to 15% (U.S. EPA/OPP 2008a, Appendix J, p. 13). As detailed further in Section 4.1.3.1.2.4, the increase in the toxicity of a formulation with increasing surfactant loading is a consequence of joint action of glyphosate and POEA surfactants.

Table 22 also summarizes the results of an acute bioassay on Rodeo. As discussed in Section 2, some glyphosate formulations, like Accord, AquaNeat, and Rodeo consist primarily of a glyphosate salt in water. Accordingly, these formulations are much less toxic than Roundup formulations to aquatic organisms. Of the many studies available on glyphosate and glyphosate IPA, the only clearly documented toxicity study on Rodeo was conducted by Mitchell et al (1987a) and reports an LC_{50} of 429 mg a.e./L for trout. As summarized in Table 4, however, Rodeo and similar formulations require the use of surfactants. As detailed further in Section 4.1.3.1.2.3, the surfactants used with Rodeo and similar formulations are less toxic than POEA surfactants. Nonetheless, even these less toxic surfactants will enhance the toxicity of glyphosate. As summarized in Table 22 and detailed in Appendix 2 (Table 2), bioassays on Rodeo with and without the X-77 surfactant appear to increase the toxicity of glyphosate by a factor of about 4 (i.e., the study by Mitchell et al. 1987a). A much more detailed consideration of the interaction of Rodeo and surfactants is given in Section 4.3.3.1.2.1 (Dose-Response Assessment for Less Toxic Formulations).

Some glyphosate formulations with the trade name of *Roundup* are far less toxic than standard Roundup formulations which contain the POEA surfactant, most notably, *Roundup Biactive*. Roundup Biactive appears to be an Australian formulation of glyphosate. Nonetheless, toxicity data on Roundup Biactive were submitted to the U.S. EPA and are covered in the recent EPA ecological risk assessment (U.S. EPA/OPP 2008a). The reported LC_{50} value for Roundup Biactive in rainbow trout is 800 mg a.e./L. In other words, this *Roundup* formulation is less toxic than Rodeo without any surfactant. Roundup Bioactive does contain a surfactant at a concentration between 10 and 20%, the identity of which is not publically disclosed (Howe et al. 2004).

Two other relatively nontoxic surfactants are referred to as "*W*" and Geronol CR/AR surfactants in U.S. EPA/OPP (2008a). It is not clear if these surfactants are included in some Roundup formulations or if the bioassays summarized in Table 22 were simply mixtures of a Roundup/POEA surfactant with the two other surfactants. In either case, these Roundup formulations do not appear to be any more toxic than Rodeo.

Most acute toxicity studies in fish are conducted on young fish at temperatures appropriate for the species being tested. The early studies by Folmar et al. (1979, Table 5) examined the effects of temperature and life stage on the toxicity of Roundup to fish. As with most pesticides as well as other chemicals, the acute toxicity of Roundup increased with increasing temperatures. With both rainbow trout and bluegill sunfish, an increase in temperature of 10 °C was associated with a decrease in the LC_{50} by about a factor of 2. Folmar et al. (1979, Table 3) also note that small

fingerlings and swim-up fry were somewhat more sensitive than larger fingerlings and much more sensitive than eyed eggs to Roundup.

4.1.3.1.2.3. Surfactants

Acute bioassays in fish using POEA surfactants which appear to be included in many glyphosate formulations are summarized in Appendix 6, Table 3. As discussed in Section 3.1.14.1 and illustrated in Figure 6, POEA surfactants are complex mixtures. Information on the variability of different POEA surfactants in different formulations is not available. The manufacturing processes for POEA surfactants are considered proprietary as are the specific chemical compositions of the POEA surfactants.

MON 0818 is Monsanto product code for the surfactant used in the original Roundup formulation. As summarized in Appendix 6, Table 3, all of the toxicity studies on the POEA surfactants involve MON 0818 (Folmar et al. 1979; Servizi et al. 1987; Wan et al. 1989). As with the Roundup formulations, the toxicity of MON 0818 increases with increasing pH. The likely cause of the relationship between pH and the toxicity of MON 0818 is the cationic nature of the surfactant in an aqueous solution, as discussed in Section 4.1.3.1.2.2. As with Roundup, Wan et al. (1989) assayed MON 0818 in five species of salmonids over a pH range of 6.3 to 8.2. Over this range, the LC_{50} values for MON 0818 decreased by factors of about 1.2 to 3.2. Folmar et al. (1979) noted a similar decrease in the LC_{50} values for bluegills (i.e., a factor of 1.3) but a much greater decrease for trout (a factor of about 11). The LC_{50} values for trout from the study by Folmar et al. (1979) define the upper and lower bound of reported LC_{50} values for MON 0818 – i.e., 0.65 mg/L to 7.4 mg/L. The typical LC_{50} values for MON 0818 are about 1 to 3 mg/L. In other words and as with the studies using Roundup, the variability in the acute toxicity of the surfactant is due primarily to differences in pH rather than apparent differences in species sensitivity.

Acute LC_{50} values for other surfactants, most of which appear to be used as surfactants added to Rodeo and other similar formulations, are summarized in Appendix 6, Table 5. Most of these toxicity values are taken from the review by McLaren/Hart (1995). As noted in the McLaren/Hart (1995) report, these toxicity values are from unpublished studies provided to McLaren/Hart by Monsanto. Almost all of these surfactants have LC_{50} values in the range of 1 to 10 mg/L, similar to the range of LC_{50} values for MON 0818 —i.e., Syndets (anionic surfactant), Activator 90, Entry II, Frigate, Induce, No Foam A, R-11, S. Spreader 200, Widespread, X-77. Based on EPA's classification system, all of these surfactants would be classified as Moderately Toxic to fish. Three surfactants are in the range of Slightly Toxic compounds (LC_{50} values ranging from >10 to 100 mg/L)—i.e., Liqua-Wet, Passage, and Spreader-Sticker. Three surfactants would be classified as Practically Nontoxic (LC_{50} values >100 mg/L)—i.e., Agri-Dex, LI 700, and Geronol CF/AR.

As discussed in Section 4.1.3.1.2.2 and summarized in Table 22, there is a toxicity study on a mixture of Roundup and Geronol CF/AR in which an LC_{50} of 450 mg a.e./L is reported. This LC_{50} is about a factor of 100 above the toxicity of Roundup—i.e., a typical LC_{50} of about 5 mg a.e./L. As indicated in Appendix 6, Table 5, the reported LC_{50} for Geronol CF/AR is >100 mg/L. Although it appears that Geronol CF/AR antagonizes the toxicity of Roundup, without

specific information about the composition of the Roundup/Geronol CF/AR mixture tested, it is impossible to make a formal analysis of the joint action.

As also discussed in 4.1.3.1.2.2 and summarized in Table 22, there are available toxicity studies on a Rodeo/X-77 mixture in which the LC_{50} values in salmonids range from about 100 to 200 mg a.e/L, which are about 2-6 times lower than the reported LC_{50} values for Rodeo (from ≈440 to 580 mg a.e./L). As indicated in Appendix 6, Table 5, the reported LC_{50} for X-77 is 4.3 mg a.e./L. In other words, the LC_{50} values for X-77 are in the range of those reported for MON 0818. Again, without additional details on the study with the Rodeo/X-77 mixture, it is impossible to make a formal analysis of the combined action of the two agents. Nonetheless, it is apparent that the toxicity of Rodeo/X-77 mixture can be attributed primarily to the toxicity of the surfactant.

4.1.3.1.2.4. Joint Action of Glyphosate and MON 0818 Surfactant

As noted in the discussion of the by Baba et al. (1989) study in Section 3.1.4.3.2, the concept of dose addition can be used to assess the joint action of the components in a mixture. In the mammalian toxicity study by Baba et al. (1989), separate bioassays on the IPA salt of glyphosate, the POEA surfactant, and a Roundup formulation could be used to suggest that the joint action of glyphosate IPA and POEA in Roundup was less than additive—i.e., the ratio of the predicted LD_{50} to the observed LD_{50} is about 0.6.

The concept of simple similar action can be applied to LC_{50} values as well as LD_{50} values. As summarized in Appendix 6, the studies by Wan et al. (1989) and Folmar et al. (1979) involved determining LC_{50} values for glyphosate, the MON 0818 surfactant, and the original Roundup formulation, which contained the MON 0818 surfactant at a concentration of 15 %. Wan et al. (1989) also tested MON 8709, a formulation of glyphosate with 10% MON 0818 surfactant.

The analyses for the data from Wan et al. (1989) are presented in Table 23 and the analyses for the data from Folmar et al. (1979) are presented in Table 24. The analyses were conducted based on the assumption of dose addition as discussed in Section 3.1.4.3.2, except that LC_{50} values rather than LD_{50} values are used.

As discussed in Section 3.1.4.3.2, the implementation of dose addition (Equation 2) can differ depending on the units in which the toxicity values are expressed. Wan et al. (1989) expresses the LC_{50} values for glyphosate in units of mg a.e./L but expresses the toxicity values for the surfactant, MON 0818, in units of POEA. Thus, using the units reported in Wan et al. (1989), the potency of POAE relative to glyphosate acid equivalents ($\rho_{ae/POEA}$) is calculated as:

$$\rho_{ae/POEA} = \frac{\mathrm{LC_{50}\ Glyphosate_{mg\ a.e./L}}}{\mathrm{LC_{50}\ POEA_{mg\ POEA/L}}} = \frac{a.e.}{POEA}$$

Equation 7

Because POEA comprises 75% of MON 0818, the proportion of POEA in the Roundup formulation is taken as $0.1125_{\text{ POEA/form}}$ [$0.15_{\text{MON 0818/form}}$ x $0.75_{\text{POEA/MON 0818}}$]. Wan et al. (1989) specifies that the Roundup formulation contained 30.5% glyphosate a.e. Based on this specification, the proportion of glyphosate a.e. in for the formulation is taken as $0.305_{\text{ae/form}}$.

Thus, for the analysis of the Wan et al. (1989) data presented in Table 23, the predicted LC_{50} for Roundup based on the assumption of dose addition is implemented as:

$$\zeta_{Roundup} = \frac{\mathrm{LC_{50}Glyphosate\ mg\ a.\,e./L}}{0.305_{ae/form} + (\rho_{ae/POEA} \times 0.1125_{POEA/form})} = LC_{50}\ mg\ form/L$$

Equation 8

For the study by Wan et al. (1989), the toxicity values given in Table 23 of the current Forest Service risk assessment are taken from Table 4 of Wan et al. (1989). Note that Table 4 in Wan et al. (1989) is sorted by water type; whereas, Table 23 in this risk assessment is sorted by species of fish. All calculations are based on rounding potency and predicted LC_{50} for the Roundup to the nearest digit following the decimal. The interaction coefficients – i.e., the ratio of the predicted to the observed LC_{50}s – are rounded to the second place following the decimal.

For example and as summarized in Table 23 of this risk assessment, Wan et al. (1989, Table 4, p. 382) report the following 96-hour LC_{50} values for Coho salmon at pH 6.3 using soft city water:

glyphosate:	27	mg a.e./L
MON 0818 as POEA:	4.6	mg POEA/L
Roundup:	32	mg formulation/L.

The potency MON 0818 expressed as POEA relative to glyphosate acid ($\rho_{ae/POEA}$) based on these LC_{50} values is calculated from Equation 7 as:

$$\rho_{ae/POEA} = \frac{27\ mg\ a.e./L}{4.6\ mg\ POEA/L} \cong\ 5.86957_{ae/POAE}$$

Equation 9

Rounding the estimated relative potency to $5.9_{ae/POEA}$, the expected LC_{50} of Roundup ($\zeta_{Roundup}$) based on the assumption of dose addition is calculated by substitution into Equation 8 as:

$$\zeta_{Roundup} = \frac{27\ \mathrm{mg\ a.\,e./L}}{0.305_{ae/form} + (5.9_{ae/POEA} \times 0.1125_{POEA/form})} \cong 27.87096\ mg\ form/L$$

Equation 10

Rounding the predicted LC_{50} of Roundup to 27.9 mg formulation/L (i.e., one significant place after the decimal), the ratio of the predicted LC_{50} to the observed LC_{50} of 32 mg formulation/L, rounded to 2 significant digits after the decimal, is 0.87 [27.9 mg formulation/L ÷ 32 mg formulation/L = 0.871875].

All other estimates of relative potency, expected LC_{50}s for Roundup, and interaction ratios given in Table 23 are calculated as in the above example and details of the these calculations are given in Attachment 3, Worksheet "Wan et al. 1989 Roundup". This worksheet also details the calculation of the average and 95% confidence intervals for the interaction ratios. Rounded to two significant figures following the decimal, the average (95% confidence interval) for the interaction ratios from Wan et al. (1989) is 0.89 (0.78 to 1.00), indicating that joint action for glyphosate and the MON 0818 is somewhat less than additive with marginal statistical significance.

As noted above, Wan et al. (1989) also assayed a formulation of glyphosate referenced as MON 8709. As with Roundup, this formulation contained glyphosate a.e. at a proportion of 0.305. Unlike Roundup, however, the formulation contained only 10% of the MON 0818 surfactant. Thus, the proportion of POEA in the MON 8709 formulation was $0.075_{POEA/form}$ [$0.10_{MON\ 0818/form}$ x $0.75_{POEA/MON\ 0818}$]. Other than this difference in the proportion of the surfactant, the analysis of joint action is identical to the above analyses for Roundup. Again taking the bioassay of Coho salmon at pH 6.3 as detailed above for Roundup, the potency of POEA to glyphosate remains the same – i.e., $5.9_{ae/POEA}$ as detailed in Equation 9 – and the calculation of the expected LC_{50} of the MON 8709 formulation is identical to that for Roundup (Equation 10) except that the proportion of POEA in the formulation is taken as $0.075_{POEA/form}$:

$$\zeta_{MON\ 8709} = \frac{27\ \text{mg a. e./L}}{0.305_{ae/form} + (5.9_{ae/POEA} \times 0.075_{POEA/form})} \cong 36.1204\ mg\ form/L$$

Equation 11

Rounding the expected LC_{50} to 36.1 mg formulation/L and using the observed LC_{50} of 55 mg formulation/L (Wan et al. 1989, Table 5), the ratio of the predicted LC_{50} to the observed LC_{50} is about 0.65 [36.1 mg formulation/L ÷ 55 mg formulation/L ≈ 0.65636], also indicating a less than additive joint action.

All other estimates of relative potency, expected LC_{50}s for MON 8709, and interaction ratios given in Table 23 are calculated as in the above example (Equation 10) and details of the these calculations are given in Attachment 3, Worksheet "Wan et al. 1989 MON 8709". As with the corresponding worksheet for Roundup, the worksheet for MON 8709 details the calculation of the average and 95% confidence intervals for the interaction ratios. Rounded to two significant figures following the decimal, the average (95% confidence interval) for the interaction ratios for MON 8709 is 0.72 (0.61 to 0.83), indicating that joint action for glyphosate and the MON 0818 is less than additive and that the less than additive joint action is statistically significant based on this data set.

The statistical analyses of the interaction ratios from the study by Wan et al. (1989) should not be overly interpreted. Wan et al. (1989) indicate that the bioassays described in the publication were conducted over a period of several months. For the assessment of joint action, it is preferable to conduct all assays – i.e., glyphosate, the surfactant, and the two formulation – at the same time. While the individual bioassays across test compounds using a single species and water type may have been conducted concurrently, this is not explicitly stated in the publication by Wan et al. (1989). In any event, it is not likely that all of the bioassays summarized in Table 23 were conducted concurrently. Nonetheless, the study by Wan et al. (1989) is the largest study in terms of the number of bioassays conducted. In addition, Wan et al. (1989) is the only study in which two different formulations of glyphosate were tested in a manner that permit an at least crude assessment of joint action. Overall, the results from the study by Wan et al. (1989) suggest that the joint action of glyphosate and the MON 0818 surfactant is less than additive.

The results of the salmonid bioassays from Wan et al. (1989) can also be used as an example of the effect of surfactant loading – i.e., the proportion of the surfactant in the formulation. Figure 8 in the current risk assessment plots the comparable LC_{50} values for salmonids, as summarized

in Table 23. The LC_{50} values for Roundup are plotted on the x-axis and the corresponding LC_{50} values for MON 8709 is plotted on the y-axis. Thus, each point illustrated in Figure 8 is defined by the LC_{50} values for one species of salmonid in water at a the same pH. The solid diagonal line designates the line of equal toxicity. In other words, if the LC_{50}s for Roundup and MON 8709 were identical, all toxicity values would fall on the solid diagonal line. Note, however, that all of the points are above the diagonal line, indicating that the Roundup formulation, which contains 15% of the MON 0818 surfactant, is more toxic than the MON 8709 formulation which contains on 10% of the MON 0818 surfactant. This relationship is to be expected because the toxicity of both the Roundup formulation and the MON 8709 formulation is dominated by the MON 0818 surfactant because the MON 0818 surfactant is much more toxic than glyphosate. As summarized in Table 23, the estimates of relative potency indicate that MON 0818 is more toxic to salmonids by factors of 3.1 (i.e., pink salmon at pH 6.3) to 135.6 (i.e., pink salmon at pH 8.2).

Folmar et al. (1979) conducted studies on the acute toxicity of glyphosate, MON 0818, and Roundup in trout and bluegills at pH 6.5 and 9.5. Folmar et al. (1979) used samples of both glyphosate and glyphosate IPA along with the surfactant and Roundup. Folmar et al. (1979), however, clearly indicate that the acute toxicity bioassays were conducted with technical grade glyphosate, and the only use of glyphosate IPA appears to have been in avoidance studies—i.e., Table 8 in Folmar et al. (1979). As noted in several entries in Appendix 6, U.S. EPA/OPP (2008a) suggests that Folmar et al. (1979) study provides LC_{50} values for glyphosate IPA, but this appears to be incorrect. The U.S. EPA/OPP (2008a) also appears to interpret the LC_{50} values for Roundup reported in Folmar et al. (1979) as being expressed in units of mg a.i./L. The publication by Folmar et al. (1979) is somewhat ambiguous and does not clearly specify the units for the Roundup LC_{50} values. Folmar et al. (1979, p. 271), however, describe Roundup as consisting of *…360.32 g/L active ingredient*. The original Roundup formulation tested by Folmar et al. (1979) contained glyphosate IPA at a concentration of approximately 480 mg glyphosate-IPA/L which corresponds to approximately 356 mg a.e./L. Based on these factors, it appears that Folmar et al. (1979) designate glyphosate acid equivalents (a.e.) as the *active ingredient*, even though common usage of the term *active ingredient* is typically used to designate glyphosate IPA. Consequently, in the current Forest Service risk assessment, the LC_{50} values for Roundup reported by Folmar et al. (1979) are interpreted as being expressed in units of glyphosate a.e./L.

As noted above, Folmar et al. (1979) indicate that the concentration of glyphosate in the Roundup formulation (identified as MON 2139) was 360.32 g a.e./L but do not state the proportion (w/w) of glyphosate a.e. in the formulation. Based on the earliest MSDS for MON 2139 that could be located (Monsanto 1992), the specific gravity of the formulation is 1.17. This specific gravity is identical to that given on a 1985 MSDS for Roundup (Monsanto 1985). Taking 1.17 g/mL as an approximate density for the Roundup formulation, the proportion (w/w) of glyphosate a.e. in the formulation is taken as $0.308_{ae/form}$ [360.32 g a.e./L ÷ 1,170 g/L ≈ 0.307966].

Unlike Wan et al. (1989), Folmar et al. (1979, Table 6, p. 276) report the LC_{50} values for MON 0818, the surfactant, in units of mg MON 0818/L rather than units of mg POEA/L. While LC_{50} values in units of mg MON 0818/L can be easily converted to units of mg POEA/L, the

analyses of joint action based on the data in the Folmar et al. (1979) are conducted based on the units reported in the study – i.e., mg MON 0818/L. Thus, the potencies of the MON 0818 surfactant relative to glyphosate acid ($\rho_{ae/MON\ 0818}$) are calculated as:

$$\rho_{ae/MON\ 0818} = \frac{\mathrm{LC_{50}\ Glyphosate_{mg\ a.e./L}}}{\mathrm{LC_{50}\ MON\ 0818_{mg\ MON\ 0818/L}}} = \frac{a.e.}{MON\ 0818}$$

Equation 12

As discussed above, Folmar et al. (1979) appear to report the LC_{50} values for Roundup in units of mg a.e./L. Using these units as reported in the study, the implementation of Equation 2 to develop predicted values for Roundup expressed in units of mg a.e./L:

$$\zeta_{Roundup} = \frac{\mathrm{LC_{50}Glyphosate\ mg\ a.e./L}}{1_{ae/ae} + (\rho_{ae/MON\ 0818} \times 0.487_{MON\ 0818/ae})} = LC_{50}\ mg\ a.e./L$$

Equation 13

Note that π_1, the proportion of glyphosate a.e. in the formulation relative to itself is, by definition, 1. The proportion of MON 0818 in the Roundup formulation relative to glyphosate a.e. is taken as $0.487_{\text{ MON 0818/ae}}$ [$0.15_{\text{MON 0818/form}} \div 0.308_{\text{ae/form}} \approx 0.487$].

As an example, Folmar et al. (1979, Table 6, p. 276) report LC_{50} values for trout at pH 6.5 of 140 mg a.e./L for glyphosate, 7.4 mg/L for MON 0818, and 7.6 mg a.e./L for Roundup. Using Equation 12, the potency of the MON 0818 surfactant to glyphosate a.e. is rounded to one significant digit following the decimal place is $18.9_{\text{ae/MON 0818}}$:

$$\rho_{ae/MON\ 0818} = \frac{140\ \mathrm{mg\ a.e./l}}{7.4\ mg\ MON\ 0818/L} \cong 18.9189\frac{a.e.}{MON\ 0818}$$

Equation 14

Substituting into Equation 13, the predicted LC_{50} for Roundup (in units of a.e./L) under the assumption of dose addition is about 13.7 mg a.e./L:

$$\zeta_{Roundup} = \frac{140\ \mathrm{mg\ a.e./L}}{1_{ae/ae} + (18.9_{ae/MON\ 0818} \times 0.487_{MON\ 0818/ae})} \cong 13.7197\ mg\ a.e./L$$

Equation 15

As noted above, the LC_{50} for the Roundup formulation is reported as 7.6 mg a.e./L. Thus, the ratio of the predicted to observed LC_{50} values is about 1.80 [13.7 mg a.e./L ÷ 7.6 mg a.e./L], suggesting a somewhat greater than additive joint action.

While the above analysis uses the units reported in Folmar et al. (1969), the derivation of the proportions is somewhat obtuse. A more intuitive analysis involves dividing the observed LC_{50}s for Roundup in units of mg a.e./L by the proportion of glyphosate a.e. in the formulation – i.e., $0.308_{\text{ae/form}}$, thus converting the LC_{50}s to units of mg formulation/L. Taking this approach, Equation 2 is implemented as:

$$\zeta_{Roundup} = \frac{\mathrm{LC_{50}Glyphosate\ mg\ a.e./L}}{0.308_{ae/form} + (18.9_{ae/MON\ 0818} \times 0.15_{MON\ 0818/form})} = LC_{50}\ mg\ formulation/L$$

Equation 16

where 0.308 (π_1 in Equation 2) is the proportion of glyphosate a.e. in the formulation and 0.15 (π_2 in Equation 2) is the proportion of MON 0818 in the formulation. Again taking the data on trout at pH 6.5 as an example, the LC_{50} for Roundup in units of mg formulation/L is estimated at about 44.5 mg a.e./L:

$$\zeta_{Roundup} = \frac{140 \text{ mg a. e./L}}{0.308_{ae/form} + (18.9_{ae/MON\ 0818} \times 0.15_{MON\ 0818/form})} \cong 44.5434\ mg\ form./L$$

Equation 17

Adjusting for the proportion of glyphosate a.e. in the formulation, the above estimate corresponds to about 13.7 mg a.e./L [44.5434 mg formulation/L x $0.308_{ae/form} \approx 13.7194$ mg a.e./L], which is identical to the estimate from Equation 15. As noted above, the LC_{50} for Roundup is reported as 7.6 mg a.e./L for Roundup, which corresponds to about 24.7 mg formulation/L [7.6 mg a.e./L ÷ $0.308_{ae/form} \approx 24.6753$ mg formulation/L]. Thus, the ratio of the predicted to the observed LC_{50}s, in units of mg formulation/L, is about 1.8 [44.5 mg form/L ÷ 24.7 mg form/L ≈ 1.8016], identical to the corresponding ratio based on the analyses from Equation 15.

A summary of the 96-hour LC_{50} values and the analyses of joint action based on these data from Folmar et al. (1979) is given in Table 24. While Equation 13 uses the formulation toxicity values reported in Folmar et al. (1979), Equation 16 is used for the calculations in Table 24 because Equation 16 is more intuitive and more closely follows the analyses used in the study by Wan et al. (1989). As discussed above, both Equation 13 and Equation 16,are mathematically equivalent. The calculations summarized in Table 24 are detailed in Attachment 3, Worksheet "Folmar et al. 1979 Fish". This worksheet also provides the calculation of the mean and 95% confidence interval for the interaction coefficients – i.e., 1.12 (0.32 to 1.91) – which indicate no significant deviation from the assumption of dose addition.

4.1.3.1.3. Acute Sublethal Toxicity

As noted in Appendix 6, NOEC concentrations are reported in some of the acute LC_{50} bioassays. These NOEC values may be regarded as information on *"sublethal"* exposures in that no lethality was observed. In terms of this risk assessment, however, the term *sublethal* is not intended to apply to endpoints that may be precursor effects leading to mortality such as various forms of necrosis or other degenerative changes in organs associated with the lethality. In addition, the term *sublethal* is not intended to apply to levels of exposure in which no mortality was observed. Such effects are referred as *nonlethal* endpoints. Rather, *sublethal* is used to designate endpoints which may lead to harmful but nonlethal effects which impair the ability of wildlife species to maintain normal populations. In other words, the term *sublethal* is intended to designate adverse effects on reproduction, behavior, or the ability to respond to other stressors.

Although several studies focus on acute effects other than mortality in fish, many of these studies involve relatively extreme exposure levels and endpoints that could be associated with lethality. The study by Szarek et al. (2000) involves very brief exposures of carp to Roundup concentrations that are far greater than the LC_{50} values—i.e., 1-hour exposures to 205 mg a.e./L and 30-minute exposures to 410 mg a.e./L. All fish died during these exposures. Changes were observed in the mitochondria of carp hepatocytes. The observed effects may be due to the uncoupling of oxidative phosphorylation (Section 3.1.2). Conversely, given that all fish died during exposure, these effects may represent normal postmortem pathology, but are not suggestive of sublethal effects on population dynamics.

Exposure to Roundup formulations may result in a broad spectrum of sublethal effects generally characterized as a stress response. Janz et al. (1991) report that short-term exposures at 5-85% of the 96-hour LC_{50} values of several glyphosate formulations did not induce indicators of physiological stress assayed as changes in biochemical parameters in blood. More recently, Cericato et al. (2008, 2009) measured changes in serum cortisol levels as an indication of stress response in catfish exposed to the LC_{50} concentration of an unspecified Roundup formulation and in a species of South American catfish exposed to one-sixth and one-third of nominal LC_{50} concentrations of the same formulation. In the earlier study, Cericato et al. (2008) noted a concentration-related decrease in cortisol levels, which was not evident in the later study (Cericato et al. 2009). Langiano and Martinez (2008) also report no significant changes in cortisol levels in fish after sublethal exposures to a Roundup formulation. Over a similar range of concentrations, Glusczak et al. (2006, 2007) observed decreases in AChE activity in the brain but not the muscle of a South American catfish and ray fin. The investigators also observed changes in hematological parameters, and suggest that the decrease in brain AChE might be attributed to the POEA surfactant rather than glyphosate and that the spectrum of responses may be viewed as indicators of a stress response. In goldfish, sublethal exposure levels resulted in an array of changes in various enzyme activities associated with oxidative stress (Lushchak et al. 2009).

Roundup formulations will cause damage to gill tissue. As with gastrointestinal tract damage in cases of suicidal ingestion, this portal of entry effect is probably associated with the corrosive effects of the surfactant used in Roundup formulations. The study by Neskovic et al. (1996b) notes histological changes in the gills, kidneys, and liver of carp, *Cyprinus carpio*. In this study, carp were exposed to *technical grade* glyphosate with a purity of only 62%, which is much lower than that used in current commercial formulations. Nonetheless, the study reports a 96-hour LC_{50} of 620 (607-638) mg/L, which is higher than values for more highly purified forms of glyphosate in trout and bluegill sunfish. The sublethal studies were conducted over 14-days of exposure to concentrations of 2.5, 5, or 10 mg a.e./L. At 10 mg/L, abnormal histopathological changes were noted in the gills and liver. At 5 mg/L, abnormal histopathological changes were noted only in the gills. These changes were accompanied by increased alkaline phosphatase activity. Histopathological changes to gill tissue were observed also in tilapia over 4-day exposures to Roundup concentrations equivalent to the LC_{50} (Jiraungkoorskul et al. 2002).

Various studies address the ability of fish to sense glyphosate in water or the tendency of fish to avoid glyphosate. All of these studies were conducted using Roundup formulations. Morgan et al. (1991) indicate that trout do not exhibit avoidance responses to glyphosate formulations at concentrations less than the 96-hour LC_{50}. Behavioral changes, including, changes in coughing and ventilation rates, changes in swimming, loss of equilibrium, and changes in coloration were observed at concentrations as low as 25% of the LC_{50} value over exposure periods of up to 96 hours. Hildebrand et al. (1982) also note that trout will avoid Roundup formulations only at relatively high concentrations of about 12 mg a.e./L. More recently, Tierney et al. (2006, 2007) demonstrated that trout can sense Roundup formulations in water at concentrations as low as 0.076 mg a.e./L, but will not avoid Roundup formulations until concentrations approach toxic levels—i.e., 7.6 mg a.e./L.

Two acute toxicity studies suggest that exposure to glyphosate formulations may impact immune function in fish (Terech-Majewska et al. 2003, 2004). These studies involve very short exposures (10 minutes) to high concentrations of Roundup (100 mg/L or about 30 mg a.e./L). While this study was conducted in Poland, the papers indicate that the 41% Roundup formulation was obtained from Monsanto, USA. The 10-minute exposures were associated with a decrease in phagocytic activity and lymphocyte induction in response to antigens (concanavalin and lipopolysaccharides) which was observed for up to 3 weeks after exposure.

Immune suppression associated with longer-term exposure to a glyphosate formulation is reported in the study by El-Gendy et al. (1998), which involved 96-hour exposures of Bolti fish (*Tilapia nilotica*) to a glyphosate formulation characterized as 48% SC (soluble concentrate) from Monsanto USA. Similar to the studies by Terech-Majewska et al. (2003, 2004), tests for immune function were conducted from 1hour to 4 weeks after exposure. The levels of exposure, which are not well-characterized, are described only as *"1/1000 of the field recommended concentration."* As detailed in Worksheet A01 of the EXCEL workbooks for terrestrial applications, field concentrations of glyphosate considered in the current Forest Service risk assessment range from approximately 5 to 24 mg a.e./L. While these concentrations cannot be applied to the El-Gendy et al. (1998) study, it appears that El-Gendy et al. (1998) may have used concentrations in the low µg/L range. Also, as in the Terech-Majewska et al. (2003, 2004) studies, El-Gendy et al. (1998) assayed immune function as proliferative responses to concanavalin and lipopolysaccharides as well as phytohemagglutinin. Decreases in cell mediated immune response included decreases in splenocyte proliferation in response to all three mitogens which progressed over the 4-week post-exposure assay period. In additional to the effects on cell mediated immune function, a decrease in humoral immune function was noted by a decrease in antibody titers after injection with sheep red blood cells. As with the cell mediated responses, effects on humoral immune function were noted from 1 hour to 3 weeks after exposure.

There are several concerns with the study by El-Gendy et al. (1998) in addition to a lack of clarity in the test concentration. First, it is stated that the assay for proliferative response of splenocytes was performed on blood samples taken at 1 and 24 hours and 2 and 4 weeks from the time of treatment. It is assumed that for each of these treatment dates, a new set of cultures would be set up. Therefore one would expect to have stimulation index (SI) values for the control for each of the mitogens tested at each time point, which is not the case, since SI values for all three mitogens are presented only once. Furthermore, it is not clear for which time point the stated SI values are associated (see Table 1 in El-Gendy et al. 1998). Second, the authors report data for the anti-sheep red blood cell titres (Table 3 in El-Gendy et al. 1998) at 1 and 24 hours and 2 and 4 weeks, and no data are presented for optimizing the number of sheep red blood cells injected. The schedule of immunization (one injection vs multiple injections) with sheep red blood cell is not stated by the authors. It is rather odd that statistically significant depressed anti-sheep red blood cell titres are noted within 1 hour following treatment. Furthermore, no data are presented on the pre-immunization level of anti-sheep red blood cell in the control and treated fish. Also only one control value is presented, and the time point to which this value applies is not specified. Finally, no control values are presented for each of the time points to which the treated groups should be compared.

The plaque forming cell assay is carried out *in vitro* using several treatment levels in µM quantities. Data from this assay are questionable for the following reasons: It is not clear whether the assay was performed in groups of fish separate from those immunized for anti-sheep red blood cell *in vivo*; there is evidence from Table 2 in El-Gendy et al. 1998 that the concentrations used in this assay are cytotoxic to spleen cells. Thus, the issue involving the direct toxicity of the chemicals in question on cells of the immune system is a very important one. Ideally there should be very little toxicity when one deals with immunological assays. In addition, the data on protein levels and serum fractions are inconclusive. Finally, and most importantly, the authors do not mention any infections of the fish and have not challenged the fish with any infectious agent to test for a potential decrease in resistance to infection due to effects on the immune system. In terms of potential ecological effects, the failure to test for susceptibility to infections greatly reduces the utility of this study. Thus, it cannot be concluded from the data presented in this study that the effects reported on the immune system represent a direct toxic effect on the immune parameters examined. Given the reported cytotoxicity, it is plausible that the reported immune effects are the result of general cytotoxicity rather than due to specific effects on immune function. The cytotoxic effects of glyphosate formulations are discussed in some detail in Section 3.1.10.1.1. In the absence of information on the precise levels of exposure, however, comparisons of the results reported by El-Gendy et al. (1998) to the many *in vitro* studies on glyphosate formulations cannot be made.

4.1.3.1.4. Longer-term Toxicity

Only one full life-cycle chronic toxicity study is available on any form of glyphosate. This is a standard life-cycle study in fathead minnows. As summarized in U.S. EPA/OPP (1993c, 2008a), no effect on mortality or reproduction was observed at a concentration of 25.7 mg/L using 87.3% pure technical grade glyphosate. As detailed in Section 4.1.3.1.2, the differences in the acute toxicity of technical grade glyphosate, glyphosate formulations, and glyphosate-surfactant mixtures are substantial, and the merit of the chronic toxicity study on technical grade glyphosate is questionable. Nonetheless, as discussed further in Section 4.2.5, the surfactants used with glyphosate are less persistent than glyphosate itself, so it is not likely that longer-term exposures following a field application of glyphosate with a surfactant will entail concurrent exposures for fish and other aquatic species to glyphosate-surfactant mixtures typical of those used in acute toxicity studies.

The four longer-term studies involving fish exposed to glyphosate formulations for periods of 2-3 months (Gabriel and George 2005; Jiraungkoorskul et al. 2003a; Li and Kole 2004; Morgan and Kiceniuk 1992) are summarized in Appendix 6, Table 7. While similar in duration, these studies involve very different types of exposure.

The Li and Kole (2004) study involves static exposure of fish to initial concentrations of 0, 1, 5, or 25 mg a.i./L a 41% (w/w) of a Chinese formulation of glyphosate. While the exposures involved a period of 65 days, no additional glyphosate formulation was added to the exposure tanks. While chronic studies using static exposure are not the most conservative, this type of exposure probably best mimics exposures expected after a field application of a glyphosate formulation. No overt signs of toxicity are noted in the study, and the only sublethal toxicity endpoints assayed were gill ATP-ase activity and liver esterase activity. No effects were noted on gill ATP-ase. Liver esterase activity was inhibited on Day 8 of the study, but the effect was

not substantial or clearly dose related. By the end of the 65-day exposure period, there was no apparent inhibition of liver esterase (Li and Kole 2004, Table II).

The studies by Gabriel and George (2005) and Jiraungkoorskul et al. (2003a) involved static renewal. Gabriel and George (2005) exposed a species of African catfish to an unspecified Roundup formulation at concentrations equivalent to 1.2, 1.6, 2.3, or 2.9 mg a.e./L for 70 days. Concentrations were roughly maintained at the nominal exposure levels by renewing a quarter of the test solution daily and half of the test solution every other day. The only endpoints assayed in detail involved changes in plasma enzyme levels indicative of liver damage (i.e., enzymes released from the liver into the plasma because of damage to or death of liver cells). Significant increases in levels of liver enzymes in plasma were noted at all concentrations. This observation is consistent with the lethal potency of Roundup formulations. As discussed in Section 4.1.3.1.2.2 and summarized in Table 22, Roundup formulations are lethal over the range of acid equivalent concentrations assayed by Gabriel and George (2005). While the study by Gabriel and George (2005) is focused on liver toxicity, it seems likely that mortality and overt signs of toxicity would have been reported. No such observations are made in the publication.

Jiraungkoorskul et al. (2003a) exposed Nile tilapia to a 48% a.e. formulation of Roundup for 3 months using a 72-hour renewal system. Details of the renewal procedure are not given in the publication, except to note that … *the requisite amount of Roundup was added in order to maintain a constant herbicide concentration*. This renewal may have involved assays of glyphosate in the test solutions; however, that is not specified in the publication. The nominal test concentrations used in this study were 5 or 15 mg formulation/L, equivalent to about 2.4 or 7.2 mg a.e./L. As in the study by Gabriel and George (2005), Jiraungkoorskul et al. (2003a) noted biochemical changes indicative of liver injury as well as dose-related pathology in gill, liver, and kidney tissue, consistent with tissue degeneration. There were no overt signs of toxicity or mortality.

The apparent lack of mortality observed in the studies by Gabriel and George (2005) and Jiraungkoorskul et al. (2003a) suggests an adaptive response by the fish to longer-term exposures to the Roundup formulations. This argument is particularly true for the Jiraungkoorskul et al. (2003a) study because the nominal concentration of 7.2 mg a.e./L is near the upper bound of reported 96-hour LC_{50} values in fish for Roundup formulations (Table 22).

The longer-term study by Morgan and Kiceniuk (1992) involved a flow-through system. Flow-through exposures involve the use of specialized pumps and diluters to maintain an at least relatively constant exposure. In the Morgan and Kiceniuk (1992) study, this type of apparatus was used to expose rainbow trout to Vision, a 356 g a.e./L formulation equivalent to Roundup, for 2 months at concentrations equivalent to 0, 4.25, 8 and 45.75 µg a.e./L. Clearly, these concentrations in units of µg/L are substantially below the LC_{50} for any Roundup formulations. No mortality or signs of overt toxicity were noted over the 2-month exposure period. The investigators assayed for but did not find any evidence of pathology or changes in growth. Nevertheless, a behavioral change, specifically a decrease in frequency of wigwag behavior, which is a form of aggressive behavior in trout, was observed at both test concentrations. The magnitude of the effect, however, was not concentration related, and the authors of the study indicated that the biological significance of the effect is unclear. This study is reviewed in U.S.

EPA/OPP (2008a), and the concentration of 45.75 µg a.e./L is classified as a LOEC, while the concentration of 4.25 µg/L is classified as a NOEC. Nonetheless, the lack of a clear concentration-response relationship as well as the questionable biological significance of the behavioral change limits the usefulness of the NOEC/LOEC determinations in the hazard identification.

4.1.3.1.5. Field Studies

Several field studies indicate that the application of glyphosate to control aquatic weeds is beneficial to fish populations (Appendix 6, Table 8). Caffrey (1996) evaluated the efficacy of glyphosate in the control of emergent weeds along the river Boyne in Ireland. Glyphosate was applied as a "5L/ha" formulated product that is not otherwise specified. In other words, the information in this publication is not sufficient to calculate exposures either as lb/acre or concentration of glyphosate in water. While no rigorous studies of fish populations were conducted, anecdotal accounts from local anglers indicate that brown trout and salmon populations were enhanced and that the fish were observed to spawn in newly cleared areas. Similarly, Olaleye and Akinyemiju (1996) report a beneficial effect on fish populations in Nigeria when Roundup (360 g/L) was used for aquatic weed control and Kruger et al. (1996) report no adverse effects when Roundup (360 g/L) was used for aquatic weed control in commercial carp production facilities. In an abstract, D'Silva et al. (1997) report that glyphosate was the least toxic herbicide, compared with 2,4-D, diquat, fluridone, endothall, in terms of sub-lethal effects in largemouth bass. This publication, however, provides little detail and a full publication was not identified in the glyphosate literature.

Folmar et al. (1979) conducted a field simulation study in which rainbow trout were subject to short-term (12-hour) exposures to either glyphosate IPA or Roundup at concentrations of 0.02, 0.2, or 2.0 mg/L. For the IPA salt, this corresponds to about 0.015, 0.15, or 1.5 mg a.e./L. For the Roundup formulation, the concentrations correspond to about 0.006, 0.06, or 0.6 mg a.e./L. After exposure, the trout were held for 30 days in uncontaminated water. No adverse effects were noted, based on the number of eggs per female and the gonadal weight in males.

4.1.3.2. Amphibians

4.1.3.2.1. Overview

The available information on the toxicity of glyphosate and glyphosate formulations to amphibians is similar to the information available on fish. Numerous studies, most of which are reviewed in U.S. EPA/OPP (20008a), address the acute lethal potency of glyphosate and glyphosate formulations to amphibians. Also, as with fish, most acute LC_{50} studies in amphibians are conducted over a 96-hour exposure period, but intermediate LC_{50} values are typically reported at 24, 48, and 72 hours. Similar to the approach taken with fish, the discussions of acute lethality focus on 96-hour LC_{50} values. Again, this simplification has no substantial impact on the hazard identification. For example, in the extensive series of bioassays conducted by Howe et al. (2004) on glyphosate, glyphosate formulations, and a POEA surfactant, the ratio of the definitive 24- to 96-hour LC_{50} values ranged from 1 to 1.3—i.e., over 24- to 96-hour periods, the relationship between exposure duration and response was insubstantial.

The skin of amphibians is highly permeable to glyphosate, at least relative to the skin of mammals (Quaranta et al. 2009). None of the available studies on glyphosate addresses its permeability in amphibian skin, relative to fish. Based on the acute toxicity data, however, there is no indication that amphibians are substantially more sensitive than fish to glyphosate, glyphosate formulations, or the POEA surfactant used in Roundup. At doses near the lower bound of the acute LC_{50} values for Roundup formulations in amphibians (i.e., 0.6-1.8 mg a.e./L), changes in thyroid function as well as in increase in intersex gonads were observed in larvae of *Rana pipiens* (Howe et al. 2004). Several outdoor microcosm studies have been conducted on glyphosate formulations at concentrations which approach or exceed the reported LC_{50} values for glyphosate formulations, and, as would be expected, document adverse effects (Relyea 2005b; Relyea 2005c; Relyea et al. 2005). NOECs from *in situ* studies using caged amphibians in field applications of Roundup or similar formulations note NOEC values of about 0.33 mg a.e./L (Thompson et al. 2004) to greater than1 mg a.e./L (Wojtaszek et al. 2004).

4.1.3.2.2. Acute Lethality

4.1.3.2.2.1. Glyphosate Acid and Salts

LC_{50} values for glyphosate acid and the IPA salt of glyphosate are summarized in Appendix 7, Table 1. An overview of these data along with comparable data on glyphosate formulations is presented in Table 25. Definitive LC_{50} values for glyphosate acid range from 75.2 to 121 mg a.e./L. This range is quite similar to the reported LC_{50} values for glyphosate acid in fish (Appendix 6, Table 1) which range from about 43 to about 100 mg a.e./L at neutral pH.

The IPA salt of glyphosate is much less toxic than glyphosate acid. As summarized in Table 25, all of the LC_{50} values reported for glyphosate IPA are non-definitive and range from >17 to >466 mg a.e./L.

Although Rodeo is a glyphosate formulation, it is essentially a solution of the IPA salt and is considered in this subsection. The only reported LC_{50} for Rodeo is 7297 mg a.e./L in *Xenopus laevis* embryos (Perkins et al. 2000). As in fish, amphibian embryos may be less sensitive than larvae to glyphosate exposure. Nonetheless, the lesser toxicity of the IPA salt relative to glyphosate acid probably reflects buffering by the IPA cation. As discussed in the following subsection, the study by Edginton et al. (2004a) indicates that frog embryos are less sensitive than frog larvae to glyphosate/surfactant exposures.

As discussed in Section 4.1.3.1.2.1, the study by Wan et al. (1989) in salmonids indicates decreasing pH (i.e., increasing acidity) leads to an increase in toxicity in young fish. Based on the study by Edginton et al. (2004b), the opposite pattern is seen in amphibian larvae. As summarized in Appendix 7, Table 2, Edginton et al. (2004b) conducted two sets of bioassays in African clawed frog (*Xenopus laevis*) embryos (Gosner Stage 8 to 10) at pH 6.5 and 8.0. The two sets of bioassays used somewhat different experimental designs (i.e., a standard 3x3 factorial and central composite rotatable designs) to specifically assess the impact of pH on the toxicity of Rodeo, Roundup, and the MON 0818 surfactant. For all three agents, toxicity increased with increasing pH. Based on 96-hour LC_{50}s, Rodeo was more toxic by a factor of about 7 to 11 at pH 8 relative to pH 6.5. In contrast, the study by Wan et al. (1989) noted a 6 to 20 fold decrease in toxicity in salmonids at pH 8.2 relative to pH 6.3. While these differences in the impact of pH on the toxicity of glyphosate might reflect the differences in life-stage, the effect of pH on the

toxicity of Roundup noted by Edginton et al. (2004b) in frog larvae is the same as that noted in fish—i.e., increasing pH (i.e., decreasing acidity) will increase toxicity (Section 4.1.3.2.2.2).

Only one study was identified on mixtures of glyphosate IPA and surfactants that may be added to glyphosate formulations such as Rodeo. U.S. EPA/OPP (2008a) summarizes a series of bioassays in tadpoles (*Ranidella signifera*) in which the Geronol CF/AR surfactant was used at concentrations of 10-45%. All LC_{50} values were indefinite and are reported as ranging from >100 to >450 mg a.e./L. U.S. EPA/OPP (2008a) indicates that these greater than values were the highest concentrations tested and that these concentrations were NOAELs. As summarized in Table 22, the definitive LC_{50} for this formulation in trout is 450 mg a.e./L. Based on this comparison, amphibians appear to be less sensitive than trout to glyphosate IPA with the Geronol CF/AR surfactant. By analogy to fish, it seems fair to speculate that more toxic surfactants will enhance the toxicity of glyphosate IPA, Rodeo, and similar formulations to amphibians.

4.1.3.2.2.2. Glyphosate Formulations

Numerous bioassays address the toxicity of glyphosate formulations to amphibians, as summarized in Table 25 and detailed further in Appendix 7, Table 2. As observed in fish bioassays (Table 22), Roundup Biactive, an Australian formulation, is relatively nontoxic to amphibians, with reported LC_{50} values ranging from >17.9 to >494 mg a.e./L (Howe et al. 2004; Mann and Bidwell 1999). In fish, the only reported definitive LC_{50} for Roundup Biactive is 800 mg a.e./L in rainbow trout (U.S. EPA/OPP 2008a, MRID 44738201). Because of the non-definitive LC_{50} values in amphibians, the relative sensitivity of amphibians to Roundup Biactive cannot be determined.

Based on the study by Howe et al. (2004), Glyfos BIO also appears to be less toxic than typical Roundup formulations to amphibians. The reported LC_{50} for Glyfos BIO is >17.9 mg a.e./L. Glyfos BIO is a formulation available from Cheminova. Howe et al. (2004) indicate that this formulation contains an unknown surfactant at a concentration of 10-20%. In its summary of the study, U.S. EPA/OPP (2008a, Table 4.11, p. 91) indicates that Glyfos BIO (referred to as Glyphos BIO in the EPA report) contains 3-7% POEA surfactant. While many other glyphosate formulations contain 15% POEA (Table 2), it is not clear that the lesser toxicity of Glyfos BIO is due to a reduced surfactant loading, to the use of a less toxic POEA surfactant, or a combination of these two factors.

Howe et al. (2004) report an LC_{50} of 8.9 mg a.e./L another Cheminova formulation, Glyfos AU, that also contains 3-7% of a POEA surfactant. As discussed further below, this LC_{50} is comparable to that of the upper bounds of reported LC_{50} values for more toxic Roundup formulations. Lajmaovich et al. (2003) also report an LC_{50} value for a Glyphos formulation (not otherwise identified) that contains a POEA surfactant at 15%. The LC_{50} value for this formulation is 0.93 mg a.e./L, which is in the range of the lower bound LC_{50} values for Roundup formulations.

The other formulation toxicity data for amphibians summarized in Table 25 involve much more toxic formulations including various Roundup, Vision, and Glyphos formulations. Roundup and Vision are generally similar formulations which contain POEA surfactants. Glyphos with a

Cosmo-Flux surfactant is a South American formulation. It is not clear that Gly***ph***os is equivalent to the Gly***f***os formulation discussed above and provided by Cheminova. The Glyphos formulation was assayed by Bernal et al. (2009a) who indicate that Glyphos is a formulation sold in Columbia. Bernal et al. (2009a) do not provide the name of the manufacturer of Glyphos but indicate that Glyphos contains a POEA surfactant.

The use of Cosmo-Flux in the study by Bernal et al. (2009a) is important in that applications of Glyphos with the Cosmo-Flux surfactant are used in studies of concern in the human health risk assessment (Sections 3.1.10.1.2). The studies on amphibians reported by Bernal et al. (2009a) constitute the only direct comparison between the toxicity of Glyphos with Cosmo-Flux and Roundup formulations which may be used in Forest Service programs.

The lowest reported LC_{50} for Glyphos with Cosmo-Flux is 1.2 mg a.e./L in *Dendrosophus microcephalus*. As summarized in Table 25, this LC_{50} is not substantially different from the lowest reported LC_{50} values for other toxic formulations—i.e., 0.8 to 2.9 mg a.e./L. The highest LC_{50} for Glyphos with Cosmo-Flux is 2.7 mg a.e./L reported in Bernal et al. (2009a), which is close to the highest reported LC_{50} for the Roundup Original Max – i.e., 3.2 mg a.e./L – from the study by Relyea and Jones (2009).

The upper bound for the other Roundup and Vision formulations summarized in Table 25 range from >8.0 to 51.8 mg a.e./L. In the absence of matched bioassays—i.e., bioassays on the other formulations by the same investigators using the same species—it cannot be determined whether the higher LC_{50} values reported in other studies for the Roundup and Vision formulations (i.e., Howe et al. 2004; Mann and Bidwell 1999; Wojtaszek et al. 2004; Edginton et al. 2004a) reflect differences in species sensitivity, experimental conditions, or simply random variability. Nonetheless, based on available information, the toxicity of Glyphos with Cosmo-Flux and Roundup Original Max to amphibians appears to be virtually the same.

The impact of pH on the toxicity of glyphosate formulations is not as well characterized in amphibians as in fish. Nonetheless, the studies by Edginton et al. (2004a,b) over a pH range of 6 to 8 indicates the same general trend observed in fish bioassays—i.e., as the pH increases, the toxicity of glyphosate formulations containing surfactants also increases. As discussed in Section 4.1.3.1.2.2, this pattern would be expected presuming that the POEA surfactants are somewhat cationic in aqueous solutions.

The study by Edginton et al. (2004a) also explicitly compares the sensitivity of amphibians at different stages of development. In all four species of frogs tested by Edginton et al. (2004), embryos (i.e., the stage prior to free-swimming larvae) were less sensitive than larvae (Gosner stage 25). Note that Gosner stages are a standard system of classification of frog development. Details of this system may be found at several sites on the Internet (e.g., http://froglet.us/Development/gosner_stages.html) The differences in sensitivity noted by Edginton et al. (2004a), however, varied among species ranging from factors of about 2 to 3 in *Bufo americanus* to factors of about 7 in *Xenopus laevis* and *Rana pipiens*. As discussed by Edginton et al. (2004a), the differences in sensitivity of embryos and larvae are probably related to the absence of fully developed gills in embryos. As with fish, damage to gills may be a sensitive indicator of damage in exposures of amphibian larvae to glyphosate and surfactants. In

addition, functioning gills could facilitate the uptake of glyphosate and/or surfactants, relative to the uptake in embryos which probably involves passive diffusion.

Differences in the sensitivity of amphibians and fish to glyphosate formulations appear to be negligible. As summarized in Table 22, the 96-hour LC_{50} values for the more toxic formulations of Roundup and other similar formulations in fish range from about 0.96 mg a.e./L (Folmar et al. 1979) to 11.26 mg a.e./L (Bidinotto 2005a). As indicated in Table 25, the corresponding 96-hour LC_{50} values in amphibians range from about 0.8 mg a.e./L (Relyea and Jones 2009) to 51.8 mg a.e./L (Mann and Bidwell 1999).

4.1.3.2.2.3. POEA Surfactant

Compared with the data on fish (4.1.3.1.2.3), relatively little information is available on the acute toxicity of surfactants to amphibians (Appendix 7, Table 3). Howe et al. (2004) assayed the POEA surfactant used in the original Roundup formulation, MON 0818, and report a 96-hour LC_{50} of 1.1 mg surfactant/L in larvae of the Green Frog, *Rana clamitans*. Perkins et al. (2000) assayed the POEA surfactant used in Roundup to determine its toxicity to embryos of African clawed frog, *Xenopus laevis*. As with the glyphosate formulations, embryos appear to be somewhat less sensitive than larvae. Perkins et al. (2000) report a 96-hour LC_{50} of 6.8 mg/L, the similar to the somewhat lower LC50 of 5 mg/L reported by Perkins (1997). Edginton et al. (2004b) also assayed African clawed frog (Gosner stage 8 to 10) and report somewhat lower LC_{50} values for MON 0818, 3.9 mg/L at pH 6.5 and 1.5 mg/L at pH 8. As with fish (Section 4.1.3.1.2.3), the toxicity of MON 0818 appear to increase with increasing pH (Edginton et al. 2004b).

While these LC_{50} values do not provide a definitive basis for comparing sensitivities in fish and amphibians, the reported range of LC_{50} values for POEA in amphibians is quite similar to that in fish—i.e., from about 1 to 3 mg/L, as discussed in Section 4.1.3.1.2.3.

4.1.3.2.2.4. Joint Action of Glyphosate and the MON 0818 Surfactant

Perkins et al. (2000, Table 1) did conduct assays using African clawed frog larvae (*Xenopus laevis*) on Rodeo, Roundup, and MON 0818, the POEA surfactant used in Roundup (Appendix 7). The 96-hour LC_{50} values as reported by Perkins et al. (2000, Table 1, p. 942) are:

Rodeo:	2796.8	mg a.e./L
MON 0818 surfactant :	6.8	mg MON 0818/L
Roundup:	9.3	mg a.e./L.

As discussed in Section 4.1.3.1.2.4 with respect to the study by Folmar et al. (1979), the assessment of joint action can be made using the LC_{50} value for Roundup reported in units of mg a.e./L but the calculations are somewhat clearer if the LC_{50} for Roundup is converted to units of mg formulation/L. Perkins et al. (2000, p. 940) identify the Round formulation as containing …*"a guarantee of 356 g glyphosate acid equivalent (AE) per liter as the ipa salt*. As summarized in Table 4 of the current risk assessment, formulations that contain the IPA salt of glyphosate at nominal concentrations of 365 g a.e./L consist of glyphosate IPA at a nominal proportion of 0.41 w/w. Using the conversion factor of 0.74 a.e./a.e for the IPA salt of glyphosate (Table 1), the proportion (w/w) of glyphosate in the formulation is taken as 0.3034

[$0.41_{ai/form}$ x $0.74 ae/ai = 0.3034_{ae/form}$]. Thus, the LC_{50} for Roundup expressed in terms of the formulation and rounded to one significant place after the decimal is taken as 30.7 mg formulation/L [9.3 mg a.e./L ÷ 0.3034 a.e./formulation ≈ 30.6522 mg formulation/L].

Rodeo is an aqueous solution of glyphosate IPA. Consequently, the LC_{50} of 2796.8 mg a.e./L is used to define the potency of MON 0818 relative to glyphosate a.e., rounded to one significant place following the decimal, as 1073.0:

$$\rho_{ae/MON\ 0818} = \frac{7296.8\ \text{mg a. e./l}}{6.8\ mg\ MON\ 0818/L} \cong\ 1073.058 \frac{a.e.}{MON\ 0818}$$

Equation 18

The predicted LC_{50} of Roundup under the assumption of dose addition is estimated at approximately 45.3 mg formulation/L:

$$\zeta_{Roundup} = \frac{7296.8\ \text{mg a. e./L}}{0.3034_{ae/form} + (1073_{ae/MON\ 0818}\ \times 0.15_{MON\ 0818/form})} = 45.251\ mg\ form./L$$

Equation 19

As noted above, the observed LC_{50} is about 30.7 mg formulation/L and the interaction ratio – i.e., the predicted LC_{50} divided by the observed LC_{50} – is about 1.48 [45.3 mg/L ÷ 30.7 mg formulation/L ≈ 1.4756], indicating a somewhat greater than additive joint action. This ratio, however, is in the range of interaction ratios seen in fish (Table 23 and Table 24).

4.1.3.2.3. Acute Sublethal Toxicity

Most studies that assay the effects of glyphosate and glyphosate formulations focus on survival as the endpoint of primary concern, although many studies report other incidental findings. As specifically noted by Edginton et al. (2004), mortality appears to be a more sensitive endpoint, relative to growth retardation in frog embryos. In studies on green frog tadpoles, Relyea (2004) notes that growth is sometimes a more sensitive endpoint than mortality; however, the differences between concentrations causing adverse effects on growth (≈1 ppm) are not substantially different than those causing mortality (≈2 ppm)—e.g., see Relyea 2004, Figure 4. Other studies by Relyea (2005a) indicate that predatory stress can enhance the toxicity of Roundup formulations. The differences, however, are most pronounced at lethal concentrations (≈1 mg/L) and much less pronounced at sublethal concentrations (≈0.1 ppm).

As discussed in Section 3.1.10.1.1, a number of *in vitro* studies indicate that some glyphosate formulations can cause chromosomal damage. Lowcock et al. (1997) note increased incidences of DNA damage in frogs in agricultural areas, compared with frogs in non-agricultural areas. Nonetheless, this study does not provide any data specifically linking glyphosate or glyphosate formulations to this effect.

The observation of hind limb deformities in free-living amphibians has substantially increased concern for the effects of xenobiotics on populations of amphibians (e.g., Quellet et al. 1997). Glyphosate IPA, Roundup, and the POEA surfactant used in Roundup have been specifically tested for malformations in the frog embryo teratogenesis assay (Perkins et al. 2000). In this assay, frog (*Xenopus laevis*) embryos were exposed to the test solution in petri dishes for 96 hours. No reported hind limb abnormalities were noted. The only abnormalities specified in the

publication include uncoiling of the gut, edema, blistering, abnormal pigmentation, and axial twisting in control embryos. No statistically significant increases in abnormalities were observed in any groups exposed to nonlethal concentrations of glyphosate IPA, Roundup, or the POEA surfactant. The precise number and nature of abnormalities in the groups exposed to lethal concentrations of glyphosate IPA, Roundup, or the POEA surfactant are not specified.

Smith (2001) assayed another formulation of glyphosate, Kleeraway Grass and Weed Killer RTU (Monsanto), which contains glyphosate IPA at 0.75% as well as an ethoxylated tallowamine surfactant. Bioassays were conducted on tadpoles (1 week post-hatching) of the western chorus frog, *Pseudacris triseriata*, and the plains leopard frog (*Rana blairi*). The concentrations used in the bioassays are specified as 0.0001, 0.001, 0.01, or 0.1 dilutions of the formulated product. A 0.75% formulation contains 7.5 g/L. Thus, the concentrations used in this study correspond to 0.75, 7.5, 75, or 750 mg IPA/L (i.e., 0.56, 5.6, 56, or 560 mg a.e./L). The test protocol involved a 24-hour exposure period followed by a 2-week observation period to detect sub-lethal toxicity. In *Pseudacris triseriata,* 100% mortality was observed at all concentrations greater than 0.56 mg a.e./L, and 55% mortality was observed at 0.56 mg a.e./L. During the post-exposure observation period, 4/9 animals died in first 2 days. In an initial experiment with *Rana blairi*, all tadpoles died at all concentrations. In a repeat experiment using older tadpoles (not otherwise specified), all animals survived at 0.56 mg a.e./L. In both of the species, normal growth and development were observed in survivors over the 2-week observation period.

As discussed previously, some data suggest avoidance reactions in terrestrial invertebrates (Section 4.1.2.4) and fish (4.1.3.1.3) exposed to glyphosate. Takahashi (2007) report that frogs may avoid laying eggs in pools contaminated with Roundup concentrations of 2.4 mg a.e./L. As summarized in Table 25, this concentration is within the range of 96-hour LC_{50} values for amphibians. Thus, as with fish, avoidance of glyphosate-surfactant mixtures by amphibians appears to occur at acutely toxic concentrations; however avoidance to sub-toxic concentrations has not been demonstrated.

Fish bioassays suggest that Roundup formulations may have an impact on immune function—i.e., cell mediated and/or humoral immunity (Section 4.1.3.1.3), but there are no studies that address the effect of glyphosate-surfactant exposures on immune function in amphibians. Rohr et al. (2008), however, assessed the impact of 3.7 mg a.e./L technical grade glyphosate on immune function in green frog tadpoles (*Rana clamitans*). No effects on immune function were observed, based on the virulence of larvae of *Echinostoma trivolvis*, the amphibian trematode larvae, or the survival of frog tadpoles.

4.1.3.2.4. Longer-term Toxicity

The longer-term toxicity studies on glyphosate and glyphosate formulations are summarized in Appendix 7, Table 5. The chronic study by Howe et al. (2004) involved 42-day exposures of Gosner stage 25 larvae of *Rana pipiens* to glyphosate IPA, two formulations of glyphosate (Roundup Original and Roundup Transorb), as well as the MON 0818 POEA surfactant used in Roundup. Glyphosate IPA was tested at only one concentration, 1.8 mg a.e./L, and no adverse effects were noted. The two Roundup formulations were assayed at concentrations of 0.6 and 1.8 mg a.e./L. Howe et al. (2004) designate the low and high concentrations of the surfactant as 0.6 and 1.8 mg a.e./L, which are intended to reflect the fact that the concentrations of the

surfactant used in the low and high exposure groups of the surfactant only studies were identical to the concentrations of the surfactant in the bioassays of Roundup Original and Roundup Transorb. Since the formulations used in the study by Howe et al. (2004) contained about 31% (w/w) glyphosate a.e. and about 15% (w/w) MON 0818, the concentration of MON 0818 in the formulations were be about half that of glyphosate a.e. – i.e., about 0.3 mg and 0.9 mg MON 0818/L.

Adverse effects were noted in all groups exposed to the surfactant – i.e., the Roundup Original, Roundup Transorb, and MON 0818 only groups. The effects included an increase in the number of days required for the larvae to reach Gosner stage 25, a decrease in the proportion of tadpoles surviving to Gosner Stage 42, and a decrease in larval length. In addition to changes in growth and survival, the Roundup formulations and the POEA surfactant were associated with an increase in the proportion of tadpoles with intersex gonads.

Based on survival to Gosner stage 42 and the number of days required to reach Gosner stage 42, Roundup Transorb appears to be somewhat more toxic than Roundup Original. As noted by Howe et al. (2004), Monsanto has indicated that Roundup Transorb contains … *surfactant blend containing POEA*. No other details on the composition of the surfactant were found in the literature. Exposures to the MON 0818 POEA surfactant alone also caused the same effects as those caused by Roundup Original and Roundup Transorb.

In the dose-response assessment conducted by U.S. EPA/OPP (2008a, Table 4.13, p. 93), the 0.6 mg a.e/L exposure levels for the two Roundup formulations in the Howe et al. (2004) study are designated at NOECs. The rationale for this designation is not clear. Based on the analysis presented in Howe et al. (2004, Figure 1, p. 1933), both formulations caused a significant decrease in survival and body length at both tested concentrations. Similarly, U.S. EPA/OPP (2008a, Table 4.14, p. 93) also appears to designate the 0.6 mg a.e/L for the POEA surfactant as an NOEC, and the rationale for this designation is unclear and is not consistent with the results presented in Howe et al. (2004).

As discussed further in Section 4.3.3.2.1.2, the study by Howe et al. (2004) is not used directly in the dose-response assessment for longer-term exposures of amphibians to more toxic formulations of glyphosate. This study, however, is important to the risk assessment in that the adverse effects observed by Howe et al. (2004), particularly the development of intersex gonads, are a concern in the risk characterization for amphibians (Section 4.4.3.2.1). In a review of an earlier draft of the current Forest Service risk assessment, Monsanto (Honegger 2010, Appendix 5) offered a detailed critique of the Howe et al. (2004) study noting the following considerations:

- The ammonia levels in the Howe et al. (2004) study may have been excessive.
- The histological evaluation of intersex gonads is questionable.
- The incidence of intersex gonads does not appear to be statistically significant.
- The primary concern with more toxic formulations is the POEA surfactant and the dissipation of POEA in water is sufficiently rapid that concerns for longer-term exposures are unwarranted.

The concern with levels of ammonia in the Howe et al. (2004) study is warranted. Howe et al. (2004, p. 1929) note that … *pH varied from 7.8 to 8.3. Total ammonia concentrations reached a maximum of 2.4 mg/L. All values fell within accepted guidelines*. The ASTM (2007) guidelines indicate that un-ionized ammonia should not exceed 35 µg/L. The chronic bioassay conducted by Howe et al. (2004) was conducted at 20°C. At 20°C and over a pH range of 7.8 to 8.3, the percentages of total ammonia that will exist as un-ionized ammonia range from 2.44% to 7.34% (U.S. EPA/ERL 1979). Thus, the maximum total ammonia of 2.4 mg/L would correspond to un-ionized ammonia concentrations of about 59 to 176 µg/L. These concentrations of un-ionized ammonia exceed the standard of 35 µg/L by factors of about 2 to 5. The statement that *values fell within accepted guidelines* does not appear to be correct. The organisms in the Howe et al. (2004) study appear to have been subjected to excessive levels of ammonia, and these levels of ammonia may have contributed to the high control mortality. Nonetheless, the intersex gonads were observed only in organisms exposed to the more toxic glyphosate formulations or the POEA surfactant. Thus, it does not seem reasonable to discount the observation of intersex gonads based on the elevated levels of ammonia.

Questions concerning the histologic evaluations presented in Howe et al. (2004) are based on a critique obtained by Monsanto from a third party reviewer. The review, however, does not appear to have involved a reevaluation of the slides. As noted in the comments from Monsanto:

> *because of low image resolution and contrast artifacts related to the printing process, published photomicrographic figures may not adequately portray changes viewed under the microscope. Therefore, diagnostic confirmation should be based on a re-evaluation of the histologic sections on glass slides*.
>
> Honegger 2010, p. 66

In the absence of a reevaluation of the slides and in the absence of another study that contradicts Howe et al. (2004), there is not a sufficient basis to discount the results presented in the peer reviewed publication by Howe et al. (2004).

The lack of statistical significance in the development of intersex gonads is partially justified. While Howe et al. (2004) provide detailed statistics for many of their observations, no statistical analysis is provided by Howe et al. (2004) for the development of intersex gonads. Honegger (2010) notes that none of the groups exposed to the more toxic formulations of glyphosate or to the POEA surfactant appear to have evidenced a statistically significant increase in the incidence of intersex gonads relative to the control group. The incidence of intersex gonads can be estimated form Figure 3 in the Howe et al. (2004) publication, and the observation by Honegger (2010) is correct. No intersex gonads were noted in any of 18 organisms in the control group. The highest incidence in any exposed group is 3/12 in the organisms exposed to Roundup Transorb at 0.6 mg a.e./L. Using the standard criterion of p=0.05, the p-value for 0/18 versus 3/12 using the Fisher Exact test is 0.054, suggesting that the incidence can be viewed as only marginally significant – i.e., 0.054 rounds to 0.05. A more conservative application of the Fisher Exact test involves combining the incidences 0/18 (control group) and 0/19 (glyphosate without surfactant). This approach is reasonable because both groups can be viewed as controls with respect to POEA exposure. Combining these two groups for a control response of 0/37, the p-values for the high dose POEA group and high dose Roundup Original group are both 0.0239 –

i.e., the incidences of intersex gonads (3/16 or about 0.142 in both groups) are statistically significant. In the high dose Roundup Transorb group, no intersex gonads were observed. Only five organisms, however, were examined in this group. Assuming that the true response rate in this group was identical to the response rates in the other high dose groups (i.e., ≈0.142) and using the binomial probability distribution, the probability of observing 0/5 responses is about 0.35 [$C_0^5 \times 0.142^0 \times (1 - 0.142)^5 = 0.353$]. Thus, because of the small number of animals sampled in the high dose Roundup Transorb group, the observation of no intersex gonads (0/5) is not unexpected.

The observation by Honegger (2010) that POEA is rapidly degraded in the environment is a reasonable factor to consider in interpreting the chronic bioassay by Howe et al. (2004). There is little doubt that agent of concern in the chronic effects noted in the study by Howe et al. (2004) is the POEA surfactant rather than glyphosate. As discussed in Section 4.2.5, the exposure assessment for aquatic organisms is based on glyphosate rather than POEA. Thus, if the POEA surfactant degrades much more rapidly than glyphosate, then the risk characterization for longer-term exposures to the more toxic formulations of glyphosate could be grossly conservative to the point that the risk characterization is distorted. In raising concern for the rapid degradation of POEA, Honegger (2010) cites the study by Wang et al. (2005) in which nine individual components in MON 0818 dissipated from aquatic microcosms with a half-life of less than one day. This statement is correct and the study by Wang et al. (2005) is well documented and well designed. Other studies, however, suggest that MON 0818 may dissipate more slowly than glyphosate. As noted in Section 1, Geisy et al. (2000) conducted an ecological risk assessment of glyphosate, and these authors had access to unpublished studies from Monsanto. Citing one of these unpublished studies (Banduhn and Frazier 1974), Geisy et al. (2000, p. 54) use aqueous half-lives for POEA ranging from of 21 to 42 days. In contrast, Giesy et al. (2000,) use half-lives for glyphosate in non-flowing water of 7 to 14 days. Thus, based on the Giesy et al. (2000) assessment, POEA may be more rather than less persistent than glyphosate at least under some circumstances. Consequently, the assessment of chronic endpoints in the study by Howe et al. (2004) seems relevant and appropriate. Lastly, it is worth noting that the static renewal schedule used by Howe et al. (2004) involved renewal (not otherwise specified) on only a weekly basis. This type of static renewal does not approach the conditions of a flow-through bioassay (in which concentrations are held reasonably constant) and more closely mimics pulse exposures which can occur as a result of periodic rainfall.

In a study similar to that of Howe et al. (2004), Cauble and Wagner (2005) exposed *Rana cascadae* larvae to Roundup at concentrations of 1 or 2 mg a.e./L for an intended exposure period of 43 days. At the 2 mg a.e./L concentration, no organisms survived to Day 43. At the 1 mg a.e./L exposure level, a substantial decrease in survival was apparent. The substantial mortality is consistent with the acute toxicity data on Roundup formulations for which the estimated 96-hour LC_{50} values range from about 1 to 2 mg a.e./L (Table 25). A somewhat unusual observation from this study is that the development of larvae appeared to be accelerated rather than retarded in the 1 mg a.e./L exposure group. Cauble and Wagner (2005) suggest that the accelerated development may be a sublethal response to stress (i.e., hormesis).

The last study summarized in Appendix 7, Table 5 is the 16-day exposure study by Relyea (2005a). As discussed in the previous subsection, this study focuses on the interaction of

exposures to Roundup and predator stress. As part of this study, however, Relyea (2005a) reports 16-day LC_{50} values for Roundup of 1.32 to 2.52 mg a.e./L for six species of frogs in the absence of predator stress. As indicated in Table 25, these 16-day LC_{50} values are somewhat higher than the 96-hour LC_{50} values reported by Relyea and Jones (2009) for Roundup Original Max, and the lower bound 16-day LC_{50} value of 1.32 mg a.e./L is within the range of lower bound 96-hour LC_{50} values for Roundup formulations reported by investigators (Bernal et al. 2009a; Howe et al. 2004; Mann and Bidwell 1999; Wojtaszek et al. 2004; Edginton et al. 2004a). As with fish (Section 4.1.3.1), there does not appear to be a substantial concentration-duration relationship for glyphosate-surfactant formulations.

4.1.3.2.5. Field/Mesocosm Studies

Field and field simulation (mesocosm) studies regarding the effects glyphosate formulations on amphibians are summarized in Appendix 7, Table 6. The field studies involving terrestrial-phase amphibians are discussed in Section 4.1.2.3. The following discussion focuses on aquatic-phase amphibians.

Relyea and coworkers (Relyea 2005b; Relyea 2005c; Relyea et al. 2005) conducted several mesocosm studies with Roundup formulations at concentrations ranging from 1.3 mg a.e./L (Relyea et al. 2005) to 2.8 mg a.e./L (Relyea 2005c). Consistent with the acute toxicity data on Roundup—i.e., 96-hour LC_{50} values ranging from about 1 to 3 mg a.e./L—decreases in survival and amphibian biomass were noted. The study by Relyea (2005c) is particularly interesting in that decreased survival was noted in mesocosms with and without sand or loam sediment over a 20-day exposure period (Relyea 2005c, Figure 1, p. 1121). Relyea (2005c) does note that loam sediment caused a significant but small increase in the survival of tree frog tadpoles. Detailed data on time to deaths are not reported; however, the study does indicate that survival in all three of the species tested was only 21% at the end of Day 1. It seems possible that the concentration of 2.8 mg a.e./L used in this study caused substantial mortality before any significant sediment binding occurred.

Relyea (2009) exposed larvae of two species of frogs to glyphosate acid at 0.009 mg/L. As might be expected, this very low concentration of glyphosate acid had no effect on mortality or growth.

Two field studies report NOECs for Roundup (Thompson et al. 2004) and Vision (Wojtaszek et al. 2004). Thompson et al. (2004) over-sprayed a wetland area with Roundup Original at application rates of about 1 lb a.e./acre to 1.7 lb a.e./acre and used caged leopard and green frogs to assay its toxicity. In over-sprayed areas, the glyphosate concentrations were 0.33 mg a.e./L, but there were no significant differences in mortality, relative to areas that were not over-sprayed, where the glyphosate concentrations were in the range of 0.03 mg a.e./L. In discussing this study, U.S. EPA/OPP (2008a) notes that the study does not provide sufficient detail to independently assess the statistical significance (or lack of significance) in frog mortalities between the areas that were and were not over-sprayed. Given the acute toxicity data on Roundup formulations to amphibians, it is possible that the concentration of 0.33 mg a.e./L was an NOEC. At third field study by Glaser (1998) reports an increase in post-hatch deformities from eggs of *Rana sylvatica* taken from small ponds after application of Vision at rates of 1.44 or 1.88 kg a.e./ha relative to eggs taken from ponds not treated with the Vision formulation. There was, however, no dose-response relationship within the Vision treatment groups.

Another *in situ* study (Wojtaszek et al. 2004) reports an NOEC for growth, mortality, and avoidance after an application of Vision that resulted in water concentrations of 1.43 mg a.e./L of glyphosate. As summarized in Table 25, this concentration is below the 96-hour LC_{50} values of 2.7- 11.47 mg a.e./L reported by Wojtaszek et al. (2004) for the Vision formulation. Thus, the field observation seems plausible. Nonetheless, given the lower LC_{50} values reported by other investigators, the reported NOEC of 1.43 mg a.e./L might not be applicable to other formulations or other populations of amphibians.

4.1.3.3. Aquatic Invertebrates

4.1.3.3.1. Overview

As is the case for most pesticides, the acute toxicity studies in aquatic invertebrates typically involve 48-hour rather than 96-hour periods of exposure. While 24-hour hour toxicity values are sometimes reported, the discussion of invertebrate toxicity values is limited to 48-hour exposures, unless otherwise specified. Particularly with smaller aquatic invertebrates, acute toxicity values are typically expressed as EC_{50} values (for immobility) rather than LC_{50} values, since most bioassays of small invertebrates do not attempt to verify that immobile invertebrates are actually dead. In practical terms, the distinction between an EC_{50} and LC_{50} is academic. In most aquatic ecosystems, an immobilized invertebrate is functionally deceased.

Most Roundup and similar glyphosate formulations are a great deal more toxic than glyphosate and salts of glyphosate to aquatic invertebrates, as is true for fish and amphibians. The acute EC_{50} values for aquatic invertebrates exposed to glyphosate or glyphosate IPA generally range from about 100 to 650 mg a.e./L; whereas, corresponding toxicity values for most Roundup formulations range from about 1 to 50 mg a.e./L. Studies that can be used to assess the joint action of glyphosate and POEA surfactants suggest a less than additive joint action. The EC_{50} values for some Accord formulations that contain surfactants range from about 20 to 25 mg a.e./L. Because so few of the toxicity studies on glyphosate can be associated with Accord formulations with surfactants, it is not clear whether Accord formulations which contain surfactants are generally less toxic than most Roundup formulations. EC_{50} values range from 50 to >500 mg a.e./L for Rodeo, a number of other non-US formulations, and Roundup formulations mixed with other surfactants.

In both fish and amphibians, no substantial duration-response relationships are apparent. Based on studies conducted by the same investigators on the same species, this is also the case with aquatic invertebrates; however, these studies are limited to glyphosate formulations. The available information on technical grade glyphosate does suggest a relationship between exposure duration and response.

4.1.3.3.2. Acute Lethality

4.1.3.3.2.1. Glyphosate Acid and Salts

Information on the acute lethal potency of glyphosate acid and salts is summarized in Appendix 8, Table 1. Studies are available on two species of daphnids (McAllister and Forbes 1978b; MRID 44320631; Pereira et al. 2009; Tsui and Chu 2003), a copepod (Tsui and Chu 2003); midge larvae (Folmar et al. 1979), and a bivalve (Bringolf et al. 2007).

Daphnia magna is a very common test species, and acute toxicity studies in this species are required for pesticide registration. The EC_{50} values for glyphosate acid in studies submitted to the U.S. EPA/OPP in support of the registration for glyphosate range from 128 mg a.e./L (MRID 44320631) to 647 mg a.e./L (MRID 00108172). The variability in these EC_{50} values is about a factor of 5, which is not uncommon in acute bioassays with daphnids. Pereira et al. (2009) report an acute EC_{50} of >2000 mg a.e./L. Although this remarkably high EC_{50} appears to be inexplicable, the test protocol used by Pereira et al. (2009) appears to be relatively standard.

In acute toxicity studies in copepods and *Ceriodaphnia*, Tsui and Chu (2003) found that glyphosate acid is somewhat more toxic than glyphosate IPA. This pattern is consistent with toxicity studies in fish and amphibians. The study by Folmar et al. (1979) indicates that midges are about equally sensitive to glyphosate acid (LD_{50} = 55 mg/L) as are *Ceriodaphnia* (LD_{50} = 147 mg/L) and copepods (LD_{50} = 35.3 mg/L) in the study by Tsui and Chu (2003).

The study by Bringolf et al. (2007) in the freshwater mussel, *Lampsilis siliquoidea*, is somewhat unusual in that it provides acute LC_{50} values for glyphosate acid, the IPA salt of glyphosate, and isopropanol amine (i.e., IPA). Glyphosate acid was relatively nontoxic with an LC_{50} of >200 mg a.e./L. Both glyphosate IPA and isopropanol amine were much more toxic, with LC_{50} values in the range of about 5-7 mg/L for larvae and juvenile mussels. As discussed further in the following subsection, Bringolf et al. (2007) also report an LC_{50} of >148 mg a.e./L for exposure to Aqua Star. As indicated in Table 2, Aqua Star is a 53.8% solution of the IPA salt of glyphosate. It is not clear, however, whether Aqua Star contains a surfactant. In any event, the greater toxicity of glyphosate IPA, relative to glyphosate acid, is an unusual observation which is not consistent with the toxicity studies in fish and amphibians. An explanation for the low toxicity of Aqua Star, relative to the IPA salt of glyphosate, is not apparent.

4.1.3.3.2.2. Glyphosate Formulations

Information on the toxicity of glyphosate formulations to aquatic invertebrates is summarized in Appendix 8, Table 2. This table includes all studies summarized in the recent ecological risk assessment by U.S. EPA/OPP (2008a) as well as studies from the open literature.

A selective overview of this information is presented in Table 26. Some studies included in Appendix 8 are not clear in terms of how units are reported (Brausch et al. 2006; Holck and Meek 1987; Linden et al. 1979). These studies are not included in Table 26. In addition, Table 26 does not include two extreme LC_{50} values – i.e., the crayfish LC_{50} of 21,633 mg a.e./L for a Roundup formulation reported by Abdelghani et al. (1997). Abdelghani et al. (1997) indicate that the Roundup formulation used in their study was obtained from Monsanto, USA. The U.S. EPA registration number given for the formulation is 524-308-AA. The 524 component of the registration number designates Monsanto as the manufacturer. The registration number, however, does not correspond to any of the formulations identified by the Forest Service (Table 2); moreover, the LC_{50} of 21,633 mg a.e./L is much higher than any other LC_{50} values for Roundup or any other glyphosate formulation.

As noted above, the study Brausch et al. (2006) is included in Table 26. This study could be interpreted as reporting the lowest LC_{50} for a Roundup formulation. Consequently, the rationale for excluding this study warrants a somewhat detailed discussion. Brausch et al. (2006)

conducted a series of bioassays on several pesticide formulations in fairy shrimp (*Thamnocephalus platyurus*). Brausch et al. (2006) indicate that one formulation of Roundup was tested. This formulation is identified as Roundup Super Concentrate in Table 1 of the publication but is not otherwise described. An MSDS for Roundup Super Concentrate from Solaris (https://www2.itap.purdue.edu/msds/docs/8890.pdf) indicates that the formulation contains 41% glyphosate IPA at a concentration. Brausch et al. (2006) suggest the formulation contained a POAE surfactant. As summarized in Appendix 8, Table 2, Brausch et al. (2006) report a 48-hour LC_{50} of 1243.38 µg/L for Roundup but do not specify the units as acid equivalents, active ingredient, or formulation. Another minor complication is that the abstract for the publication (Brausch et al. 2006, p. 309) gives the LC_{50} for Roundup as 1,248 mg/L rather than 1,248 µg/L. Throughout the text of the publication, however, references to concentrations of Roundup are given in units of µg/L and the entry in the abstract appears to be a typographical error. If it were assumed that the LC_{50} is reported in units of formulation and that the formulation contained 41% glyphosate IPA, the LC_{50} of 1,248 µg formulation/L would correspond to about 0.38 mg a.e./L [1.248 mg formulation/L x $0.41_{IPA/form}$ x $0.74_{a.e./IPA}$ = 0.379 mg a.e./L]. While this LC_{50} would be the lowest LC_{50} identified for any Roundup formulation in aquatic invertebrates, this toxicity value is not used directly in the current risk assessment because identity and source of the formulation is not clear and the units for the LC_{50} are cannot be identified as being expressed in acid equivalents, active ingredient, or formulation.

Table 26 is organized in roughly the same manner as Table 22, the corresponding table of formulation toxicity values for fish. As with fish, Roundup formulations are the most extensively assayed of the glyphosate formulations and appear to be the most toxic with LC_{50} values ranging from about 1.5 to 62 mg a.e./L. In addition, these values are similar to the corresponding toxicity values for fish—i.e., 0.96-10 mg a.e./L. Data on the toxicity of specific formulations to aquatic invertebrates are somewhat more abundant than the corresponding data on fish. Nonetheless, the overall pattern of toxicity is similar with Rodeo (i.e., essentially an aqueous solution of the IPA salt of glyphosate) and other equivalent formulations being among the least toxic formulations, with LC_{50} values ranging from about 200 to over 4,000 mg a.e./L. As with the corresponding LC_{50} values in fish, Rodeo is much less toxic to aquatic invertebrates than Roundup formulations and other formulations of glyphosate that contain surfactants.

Some LC_{50} values for certain Roundup formulations are at the lower bound of the range for Roundup formulations which cannot be identified specifically—i.e., the LC_{50} values of 2.7 mg a.e./L for MON 65005 (an older product code for Roundup Pro) and 3.2 mg a.e./L for MON 77360 (Roundup Ultra). The LC_{50} values for two Accord formulations—i.e., GF-1279 and GF-128—range from about 20 to 25 mg a.e./L, near the upper bound for LC_{50} values reported for some Roundup formulations. It is not clear if toxicity values for the Accord formulations indicate that these formulations are somewhat less toxic (e.g., different surfactants) or if the differences are simply due to random variation in the bioassays or other unidentified factors.

Also as with fish, several toxicity studies indicate that Roundup Biactive) and some other non-US formulations (e.g., Ron-Do and Spasor) as well as blends of Roundup with other surfactants are much less toxic than traditional Roundup to aquatic invertebrates. One 62.4% glyphosate IPA formulation without a surfactant has a reported LC_{50} of 401 mg a.e./L (MRID 78663), which

is in the range of LC_{50} values for Rodeo, and the specific formulation may be essentially equivalent to Rodeo.

As noted in Table 2 and discussed in the Program Description (Section 2), the Forest Service may use some formulations of the monoammonium salt of glyphosate, for which relatively little information is available. MON 14420 is a product code for a granular formulation of the monoammonium salt. The LC_{50} for this formulation in *Daphnia magna* is 28.8 mg a.e./L (MRID 45777401). This toxicity value is mid-point in the range for standard Roundup formulations, and this relationship suggests that the formulation contains a surfactant that is as toxic as the POEA surfactant used in some Roundup formulations.

4.1.3.3.2.3. Surfactants

Information on the toxicity of surfactants in glyphosate formulations is summarized in Appendix 8, Table 5. Corresponding information on surfactants that may be added to glyphosate formulations is presented in Appendix 8, Table 6.

Several bioassays address the toxicity of MON 0818, the surfactant used in at least some Roundup formulations. The LC_{50} values for invertebrate exposure to MON 0818 range from 0.5 to 13 mg/L. As discussed in Section 4.1.3.1.2.3, the corresponding range in fish bioassays is 0.65-7.4 mg/L. Despite the similarity in the LC_{50} values, the fish bioassays include studies conducted at various pH values. Such studies have not been conducted in aquatic invertebrates. For example, the lowest LC_{50} for invertebrates, 0.5 mg/L, is for larvae of a freshwater mussel (Bringolf et al. 2007), in which the pH ranged from 8.22 to 8.76. As discussed in Section 4.1.3.1.2.3, the toxicity of MON 0818 to fish increases with increasing pH. The highest pH used in the assays in fish is 8.2. Thus, the LC_{50} of 0.5 mg/L from the Bringolf et al. (2007) study may reflect the higher pH used in this study rather than a greater sensitivity of invertebrates, relative to fish, to the toxicity of POEA.

The upper bound toxicity value of 13 mg a.e./L for midge larvae is from the study by Folmar et al. (1979), which appears to have been conducted at a pH of 7.2 (Folmar et al. 1979, p. 271). A pH of 7.2 was also used in the salmonid bioassays by Wan et al. (1989) in which the LC_{50} values for POEA ranged from 2.4 to 2.8 mg/L (Appendix 6, Table 4). Thus, it appears that midge larvae may be somewhat more tolerant than salmonids under similar conditions of exposure. Nonetheless, the differences in LC_{50} values—i.e., about 2 mg a.e./L, compared to about 13 mg a.e./L—are not substantial and could be due to factors other than differences in species sensitivity.

Brausch et al. (2007) assayed POEA surfactants using oxide to tallow ratios of 5:1 to 15:1. In assays with *Daphnia magna*, the LC_{50} values range from about 0.1 to 0.8 mg/L with decreasing toxicity as the oxide to tallow ratio increased. The upper bound LC_{50} of 0.8 mg/L is only modestly below the reported LC_{50} values of MON 0818 in *Daphnia magna*—i.e., 2.00 mg/L (Servizi et al. 1987) and 2.9 mg/L (Wang et al. 2005).

Brausch and Smith (2007) also conducted similar assays with fairy shrimp (*Thamnocephalus platyurus*). As summarized in resulted in much lower 48-hour LC_{50} values ranging from 0.00201 mg/L to 0.00517 mg/L. These LC_{50} values are much lower than any other reported

LC_{50}s in aquatic invertebrates. As summarized in Appendix 8, Table 5, the lowest LC_{50} reported for a POEA in any other study is the LC_{50} of 0.57 mg/L for the copepod, *Acartai tonsa*, from the study by Tsui and Chu (2003). This LC_{50}, in turn, is only modestly higher than the LC_{50} of 0.5 mg/L for MON 0818 reported by Bringolf et al. (2007) in mussel larvae. Taking 0.75 as the proportion of POEA in MON 0818, the LC_{50} of 0.5 mg MON 0818/L corresponds to an LC_{50} of 0.375 mg POEA/L [0.5 mg MON 0818/L x $0.75_{POEA/MON\ 0818}$]. Note that 0.375 mg POEA/L is lower than the upper bound LC_{50} of 0.00517 mg POEA/L reported by Brausch and Smith (2007) by a factor of over 70 [0.375 mg /L ÷ 0.00517 mg/L ≈ 72.53].

As discussed in Section 4.1.3.3.2.2, Brausch et al. (2006) also conducted bioassays using the same species of fairy shrimp on a formulation of Roundup and report an LC_{50} of 1.248 mg/L. The Brausch et al. (2006) study is not used quantitatively in the current risk assessment because it is not clear whether the LC_{50} is reported as a.e., a.i., or formulation. Making the conservative assumption that the LC_{50} is reported as formulation and that the formulation contained 15% MON 0818 (equivalent to 11.25% POEA), LC_{50} of 1.248 mg formulation/L would correspond to about 0.14 mg POEA/L [1.248 mg formulation/L x $0.1125_{POEA/form}$ = 0.1404 mg POEA/L]. This LC_{50} of 0.14 mg POEA/L is about a factor of 27 higher than the upper bound LC_{50} of 0.00517 mg/L reported in Brausch and Smith (2007) [0.14 mg /L ÷ 0.00517 mg/L ≈ 27.08].

The apparent extreme sensitivity of fairy shrimp to POEA in the study by Brausch and Smith (2007) is the only example seen of a substantial difference in species sensitivity of aquatic animals to glyphosate formulations or surfactants. This extreme sensitivity, however, is not apparent in the study by Brausch et al. (2006) using a Roundup formulation.

Appendix 8, Table 5 also includes a bioassay on Geronol CF/AR surfactant. It is not clear if this surfactant is used in some glyphosate formulations, used as adjuvant to glyphosate formulations, or both. The EC_{50} for this surfactant in *Daphnia magna* is 48 mg/L (MRID 44738201). As demonstrated in fish bioassays, Geronol CF/AR surfactant is much less toxic than the MON 0818 surfactant.

As summarized in Appendix 8, Table 6, the toxicity of surfactants that can be added to glyphosate formulations are addressed in several studies (Abdelghani et al. 1997; Buhl and Faerber 1989; Henry et al. 1994; McLaren/Hart 1995). As with the corresponding data on fish (Appendix 6, Table 5), the data from McLaren/Hart (1995) come from studies provided by Monsanto. Also, as noted in the fish data, some of the surfactants are similar in toxicity to MON 0818 (e.g., Activator 90, Entry II, X-77), and one surfactant, Agri-Dex, is virtually nontoxic. The EC_{50} values for other surfactants range from about 10 to 100 mg/L.

4.1.3.3.2.4. Joint Action of Glyphosate and Surfactants

The joint action of glyphosate and the surfactant used in Roundup can be assessed based on the studies by Folmar et al. (1979) and Tsui and Chu (2003).

As discussed in Section 4.1.3.1.2.4, Folmar et al. (1979), assayed glyphosate acid, MON 0818 (the POEA surfactant used in the original Roundup), and the original Roundup formulation in two species of fish. These investigators also conducted a similar set of bioassays in midge larvae (*Chironomous plumosus*). The reported 48-hour LC_{50} values are 55 mg a.e./L for glyphosate

acid, 18 mg a.e./L for Roundup, and 13 mg/L for the MON 0818 surfactant. Based on these LC_{50} values, the potency of the MON 0818 surfactant relative to glyphosate acid ($\rho_{ae/MON\ 0818}$) is calculated as about $4.2_{ae/MON\ 0818}$:

$$\rho_{ae/MON\ 0818} = \frac{55\ \text{mg a. e./L}}{13\ \text{mg MON 0818/L}} \cong\ 4.2308 \frac{a.e.}{MON\ 0818}$$

Equation 20

As discussed in Section 4.1.3.1.2.4, the expected LC_{50} of Roundup under the assumption of dose addition can be estimated in units of mg formulation/L as:

$$\zeta_{Roundup} = \frac{\text{LC}_{50}\text{Glyphosate mg a. e./L}}{0.308_{ae/form} + (\rho_{ae/MON\ 0818} \times 0.15_{MON\ 0818/form})} = LC_{50}\ mg\ formulation/L$$

Equation 21

where 0.308 is the proportion of glyphosate a.e. in the formulation and 0.15 is the proportion of the MON 0818 surfactant in the formulation. Substituting the LC_{50} values for glyphosate and MON 0818 reported by Folmar et al. (1979) into the above equation, the predicted LC_{50} for Roundup is about 58.6 mg formulation/L:

$$\zeta_{Roundup} = \frac{55\ \text{mg a. e./L}}{0.308_{ae/form} + (4.2_{ae/MON\ 0818} \times 0.15_{MON\ 0818/form})} \cong 58.6354\ mg\ from./L$$

Equation 22

As noted above, the observed LC_{50} for the Roundup formulation is reported as 18 mg a.e./L, which is equivalent to about 58.4 mg formulation/L [18 mg a.e./L ÷ 0.308 a.e./formulation ≈ 58.4416 mg formulation/L]. Note that the observed and predicted LC_{50}s are virtually identical and this concordance indicates additive joint action – i.e., the ratio of the predicted to observed LC_{50} values for Roundup is about 1.00342.

Tsui and Chu (2003) conducted similar bioassays with both a daphnid, *Ceriodaphnia dubia*, and a copepod, *Acartia tonsa*. In the daphnid assay, the 48-hour LC_{50} values (in units of mg a.e./L) are 415 mg a.e./L for glyphosate IPA, 1.15 mg a.e./L for POEA, and 5.39 mg a.e./L for Roundup. Based on these LC_{50} values, the potency of the POEA surfactant relative to glyphosate acid ($\rho_{ae/POEA}$) is calculated as approximately $361_{ae/POEA}$:

$$\rho_{ae/POEA} = \frac{415\ \text{mg a. e./L}}{1.15\ \text{mg POEA/L}} \cong\ 360.8696 \frac{a.e.}{POEA}$$

Equation 23

The formulation of Roundup used in the Tsui and Chu (2003) is characterized as a formulation that contains 41% a.i. (glyphosate IPA) from Monsanto, USA. As summarized in Table 2, this description could include any of several 41% glyphosate IPA formulations that are available from Monsanto. Using the 0.74 a.e./a.i. conversion factor for the IPA salt of glyphosate, the proportion of glyphosate a.e. in the formulation is taken as $0.3034_{ae/form}$ [$0.41 ai/form$ x 0.74 $_{ae/ai}$]. Tsui and Chu (2003, p. 1190, column 2) used a commercially available POEA surfactant characterizes as *…polyoxyethylene amine (POEA) (CAS: 61791-26-2; 100% a.i.)*. Consequently, the proportion of POEA in the formulation tested by Tsui and Chu (2003) is assumed to be 0.1125 [0.15 x 0.75]. Based on the assumption of additivity, the predicted LC_{50} for Roundup is about 10.1 mg formulation/L:

$$\zeta_{Roundup} = \frac{415\ mg\ a.e./L}{0.3034_{a.e./form} + (361_{a.e./POEA} \times 0.1125_{POEA/form})} \cong 10.1448\ mg\ form./L$$

Equation 24

As noted above, the observed LC_{50} for the Roundup formulation is reported as 5.39 mg a.e./L, corresponding to about 17.8 mg formulation/L [5.39 mg a.e./L ÷ 0.3034 a.e./formulation ≈ 17.765 mg formulation/L]. The ratio of the predicted to observed LC_{50} for Roundup is about 0.57 [10.1 mg formulation/L ÷ 17.8 mg formulation/L ≈ 0.5674], indicating a less than additive joint action.

In the copepod (*Acartia tonsa*) assay by Tsui and Chu (2003), the 48-hour LC_{50} values (also expressed in units of mg a.e./L) are 49.3 mg a.e./L for glyphosate IPA, 0.57 mg a.e./L for POEA, and 1.77 mg a.e./L for Roundup. Based on these LC_{50} values, the potency of the POEA surfactant relative to glyphosate acid ($\rho_{ae/POEA}$) is calculated as approximately $86_{ae/POEA}$:

$$\rho_{ae/POEA} = \frac{49.3\ \text{mg a. e./L}}{0.57\ \text{mg POEA/L}} \cong 86.4912$$

Equation 25

Using the same approach taken with the daphnid study, the expected LC_{50} for Roundup based on the assumption of dose addition is about 4.9 mg formulation/L.

$$\zeta_{Roundup} = \frac{49.3\ mg\ a.3./L}{0.3034_{a.e./form} + (86_{a.e./POEA} \times 0.1125_{POEA/form})} \cong 4.9407\ mg\ form./L$$

Equation 26

The observed LC_{50} for the Roundup formulation in the copepod assay is 1.77 mg a.e./L which corresponds to about 5.8 mg formulation/L [1.77 mg a.e./L ÷ $0.3034_{a.e./formulation}$ ≈ 5.834 mg formulation/L]. The ratio of the predicted to observed LC_{50}s for Roundup is about 0.84 [4.9 mg formulation/L ÷ 5.8 mg formulation/L ≈ 0.3448], indicating a less than additive joint action.

As with the assessments of joint action discussed in other parts of the current risk assessment, the above calculations of joint action for the studies by Folmar et al. (1979) and Tsui and Chu (2003) are included in Attachment 3 – i.e., Worksheets "Folmar et al. 1979 Midge", "Tsui and Chu 2003 daphnid", and "Tsui and Chu 2003 copepod".

4.1.3.3.3. Other Acute Toxicity Studies

Most acute toxicity studies on aquatic invertebrates look at lethality or immobility as the endpoint, as opposed to truly sublethal effects. The toxicity studies summarized in this subsection focus on the factors which may have an impact on the toxicity of glyphosate and glyphosate formulations to aquatic invertebrates.

An early study by Hartman and Martin (1984) examines the impact of suspended clay (50 mg/L) on the toxicity of Roundup to *Daphnia pulex* and notes that increasing concentrations of suspended clay in water enhance the toxicity of Roundup. The 48-hour LC_{50} is 7.9 (7.2-8.6) mg a.i./L in the absence of suspended clay and 3.2 (3.0-3.4) mg a.i./L with suspended clay.

More recently, Tsui and Chu (2003) conducted a series of bioassays in *Ceriodaphnia dubia* exposed to Roundup (NOS) concentrations ranging from 50 to 200 mg/L on Roundup (NOS) with and without suspended clay. The study demonstrates a concentration-related increase in the toxicity of Roundup with 48-hour LC_{50} values ranging from 5.38 mg a.e./L without clay to 0.59 mg a.e./L with clay at 200 mg/L. It is not clear why increasing concentrations of clay increased the toxicity of Roundup. As discussed by Hartman and Martin (1984), daphnids are efficient filter feeders and they may ingest and absorb glyphosate and/or POEA from suspended sediment, thus increasing their exposure to the toxicants.

As discussed in Section 4.1.3.3.2.2, Roundup Biactive is a relatively nontoxic formulation, at least with respect to the original Roundup and similar formulations with the POEA surfactant. Tsui and Chu (2004) conducted a comparative study of Roundup Biactive and a standard Roundup formulation. In comparative sediment assays with *Ceriodaphnia dubia*, Roundup Biactive was much less toxic than Roundup; however, the toxicity of Roundup Bioactive was less affected by sediment binding, compared with Roundup. As discussed by Tsui and Chu (2004), this result suggests that the surfactant used in Roundup Bioactive has a lesser affinity to sediment, relative to the surfactant in Roundup.

Tsui et al. (2005) assayed the impact of glyphosate and Roundup on the toxicity of heavy metals to *Ceriodaphnia dubia*. For most metals (Cd, Cu, Cr, Ni, Pb, Se and Zn), the joint action of glyphosate and Roundup with the heavy metals suggested an antagonism, probably associated with chelation of the metals by glyphosate.

Whereas the above studies focus on factors affecting acute lethal potency, other studies focus on sublethal toxicity. As discussed in Section 3.1.2 (Mechanisms of Action), glyphosate appears to inhibit cytochrome P450 in mammals. At least for mosquito larvae, the opposite occurs. Pre-exposure to nonlethal concentrations of glyphosate result in a significant (i.e., about a factor 2) increase in levels of P450 in mosquito larvae after 72 hours (Riaz et al. 2009).

As discussed in Section 4.1.3.1.3, some glyphosate formulations may impair immune function in fish. In studies on the infectivity of the protozoan parasite, *Perkinsus olseni* to clams (*Ruditapes decussatus*), Elandalloussi et al. (2008) report a concentration-related decrease in infected clams at Roundup concentrations of 10 and 25 mg/L over 5-day periods of exposure. As discussed by Elandalloussi et al. (2008), this effect is most likely due to the direct toxicity of Roundup on the parasite rather than any impact of Roundup on immune function. Other studies on the toxicity of glyphosate and glyphosate formulations to aquatic microorganisms are discussed in Section 4.1.3.4.3.

In studies on the freshwater annelid, *Lumbriculus variegatus*, sublethal exposures to both glyphosate and Roundup Ultra are associated with an induction of superoxide dismutase (Contardo-Jara et al. 2009). Consistent with observations in fish (Section 4.1.3.1.3), the induction of superoxide dismutase is an indicator of general oxidative stress.

As discussed in Section 3.1.13.1, Marc et al. (2004b) assayed the effects of several glyphosate formulations apparently used in France on the development of sea urchin eggs over extremely short durations of exposure—i.e., up to about 4 hours. Concentrations of 0.1 mM or about 17 mg

a.e./L of any of the formulations tested did not cause adverse effect; whereas, concentrations of 1-30 mM or from about 170 to about 5000 mg a.e./L resulted in abnormal or completely arrested development. The concentrations causing adverse effects are within the range of EC_{50} values for less toxic glyphosate formulations, like Aqua Star, Rodeo, and Spasor (Table 26).

4.1.3.3.4. Longer-term Toxicity

Information on the longer-term toxicity of glyphosate acid, salts, and formulations to aquatic invertebrates is summarized in Appendix 8, Table 4. The standard chronic invertebrate bioassay for pesticides is the life-cycle study in *Daphnia magna*. This study is available for the IPA salt of glyphosate (McKee et al. 1982). Glyphosate IPA was assayed at concentrations of 0, 25, 50, 99, 199, or 397 mg a.i./L using a flow-through system with a standard 21-day period of exposure. Based on a reduction in the number of young, the NOEC is 50 mg a.i./L (37 mg a.e./L) with a corresponding LOEC of 100 mg a.i./L (74 mg a.e./L). As discussed in Section 4.1.3.3.2.1, no acute toxicity studies are available on the toxicity of glyphosate IPA to *Daphnia magna*. The 48-hour LC_{50} of technical grade glyphosate to *Daphnia magna* is 647.4 mg a.e./L with a corresponding NOEC of 464.8 mg a.e./L (McAllister and Forbes 1978b). The 48-hour LC_{50} of glyphosate IPA to *Ceriodaphnia dubia* is 415 mg a.e./L (Tsui and Chu 2004). Based on these comparisons, there appears to be a duration-response relationship in daphnids.

As with the acute lethality studies, the population studies indicate a greater response with suspended clay, relative to cultures without suspended clay. Hartman and Martin (1984) exposed breeding populations of *Daphnia pulex* to Roundup at concentrations of 1, 2, or 4 mg a.i./L with and without suspended clay. These exposures appear to be static without renewal. At all concentrations, the populations of the cultures were reduced 1 week after exposure and the magnitude of the reductions was generally concentration-related. As in the acute toxicity study discussed in the previous subsection, the magnitude of the reductions in offspring was greater in the presence of suspended clay, relative to exposures without clay. By 2 weeks after the initial exposure, however, no significant differences were noted between control and Roundup treated organisms at any concentration. Thus, Roundup at 4 mg a.i./L (about 3 mg a.e./L) had only a transient effect on daphnid reproduction. Given that the 48-hour LC_{50} for Roundup in daphnids ranges from about 2.7 to 3.2 mg a.e./L (Drottar and Krueger 2000c; MRID 44538201 as summarized in Appendix 8, Table 2), the reproduction study by Hartman and Martin (1984) does not suggest a substantial duration-response relationship.

As with the Hartman and Martin (1984) study, a duration-response relationship is not apparent in the matched 48-hour and 28-day LC_{50} values in mussels reported in the Bringolf et al. (2007) study. For glyphosate acid, the 48-hour and 28-day LC_{50} values were both >200 mg a.e./L. For glyphosate IPA, the 48-hour and 28-day LC_{50} values were 5.0 and 4.8 mg/L, respectively. Similarly, Bringolf et al. (2007) did not observe a duration-response relationship for the POEA surfactant, with 48-hour and 28-day LC_{50} values of 0.5 and 1.7 mg/L, respectively. Bringolf et al. (2007) also provide both 48-hour and 28-day LC_{50} values for two glyphosate formulations, Aqua Star and Roundup Ultramax. For Aqua Star, a duration-response relationship is apparent with a 48-hour LC_{50} of greater than 148 mg a.e./L and a 21-day LC_{50} of 43.8 mg a.e./L. For Roundup Ultramax, however, no duration-response relationship is apparent with a 48-hour LC_{50} of 2.9 mg a.e./L and a 28-day LC_{50} of 3.7 mg a.e./L.

The sublethal effects associated with longer-term exposure to technical grade glyphosate have also been determined in an aquatic snail, *Pseudosuccinea columella*, an intermediate host of the sheep liver fluke. Tate et al. (1997) assayed glyphosate acid for sub-lethal effects on egg production at concentrations of 0.1, 1, or 10 mg a.e./L for 3 generations. No marked effects were noted on the first or second generations. In the third generation, snail embryos exposed to 1 mg/L developed much faster than those exposed to 0.1 or 10 mg/L or control snails. Hatching, however, was inhibited at 10 mg/L and inhibited slightly at 0.1 mg/L; however, egg-laying capacity increased at both of these concentrations. In a follow up study, Tate et al. (2000) noted effects on concentrations of amino acids in snails (specifically alanine, glycine, glutamic acid and threonine) at the same concentrations. Effects on concentrations of some proteins are noted also by Christian et al. (1993) for this species of snail. The mechanism for the effect of glyphosate on amino acid and protein metabolism in aquatic invertebrates is not well characterized. In terms of potentially significant reproductive effects, the Tate et al. (1997) study suggests that some changes might be observed at concentrations as low as 0.1 mg/L but that the mixed effects of glyphosate on egg-laying capacity (stimulation) and hatching (inhibition) could be off-setting, in terms of total reproductive capacity.

4.1.3.3.5. Field/Mesocosm Studies

Various field studies indicate that applications of Rodeo or Roundup (Appendix 8, Table 8) have no remarkable adverse effects on aquatic invertebrates. Gardner and Grue (1996) observed no adverse effects on aquatic invertebrates at application rates of 1 L Rodeo/ha (0.48 g a.e./ha) for the control of purple loosestrife. At application rates of 0.94 or 1.48 kg a.i./ha as glyphosate IPA (Rodeo), Hagg (1986) found no indication of lethality in two water hyacinth weevils, *Neochetin eichhorniae* and *N. bruchi*. Finally, no indication of short- or long-term (119 days) effects were noted after the application of a Rodeo and X-77 mixture to control smooth cordgrass in a marine estuary. In this study, Rodeo was applied at a rate of 4.7 L/ha (≈2.2 kg a.e./hr) and X-77 was applied at a rate of 1 L/ha (Simenstad et al. 1996).

For Roundup, Hildebrand et al. (1980) found no differences in invertebrate survival over an 8-day period after sprays of 2.2, 22, or 220 kg/ha in a forest pond mesocosm. Similarly, the aquatic mesocosm study by Relyea (2005b) reports no effects on predatory insects or snails after Roundup applications resulting in a water concentration of 3.5 mg a.i./L (≈ 3 mg ae/L). A significant reduction was noted, however, in some species of dragonfly and backswimmers. In an artificial stream mesocosm treated with Vision formulation, Austin et al. (1991) observed an increase in periphyton populations, which was attributed to the use of glyphosate as a nutrient by the organisms.

4.1.3.4. Aquatic Plants

4.1.3.4.1. Overview

Summaries of the toxicity of glyphosate and glyphosate formulations are given in Table 27 for algae and Table 28 for macrophytes. For the hazard identification, the emphasis is on comparative toxicity and the toxicity values for both groups of organisms are typically expressed as EC_{50} values. The endpoints for the EC_{50} values involve growth inhibition. The specific endpoints—e.g., cells mass, chlorophyll content, frond count or growth—vary according to the cited studies. For the most part, only the most sensitive endpoints are discussed, unless the nature of the data requires a specific discussion of specific endpoints. Most of the EC_{50} values

for algae are based on 48-hour exposures. Most bioassays on macrophytes are typically conducted over periods of 7-14 days. As with aquatic animals, duration-response relationships are not pronounced in macrophytes.

As with aquatic invertebrates, differences in species sensitivities are apparent for both algae and aquatic macrophytes. For glyphosate acid, the EC_{50} values range from about 2 to 600 mg a.e./L for algae and from 10 to nearly 200 mg a.e./L for macrophytes. The more extreme values for algae, compared with those for macrophytes, may reflect the greater number of studies conducted on algae. While fewer bioassays on glyphosate formulations have been conducted on algae, relative to aquatic animals, the patterns in the toxicity of glyphosate formulations to algae are similar to those for aquatic animals. Roundup and similar formulations are more toxic than Rodeo and comparable formulations. Also, as with aquatic animals, some surfactants such as Geronol CF/AR appear to decrease the toxicity of Roundup.

4.1.3.4.2. Glyphosate Acid and Salts

4.1.3.4.2.1. Algae

As discussed in previous subsections on aquatic animals, substantial differences in species sensitivities to glyphosate are uncommon. This is not the case for the sensitivity of algae to glyphosate acid or glyphosate IPA. As summarized in Appendix 9, Table 1, the reported 96-hour EC_{50} values for glyphosate acid range from 2.27 mg a.e./L (*Skeletonema costatum* from the study by Tsui and Chu 2003) to 590 mg a.e./L (*Chlorella pyrenoidosa* from the study by Maul and Wright 1984). These differences for glyphosate acid span a factor of about 260.

To some extent, the differences in EC_{50} values probably reflect differences in experimental methods or organism populations or conditions or even random error rather than true differences in species sensitivity. The greatest intraspecies difference occurs for *Anabaena flosaquae*. For this species, the reported 4- and 5-day EC_{50} values span a factor of about 30 ranging from 11.4 mg a.e./L (MRID 40236904 in U.S. EPA/OPP 2008a) to 304 mg a.e./L (Maul and Wright 1984).

The greatest number of bioassays (n=5) are available in *Selenastrum capricornutum*, the older name for a species currently designated as *Pseudokirchneriella subcapitata*. Four of the five bioassays on this species are on glyphosate acid. For this species, the reported EC_{50} values for glyphosate acid span a factor of about 20, ranging from the 5-day EC_{50} of 13.4 mg a.e./L (MRID 44320637) to the 2-day EC_{50} of 270 mg a.e./L (Cedergreen and Streibig 2005) [270 mg a.e./L ÷ 13.4 mg a.e./L ≈ 20.15]. Ignoring the relatively brief 2-day exposure, the EC_{50} values for *Selenastrum capricornutum* span a factor of only about 2 [24.7 mg a.e./L ÷ 13 mg a.e./L = 1.9], with the higher EC_{50} from the study by Maul and Wright (1984). Thus, considering the intraspecies variability, the true interspecies variability in the toxicity of glyphosate to algae appears to encompass a factor of about 13 to over 100.

Differences in the toxicity of glyphosate acid and glyphosate IPA to algae are inconsistent and less substantial. The only matched bioassays are those of Tsui and Chu (2003) in the diatom, *Skeletonema costatum*, and the green alga, *Selenastrum capricornutum*. In both cases, glyphosate acid was more toxic than the IPA salt by a factor of about 2. As discussed in previous subsections on aquatic animals, glyphosate IPA is typically less toxic than glyphosate acid. In unmatched studies—i.e., different investigators and/or species—the IPA salt was modestly more toxic to one species of Chlorella—i.e., a 1-day EC_{50} of ≈280 mg a.e./L for the

IPA salt in *Chlorella fusca* (Faust et al. 1994) compared with a 4-day EC_{50} of 590 mg a.e./L for glyphosate acid in *Chlorella pyrenoidosa* (Maul and Wright 1984). For two species of *Scenedesmus*, colonial algae, Saenz et al. (1997) report EC_{50} values for the IPA salt of glyphosate—i.e., 7.2-10.2 mg a.e./L—that are somewhat lower than those reported for *Scenedesmus* with glyphosate acid—i.e., 26 mg a.e./L by Vendrell et al. (2009) and 55.85 mg a.e./L by Ma (2002).

Given the interspecies and intraspecies variability in the data on algae, duration-response relationships are somewhat difficult to assess. The best data set for assessing duration appear to be the four bioassays on glyphosate acid in *Selenastrum capricornutum* that range in duration from 2 to 5 days with a 2-day EC_{50} of 220 mg a.e./L (Cedergreen and Streibig 2005), two 4-day EC_{50} values of 12.1 mg a.e./L (MRID 40236901) and 24.7 mg a.e./L (Tsui and Chu 2003), and a 5-day a EC_{50} of 13.4 mg a.e./L (MRID 44320637). At least with respect to the 2- and 4-day durations, a substantial duration-response relationship is apparent.

4.1.3.4.2.2. Macrophytes

Compared with the algae, relatively little information is available on the toxicity of glyphosate acid or salts to macrophytes. An overview of the toxicity to aquatic macrophytes of glyphosate acid as well as glyphosate formulations is given in Table 28 and additional details are given in Appendix 9 (Table 2). The first row of Table 28 includes the overview of toxicity data on glyphosate acid and this row is atypical in that it includes not only lower and upper bound values but also intermediate toxicity values. This approach is taken because the available data on *Lemna*, a genus of free-floating aquatic macrophytes that are commonly used in bioassays for the U.S. EPA, are bracketed by toxicity values for submerged vascular macrophytes.

Table 28 does not include the efficacy study on giant salvinia by Fairchild et al. (2002). Giant salvinia is a noxious aquatic floating weed. Fairchild et al. (2002) noted about 85% to 90% inhibition growth inhibition relative to controls in giant salvinia at concentrations of 4500 mg a.e./L to 36,500 mg a.e./L. Fairchild et al. (2002) did not attempt to estimate NOEC or EC_{50} values comparable to those given for other species in Table 28.

The standard macrophytes used in toxicity tests submitted to the U.S. EPA/OPP are Lemna gibba and Lemna minor, two species of duckweed. As summarized in Appendix 9, Table 1, standard 7- to 10-day EC50 values in these species using glyphosate acid range from about 10 mg a.e./L (Lemna gibba in MRID 44320638) to about 47 mg a.e./L (Lemna minor from the open literature study by Cedergreen and Streibig 2005). One bioassay is available on the toxicity of glyphosate IPA (Michel et al. 2004). This study was conducted with another species of duckweed, Lemna paucicostata, and the 7-day EC50 of 42 mg a.e./L is in the range of EC50 values for the more common test species of Lemna. The EC50 values for Lemna are remarkably close to the geometric mean of the range of EC50 values for algae—i.e., (2.27 mg a.e./L x 590 mg a.e./L)0.5 ≈ 37 mg a.e./L (Section 4.1.3.4.2.1).

Only two studies using glyphosate acid are available on submerged macrophytes: the bioassay by Perkins (1997) using watermilfoil and the bioassay by Nielsen and Dahllof (2007) using eelgrass. Both of these bioassays involve exposure periods of 14 days similar to the bioassays in Lemna. The bioassay on watermilfoil, however, yielded an EC50 for a reduction in root length of 1.56

mg a.e./L. This EC50 is below the lower bound of the EC50s for Lemna by about a factor of 6 [10 mg a.e./L ÷ 1.56 mg a.e./L ≈ 6.41]. No EC50 is available for eelgrass but Nielsen and Dahllof (2007), report an NOAEC for growth inhibition of 170 mg a.e./L for this species with a stimulation of growth at 17 mg a.e./L. While stimulation of plant growth a low levels of exposure is not an uncommon observation for herbicides, the lack of any growth inhibition at 170 mg a.e./L suggests that eelgrass is much more tolerant than either watermilfoil or Lemna.

4.1.3.4.3. Glyphosate Formulations

4.1.3.4.3.1. Algae

An overview of the studies regarding the toxicity of glyphosate formulations to algae is given in Table 27, and additional details are provided in Appendix 9 (Table 2). As with similar tables on other groups of aquatic organisms, Table 27 gives toxicity values for different formulations or groups of formulations with lower and upper bounds, when possible.

The toxicity data on algae for glyphosate formulations are not as abundant as the data for aquatic animals. A Glyphos (IPA) formulation appears to be the most toxic formulation—i.e., EC_{50} values of 0.12 mg a.e./L to 0.68 mg a.e./L. In general, the pattern of potency among formulations is similar to that for aquatic animals with most glyphosate-surfactant formulations being more toxic than Rodeo without a surfactant and technical grade glyphosate. As discussed in Section 4.1.3.4.2.1, however, numerous species have been assayed with technical grade glyphosate, and substantial species differences are apparent with EC_{50} values ranging from 2.27 to 590 mg a.e./L. Thus, in terms of the lowest EC_{50} values, the differences between technical grade glyphosate and the most toxic formulation, Glyphos, are only about a factor of 20 [2.27 mg a.e./L ÷ 0.12 mg a.e./L ≈ 18.9]. As with aquatic animals, mixtures of Roundup with the Geronol CF/AR surfactant are less toxic than most standard Roundup formulations and similarly toxic formulations.

4.1.3.4.3.2. Aquatic Macrophytes

An overview of the toxicity of glyphosate formulations to aquatic macrophytes is given in Table 28 and additional details are given in Appendix 9 (Table 2). Except for the bioassays of Rodeo and Roundup in watermilfoil (Perkins 1997) and a bioassay with sago pondweed using an unspecified formulation (Hartman and Martin 1985), all studies on glyphosate formulations are limited to species of *Lemna*. As discussed in Section 4.1.3.4.2.2 and also summarized in Table 28, bioassays of glyphosate acid in watermill foil (Perkins 1997) and eelgrass (Nielsen and Dahllof 2007) suggests that submerged rooted aquatic macrophytes may exhibit a wider range of sensitivities to glyphosate than is evident in species of *Lemna*.

Data are available on the toxicity of only Rodeo, Roundup and Glyphos to *Lemna*. Based on the 7-day EC_{50} values, the differences between the Roundup and Glyphos formulations are vary by only a factor of about 2—i.e., an EC_{50} of 7.7 mg a.e./L for Glyphos and 3.4 mg a.e./L for Roundup. Based on the 14-day bioassays conducted by Perkins (1997) in both watermilfoil and *Lemna gibba*, the differences between Rodeo and Roundup also insubstantial and vary by only a factor of about 1.5 in watermilfoil [1.22 mg a.e./L ÷ 0.84 mg a.e./L ≈ 1.452] and 1.7 in *Lemna gibba* [7.60 mg a.e./L ÷ 4.58 mg a.e./L ≈ 1.659]. Hartman and Martin (1984) report a somewhat lower 14-day EC_{50} of 1.5 mg a.e./L for Roundup in *Lemna minor*. This LC_{50}, however, is only

about a factor of 3 less than the EC_{50} for Roundup in Lemna gibba reported by Perkins (1997) [4.58 mg a.e./L ÷ 1.5 mg a.e./L ≈ 3.053].

In the study by Sobrero et al. (2007) in *Lemna*, the EC_{50} at Day 2 (9.2 mg formulation/L or 6.5 mg a.e./L) was somewhat lower than the EC_{50} at Day 10 (11.6 mg formulation/L or 8.2 mg a.e./L). These EC_{50} values are for growth rate inhibition, the most sensitive endpoint for the EC_{50} values. Other endpoints and other response rates given in the Sobrero et al. (2007, Table 2) publication suggest only a modest duration-response relationship over the 10-day period of exposure. Based on unmatched observations—i.e., EC_{50} values from different studies—only a modest duration-response relationship is apparent. For example, the 7-Day EC_{50} for Roundup is 3.4 mg a.e./L for *Lemna minor* in the study by Cedergreen and Streibig (2005). The 14-day EC_{50} for the same species is about 1.5 mg a.e./L (2.0 mg a.i./L for the IPA salt) in the study by Hartman and Martin (1984).

As noted in Table 28, the 14-day EC_{50} of 2.0 mg a.i./L in the study by Hartman and Martin (1984) involved water without suspended clay. In a parallel study with suspended clay (50 mg/L), Roundup was much less toxic with an NOEC of 10 mg a.i./L. The effect of suspended clay on the toxicity of Roundup to macrophytes reflects the binding of glyphosate and the POEA surfactant to clay with the consequent decrease in bioavailability to macrophytes. As discussed in Section 4.1.3.3.3, a very different pattern is apparent with daphnids. For these filter feeders, suspended clay appears to enhance the toxicity of Roundup.

4.1.3.4.4. Surfactants

Information on the toxicity of surfactants to algae is limited to bioassays on the POEA surfactant used in some formulations of Roundup to two species of algae, *Selenastrum capricornutum* and *Skeletonema costatum*. As summarized in Appendix 9, Table 3, the 96-hour EC_{50} values are remarkably similar, ranging from 3.35 mg/L (Tsui and Chu 2003) to 4.1 mg/L (Van Ginkel et al. 1993). The EC_{50} values for algae are within the range of those for Roundup formulations to algae (Table 27) as well as the those for POEA to fish (1-3 mg/L, Section 4.1.3.1.2.3.), amphibians (2.2 to 6.8 mg/L, Section 4.1.3.2.2.3), and aquatic invertebrates (0.5 to 13 mg/L, Section 4.1.3.3.2.3).

In the efficacy study of glyphosate for the control of giant salvinia, Fairchild et al. (2002) noted that several surfactants, including MON 0818, were not toxic to salvinia at a concentration of 2500 mg surfactant/L. Only one surfactant, Optima, was effective in enhancing the efficacy of glyphosate for the control of salvinia.

4.1.3.4.5. Field Studies

Glyphosate is an effective herbicide, and its formulations, particularly Rodeo and similar formulations, are registered for the control of aquatic weeds. No attempt is made to review efficacy studies on these formulations. As summarized in Appendix 9 (Table 4), a few field and field simulation studies focus primarily on the assessment of unintended effects on aquatic plants. While growth inhibition of algae was observed at high concentrations (e.g., 44.4-69.7 mg/L as Roundup), several studies note stimulation of growth at glyphosate concentrations of about 10 mg a.e./L (Goldsborough and Brown 1988; Schaffer and Sebetich 2004).

Perez et al. (2007) observed a decrease in the abundance of phytoplankton in a mesocosm study with Roundup concentrations of 6 and 12 mg a.e./L. Based on the toxicity of Roundup to algae (Table 27), decreases in phytoplankton would be expected. Nonetheless, the primary productivity of the mesocosms decreased only on Day 1 of the study and then increased at both concentrations by Day 11 of the study (Perez et al. 2007, Figure 3).

As with effects on periphyton (Austin et al. 1991), the stimulation of algal growth could be associated with their use of glyphosate as a source of nitrogen and/or phosphorous (Schaffer and Sebetich 2004). Other studies in which exposures are expressed as application rates rather than water concentrations report no or only equivocal effects on algae at application rates ranging from 0.4 to 2 lbs/acres (Gardner and Grue 1996; Perschbacher et al. 1997; Sullivan et al. 1981).

4.1.3.5. Aquatic Microorganisms

As discussed in Section 4.1.2.6, there is little indication that glyphosate or glyphosate formulations are toxic to terrestrial microorganisms. Nonetheless, toxicity to microorganisms might be expected because microorganisms use the shikimate pathway for the production of aromatic amino acids (Issa 1999).

As discussed in previous subsections, the study by Tsui and Chu (2003) assayed aquatic invertebrates and algae. In addition, Tsui and Chu (2003, Table 2) conducted parallel bioassays on three aquatic ciliates, *Vibrio fischeri* (a bioluminescent marine bacterium), *Tetrahymena pyriformis* (a freshwater protozoan), and *Euplotes vannu* (a marine protozoan). For *Vibrio fischeri*, differences between the toxicity of glyphosate acid (EC_{50} = 17.5 mg a.e./L) and Roundup (EC_{50} = 24.9 mg a.e./L) were slight. Similar EC_{50} values for this species are reported for both glyphosate acid (EC_{50} = 44.2 mg a.e./L in the study by Hernando et al 2007) and Roundup (EC_{50} = 36.4 mg a.e./L in the study by Amoros et al. 2007). All of the studies in *Vibrio fischeri* are relatively short-term (15-30 minutes) and involve assays for bioluminescence.

The 48-hour bioassays in the other two ciliates report remarkable differences in sensitivity to glyphosate acid—i.e., an EC_{50} of 10.1 mg a.e./L for *Euplotes vannu* and an EC_{50} of 648 mg a.e./L for *Tetrahymena pyriformis*. Similar toxicity values were obtained for glyphosate IPA. The sensitivity of these two organisms to Roundup, however, were similar—i.e., an EC_{50} of 23.5 mg a.e./L for *Euplotes vannu* and an EC_{50} of 29.5 mg a.e./L for *Tetrahymena pyriformis* (Tsui and Chu 2003). This study suggests that the sensitivity of aquatic microorganisms to glyphosate acid is similar to that of aquatic algae—i.e., about 2 mg a.e./L to 600 mg a.e./L for glyphosate acid, as summarized in Table 27. The EC_{50} values for Roundup in these aquatic microorganisms suggest that these microorganisms are less sensitive than algae based on EC_{50} values for Roundup and other glyphosate formulations—i.e., EC_{50} values for algae of about 0.12 mg a.e./L (Glyphos) to 19 mg a.e./L (Roundup), as summarized in Table 27.

In the aquatic mesocosm study by Perez et al. (2007), cyanobacteria increased up to a factor of 40 at Roundup concentrations of 6 and 12 mg a.e./L; however, other bacteria were not substantially affected, which is similar to observations on the impact of glyphosate applications on terrestrial organisms (Section 4.1.2.6) and may reflect the secondary effects of the use of glyphosate as a nutrient source.

Enrich-Prast (2006) reports that glyphosate (NOS) as well as several other pesticides caused a decrease in nitrification at concentrations of 0.1 mg/L (≈20%) and 0.3 mg/L (≈66%) in sediment from a eutrophic lake. The apparent NOEC was 0.03 mg/L (Enrich-Prast 2006, Figure 1). No measures of variability in nitrification are given in the publication. In addition, the experiment appears to have lasted for only 2 hours. Thus, it cannot be determined if this effect was transient. Pesce et al. (2009) observed no effect on an aquatic microbial community over a 2-week exposure period to a concentration of 0.01 mg/L glyphosate (NOS). This was the only concentration tested in the study.

In a 7-day marine aquatic microcosm study, Stachowski-Haberkorn et al. (2008) observed changes in microbial community structure, based on differences in ribosomal DNA, after exposures to Roundup concentrations as low as 0.001 mg/L. It is not clear from the study that these effects would be associated with perturbations in the function of aquatic communities. While a decrease in microbial species diversity was noted at both 0.001 and 0.01 mg a.e./L, the decrease was not concentration related (Stachowski-Haberkorn et al. 2008, Table 1). In a similar study, Widenfalk et al. (2008) observed that glyphosate concentrations of 0.150 and 150 mg/kg dry weight had no effect on microbial biomass in freshwater sediment. Based on assays of ribosomal RNA, however, changes in microbial composition were noted. The functional significance of these changes is not clear. As noted in the discussion by the authors:

> *The large functional redundancy in sediment microbial communities may likely constitute an inherent buffer against the loss of important ecological functions due to environmental constraints.*
>
> Widenfalt et al. 2008, p. 583

In other words, genetic fingerprinting techniques may provide sensitive assays for changes in microbial communities that reflect exposures to pesticides. It is less clear, however, that these changes can be associated with adverse impacts on the microbial communities.

4.2. EXPOSURE ASSESSMENT

4.2.1. Overview

For terrestrial applications, a standard set of exposure assessments is given for backpack foliar applications (Attachment 1a), ground broadcast foliar applications (Attachment 1b), and aerial foliar applications (Attachment 1c). A subset of the standard exposure scenarios is provided for aquatic applications (Attachment 2). All workbooks use a unit application rate of 1 lb a.e./acre. The use of other application rates is discussed in the risk characterization. As in the human health risk assessment, three general types of exposure scenarios are considered: accidental, acute non-accidental, and longer-term.

Exposure assessments for mammals and birds are summarized in Worksheet G01 of the EXCEL workbooks that accompany this risk assessment. At the unit application rate of 1 lb a.e./acre, accidental exposure scenarios lead to upper bound estimates of exposure ranging from about 0.7 mg/kg bw (the consumption of contaminated water by a bird after an accidental spill) to about 24 mg/kg bw (dermal exposure for a small mammal after direct spray, assuming 100% absorption). The highest acute non-accidental exposures are associated with the consumption of contaminated insects by a small bird (112 mg/kg bw) and the consumption of contaminated grasses by mammals (≈40 mg/kg bw). Scenarios for the consumption of contaminated vegetation also lead to the highest longer-term exposures, up to about 12 mg/kg bw/day for a large bird consuming contaminated grasses. For both acute and chronic exposures, contaminated water leads to dose estimates far below those associated with contaminated vegetation. This is a common pattern that is observed with many herbicides following terrestrial application and reflects the direct application of the herbicide to vegetation.

For terrestrial plants, five exposure scenarios are considered quantitatively: direct spray, spray drift, runoff, wind erosion, and the use of contaminated irrigation water. Unintended direct spray is expressed simply as the application rate. As with terrestrial animals, all exposure assessments used in the workbooks that accompany this risk assessment are based on a unit application rate of 1 lb a.e./acre. The consequences of using other application rates are discussed in the risk characterization. Exposures of aquatic plants and animals to glyphosate are based on essentially the same information used to assess the exposure to terrestrial species from contaminated water.

4.2.2. Mammals and Birds

All exposure scenarios for terrestrial animals are summarized in Worksheet G01 in the EXCEL workbooks that accompany this risk assessment (Attachments 1a-c for terrestrial applications and Attachment 2 for aquatic applications).

For terrestrial applications of glyphosate, mammals and birds might be exposed to any applied pesticide from direct spray, the ingestion of contaminated media (e.g., vegetation, prey species, or water), grooming activities, or indirect contact with contaminated vegetation. In the exposure assessments for the ecological risk assessment, estimates of oral exposure to mammals and birds are expressed in the same units as the available toxicity data. 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 for terrestrial animals. **Unless otherwise specified, all exposure estimates for glyphosate are expressed as mg a.e. (acid equivalents).**

For dermal exposure of mammals and birds to an applied pesticide, the units of exposure are expressed in mg of agent per cm^2 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 taken in or absorbed by the animal.

Because of the relationship of body weight to surface area as well as to the consumption of food and water, small animals will generally receive a higher dose, in terms of mg/kg body weight, relative to large animals, for a given type of exposure. Consequently, some exposure scenarios, including direct spray (F01 and F02) and the consumption of contaminated insects (F14a,b), are based on a small mammal or a small bird. Most other exposure scenarios are conducted for animals of various sizes. These exposure scenarios include the consumption of contaminated water following an accidental spill (F05a-e) and the consumption of contaminated water at expected short-term concentrations (F06a-e) as well as expected longer-term concentrations (F07a-e). Both small and large animals are also assessed for the consumption of contaminated vegetation for both acute exposures (F03a-b, F10 and F12) and longer-term exposures (F04a-b, F11a-b, and F13a-b). Generally, pesticide concentrations on grasses will be higher than concentrations on fruits and other types of vegetation (Fletcher et al. 1994). Although small mammals do not typically consume large amounts of grass over prolonged periods of time, small mammals, like the meadow vole (*Microtus pennsylvanicus*), may consume grasses as a substantial proportion of their diet, at certain times of the year. Consequently, the acute consumption of contaminated grass by a small mammal is considered in this risk assessment (F03b). Large mammals may consume grasses over a long period of time, and these scenarios are included both for acute exposures (Worksheet F10) and longer-term exposures (Worksheets F11a and F11b). Other exposure scenarios for mammals involve the consumption of small mammals contaminated by direct spray by a large mammalian carnivore (Worksheet F16a). The corresponding exposure scenarios for birds involve the consumption of contaminated fish by a predatory bird (Worksheets F08 and F09) and the consumption of small mammals contaminated by direct spray by a predatory bird (F16b).

For aquatic applications, the exposure assessments for terrestrial animals are a subset of those included for terrestrial applications. In aquatic applications, glyphosate will be applied directly to surface water; consequently exposure scenarios concerning the consumption of contaminated vegetation or fruit, the direct spray of a small mammal, and the consumption of a sprayed small mammal by a predator are not included for aquatic applications.

4.2.2.1. Direct Spray

The unintentional direct spray of wildlife during broadcast applications of a pesticide is a credible exposure scenario similar to the accidental exposure scenarios for the general public discussed in Section 3.2.3.2. In a scenario involving exposure to direct spray, the amount of pesticide absorbed depends on the application rate, the surface area of the organism, and the rate of absorption.

For this risk assessment, two direct spray or broadcast exposure assessments are conducted for terrestrial applications. The first spray scenario (Worksheet F01) concerns the direct spray of

half of the body surface of a 20 g mammal as the chemical is being applied. This exposure assessment assumes first-order dermal absorption. The second exposure assessment (Worksheet F02) assumes complete absorption over Day 1 of exposure. This assessment is included in an effort to encompass the increased exposure due to grooming.

There are no exposure assessments for the direct spray of large mammals, principally because allometric relationships dictate that according to body weight, the amount of a compound per unit body weight to which large mammals will be exposed as a result of direct spray is less than the amount per unit body weight to which smaller mammals will be exposed.

4.2.2.2. Dermal Contact with Contaminated Vegetation

As discussed in the human health risk assessment (Section 3.2.3.3), the only approach for estimating the potential significance of dermal contact with contaminated vegetation is to assume a relationship between the application rate and dislodgeable foliar residue. Unlike the human health risk assessment, in which estimates of transfer rates are available, there are no transfer rates available for wildlife species. Wildlife species are more likely than humans to spend long periods of time in contact with contaminated vegetation. It is reasonable to assume that for prolonged exposures, equilibrium may be reached between pesticide levels on the skin, rates of dermal absorption, and pesticide levels on contaminated vegetation. Since data regarding the kinetics of this process are not available, a quantitative assessment for this exposure scenario cannot be made in the ecological risk assessment.

4.2.2.3. Ingestion of Contaminated Vegetation or Prey

In foliar applications, the consumption of contaminated vegetation is an obvious concern. Separate exposure assessments are developed for acute and chronic exposure scenarios involving a small mammal (Worksheets F03a, F03b, F04a and F04b), a large mammal (Worksheets F10, F11a, and F11b), and large birds (Worksheets F12, F13a, and F13b). Similarly, the consumption of contaminated insects is modeled for a small mammal (Worksheet 14a) and a small bird (Worksheet 14b). As detailed in the exposure assessment for human health (Section 3.2.3.3), the empirical relationships based on those recommended by Fletcher et al. (1994) are used to estimate residues in contaminated insects (Worksheets F14a and F14b). For all exposure scenarios involving contaminated vegetation or insects, residues rates for broadcast foliar liquid applications are higher than those for broadcast granular applications, as indicated in Table 18.

A similar set of scenarios is provided for the consumption of small mammals by either a predatory mammal (Worksheet 16a) or a predatory bird (Worksheet 16b). In addition to the consumption of contaminated vegetation, insects, and other terrestrial prey, exposure pathways for glyphosate may be associated with ambient water and fish. Thus, a separate scenario is developed for the consumption of contaminated fish by a predatory bird, which involves acute (Worksheet F09a) and chronic (Worksheet F09b) exposure.

4.2.2.4. Ingestion of Contaminated Water

The methods for estimating glyphosate concentrations in water are identical to those used in the human health risk assessment (Section 3.2.3.4). The only major differences in the estimates of exposure involve the weight of the animal and the amount of water consumed. These differences are detailed and documented in the worksheets that address the consumption by mammals and

birds of contaminated water from accidental spills (Worksheets F05a-e), peak expected concentrations (Worksheets F06a-e), and longer-term concentrations (Worksheets F07a-e).

Unlike the human health risk assessment, estimates of the variability of water consumption are not available. Thus, for the acute scenario, the only factors affecting the estimate of the ingested dose include the field dilution rates (i.e., the concentration of the chemical in the solution that is spilled) and the amount of solution that is spilled. As in the acute exposure scenario for the human health risk assessment, the central estimate of the amount of the spilled solution is taken as 100 gallons of a field solution with a range of 20-200 gallons (Worksheets F05a-e).

In the exposure scenario involving ponds or streams contaminated by runoff or percolation, the only variable factors are the water contamination rates (Section 3.2.3.4.2) and the application rates.

4.2.3. Terrestrial Invertebrates

4.2.3.1. Direct Spray and Drift

Estimated levels of exposure associated with broadcast terrestrial applications of glyphosate are detailed in Worksheet G02b. Honeybees are used as a surrogate for other terrestrial insects, and honeybee exposure levels associated with broadcast applications are modeled as a simple physical process based on the application rate and surface area of the bee. The surface area of the honeybee (1.42 cm^2) is based on the algorithms suggested by Humphrey and Dykes (2008) for a bee with a body length of 1.44 cm.

The amount of a pesticide deposited on a bee during or shortly after application depends on how close the bee is to the application site as well as foliar interception of the spray prior to deposition on the bee. The estimated proportions of the nominal application rate at various distances downwind given in G02b are based on Tier 1 aerial estimates from AgDrift (Teske et al. 2002) for distances of 0 (direct spray) to 900 feet downwind of the treated site.

In addition to drift, foliar interception of a pesticide may occur. The impact of foliar interception would vary depending on the nature of the canopy above the bee. For example, in studies investigating the deposition rate of diflubenzuron in various forest canopies, Wimmer et al. (1993) noted that deposition in the lower canopy, relative to the upper canopy, generally ranged from about 10% (90% foliar interception in the upper canopy) to 90% (10% foliar inception by the upper canopy). In Worksheet G02b, foliar interception rates of 0% (no interception), 50%, and 90% are used.

During broadcast applications of a pesticide, it is likely that terrestrial invertebrates other than bees will be subject to direct spray. As discussed in further detail in Section 4.3.2.3 (dose-response assessment for terrestrial invertebrates), the available toxicity data on terrestrial invertebrates do not support the derivation of separate toxicity values for different groups of terrestrial insects. Thus, the honeybee is used as a surrogate for other insect species.

4.2.3.2. Ingestion of Contaminated Vegetation or Prey

Like terrestrial mammals and birds, terrestrial invertebrates may be exposed to glyphosate through the consumption of contaminated vegetation or contaminated prey. As discussed in Section 4.1.2.4.2, concern for this exposure pathway is raised in the studies by Benamu et al. (2010) and Schneider et al. (2009) in which arthropods were fed prey contaminated with glyphosate formulations and a spectrum of adverse effects were noted.

For broadcast foliar applications, estimates of residues on contaminated vegetation or prey are based on estimated residue rates (i.e., mg/kg residues per lb a.i. applied) from Fletcher et al. (1994), which is a reanalysis of residue rates derived by Hoerger and Kenaga (1972). These residue rates are the same ones used in Forest Service risk assessments and the ecological risk assessments conducted by the U.S. EPA/EFED (2001).

The original analysis by Hoerger and Kenaga (1972) as well as the reanalysis by Fletcher et al. (1994) give only central and upper bound estimates of residues rates. For the current analysis, lower limits on residue rates are calculated under the assumption that variability in the residue rates are distributed proportionately (i.e., the ratio of the central estimate to the upper limit will be the same as the ratio of the lower limit to the central estimate). The specific residue rates used to estimate plausible concentrations of glyphosate in food items are summarized in Table 18.

An estimate of food consumption is necessary to calculate a dose level for a foraging herbivorous insect. Insect food consumption varies greatly, depending on the caloric requirements in a given life stage or activity of the insect and the caloric value of the food to be consumed. The derivation of consumption values for specific species, life stages, activities, and food items is beyond the scope of the current analysis. Nevertheless, general food consumption values, based on estimated food consumption per unit body weight, are available.

Reichle et al. (1973) studied the food consumption patterns of insect herbivores in a forest canopy and estimated that insect herbivores may consume vegetation at a rate of about 0.6 of their body weight per day (Reichle et al. 1973, pp. 1082 to 1083). Higher values (i.e., 1.28-2.22 in terms of fresh weight) are provided by Waldbauer (1968) for the consumption of various types of vegetation by the tobacco hornworm (Waldbauer 1968, Table II, p. 247). The current risk assessment uses food consumption factors of 1.3 (0.6 to 2.2) kg food /kg bw. The lower bound of 0.6 is taken from Reichle et al. (1973), and the central estimate and upper bound are taken from the range of values provided by Waldbauer (1968).

Details concerning estimated exposure levels for the consumption of contaminated vegetation by herbivorous insects are provided in Worksheets G07a, G07b, G07c, and G07d. These levels pertain to the four food items included in the standard residue rates provided by Fletcher et al. (1994). The exposure estimates are included in the EXCEL workbooks only for foliar broadcast applications (Attachments 1a-c).

4.2.4. Terrestrial Plants

Generally, the primary hazard to nontarget terrestrial plants associated with the application of most herbicides is unintended direct deposition or spray drift. In addition, herbicides may be transported off-site by percolation or runoff or by wind erosion of soil. As noted in Section 4.1.2.5 (Hazard Identification for Terrestrial Plants) and discussed further in Section 4.3.2.5

(Dose-Response Assessment for Terrestrial Plants), the toxicity data on glyphosate are sufficient to interpret risks associated with these exposure scenarios. Consequently, exposure assessments are developed for each of these exposure scenarios, as detailed in the following subsections.

4.2.4.1. Direct Spray

Unintended direct spray will result in an exposure level equivalent to the application rate. For many types of herbicide applications, it is likely that nontarget plants immediately adjacent to the application site could be sprayed directly. This type of scenario is modeled in the worksheets that assess off-site drift, as discussed in the following subsection.

4.2.4.2. Off-Site Drift

Because off-site drift is more or less a physical process that depends primarily on droplet size and meteorological conditions rather than specific properties of the compound being sprayed, estimates of off-site drift can be modeled using AgDrift. These estimates are summarized in Worksheet G05 of the EXCEL workbooks for terrestrial applications (Attachments 1a-c). The estimates of drift used for terrestrial plants are identical to those used for the exposure assessment of the honeybee (Section 4.2.3.1.).

The estimates of drift should be regarded as little more than generic estimates similar to the water concentrations modeled using GLEAMS (Section 3.2.3.4.3). Actual drift will vary according to a number of conditions—e.g., the topography, soils, weather, and the pesticide formulation. All of these factors cannot be considered in this general risk assessment.

The drift estimates used in the current Forest Service risk assessment are based on AgDRIFT (Teske et al. 2002) using Tier 1 analyses for aerial and ground broadcast applications. The term *Tier 1* is used to designate relatively generic and simple assessments that may be viewed as plausible upper limits of drift. Aerial drift estimates are based on Tier 1 using ASAE Fine to Medium drop size distributions. Tier 1 estimates of drift for ground broadcast applications are modeled using both low boom and high boom options in AgDRIFT. For both types of applications, the values are based on Very Fine to Fine drop size distributions and the 90^{th} percentile values from AgDrift.

Drift associated with backpack applications (directed foliar applications) are likely to be much less than drift from ground broadcast applications. Few studies, however, are available for quantitatively assessing drift after backpack applications. For the current Forest Service risk assessment, estimates of drift from backpack applications are based on an AgDRIFT Tier 1 run of a low boom ground application using Fine to Medium/Coarse drop size distributions (rather than very fine to fine) as well as 50^{th} percentile estimates of drift (rather than the 90^{th} percentile used for ground broadcast applications).

4.2.4.3. Runoff and Soil Mobility

Any pesticide can be transported from the soil at the application site by runoff, sediment loss, or percolation. Runoff, sediment loss, and percolation are considered in estimating contamination of ambient water. Only runoff and sediment loss are considered in assessing off-site soil contamination. This approach is reasonable because off-site runoff and sediment transport will contaminate the off-site soil surface and could impact non-target plants. Percolation, on the other hand, represents the amount of the herbicide that is transported below the root zone and

thus may impact water quality but should not affect off-site vegetation. The GLEAMS modeling used to estimate concentrations in water provides data on loss by runoff. As with the estimates of glyphosate in surface water, runoff estimates are modeled for clay, loam, and sand at nine sites which are representative of different temperatures and rainfall patterns.

For glyphosate, the results of the standard GLEAMS modeling of runoff and sediment losses are summarized in Table 1 of Appendix 10. Note that the proportion of runoff as a fraction of the application rate will vary substantially with different types of soils as well as climates—i.e., temperature and rainfall. For this generic risk assessment, the average runoff is taken as 0.00315 which is the average of the central estimates from the 27 Gleams-Driver simulations conducted for the current Forest Service risk assessment. The upper bound of 0.089 lb a.e./acre is the maximum value for all of the simulations conducted. For glyphosate, this maximum is the highest runoff proportion in the 100 individual simulations for an area with predominantly clay soils, cool temperatures, and high rainfall. The lower bound value of 0.0000001 lb a.e./acre of the application rate would be expected in arid areas with predominantly loam or sandy soils.

The amount of pesticide not washed off in runoff or sediment will penetrate into the soil column, and the depth of penetration will depend on the properties of the chemical, the properties of the soil, and the amount of rainfall. The GLEAMS model provides estimates of pesticide concentrations in soil layers of varying depths. These concentrations are output by GLEAMS in mg pesticide/kg soil (ppm). The minimum non-zero value that GLEAMS will output is 0.000001 mg/kg, equivalent to 1 nanogram/kg soil or 1 part per trillion (ppt).

The deepest penetration of glyphosate in clay, loam, and sand modeled using GLEAMS is summarized in Table 4 of Appendix 10. Based on GLEAMS modeling, the maximum penetration of glyphosate into clay or loam soils is an estimated 4-12 inches, with the depth of penetration increasing as rainfall rates increase. In predominantly sand soils, glyphosate may penetrate to a depth of about 8-18 inches, depending on rainfall rates.

4.2.4.4. Contaminated Irrigation Water

Unintentional direct exposure of nontarget plants is possible from the use of contaminated ambient water for irrigation, as observed by Bhandary et al. (1991) for certain herbicides. The levels of exposure associated with this scenario will depend on the pesticide concentration in the ambient water used for irrigation and the amount of irrigation water used. Concentrations in ambient water are generally based on the concentrations modeled in the human health risk assessment (Section 3.2.3.4). The amount of irrigation used will depend on the climate, soil type, topography, and plant species under cultivation. Thus, the selection of an irrigation rate is somewhat arbitrary.

In the absence of any general approach for determining and expressing the variability of irrigation rates, the application of 1 inch of irrigation water is used in this risk assessment. Details of the calculations used to estimate the functional application rates based on irrigation using contaminated surface water are provided in Worksheet F15. At a unit application rate of 1 lb a.e./acre, the functional application rate associated with the use of contaminated surface water for irrigation is about 0.0025 (0.00007 to 0.038) lb a.e./acre. The central and upper bound of these functional application rates are below those associated with runoff (Worksheet G04). Consequently, the risks of contaminated irrigation water are not considered further.

4.2.4.5. Wind Erosion

Wind erosion is a major transport mechanism for soil (e.g., Winegardner 1996), and wind erosion is also associated with the environmental transport of herbicides (Buser 1990). Wind erosion leading to off-site movement of pesticides is likely to be highly site-specific. The amount of glyphosate that might be transported by wind erosion depends on several factors, including application rate, depth of incorporation into the soil, persistence in the soil, wind speed, and topographical and surface conditions of the soil. Under desirable conditions—e.g., relatively deep (10 cm) soil incorporation, low wind speed, and surface conditions which inhibit wind erosion—it is likely that the amount of glyphosate transported by the wind would be insubstantial.

For this risk assessment, the potential effects of wind erosion are estimated in Worksheet G06. In this worksheet, it is assumed that glyphosate is incorporated into the top 1 cm of soil, which is identical to the depth of incorporation used in GLEAMS modeling. Average soil losses are estimated to range from 1 to 10 tons/ha/year with a typical value of 5 tons/ha/year. These estimates are based on the results of agricultural field studies which found that wind erosion may account for annual soil losses ranging from 2 to 6.5 metric tons/ha (Allen and Fryrear 1977).

As noted in Worksheet G06, offsite losses are estimated to reach as much as 0.014% of the application rate. Larney et al. (1999), however, report that wind erosion of other herbicides could be associated with losses up to 1.5% of the nominal application rate following soil incorporation or 4.5% following surface application. This difference appears to be at least partially due to the much higher soil losses noted by Larney et al. (1999)—i.e., up to 56.6 metric tons/ha from a fallow field. The losses reflected in Worksheet G06 may be somewhat more realistic for forest or rangeland applications, because herbicide applications are rarely made to fallow areas. In any event, the higher offsite losses reported by Larney et al. (1999) are comparable to exposures associated with offsite drift at distances of 100-300 feet from the application site (G05). All of these estimates for wind erosion and offsite drift are likely to vary dramatically according to site conditions and weather conditions.

4.2.5. Aquatic Organisms

The plausibility of effects on aquatic species is assessed based on estimated concentrations of glyphosate in water which are identical to those used in the human health risk assessment. These values are summarized in Table 17 and discussed in Section 3.2.3.4.6 for both terrestrial and aquatic applications of glyphosate.

4.3. DOSE-RESPONSE ASSESSMENT

4.3.1. Overview

Overviews of the dose-response assessments for the ecological risk assessment are given in Table 29 for more toxic formulations of glyphosate and Table 30 for less toxic formulations of glyphosate. As discussed in the human health risk assessment (Section 3) as well as the hazard identification for ecological effects (Section 4.1), there are obvious and in many cases substantial differences between the toxicity of technical grade glyphosate, glyphosate formulations that do not contain a surfactant, and some glyphosate formulations that contain POEA surfactants. While the available information does not permit formulation-specific toxicity values, an attempt is made to discriminate between less toxic and more toxic formulations, when possible.

With the human health risk assessment, Forest Service risk assessments attempt to maintain consistency with ecological risk assessments conducted by U.S. EPA/OPP. Thus, in each of the following subsections, the approach taken in the most recent EPA ecological risk assessment (U.S. EPA/OPP 2008a) is discussed. One area of difference, however, involves the use of LD_{50} and LC_{50} values. U.S. EPA/OPP uses LD_{50} and LC_{50} values with varying levels of concern (U.S. EPA/OPPTS 2004). The Forest Service, however, prefers to use NOAEL or NOAEC values with a fixed level of concern (HQ=1), whenever NOAEL or NOAEC values are available for a receptor group. So, despite every attempt to maintain consistency in study selection, the actual values used in Forest Service risk assessments often differ from those used by U.S. EPA. Specific instances of these types of difference are noted in the following subsections.

For most ecological receptors, with the exception of plants, separate toxicity values can be derived for more and less toxic glyphosate formulations, as indicated in Table 29 and Table 30. The dose-response assessment for terrestrial plants assumes that the surfactants added to all formulations of glyphosate will result in equal efficacy among formulations.

An issue in using the separate toxicity values for more and less toxic formulations involves the categorization of formulations as more toxic versus less toxic. Ideally, there would be a complete set of equally diverse studies on or associated with each formulation of glyphosate; however, discussed in Section 2, this is not the case. A general classification of formulations is given in Table 5. Formulations identified as *Low Toxicity* in Table 5 can be regarded as *less toxic formulations*. Other formulations should be regarded as *more toxic formulations* unless data on the formulation are available to justify a different classification. Additional formulations may become available subsequent to the release of this risk assessment, which may require the use of judgment to classify new formulations as more or less toxic. In general, it would be prudent to classify any formulation that contains a POEA surfactant as more toxic, except when there is a compelling reason to do otherwise. If the presence and/or toxicity of the surfactants in the formulation cannot be determined, it would be prudent to classify that the formulation as more toxic.

The above guidance is not intended to be prescriptive, especially since the classifications designated in Table 5 are based on incomplete information. As discussed in the following subsections, some glyphosate formulations which contain surfactants appear to be less toxic than others, which might be due to a lower concentration of the surfactant or the use of a less toxic

surfactant. For some formulations, like Roundup Biactive, the lower toxicity of the formulation is well documented; whereas, for other formulations, the apparently lower toxicity is not well documented. The possibly less toxic formulations are identified as Medium Toxicity in Table 5. As additional information becomes available, a separate set of toxicity values might be warranted for some of these formulations. At this time, however, these formulations are considered with the more toxic formulations.

In practical terms, the less toxic formulations of glyphosate are those that do not contain a surfactant—e.g., Foresters, Rodeo and Accord. As summarized in Table 4, however, the labels for these formulations specify that a surfactant must be added to the field solution prior to application. Depending on the toxicity of the surfactant, the surfactant may be the dominant concern at least for effects on aquatic species. The impact of using surfactants with less toxic formulations of glyphosate is discussed in the risk characterization. The dose-response assessments for the less toxic surfactants are based on the toxicity of glyphosate, salts of glyphosate, and the information on the toxicity of the less toxic formulations of glyphosate.

4.3.2. Toxicity to Terrestrial Organisms

4.3.2.1. Mammals

Forest Service risk assessments generally base the dose-response assessment for mammalian wildlife on the acute and chronic NOAELs used to derive the acute and chronic RfDs. As discussed in Section 3.3, only a single RfD, 2 mg/kg bw/day, is used for glyphosate (U.S. EPA/OPP 1993a,b, 2000). This RfD is based on a NOAEL of 175 mg a.e./kg bw/day from a developmental study in rabbits (Rodwell et al. 1980b), and the RfD is applied to both acute and chronic exposures. While there is little reservation with regard to this RfD as it applies to technical grade glyphosate, a recent study by Dallegrave et al. (2007) using a South American formulation of glyphosate suggests doses below 175 mg a.e./kg bw could have an impact on testosterone levels. As discussed at some length in Section 3.3.3.3, however, the Dallegrave et al. (2007) study does not provide a sufficient basis for rejecting the current RfD from U.S. EPA/OPP (1993a,b, 2000).

The use of the NOAEL of 175 mg a.e./kg bw/day is somewhat more conservative than the approach taken in the recent EPA ecological risk assessment (U.S. EPA/OPP (2008a). In discussing the rabbit study conducted by Rodwell et al. (1980b), U.S. EPA/OPP (2008a, p. 114) notes that the study involves gavage exposure and that bolus dosing is atypical of environmental exposures to wildlife. As an alternative, U.S. EPA/OPP (2008a) uses the NOAEL of 500 mg/kg bw/day from the multi-generation study by Reyna (1985) which involves dietary exposures.

The approach taken in U.S. EPA/OPP (2008a) has merit at least with respect to less toxic formulations of glyphosate. The NOAEL of 500 mg/kg bw/day is substantially above the acute LD_{50} values for glyphosate as well as most glyphosate formulations, and, except for the developmental studies in rabbits, the NOAEL of 500 mg/kg bw/day is supported by a number of subchronic and chronic toxicity studies on technical grade glyphosate. For the current Forest Service risk assessment, the NOAEL of 500 mg/kg bw/day is used to characterize risks associated with applications of less toxic glyphosate formulations.

For more toxic formulations—i.e., those that contain or may contain a POEA or similarly toxic surfactant—the NOAEL of 500 mg/kg bw/day does not seem sufficiently protective. The dose of 500 mg a.e./kg bw/day is greater than the dose of 487 mg/kg bw/day associated with liver pathology from the study by Benedetti et al. (2004) on another South American formulation of glyphosate with a POEA surfactant. Given the impact of toxic surfactants on the toxicity of glyphosate, the NOAEL of 175 mg a.e./kg bw/day is maintained for more toxic glyphosate formulations. As noted above, this is the NOAEL used as the basis for the U.S. EPA/OPP (1993a,b, 2000) RfD on glyphosate and thus the use of this NOAEL for mammalian wildlife is consistent with the approach used in most Forest Service risk assessments.

4.3.2.2. Birds

As discussed in Section 4.1.2.2.1, a relatively standard set of acute dietary studies are available for both technical grade glyphosate and glyphosate formulations. These studies demonstrate that there are no differences in the toxicity of technical grade glyphosate and glyphosate formulations to birds. All of the acute dietary LC_{50} studies are non-definitive and yield NOAECs of about 5000 ppm a.e. for glyphosate acid and about 1800 ppm a.e. for a glyphosate formulation that appears to be equivalent to Roundup Pro (Appendix 3, Table 2). The differences in these NOAECs reflect the maximum doses used in the studies and cannot be used to infer that the formulations are more toxic than technical grade glyphosate. Based on a standard avian reproduction study, U.S. EPA/OPP (2008a, p. 110) selected the reproductive NOAEL of 830 ppm a.e. in bobwhite quail (MRID 108207) for risk characterization.

U.S. EPA/OPP (2008a) includes reviews of some of the open literature on birds but does not discuss the study by Kubena et al. (1981) in broilers. As summarized in Appendix 3, Table 3, Kubena et al. (1981) noted reduced body weight and changes in bone chemistry in broilers, during a 21-day dietary study using Roundup. The NOAEL for these effects was 608 ppm a.i. or about 450 ppm a.e.

A review of the Oliveira et al. (2007) study conducted with mallard drakes is given in U.S. EPA/OPP (2008a). As discussed in Section 4.1.2.2.2, this 15-day study noted substantial decreases in plasma testosterone as well as testicular pathology at Roundup doses of 5 and 100 mg/kg bw/day. As with the rat study conducted by Dallegrave et al. (2007), the study by Oliveira et al. (2007) tested a Brazilian formulation of Roundup, and the extent to which this formulation is applicable to formulations used in the United States is unclear. The conclusion reached by the U.S. EPA/OPP (2008a) concerning the merit of the Oliveira et al. (2007) study is similar to the conclusion reached in the current Forest Service risk assessment concerning the use of the Dallegrave et al. (2007) study. U.S. EPA/OPP (2008a) notes:

> *Further studies would be needed to determine whether or not these observed effects would affect avian (or, in this case, terrestrial-phase amphibian) reproduction.*
>
> U.S. EPA/OPP 2008a, p. 111

The current Forest Service risk assessment concurs with the EPA assessment made in U.S. EPA/OPP (2008a) and makes a similar argument for the Dallegrave et al. (2007) study discussed in detail in Section 3.3.3.3.

For acute toxicity values, the current Forest Service risk assessment adopts the same basic approach taken in U.S. EPA/OPP (2008a), and uses the NOAECs for acute dietary studies. For less toxic formulations, the NOAEC of 5000 ppm a.e. is used. As discussed above, the acute dietary studies, because they do not define adverse effects levels, cannot be used to substantiate an argument that Roundup formulations are more toxic than technical grade glyphosate. Nonetheless, the formulation studies were tested at lower concentrations (when expressed as acid equivalents). Given the general patterns in the toxicity of glyphosate versus Roundup formulations, the acute dietary studies conducted with the formulation do not provide assurance that 5000 ppm a.e. would be a NOAEC for the more toxic Roundup formulations. Consequently, for the more toxic glyphosate formulations, the acute dietary concentration of 1800 ppm a.e. is used as an acute NOAEC.

Food consumption rates are not reported for the studies used by U.S. EPA/OPP (2008a). Based on recent acute dietary studies in birds on another herbicide, aminopyralid, acute food consumption factors—i.e., kg food/kg body weight per day—for mallard ducks and bobwhite quail are in the range of 0.3 for quail and 0.42 for mallards (SERA 2007c). Using the lower factor for quail, which results in a lower and more conservative NOAEL, the NOAEC of 5000 ppm a.e. for technical grade glyphosate corresponds to a NOAEL of 1500 mg a.e./kg bw [5000 mg a.e./kg diet x 0.3 kg diet/kg bw]. Using the same approach, the NOAEC of 1800 ppm a.e. for the Roundup formulation corresponds to a NOAEL of 540 mg a.e./kg bw [1800 mg a.e./kg diet x 0.3 kg diet/kg bw].

For longer-term exposures to less toxic formulations, the current Forest Service risk assessment adopts the EPA reproductive NOAEL of 830 ppm a.e. in bobwhite quail exposed to technical grade glyphosate (MRID 108207) cited in U.S. EPA/OPP (2008a) for risk characterization. Again using food consumption data from recent reproduction studies on aminopyralid, food consumption factors for mallard ducks and bobwhite quail in longer-term dietary studies are about 0.07 for both mallards and quail (SERA 2007b). Thus, the dietary NOAEC of 830 ppm a.e. corresponds to an NOAEL of about 58 mg a.e./kg bw [830 mg a.e./kg diet x 0.07 kg diet/kg bw = 58.1 mg a.e./kg bw]. This toxicity value is based on technical grade glyphosate and is applied to the less toxic glyphosate formulations.

For the more toxic formulations, the NOAEC of 450 ppm a.e. for Roundup from the study by Kubena et al. (1981) is used. Kubena et al. (1981) reported reduced body weight (about 45% relative to controls) by the end of the study but do not provide information on food consumption. By analogy to the dietary studies on Zebra finches by Evans and Batty (1986), it is reasonable to suppose that the broilers in the study by Kubena et al. (1981) may have consumed less food than would be expected based on general allometric relationships. Nonetheless, no decrease in body weight was noted in the 450 ppm a.e. exposure group and it seems reasonable to assume that the birds in this group displayed typical food consumption. Based on the general approach recommended by U.S. EPA/ORD (1993, p. 3-4, Eq. 3-3), food consumption in birds can be estimated as:

$$F_{kg/day} = 0.0582 \times W_{kg}^{0.651}$$

For the 450 ppm a.e. groups, Kubena et al. (1981, Table 1, p. 133) report a control body weight of about approximately 0.235 kg for males and females combined. Based on the above allometric relationship, the food consumption would be about 0.0223 kg and the corresponding food consumption factor would be about 0.095 kg food/kg bw [0.0223 kg food ÷ 0.235 kg bw]. Thus, the 450 ppm a.e. NOAEC corresponds to a NOAEL of about 43 mg a.e./kg bw [450 mg a.e./kg diet x 0.095 kg diet/kg bw = 42.75 mg a.e./kg bw]. This dose level is not substantially different from the NOAEL of 58 mg a.e./kg bw used for less toxic formulations. Nonetheless, this NOAEL of 43 mg a.e./kg bw is at least based on a defined LOAEC and is used to characterize longer-term risks to birds associated with more toxic formulations.

4.3.2.3. Reptiles and Amphibians (Terrestrial-phase)

As noted in Section 4.1.2.3, the U.S. EPA does not require standard toxicity studies on terrestrial-phase amphibians, and no toxicity data are available regarding the effects of glyphosate on reptiles. The recent EPA ecological risk assessment on glyphosate (U.S. EPA/OPP 2008a) does not address the issue of reptile exposure and does not develop a dose-response assessment for terrestrial-phase amphibians. The EPA notes that: *No toxicity studies on glyphosate are available for terrestrial-phase amphibians* (U.S. EPA/OPP 2008a, p. 111). As discussed in Section 4.1.2.3, some toxicity studies on terrestrial-phase amphibians have been published since the U.S. EPA/OPP (2008a) assessment. Nonetheless, these studies do not lend themselves to the types of dose-response assessments that are conducted for mammals and birds. No dietary or gavage toxicity studies are available.

Typically, U.S. EPA/OPP and Forest Service risk assessments characterize risks to terrestrial-phase amphibians based on the risk characterization for birds (e.g., U.S. EPA/OPPTS 2004). Given the lack of standard dietary toxicity studies, no formal dose-response assessment is developed for terrestrial-phase amphibians. The available mesocosm and field studies along with the risk characterizations for mammals and birds are considered further in the risk characterization for terrestrial-phase amphibians (Section 4.4.2.3).

4.3.2.4. Terrestrial Invertebrates

Most ecological risk assessments conducted by the U.S. EPA/OPP use the honeybee as a surrogate for other terrestrial insects. U.S. EPA/OPP (2008a, Table 4.34, p. 116) uses an indefinite oral LC_{50} of >100 µg/bee for the honeybee. As discussed in Section 4.1.2.4.1 and detailed in Appendix 4, a relatively large number of acute toxicity studies have been conducted on bees and other species of terrestrial insects using both technical grade glyphosate as well as various glyphosate formulations.

For technical grade glyphosate, the oral and contact LD_{50} values are >100 µg/bee. Consistent with the EPA approach in U.S. EPA/OPP (2008a), these toxicity values are used for less toxic glyphosate formulations. Typical body weights for worker bees range from 81 to 151 mg (Winston 1987, p. 54). Taking 116 mg as an average body weight, the dose of 100 µg/bee corresponds to about 860 mg/kg bw [0.1 mg ÷ 0.000116 kg ≈ 862.07 mg/kg bw].

For glyphosate formulations, the most relevant and sensitive toxicity studies are the oral and contact bioassays in honeybees by Palmer and Krueger (2001a,b) conducted with MON 77360. As summarized in Table 3, this Monsanto code corresponds to several glyphosate formulations that contain a POEA surfactant including, Roundup Ultra Herbicide; Roundup Ultra RT

Herbicide; Roundup Pro Herbicide; Roundup Original II CA; MON 77360 Herbicide; Roundup W Herbicide; Gly 41 Herbicide. As discussed in Section 4.1.2.4.1, the contact study by Palmer and Krueger (2001a) yielded a NOAEC of 30µg/bee. This NOAEC is confirmed in U.S. EPA/OPP (2008a, Table 4.35, p. 118). Using the body weight of 116 mg for the average worker bee, this NOAEC corresponds to a dose of about 260 mg a.e./kg bw [0.03 mg ÷ 0.000116 kg ≈ 258.62 mg/kg bw]. For the oral study, the U.S. EPA/OPP (2008) designates an NOAEC of 15 µg/bee. The source of this NOAEC is not clear. As discussed in Section 4.1.2.4.1, the NOAEC from this study appears to be 50 µg/bee, corresponding to a dose of about 430 mg/kg bw [0.05 mg ÷ 0.000116 kg ≈ 431.03 mg/kg bw]. The dose of 430 mg/kg bw is used to characterize risks associated with oral exposures to more toxic formulations.

As discussed in Sections 4.1.2.4.2 and 4.1.2.4.3, several studies are available on other arthropods and other terrestrial invertebrates. These studies do not lend themselves to the development of toxicity values or HQs but are considered qualitatively in the risk characterization (Section 4.4.2.4). Nonetheless, the study by Benamu et al. (2010) using an Argentinean formulation of glyphosate suggests adverse effects on spiders fed contaminated prey. The dosing method, however, does not appear to be directly applicable to likely environmental exposures. No toxicity values in units of mg/kg bw are available on insects other than the honeybee. In an attempt to assess the consequences of the effects of glyphosate on insect diets, the oral toxicity values for the honey are used as a surrogate for herbivorous insects. Risks to this group of organisms are considered further in the risk characterization (Section 4.4.2.4).

4.3.2.5. Terrestrial Plants (Macrophytes)

4.3.2.5.1. Foliar Exposures

As discussed in Section 4.1.2.5 and summarized in Appendix 5, toxicity studies in terrestrial plants are available on both technical grade glyphosate as well as formulations of glyphosate. As would be expected, the glyphosate formulations are more toxic than technical grade glyphosate, and it is reasonable to assume that the increased toxicity is attributable to the surfactants in the formulations. While some glyphosate formulations do not contain surfactants, the product labels for these formulations recommend the use of surfactants in field solutions prior to application. While some surfactants may be more toxic than others to nontarget species, the current Forest Service risk assessment assumes that all surfactants that might be used in Forest Service programs are effective. Thus, for terrestrial vegetation, no distinction is made between less toxic and more toxic surfactants, and the dose-response assessment is based only on the toxicity data involving glyphosate formulations.

As summarized in Appendix 5, Table 2, the most sensitive species based on NOAECs in the standard toxicity studies submitted to the U.S. EPA/OPP in support of the registration of glyphosate is *Rhaphanus sativus* (a species of radish) with an NOAEC of 0.02 lb a.e./acre for a formulation specified as 80WDG, 75% a.i. (MRIDs 44125715 and 45045101). The least sensitive species based on NOAEC values is *Cyperus rotundus* (purple nutsedge) with an NOAEC of 0.445 lb a.e./acre for a formulation specified as 80WDG, 48.3% a.i. (Everett et al. 1996b, MRID 44320636).

Typically, the NOAECs of 0.02 lb a.e./acre and 0.445 lb a.e./acre would be used for sensitive and tolerant species, respectively. A reservation with this approach for sensitive species,

however, involves the open literature study by Boutin et al. (2004). As discussed in Section 4.1.2.5 and summarized in Appendix 5, Table 4, Boutin et al. (2004) assayed a European formulation of glyphosate, Roundup Bio, on 15 species of nontarget terrestrial plants. Boutin et al. (2004) report only EC_{50} values, and the lowest EC_{50} is 14.26 g a.i./ha for *Bellis perennis*, a European species of daisy. The application rate of 14.26 g a.i./ha corresponds to about 0.0094 lb a.e./acre [0.01426 kg a.i./ha x 0.892 ha/acre x 0.74 a.e./a.i. for an IPA salt ≈ 0.009413 lb a.e./acre]. The EC_{50} of 0.0094 lb a.e./acre is about a factor of 2 below the NOAEC 0.02 lb a.e./acre for radish.

Boutin et al. (2004) do not provide an NOAEC for *Bellis perennis* but they conduct a probabilistic analysis of the species sensitivity distribution for glyphosate and derive an HC_5 (concentration hazardous to the 5th percentile based on the EC_{50}) of about 5.5 g a.i./ha based on the species covered in their study and 82 g a.i./ha based on the EPA data for the 48.3% a.i. formulation considered by EPA (see Boutin et al. 2004, Table 3, p. 360). In other words, EC_{50} values for the species and formulation used in the Boutin et al. (2004) study are lower than the EPA toxicity values by a factor of about 15 [82 g a.i./ha ÷ 5.5 g a.i./ha ≈ 14.91].

The study by Boutin et al. (2004) suggests that the NOAEC of 0.02 lb a.e./acre from the registrant submitted studies might not be sufficiently protective of sensitive nontarget species of terrestrial vegetation. Consequently, the NOAEC of 0.02 lb a.e./acre is adjusted downward by a factor of 15 based on the probabilistic analysis by Boutin et al. (2004), and an estimated NOAEC of 0.0013 lb a.e./acre is used for characterizing risks to potentially sensitive species of terrestrial vegetation.

As also noted in Section 4.1.2.5.2, exposures in the range of 0.7 lbs/acre may have long-term impacts on bryophyte and lichen communities (Newmaster et al. 1999). This endpoint is not highly sensitive, compared with the much lower NOEC values used above for the quantitative dose-response assessment.

4.3.2.5.2. Soil Exposures

While soil applications are not conducted with glyphosate, soil exposure may occur, primarily through transport of glyphosate in runoff or sediment. The effects of soil exposure are assessed with studies on seedling emergence. As summarized in Appendix 5 (Table 3), glyphosate is much less toxic and less effective as an herbicide in soil exposures. Based on standard Tier 1 seedling emergence assays, the range of reported NOAECs is modest—i.e., from 3.6 lb a.e./acre (Everett et al. 1996a, MRID 44320635) to > 5 lb a.e./acre. Following the same reasoning applied to foliar exposures, NOAECs of 3.6 and 5 lb a.e./acre are used for sensitive and tolerant species of terrestrial vegetation for both less toxic and more toxic formulations.

4.3.2.6. Terrestrial Microorganisms

As noted in Section 4.1.2.6, soil microorganisms possess the shikimate pathway, and a number of studies demonstrate that glyphosate inhibits microbial growth in laboratory culture. This effect is consistent with transient decreases in populations of soil fungi and bacteria after field applications of 0.54 kg/ha or about 0.5 lbs/acre glyphosate (Chakravarty and Chatarpaul 1990), which are substantially lower than the application rates used in Forest Service programs. Also, several field studies report an increase rather than decrease in soil microorganisms or microbial activity, including populations of fungal plant pathogens, after exposure to glyphosate (Section

4.1.2.6). Although the mechanism for this apparent enhancement is unclear, it is possible that glyphosate is used as a nutrient source by soil microorganisms or that glyphosate increases the nutrients in soil, secondary to plant damage. In either case, glyphosate does not pose a clear hazard to soil microorganisms, and a dose-response assessment is not developed for this group of nontarget organisms.

4.3.3. Aquatic Organisms

4.3.3.1. Fish

The most recent EPA ecological risk assessment on glyphosate (U.S. EPA/OPP 2008a), identifies the acute LC_{50} values used as the basis for RQs as: 43 mg a.e./L for technical grade glyphosate (U.S. EPA/OPP 2008a, Table 4.4, p. 80), 1 mg a.e./L for a toxic formulation, and 224 mg a.e./L for a less toxic formulation (U.S. EPA/OPP 2008a, Table 4.5, p. 82). The chronic life-cycle study in fathead minnows with technical grade glyphosate, which identifies an NOEC of 25.7 mg a.e./L, is cited but not used to generate an RQ. An RQ, which is the abbreviation for *Risk Quotient*, is the ratio of an exposure level to a toxicity value and is analogous to the HQ (Hazard Quotient) used in Forest Service risk assessments in the quantitative expression of a risk characterization.

4.3.3.1.1. More Toxic Formulations

4.3.3.1.1.1. Acute Exposures

As summarized in Table 22, the LC_{50} values for more toxic glyphosate formulations range from 0.96 mg a.e./L (Folmar et al. 1979) to 10 mg a.e./L (Wan et al. 1989), and the lower bound of this range is basically equivalent to the 1 mg a.e./L LC_{50} cited by U.S. EPA/OPP (2008a). As discussed in Section 4.1.3.1.2.2, this range of toxicity values is based more on the conditions of exposure, particularly pH, than on species differences. In the absence of information on NOEC values, these LC_{50} values would be multiplied by a factor of 0.05 to reflect the U.S. EPA/OPP (2008a) level of concern, 0.05, for endangered aquatic species. Thus, the surrogate NOAECs would be 0.048 mg a.e./L to 0.5 mg a.e./L.

As summarized in Appendix 6 (Table 3), many acute toxicity studies report sublethal effects at much higher concentrations than the surrogate NOECs that can be derived from the studies by Folmar et al. (1979) and Wan et al. (1989). No studies, however, report sublethal effects at concentrations below 0.048 mg a.e./L. Tierney et al. (2007) notes that trout may be able to sense glyphosate, applied as Roundup, at about 0.074 mg a.e./L but will not exhibit an avoidance response at this concentration. Cericato et al. (2008, 2009) report differing results for evidence of a stress response in catfish at about 0.4 mg a.e./L but did not assay for this response at lower concentrations. Thus, the lower bound toxicity value of 0.048 mg a.e./L is not contradicted by any studies on sublethal toxicity.

As summarized in Appendix 6 (Table 2), several studies suggest that the upper bound toxicity value of 0.5 mg a.e./L may be overly conservative. For example, the study by Forbis et al. (1982a, MRID 124760) reports an LC_{50} of 1.8 mg a.e./L with a corresponding NOEC of 0.7 mg a.e./L for a Roundup formulation in a bioassay using bluegill sunfish. The ratio of the NOEC to the LC_{50} is only about 0.4, suggesting that the 0.05 factor used above to derive the surrogate NOEC is grossly conservative. Nonetheless, many registrant-submitted studies report NOECs

for mortality— i.e., no fish died, but these NOECs may not encompass concerns for sublethal effects.

In some cases, registrant-submitted studies include more detailed observations. For example, a DER (Data Evaluation Record) is available for the study by Swarbrick and Shillabeer (1999a) on rainbow trout which reports an LC_{50} of 824 mg formulation/L, an NOEC for mortality of 587.2 mg formulation/L, and an NOEC for sublethal effects of 183.5 mg formulation/L. The nature of the sublethal effects, however, is described in the DER as … *weak swimming, loss of balance and dark discoloration*. While these are sublethal effects, the nature of the effects are severe in terms of the ability of the fish to survive, and an NOEC for these types of effects does not rule out the occurrence of other more subtle but significant effects such as those noted in the other acute toxicity studies summarized in Appendix 6, Table 3. It is noted that the formulation used in the study by Swarbrick and Shillabeer (1999a) is obviously a less toxic formulation, but the issue concerning the nature of sublethal effects reported in routine acute toxicity bioassays is still valid.

A more serious concern with what may be viewed as an overly-conservative dose-response assessment for acute effects in fish is raised in the study Holdway and Dixon (1988) concerning differences in LC_{50} values between fasted and fed fish. As discussed in Section 4.1.3.1.2.1, Holdway and Dixon (1988) noted a 10-fold higher LC_{50} for technical grade glyphosate in fed fish, relative to fasted fish. As also noted in Section 4.1.3.1.2.1, virtually all acute toxicity studies in fish involve fasting prior to and during testing. This is an inherently conservative procedure that applies to all pesticides. The difference between fed and fasted fish may account for some of the lack of adverse effects observed in fish after applications of glyphosate formulations in field studies (e.g., Caffrey 1996; Hildebrand et al. 1982; Olaleye and Akinyemiju 1996; Tsui and Chu 2008). Nonetheless, fasting prior to and during testing may be appropriate to account for exposure levels in stressed populations of fish.

For the current Forest Service risk assessment, the surrogate NOECs of 0.048 and 0.5 mg a.e./L derived from the acute LC_{50} value reported by Folmar et al. (1979) [0.96 mg a.e./L x 0.05 = 0.048 mg a.e./L] and Wan et al. (1989) [10 mg a.e./L x 0.05 = 0.5 mg a.e./L] are used to characterize the risks associated with peak exposures to the more toxic glyphosate formulations.

4.3.3.1.1.2. Longer-term Exposures

As discussed in Section 4.1.3.2.4, there is no indication of a pronounced duration-response relationship for glyphosate or glyphosate formulations. Two of the four longer-term studies on glyphosate formulations report sublethal effects at concentrations in the range of about 1.2 mg a.e./L (biochemical changes indicative of liver damage in the study by Gabriel and George 2005) to 7.2 mg a.e./L (tissue degeneration but not mortality in the study by Jiraungkoorskul et al. 2003a). All of these concentrations are in the range of reported LC_{50} values for the more toxic glyphosate formulations —i.e., 0.96-10 mg a.e./L as summarized in Table 22. As discussed in the previous subsection, the relatively mild effects noted in these longer-term studies at concentrations that are lethal in acute studies may be related to the lack of fasting in the longer-term studies.

The study by Li and Kole (2004) notes that concentrations as low as 1 mg a.i./L (≈0.74 mg a.e./L) caused a transient inhibition of liver esterase on Day 8 of the 65-day exposure, which was not evident by the end of the study. In other words, the transient liver effect can be regarded as an acute sublethal response which is encompassed by the 0.048 and 0.5 mg a.e./L surrogate NOECs derived in the previous section.

The only other longer-term study with more toxic glyphosate formulations is the 2-month study conducted by Morgan and Kiceniuk (1992) in which a transient increase in aggressive behavior was noted at a concentration of about 0.046 mg a.e./L in Month 1 of the study. The toxicological significance of the decrease in aggressive behavior at Month 2 at a concentration of about 0.004 mg a.e./L is questionable, because the effect was not seen at higher concentrations and there were no effects on fish growth or evidence of gill pathology. Thus, while U.S. EPA/OPP (2008a, Table 4.9, p. 87) classifies 0.046 mg a.e./L as a LOAEC, the severity of the effect appears to be marginal.

Because of the lack of any substantial duration-response relationship, the 0.048 and 0.5 mg a.e./L surrogate NOECs derived in the previous section for acute exposure are maintained and used to characterize risks associated with longer-term exposures to more toxic formulations.

4.3.3.1.2. Less Toxic Formulations

4.3.3.1.2.1. Acute Exposures

As discussed in Section 4.1.3.1.2.1 and detailed in Appendix 6 (Table 6), there are numerous LC_{50} determinations for technical grade glyphosate, and the 96-hour LC_{50} values range from 10 mg a.e./L for chum salmon at pH 6.3 from the study by Wan et al. (1989) to 240 mg a.e./L for rainbow trout from the study by Folmar et al. (1979). Notably, this range does not include the LC_{50} of 620 mg a.e./L reported by Neskovic et al. (1996). The test material used in this study is described as *technical grade* but the purity of the test material is reported as only 62%.

The study conducted by Mitchell et al. (1987a) which reports an LC_{50} of 580 mg a.i./L or about 430 mg a.e./L [580 mg a.i./L x 0.74 a.e./a.i. = 429.2 mg a.e./L] in rainbow trout exposed to Rodeo is clearly relevant. Although the study does not specify the pH for the bioassay involving only Rodeo, in the discussion of bioassays involving a mixture of Rodeo and X-77 surfactant, Mitchell et al. (1987a, p. 1032) note that the pH in these studies was 7.8. Wan et al. (1989) report an LC_{50} of 93 mg a.e./L glyphosate in rainbow trout at the same pH. While LC_{50} of 93 mg a.e./L glyphosate is about a factor of 4 less than the LC_{50} of Rodeo (as a.e. equivalents) assayed by Mitchell et al. (1987a), this discrepancy is not uncommon in toxicity studies conducted by different investigators at different times. Mitchell et al. (1987a, 1031) discuss the differences between their study and another study on glyphosate and note: *The reason for this difference in toxicity is unknown, but could be due to differences in bioassay testing methodology*.

Given that both Folmar et al. (1979) and Wan et al. (1989) controlled pH in their studies, there is no reason not to apply the toxicity values from these studies to less toxic formulations of glyphosate. In the EPA dose-response assessment (U.S. EPA/OPP 2008a, Table 4.4, p. 80), the toxicity value for technical grade glyphosate is 30 mg a.e./L (MRID 44320630). As summarized in Appendix 6 (Table 6), this LC_{50} is from an assay in bluegill sunfish from an unpublished registrant-submitted study. The somewhat lower LC_{50} of 10 mg a.e./L from the study by Wan et al. (1989) is reasonably close to the toxicity value used by the. EPA and may better reflect the

toxicity of glyphosate to sensitive species of fish at a low but environmentally realistic pH. Consequently, the LC_{50} of 10 mg a.e./L is used to derive a toxicity value for sensitive species of fish. The upper bound LC_{50} is taken as 429.2 mg a.e./L from the study by Mitchell et al. (1987a). As discussed above, this study is clearly relevant to the assessment of risks associated with exposures to less toxic formulations of glyphosate.

For the dose-response assessment for fish, the LC_{50} values of 10 and 429.2 mg a.e./L are multiplied by a factor of 0.05 and rounded to two significant places to derive surrogate NOAECs of 0.5 and 21 mg a.e./L. The factor of 0.05 is consistent with the general approach taken by U.S. EPA/OPPTS (2004) in the use of a level of concern for endangered species of fish in the interpretation of RQs based on LC_{50} values for aquatic species. While these surrogate NOAECs are applied to sensitive and tolerant species of fish, the variability in the NOAECs may more properly reflect differences in exposure conditions, particularly the pH of surface water.

As is the case with the dose-response assessment for acute exposures to the more toxic formulations, there are no studies regarding the acute sublethal toxicity of glyphosate or the less toxic glyphosate formulations to suggest that the surrogate NOAECs will not be sufficiently protective. To the contrary, and as with the surrogate NOAECs for the more toxic formulations, the study by Holdway and Dixon (1988) on fasted versus fed fish suggests that the surrogate NOAECs could be overly conservative.

4.3.3.1.2.2. Longer-term Exposures

As discussed in Section 4.1.3.1.4 and summarized in Appendix 6 (Table 7), the only longer-term toxicity study on technical grade glyphosate is the life-cycle study in fathead minnows in which the NOEC is 25.7 mg a.e./L and a LOEC was not determined. The NOEC of 25.7 mg a.e./L is in the lower part of the range of acute LC_{50} values for technical grade glyphosate. In life-cycle studies, fish are obviously fed. That the NOEC from the life-cycle study is within the range of acute LC_{50} values reinforces concern that the use of fasted fish in acute lethality studies may substantially overestimate the sensitivity of many field populations of fish to glyphosate. Nonetheless, the acute LC_{50} values may be reasonable approximations of the toxicity of glyphosate to stressed populations of fish.

In any event, the longer-term NOEC of 25.7 mg a.e./L clearly supports the lack of a duration-response relationship for fish. As with the more toxic formulations of glyphosate, the surrogate acute NOAECs are applied to longer-term exposure scenarios.

4.3.3.2. Amphibians, Aquatic-Phase

In the most recent EPA risk assessment on glyphosate (U.S. EPA/OPP 2008a), the only toxicity values clearly designated for the derivation of risk quotients are a chronic NOAEC of 1.8 mg a.e./L for technical grade glyphosate IPA and the NOAEC/LOAEC of 0.6/1.8 mg a.e./L for Roundup Original and Roundup Transorb (U.S. EPA/OPP 2008a, Table 4.13, p. 93). All of these toxicity values are taken from the open literature publication by Howe et al. (2004). The data referenced by U.S. EPA/OPP (2008a) are illustrated in Figure 1 of the Howe et al. (2004, p. 1933) publication. U.S. EPA/OPP (2008a) does not identify acute toxicity values for the risk characterization of glyphosate, glyphosate IPA, or glyphosate formulations in amphibians.

As summarized in Appendix 7 (Table 5), the NOAEC for technical grade glyphosate IPA is from a 42-day exposure study of Gosner stage 25 larvae in which no statistically significant effects were noted on the number of days required to reach Gosner stage 42, percent survival to Gosner stage 42, or larval length. This NOAEC is consistent with the data in Howe et al. (2004).

The NOAEC of 0.6 mg a.e./L for Roundup identified in U.S. EPA/OPP (2008a) does not appear to be consistent with the data in Howe et al. (2004). As illustrated in Figure 1 of Howe et al. (2004), a decrease in length as well as a decrease in survival to Gosner stage 42 was noted at concentrations of 0.6 and 1.8 mg a.e./L for both Roundup Original and Roundup Transorb. The only NOAEC identified in the study appears to be for the number of days required to reach Gosner stage 42 at a concentration of 0.6 mg a.e./L for Roundup Original. Given the effects on decreased length and survival, the 0.6 mg a.e./L exposure to Roundup Original cannot be classified as an NOAEC. In addition, as illustrated in Figure 2 of the Howe et al. (2004) publication, an increase in intersex gonads was noted for Roundup Original at concentrations of 0.6 and 1.8 mg a.e./L. For both Roundup formulations tested in the 42 day exposures by Howe et al. (2004), the concentration of 0.6 mg a.e./L appears to be an adverse effect level. Given that the endpoints involved mortality and the development of intersex gonads, the exposure should probably be classified as a frank effect level (FEL) rather than an LOEC.

4.3.3.2.1. More Toxic Formulations

4.3.3.2.1.1. Acute Exposures

As summarized in Table 25 and discussed in Section 4.1.3.2.2.2, the acute LC_{50} values for more toxic formulations of glyphosate range from about 0.8 mg a.e./L (Relyea and Jones 2009) to 51.8 mg a.e./L (Mann and Bidwell 1999). This range of LC_{50} values is very similar to the range of LC_{50} values in fish for more toxic formulations of glyphosate —i.e., 0.96-10 mg a.e./L (Section 4.3.3.1.1.1). In other words, based on the acute bioassays with the more toxic formulations of glyphosate, the sensitivities of fish and aquatic-phase amphibians to glyphosate appear to be virtually identical. For amphibians, the more toxic formulations of glyphosate on which toxicity data are available include various formulations of Roundup, Vision, and Glyfos.

The dose-response assessment for amphibians is developed in the same manner as for fish, and the rationale for this approach is identical to that for fish (Section 4.3.3.1.1.1). As a first approximation, the LC_{50} values of 0.8 and 51.8 mg/L are multiplied by 0.05 and rounded to two significant places following the standard LOC approach from U.S. EPA/OPPTS (2004). Using this approach, surrogate NOAECs are estimated at 0.040 and 2.6 mg a.e./L.

As with fish, a number of acute bioassays report NOAECs or low mortality rates such as 5 or 10% response rates (e.g., Bernal et al. 2009a; Edginton et al. 2004a; Perkins et al. 2000; Relyea and Jones 2009; Wojtaszek et al. 2004). For some pesticides, NOAECs for mortality may be used directly in the dose response assessment. In other cases, low response rates for mortality (e.g., LC_1, LC_5, or even LC_{10} values) may be treated as surrogate NOAECs and used directly in the dose-response assessment. If this approach were taken with more toxic glyphosate formulations, the factor of 0.05 (which amounts to an uncertainty factor of 20) could be viewed as grossly conservative.

For the more toxic glyphosate formulations, many of the dose-response curves appear to be very steep. For example, in the study by Edginton et al. (2004), the maximum ratio of the LC_{50} to the

LC_{10} for any species and life stage is about 2.5—i.e., *Xenopus laevis* larvae in Table 2 of the publication. In most cases, the ratio of the LC_{50} to the LC_{10} as reported by Edginton et al. (2004) is less than a factor of 2. As summarized in Appendix 7 (Table 2), the maximum ratio of the LC_{50} to the LC_{10} for any species is about 8—i.e., the ratio for spring peeper (*Pseudacris crucifer*) from the study by Relyea and Jones (2009). Thus, an argument could be made for using a higher factor than 0.05 to derive a surrogate NOAEC.

The direct use of NOAECs, however, should be done in the expectation that the surviving animals are not adversely affected. As with fish, there are concerns that this is not the case for surviving amphibians in acute toxicity studies with the more toxic glyphosate formulations. For example, in the study by Lajmanovich et al. (2003), various malformations were noted in surviving tadpoles, including ocular and other facial malformations as well as deformed tails. Similarly, in the study by Edginton et al. (2004, Table 3, p. 820), observations in some groups of organisms surviving the acute toxicity bioassays indicated significant ($p < 0.05$) growth inhibition relative to controls – i.e., embryos of *Xenopus laevis* and *Rana clamitans* as well as both embryos and larvae of *Rana pipiens*. Of the observations involving statistically significant levels of growth inhibition, the most pronounce inhibition (i.e., 68% growth relative to controls) was observed in larvae of *Rana pipiens*. Thus, while the use of the 0.05 factor from U.S. EPA/OPPTS (2004) may be somewhat conservative, this seems justified in view of the adverse effects noted in amphibians surviving acute bioassays.

A converse concern with application of the 0.05 factor to LC_{50} values is that the surrogate NOAECs may not be sufficiently protective—i.e., sublethal but significant effects could occur below the surrogate NOAEC. For the more toxic formulations of glyphosate, no information is available that supports this concern. As discussed in Section 4.1.3.2.3 and summarized in Appendix 7 (Table 4), most sublethal toxicity studies in amphibians have been conducted at concentrations that are in the range of or close to the lower bound of LC_{50} values—i.e., 0.8 mg a.e./L. The lowest acute *LOAEC* listed in Appendix 7 is 0.55 mg a.e./L from the study by Smith (2001). In this study, however, the effect is mortality, and mortality at a concentration of 0.55 mg a.e./L is not a peculiar observation in that this concentration is close to the lower bound of the LC_{50} values for amphibians. Nonetheless, no reports of sublethal effects near the lower bound of the estimated NOAEC of 0.040 mg a.e./L are available.

Based on the above discussion, the general approach used by U.S. EPA/OPPTS (2004) seems appropriate—i.e., a level of concern of 0.05 (RQ=0.05) based on ratio of exposure to the LC_{50} appears to be an appropriate basis for the dose-response assessment for acute exposures to more toxic formulations of glyphosate. Because the Forest Service prefers a fixed level of concern (HQ=1), the LC_{50} values of 0.8 mg a.e./L (Relyea and Jones 2009) and 51.8 mg a.e./L (Mann and Bidwell 1999) are multiplied by a factor of 0.05 and the surrogate NOAECs are taken as 0.04 mg a.e./L for sensitive species [0.8 mg a.e./L x 0.05] and 2.6 mg a.e./L for tolerant species [51.8 mg a.e./L x 0.05 = 2.59 mg a.e./L ≈ 2.6 mg/L].

4.3.3.2.1.2. Longer-term Exposures

The dose-response assessment for longer-term exposures to more toxic formulations of glyphosate is problematic. As discussed in Section 4.3.3.2, the EPA identified a chronic NOAEC for amphibians of 0.6 mg a.e./L for Roundup Original from the study by Howe et al.

(2004) (U.S. EPA/OPP 2008a). While Forest Service risk assessments will typically defer to the U.S. EPA/OPP at least in terms of study selection, the designation of 0.6 mg a.e./L appears to be an error, and the concentration of 0.6 mg a.e./L appears to be a frank effect level based on several endpoints, including survival, growth, and the development of intersex gonads.

Based on acute LC_{50} values of 0.8 and 42.2 mg a.e./L, acute NOAECs of 0.04 and 2.2 mg a.e./L are used for sensitive and tolerant species of amphibians. In the study by Howe et al. (2004, Figure 1), the 42-day exposures to Roundup Original at concentrations of 0.6 and 1.8 mg a.e./L resulted in about 50% survival to Gosner stage 42 at both concentrations. For Roundup Transorb, however, the survival rate appears to be about 40% at 0.6 mg a.e./L and 20% at 1.8 mg a.e./L. The survival rate in the control group, however, appears to have been about 80%; thus, the survival rates in the exposed groups are not directly comparable to the acute LC_{50} values.

Typically, a LOAEL might be divided by a factor of 10 to approximate an NOAEC. This approach is analogous to the use of uncertainty factors in the human health risk assessment. Thus, the 0.6 mg a.e./L effect level concentration would be adjusted to 0.06 mg a.e./L to approximate an NOAEC. The lower bound of the acute NOAECs is 0.04 mg a.e./L and may be considered sufficiently protective for longer-term exposures. The only residual concern with this approach is the severity of the effects seen in the Howe et al. (2004) study at 0.6 mg a.e./L.

The 16-day study by Relyea (2005a), however, does provide some level of reassurance that a surrogate NOAEC of 0.04 mg a.e./L is sufficiently protective. In addition to the 16-day LC_{50} studies summarized in Appendix 7 (Table 5), Relyea (2005a) examined the effect of predator stress on survival. For the most sensitive species, the wood frog, the exposure of 0.1 mg a.e./L evidenced lower survival; however, this trend was not close to statistical significance ($p=0.304$). Thus, the study by Relyea (2005a) provides a relevant measure of a reasonably subtle and environmentally relevant endpoint, and the NOEC of 0.1 mg a.e./L supports the use of the surrogate NOAEC for acute exposures of 0.04 mg a.e./L.

Accordingly, as with the dose-response assessment for fish, the surrogate acute NOAECs of 0.04 and 2.6 mg a.e./L are applied to longer-term exposures.

4.3.3.2.2. Less Toxic Formulations

4.3.3.2.2.1. Acute Exposures

In many respects, the dose-response assessment for acute exposures of amphibians to less toxic formulations is similar to that of fish. In terms of glyphosate acid, few acute bioassays are available on amphibians, and the range of acute LC_{50} values in amphibians is narrow, from about 75 mg a.e./L (MRID 43839601) to about 120 mg a.e./L (Mann and Bidwell 1999). As discussed in Section 4.3.3.1.2.1, the range of LC_{50} values for glyphosate acid in fish is much broader, from10 to about 240 mg a.e./L. This difference, however, may simply reflect the greater number of acute toxicity studies on fish as well as the more extreme conditions (particularly in terms of pH) in the fish bioassays by Folmar et al. (1979) and Wan et al. (1989).

A difference between the data base on fish and amphibians, however, involves differences in the data on glyphosate IPA. As discussed in Section 4.3.3.1.2.1, the study by Mitchell et al. (1987a) suggests that glyphosate IPA may be less toxic than glyphosate acid, even under conditions in which pH is controlled. In amphibians, the lesser toxicity of glyphosate IPA is more fully

documented. The documentation for the lesser toxicity of glyphosate IPA relative to glyphosate acid, is most strongly supported in the study by Mann and Bidwell (1999) which involves three species of amphibians. As detailed in Appendix 7 (Table 1), the LC_{50} values for glyphosate acid ranged from 81.2 to 121 mg a.e./L. In the corresponding acute bioassays with glyphosate IPA, all of the LC_{50} values are non-definitive and are reported as >343 to >466 mg a.e./L. Although the bioassays are matched in only one species (*Litoria moorei*), these bioassays along with the supporting data from Howe et al. (2004) summarized in Appendix 7 (Table 1) clearly indicate that glyphosate IPA is less acutely toxic than glyphosate acid to amphibians. The lower toxicity of glyphosate IPA relative to glyphosate acid is also supported by the definitive LC_{50} of 7297 mg a.e./L for Rodeo in frog embryos (Perkins et al. 2000).

Mann and Bidwell (1999, p. 197) classify glyphosate IPA as essentially nontoxic and indicate that … *no mortality was observed in equivalent concentrations of glyphosate IPA*. The term *equivalent* refers to other bioassays on Roundup Biactive. As discussed by Mann and Bidwell (1999), the differences between the toxicity of glyphosate IPA and glyphosate acid relates to pH. Unlike the studies by Folmar et al. (1979) and Wan et al. (1989) on glyphosate acid in fish, pH was not controlled in the bioassays on glyphosate acid, and the amphibians were subject to a pH of less than 3. The contributions of the individual stressors – i.e., glyphosate and pH – or a joint action of the two stressors cannot be determined.

Unlike the case with fish, the above data are sufficiently compelling to assert that the lower toxicity values for glyphosate acid are not appropriate for the dose-response assessment. All of the less toxic formulations of glyphosate likely to be used in Forest Service programs (Table 2 and Table 4) contain glyphosate IPA as the active ingredient. Consequently, for amphibians, the dose-response assessment for less toxic formulations is based on studies using glyphosate IPA.

While the indefinite LC_{50} values of >343 mg to 466 mg a.e./L can be clearly viewed as NOAECs for lethality, it is less clear that these concentrations are NOAECs for more subtle endpoints. No sublethal toxicity studies have been identified on glyphosate IPA, Rodeo, or equivalent formulations. The lack of more detailed sublethal toxicity studies on glyphosate IPA, Rodeo, and other similar formulations is treated qualitatively as a data gap. There is no apparent reason to apply the 0.05 factor or any other arbitrary uncertainty factor to the indefinite LC_{50} values. Thus, the NOECs of 343 and 466 mg a.e./L are accepted without modification. These NOAECs are rounded to two significant places—i.e., 340 and 470 mg a.e./L—and are used as NOECs for sensitive and tolerant species of amphibians, respectively.

4.3.3.2.2.2. Longer-term Exposures

The dose-response assessment for longer-term exposures of amphibians to less toxic formulations is extremely simple. As discussed in Section 4.1.3.2.4 and summarized in Appendix 7 (Table 5), only one longer-term study is available (Howe et al. 2004). In this study, leopard frogs (*Rana pipiens*) were exposed to glyphosate IPA at a concentration of 1.8 mg a.e./L for 42 days from Gosner stage 25 through Gosner stage 42, and no adverse effects were noted on growth, development (including the lack of any intersex gonads), or survival.

Because the longer-term NOAEC of 1.8 mg a.e./L is the only data available, risks to sensitive and tolerant species cannot be distinguished. While this data gap adds some uncertainty to the

risk assessment for amphibians, the acute data on glyphosate IPA and Rodeo as well as the lack of a concentration-duration relationship for other aquatic organisms suggest that the free-standing NOAEC of 1.8 mg a.e./L is a highly conservative NOAEC—i.e., it is likely that no adverse effects would be observed at higher and possibly much higher concentrations of glyphosate IPA.

4.3.3.3. Aquatic Invertebrates

4.3.3.3.1. More Toxic Formulations

4.3.3.3.1.1. Acute Exposures

For exposures of aquatic invertebrates to glyphosate formulations, U.S. EPA/OPP (2008a, Table 4.17, p. 96) identifies acute 48-hour EC_{50} values from which to derive risk quotients ranging from 2.2 to 44.8 mg a.e./L for glyphosate formulations. Both of these EC_{50} values are from bioassays conducted using *Daphnia magna*. As summarized in Appendix 8 (Table 2), the lower bound EC_{50} is from the study by Folmar et al. (1979) using the original Roundup formulation. In Appendix 8, Table 2, the 48-hour EC_{50} from Folmar et al. (1979) is given as 3 mg a.e./L rather than 2.2 mg a.e./L. This discrepancy is due to the interpretation by the U.S. EPA/OPP (2008a) that the LC_{50} values for the formulation are reported in units of mg a.i./L. As detailed in Footnote 2 to this table in Appendix 8, the current Forest Service risk assessment interprets the formulation LC_{50} values reported in Folmar et al. (1979) as reported in units of mg a.e./L. The upper bound LC_{50} of 44.8 mg a.e./L is from the registrant submitted study by Swarbrick and Shillabeer (1999b, MRID 45374003). The formulation used in the study by Swarbrick and Shillabeer (1999b) is specified only as YF11357. This formulation appears to contain glyphosate IPA at a concentration of 27.24% and does not correspond to any of the formulations identified by the Forest Service (Table 2).

As summarized in Table 26, the current Forest Service risk assessment has identified a similar but somewhat broader range of acute toxicity values—i.e., a 48-hour LC_{50} of 1.5 to 46 mg a.e./L. Both of these LC_{50} values are for amphipods. The lower toxicity value is from the study by Tsui and Chu (2004) using a Roundup formulation from Monsanto USA and the higher LC_{50} is from the study by Folmar et al. (Folmar et al. 1979) using the original Roundup formulation. For the current Forest Service risk assessment, the modestly broader range of toxicity values from Table 26 is used as the basis for the dose-response assessment.

As discussed in Section 4.1.3.3.2.2, toxicity data on some formulations of glyphosate that contain surfactants, including Accord XRT, Accord XRT II, MON 14420, have EC_{50} values near the upper bound of the reported EC_{50} values for Roundup formulations. These formulations may be less toxic than the original Roundup and some of the other current formulations of glyphosate/surfactant. Few toxicity studies, however, are available on these potentially less toxic glyphosate/surfactant formulations. Consequently, a separate and higher set of toxicity values is not derived for these formulations.

As with fish and amphibians, the first approximation to estimating NOAECs is made by multiplying the range of acute EC_{50} values by the factor of 0.05 (U.S. EPA/OPPTS 2004) to approximate surrogate NOAECs of 0.075 mg a.e./L [1.5 mg a.e./L x 0.05] to 2.3 mg a.e./L [46 mg a.e./L x 0.05]. Also as with fish and amphibians, several studies summarized in Appendix 8 (Table 2) indicate very steep concentration-response curves and ratios of NOAEC to EC_{50} values

that are much greater than 0.05. Again, this suggests that the application of the 0.05 factor (i.e., equivalent to a safety factor of 20) may be overly conservative.

Also as with fish and amphibians, however, information on glyphosate suggests that the direct use of NOAECs from standard acute toxicity studies may not be sufficiently protective. As discussed in Section 4.1.3.3.3, the early study by Hartman and Martin (1984) in *Daphnia pulex* and the more recent and detailed study by Tsui and Chu (2003) in *Ceriodaphnia dubia* indicate that elevated concentrations of suspended sediments will enhance the toxicity of glyphosate formulations to filter feeders. Normal bioassays in aquatic invertebrates do not involve substantial concentrations of suspended sediments. The study by Tsui and Chu (2003) indicates that high concentrations of suspended sediments may increase the toxicity of Roundup to filter feeders by a factor of up to about 10—i.e., an EC_{50} of 5.38 mg a.e./L versus and EC_{50} of 0.59 mg/L with suspended clay at a concentration of 200 mg/L. While somewhat speculative, this finding also suggests that benthic organisms could be more sensitive than generally pelagic organisms.

Another reservation with the use of NOAECs from standard acute toxicity studies is based on the observations by Achiorno et al. (2008) in horsehair worms (*Chordodes nobilii*) exposed to a *Roundup-like* formulation. In this species, the LC_{50} for adult worms is 1.76 mg a.e./L. While much lower concentrations had no impact on larval development in short-term exposures, the exposure of eggs to concentrations as low as 0.1 mg a.e./L, a factor of 0.056 of the adult LC_{50}, was associated with a subsequent decrease in larval infectivity.

Because of concerns with exposure factors that could enhance the toxicity of glyphosate formulations to some groups of invertebrates, such as filter feeders, as well as concerns for sublethal effects that may not be adequately reflected in NOAECs from acute bioassays, the adjustment factor of 0.05 to reflect the standard level of concern from U.S. EPA/OPPTS (2004) is maintained, and the surrogate acute NOAECs of 0.075 and 2.3 mg a.e./L are used to characterize the risks to aquatic invertebrates associated with applications of more toxic glyphosate formulations.

4.3.3.3.1.2. Longer-term Exposures

The U.S. EPA/OPP (2008a) does not identify a chronic study on more toxic glyphosate formulations in the dose-response assessment for aquatic invertebrates. As discussed in Section 4.1.3.3.4 and summarized in Appendix 8 (Table 4), longer-term toxicity studies in Roundup or essentially equivalent Vision formulations do not suggest any substantial duration-response relationship for the more toxic formulations of glyphosate.

As discussed in the previous subsection, surrogate NOAECs of 0.075 mg a.e./L and 2.3 mg a.e./L are used for acute exposures. Chen et al. (2004) note decreased reproductive performance in a cladoceran (*Sirnocephalus vetulus*) exposed for 8 days to 0.75 mg a.e./L of a Vision formulation and pH 7.5. This effect was not noted at pH 5.5. The failure to detect a significant effect, relative to the control group, at pH 5.5 may have resulted from lower reproduction rates in the control group at this pH, due to pH stress. In any event, the concentration of 0.75 mg a.e./L is close to the lower bound of the acute LC_{50} for Roundup and Vision formulations (i.e., 1.5 mg a.e./L); moreover, Chen et al. (2004) noted substantial mortality in *Sirnocephalus vetulus* adults

at this concentration. In other words, adverse reproductive effects are to be expected at lethal concentrations. The results from Chen et al. (2004) are remarkably similar to the results in the earlier study by Hartman and Martin (1984) in which a transient decrease in reproductive capacity was noted in *Daphnia pulex* exposed to concentrations as low as 1 mg a.i./L or about 0.74 mg a.e./L. Risks to these species, all of which would be classified as sensitive, are encompassed by the acute NOAEC of 0.075 mg a.e./L.

Given the above considerations and in the absence of studies that demonstrate a substantial duration-response relationship for more toxic glyphosate formulations, the surrogate acute NOAECs 0.075 mg a.e./L for sensitive species and 2.3 mg a.e./L for tolerant species are used to characterize risks associated with longer-term exposures.

4.3.3.3.2. Less Toxic Formulations

For the calculations of RQs associated with acute exposures of aquatic invertebrates to glyphosate, the EPA selected the acute LC_{50} of 53.2 mg a.e./L in midge larvae from the study by Folmar et al. (1979) (U.S. EPA/OPP 2008a, Table 4.16, p. 96). For longer-term exposures, the EPA selected the life-cycle NOAEC of 49.9 mg a.e./L in *Daphnia magna* from the study by McKee et al. (1982) (U.S. EPA/OPP 2008a, Table 4.20, p. 99). While U.S. EPA/OPP (2008a) identifies the test compound as glyphosate IPA, the DER for this study is ambiguous. A comparison of the nominal and measured (glyphosate) concentrations suggests that the technical material may have been glyphosate acid.

4.3.3.3.2.1. Acute Exposures

A discussed above, the acute toxicity data on glyphosate acid and glyphosate IPA in amphibians indicate that glyphosate IPA is less toxic than glyphosate acid, probably due to effects on pH (Section 4.3.3.2.2.1). For aquatic invertebrates, the studies on the toxicity of glyphosate acid relative to glyphosate IPA are not consistent (Appendix 8, Table 1).

In a bioassay using juvenile and larval stages of a species of freshwater mussel, Bringolf et al. (2007) found that glyphosate IPA is much more toxic than glyphosate acid and that the toxicity of glyphosate IPA is probably attributable to isopropanol amine. In matched bioassays on two species of aquatic arthropods, however, Tsui and Chu (2003) found that glyphosate IPA is less toxic than glyphosate acid by factors of about 1.4-2.8. Tsui and Chu (2003) report a 48-hour LC_{50} of 415 mg a.e./L in *Ceriodaphnia dubia*. As summarized in Table 26, the LC_{50} values in aquatic arthropods for less toxic formulations of glyphosate in which the IPA salt of glyphosate is the active ingredient (a.i.) range from 218 mg a.e./L (*Daphnia magna*, Henry et al. 1994) to 4140 mg a.e./L (*Chironomus riparius* larvae, Buhl and Faerber 1989). In addition, Bringolf et al. (2007) reports LC_{50} values >148 mg a.e./L in juvenile and larval mussels for Aqua Star, a formulation of glyphosate IPA that does not contain a surfactant. Finally, the study by Bringolf et al. (2007) also assayed toxic formulations of glyphosate in the freshwater mussel and, the LC_{50} values in these assays suggest that the mussel is no more sensitive than aquatic arthropods.

The study by Bringolf et al. (2007) could be used to propose a relatively low toxicity value for glyphosate IPA, based on the reported LC_{50} values of 5 mg a.e./L in larvae and 7.2 mg a.e./L in juvenile mussels, which would be the most conservative approach. The study by Bringolf et al. (2007) is published in the open literature, is well reported, and appears to have been well conducted. The very low LC_{50} values, however, are clearly contrary to the very high LC_{50} value

for the Aqua Star formulation. In discussing these conflicting results, Bringolf et al. (2007) note the following:

> *Further research is needed to understand why technical-grade glyphosate IPA was toxic but a formulation based on the same active ingredient was not. Other components of the formulation may have influenced the liberation of ammonia, which resulted in the low toxicity of Aqua Star.*
>
> Bringolf et al. 2007, p. 2098.

This section of the current Forest Service risk assessment is concerned with relatively nontoxic glyphosate formulations, such as Aqua Star, Accord, and Rodeo. Given the high and indefinite LC_{50} for Aqua Star, it does not seem appropriate to use the very low LC_{50} values for glyphosate IPA from the study by Bringolf et al. (2007) in the dose-response assessment of formulations that are less toxic to aquatic invertebrates.

As noted above, the LC_{50} values for less toxic formulations of glyphosate IPA (i.e., Rodeo) range from 218 to 4140 mg a.e./L (Table 26). This range is the most appropriate set of values on which to base a dose-response assessment for less toxic formulations of glyphosate. As noted at the start of Section 4.3.3.3.2, however, the EPA selected the acute LC_{50} of 53.2 mg a.e./L in midge larvae (*Chironomous plumosus*) from the study by Folmar et al. (1979) as the basis for the risk characterization (U.S. EPA/OPP 2008a). Unless there is a compelling reason to do otherwise, Forest Service risk assessments do not adopt an approach that is less conservative/protective than that used by the U.S. EPA. The LC_{50} of 53.2 mg a.e./L is only about a factor of 4 below the lower bound of the LC_{50} values for less toxic formulations of glyphosate. While the use of the very low LC_{50} values from Bringolf et al. (2007) could substantially distort the risk characterization, the modestly lower LC_{50} used in U.S. EPA/OPP (2008a) would not. Consequently, the dose-response assessment is based on LC_{50} values ranging from 53.2 (Folmar et al. 1979) to 833 mg a.e./L (Buhl and Faerber 1989).

Applying the adjustment factor of 0.05 (U.S. EPA/OPPTS 2004) and rounding to two significant places, the surrogate NOAECs are estimated as 2.7 mg a.e./L [53.2 mg a.e./L x 0.05 = 2.66 mg a.e./L] sensitive species and 210 mg a.e./L [4140 mg a.e./L x 0.05 = 207 mg a.e./L] for tolerant species. Note that the upper bound value of 210 mg a.e./L is taken as 207 mg a.e./L rounded upward to 2 significant digits. While this modestly increases the toxicity value, this increase has no impact on the risk characterization (Section 4.4.3).

The only reservation with the above surrogate NOAECs is associated with the study by Achiorno et al. (2008) in horsehair worms (*Chordodes nobilii*). As discussed in Section 4.3.3.3.1.1, Achiorno et al. (2008) tested an unspecified *Roundup-like* formulation of glyphosate and noted a decrease in infectivity of horsehair worm larvae at concentrations as low as 0.1 mg a.e./L. Achiorno et al. (2008) also assayed technical grade glyphosate and reported essentially identical results. This is the only study reporting a similarity between the potency of technical grade glyphosate and more toxic glyphosate formulations. As with the study by Bringolf et al. (2007), the publication is clearly reported with no substantial deficiencies. In addition, the effects are clearly concentration dependent. This study raises concern for effects in this species exposed to less toxic formulations of glyphosate. It is far less clear that these or comparable effects would

be seen in other species. Consequently, this study is not incorporated into the dose-response assessment.

4.3.3.3.2.2. Longer-term Exposures

As noted in Section 4.3.3.3.2, U.S. EPA/OPP (2008a) uses the daphnid chronic NOAEC of 49.9 mg a.e./L to derive RQs for longer-term effects in aquatic invertebrates. This clearly a relevant study for longer-term effects associated with the use of less toxic formulations of glyphosate. Nonetheless, as noted above, this toxicity value is higher than the surrogate acute NOAECs of 2.7 and 42 mg a.e./L. In other words, as with most other groups of aquatic organisms, there is no evident duration-response relationship for glyphosate.

As summarized in Appendix 8 (Table 4), daphnids do not appear to be the most sensitive aquatic invertebrates. As with acute toxicity studies, there is a suggestion that mollusks may be more sensitive. Christian et al. (1993) note that exposure to concentrations of 0.1 to 10 mg a.e./L for 4 weeks caused biochemical changes suggestive of effects on liver function in snails. None of these changes, however, were clearly concentration dependent. Over the same range of concentrations, Tate et al. (1997) observed adverse reproductive effects in snails in a multigeneration study. Most of the effects, however, do not appear to be concentration related. Nonetheless, a substantial decrease in egg hatchability was noted at a concentration of 10 mg a.e./L in third generation snails (Tate et al. 1997, Figure 2, p. 288). The NOAEC for this effect was 1 mg a.e./L. This NOAEC is somewhat lower than the surrogate NOAEC derived from LC_{50} values—i.e., the subchronic toxicity data indicate that the surrogate NOAEC is not sufficiently protective.

For the current Forest Service risk assessment, the lower bound of the NOAEC is taken as 1 mg a.e./L from the study by Tate et al. (1997). This NOAEC is used to characterize risks for sensitive species of aquatic invertebrates. For tolerant species, the surrogate acute NOAEC of 210 mg a.e./L is maintained for longer-term exposures. This concentration is very close to and supported by the NOAEC of 50 mg a.e./L in the chronic daphnid study.

4.3.3.4. Aquatic Plants

4.3.3.4.1. Algae

4.3.3.4.1.1. More Toxic Formulations

For the risk characterization of algae, U.S. EPA/OPP (2008a) uses an EC_{50} of 0.12 mg a.e./L for more toxic glyphosate formulations (MRID 45666701). As summarized in Table 27, this is the lowest reported EC_{50} in algae and is obtained from a study in *Navicula pelliculosa* using Glyphos.

For algae as well as macrophytes, the adjustment factor of 0.05 is not used by U.S. EPA/OPP. As an alternative, risks to non-endangered species are characterized with an EC_{50} and an LOC of 1 and risks to threatened and endangered species are based on an NOAEC or an EC_5. The Forest Service has elected not to use an EC_{50} for risk characterization. Analogous to the approach for aquatic animals, risk characterizations in Forest Service risk assessments treat all species as if they were endangered. Thus, risks to algae are characterized based on an NOAEC.

As detailed in Appendix 9 (Table 2), an NOAEC of 0.082 mg a.e./L is reported for *Navicula pelliculosa* in the registrant-submitted study on Glyphos with the EC_{50} of 0.12 mg a.e./L (MRID 45666701). No studies in algae report an adverse effect level below 0.082 mg a.e./L. Wong (2000) reports a stimulation of algal growth at a concentration of 0.02 mg a.e./L for an unspecified Monsanto formulation of glyphosate. A stimulation of growth at sub-toxic concentrations (i.e., hormesis) is a common observation in algae and is not classified as an adverse effect. The stimulation of growth was noted also at much higher concentrations in more tolerant species of algae (e.g., Kish et al. 2006). Thus, the NOAEC of 0.082 mg a.e./L is used to characterize risks to sensitive species of algae for the more toxic formulations of glyphosate.

As summarized in Appendix 9 (Table 2), studies are available on many species of algae and some species are clearly less sensitive to glyphosate formulations. For Roundup, the highest reported EC_{50} is 19 mg a.e./L for *Pseudokirchneriella subcapitata* from the study by Cedergreen and Streibig (2005). Cedergreen and Streibig (2005) report an EC_{10} of 3.78 mg a.e./L. While an EC_5 could be approximated, the EC_{10} of 3.78 mg a.e./L is accepted as a reasonable approximation of a minimal effect level. This concentration is rounded to 3.8 mg a.e./L and is used for the risk characterization of tolerant species of algae. As discussed further in the risk characterization (Section 4.4.3.4.1), plausible exposures to glyphosate are substantially below 3.78 mg a.e./L and the use of the EC_{10} rather than the EC_5 has no impact on the risk assessment for tolerant species of algae.

4.3.3.4.1.2. Less Toxic Formulations

U.S. EPA/OPP (2008a, Table 4.21, p. 100) uses the EC_{50} of 12.1 mg a.e./L for technical grade glyphosate (MIRD 40236901). As summarized in Appendix 9 (Table 1), this study used the green alga, *Pseudokirchneriella subcapitata*. The rationale for selecting this study is not clear. Also in Table 4.21 of U.S. EPA/OPP (2008a), a lower EC_{50} of 11.4 mg a.e./L with a shallower slope (i.e., higher risk at lower doses) is cited for the bluegreen algae (*Anabaena flos-aquae*).

As summarized in Table 27, a much lower EC_{50} of 2.27 mg a.e./L in *Skeletonema costatum* is given for technical grade glyphosate in the study by Tsui and Chu (2003). As discussed in Section 4.1.3.4.2.1, the differences in the response of algae to technical grade glyphosate and glyphosate IPA are inconsistent and insubstantial. Thus, the EC_{50} of 2.27 mg a.e./L appears to be most appropriate study for sensitive species of algae. Tsui and Chu (2003) do not provide information on the slope of the concentration-response curve or an NOAEC. Based on other studies that report both EC_{50} values and NOAECs (Appendix 9, Table 1), the greatest difference between the EC_{50} and NOAEC is a factor of about 9.3 from the study by Saenz et al. (1997) in *Scenedesmus quadricauda* [7.2 mg a.e./L ÷ 0.77 mg a.e./L≈ 9.351]. As a conservative approximation, the EC_{50} of 2.27 mg a.e./L in *Skeletonema costatum* is divided by a factor of 10 and rounded to two significant place and the toxicity value for sensitive species of algae is taken as 0.23 mg a.e./L.

As summarized in Table 27, the most tolerant algal species appears to be *Chlorella pyrenoidosa* with an EC_{50} of 590 mg a.e./L from the open literature study by Maul and Wright (1984). As with the study by Tsui and Chu (2003), Maul and Wright (1984) do not report a NOAEC or slope of the concentration-response curve. Adopting the same approach used above for sensitive

species, the EC_{50} of 590 mg a.e./L is divided by a factor of 10 and the NOAEC is estimated at 59 mg a.e./L.

4.3.3.4.2. Macrophytes

As discussed in Section 4.1.3.4.3.2.and summarized in Table 28, there are no substantial differences between the sensitivity of macrophytes to the formulations of glyphosate that are generally classified as more toxic or less toxic formulations in the current risk assessment. Consequently and as with terrestrial macrophytes (Section 4.1.2.5), separate dose-response assessments for more and less toxic formulations of glyphosate are not developed for aquatic macrophytes.

The lowest toxicity value reported in U.S. EPA/OPP (2008a, Table 4.22, p. 101) for the effects of glyphosate formulations on macrophytes is a 14-day EC_{50} of 1.5 mg a.e./L in duckweed (*Lemna minor*). The EPA summary indicates that an NOAEC is not reported. This toxicity study is cited as MRID 44125714. This submission appears to refer to the open literature publication by Hartman and Martin (1984). Hartman and Martin (1984) report the EC_{50} as 2 mg a.i./L, which corresponds to about 1.5 mg a.e./L [2 mg a.i./L x 0.74 = 1.48 mg a.e./L]. Hartman and Martin (1984, Figure 1, p. 358) provide the concentration-response points for *Lemna minor*. Based on the concentration-response points, the NOAEC for the decrease in frond counts in the absence of suspended sediment appears to be about 0.7 mg a.e./L.

U.S. EPA/OPP (2008a), however, does not cite Perkins (1989). As summarized in Table 28, Perkins (1989) reports an EC_{50} of 0.84 mg a.e./L in watermilfoil in a bioassay using Rodeo. Perkins (1989) does not report a NOAEL and does not provide any information on the dose-response relationship – e.g., the slope of the dose-response curve. The Forest Service does not use EC_{50} values for risk characterization. In the absence of other relevant information, the Forest Service prefers to estimate an NOAEC from an EC_{50} by multiplying the EC_{50} by a factor of 0.05. This is analogous to the U.S. EPA/OPP approach of using an RQ of 0.05 as a level of concern for threatened and endangered aquatic species. Using this approach, the 0.84 mg a.e./L in watermilfoil could be used to estimate a NOAEC of 0.04 mg a.e./L [0.84 mg a.e./L x 0.05 = 0.042 mg a.e./L].

The data from Hartman and Martin (1984), however, suggests that this approach would be overly conservative. As noted about, the study by Hartman and Martin (1984) suggests that a factor of about 0.5 would be more appropriate for glyphosate [0.7 mg a.e./L ÷ 1.5 mg a.e./L ≈ 0.466]. The factor of 0.5 is identical to the factor used by the U.S. EPA/OPP for acute risk (e.g., U.S. EPA/OPP 2008a). If this approach were taken, the estimated NOAEC would be 0.4 mg a.e./L. This approach, however, would assume that the dose-response function for watermilfoil is reasonably similar to that for species of *Lemna* and there is no data available to support this supposition.

As discussed in Section 4.3.3.4.1.1, a well-document NOAEC of 0.082 mg a.e./L is available for algae and no studies in algae report an adverse effect level below 0.082 mg a.e./L for any glyphosate formulation. This NOAEC is a factor of about 10 below the EC_{50} for watermilfoil in the study by Perkins (1989). Given these relationships and the very extensive data base on the effects of glyphosate on algae, it seems reasonable to assert that the NOAEC of 0.082 mg a.e./L in algae may serve as a reasonably protective surrogate NOAEL for sensitive species of aquatic

macrophytes and this approach is taken in the current risk assessment. This approach is also virtually identical to the U.S. EPA/OPP (2008a) selection of the EC_{50} of 1.48 mg a.e./L using a level of concern of 0.05 [1.48 mg a.e./L x 0.05 = 0.074 mg a.e./L].

As noted in Table 28, reported EC_{50} values in *Lemna* species range up to 47 mg a.e./L. The study by Nielsen and Dahllof (2007) in eelgrass, however, indicates that this species, with an NOAEC of 170 mg a.e./L, is much more tolerant than *Lemna* species. Consequently, the NOAEC of 170 mg a.e./L is used for the risk characterization of tolerant species of aquatic macrophytes.

4.4. RISK CHARACTERIZATION

4.4.1. Overview

As in other sections of this risk assessment, this risk characterization of glyphosate is designed to clearly differentiate between the more toxic and less toxic formulations. While some formulations cannot be easily classified as more or less toxic, the general approach discussed in the dose-response assessment (Section 4.3.1) is applicable to the risk characterization: any formulation that contains a POEA surfactant should be regarded as more toxic, unless there is compelling evidence to the contrary. If the presence and/or toxicity of the surfactants in the formulation cannot be determined, it is prudent to classify that the formulation as more toxic.

The only notable exception to the classification of glyphosate formulations involves risks to terrestrial plants and aquatic macrophytes. Glyphosate is an effective postemergence herbicide. Foliar applications of glyphosate with an effective surfactant (POEA or otherwise) may pose a risk to terrestrial plants. The direct spray of a nontarget terrestrial plant at an effective application rate is likely to kill or seriously injure most plants. Nonetheless, substantial differences in sensitivity to glyphosate are apparent among different species of plants. For sensitive species, offsite drift of glyphosate can pose a risk. The nature of the risk depends on the application rate, application method, and site-specific conditions that affect the extent of drift. Terrestrial applications of the more toxic formulations of glyphosate may pose a risk to sensitive species of aquatic plants with an upper bound HQ of 1 at the unit application rate of 1 lb a.e./acre and an HQ of 8 at an application rate of 8 lb a.e./acre. Aquatic applications of less toxic formulations of glyphosate are used to control aquatic macrophytes and such applications are likely to damage sensitive species of aquatic macrophytes. Even at the maximum application rate of 3.75 lb a.e./acre, however, some tolerant species of aquatic macrophytes might not evidence any adverse effects.

For nontarget organisms, other than terrestrial plants and aquatic macrophytes, the risk characterization differs according to the more toxic and less toxic formulations, as detailed in the following subsections of this overview.

4.4.1.1. More Toxic Formulations

For terrestrial organisms other than plants, applications of up to 2.5 lb a.e./acre of the more toxic formulations do not present any apparent risks, based on upper bound estimates of exposure levels. At application rates greater than 2.5 lb a.e./acre, risks to mammals cannot be ruled out, based on upper bound estimates of exposure; however, no risks are apparent, based on central estimates of exposure. At application rates greater than approximately 3.3 lb a.e./acre, the HQs for birds modestly exceed the level of concern; however, there is no demonstrated evidence that these exposure levels will cause overt toxicity in birds. Risks to terrestrial insects are a greater concern based on dietary exposures, relative to direct spray. Based on upper bound estimates of exposure at the maximum application rate of 8 lb a.e./acre, the HQs for terrestrial insects can reach a value of 10. Concern for terrestrial invertebrates is enhanced by two toxicity studies using South American formulations of glyphosate in which adverse effects on reproduction and development were noted. While most field studies suggest that effects on terrestrial invertebrates are due to secondary effects on vegetation, the field studies do not directly contradict the South American toxicity studies or the HQs.

The risk characterization for aquatic organisms suggests that amphibians are the group at greatest risk both in terms of sensitivity and severity of effects. At an application rate of 1 lb a.e./acre, the upper bound HQ for amphibians is 2. The corresponding HQs for other groups of aquatic organisms are 1.7 for fish, 1.1 for invertebrates, 1.0 for algae, and 0.008 for aquatic macrophytes. Concern for amphibians is enhanced by the Howe et al. (2004) study which indicates that two formulations of Roundup as well as the POAE surfactant used in some of the more toxic formulations of glyphosate are associated with the development of intersex gonads. The HQs for aquatic species will increase linearly with the application rate. Because the upper bound HQs for most groups of aquatic organisms exceeds or reaches the level of concern at the relatively low application rate of 1 lb a.e./acre, care should be exercised when applying more toxic formulations of glyphosate near surface water.

4.4.1.2. Less Toxic Formulations

The less toxic formulations of glyphosate do not appear to present any risks to terrestrial organisms other than terrestrial plants.

Unlike the case with more toxic formulations, risks to amphibians and aquatic invertebrates appear to be insubstantial. Algae appear to be the most sensitive group of nontarget aquatic organisms. At an application rate of 1 lb a.e./acre, the upper bound of the HQ for sensitive species of algae is 0.8. At the maximum aquatic application rate of 3.75 lb a.e./acre, the corresponding HQ is 3. At this upper bound HQ, some inhibition of growth might be observed, but the extent of inhibition could be minor. Risks to fish cannot be ruled out based on standard and conservative assumptions and methods for applications of less toxic formulations of glyphosate at rates in excess of about 2.5 lb a.e./acre (acute effects). It seems most likely, however, that adverse effects would be observed in stressed populations of fish and less likely that effects would be noted in otherwise healthy populations of fish.

The less toxic formulations of glyphosate require the use of a surfactant. Some surfactants such as Agri-Dex (LC_{50} >1000 mg/L) are virtually nontoxic, and the use of a nontoxic surfactant would have no substantial impact on the risk characterization. Based on the available toxicity data in fish and aquatic invertebrates, some surfactants that may be used with the less toxic formulations of glyphosate could pose a much greater risk than the glyphosate formulation itself. An approach to assessing risks associated with toxic surfactants is illustrated for fish (Section 4.4.3.1.3) and aquatic invertebrates (Section 4.4.3.3.3). For a fixed concentration of the surfactant in a field solution, reducing the application volume will diminish the impact of the surfactant.

4.4.2. Terrestrial Organisms

4.4.2.1. Mammals

4.4.2.1.1. More Toxic Formulations

The risk characterization for mammals and birds is summarized in Worksheet G02 of the EXCEL workbooks that accompany this risk assessment (Attachments 1a-c). At the unit application rate of 1 lb a.e./acre, none of the hazard quotients for mammals exceed the level of concern (HQ=1). The HQs are linearly related to the application rate. For example, HQs at an application rate of 2 lb a.e./acre will be twice those of HQs at an application rate of 1 lb a.e./acre.

For the unit application rate of 1 lb a.e./acre, the highest HQ for mammals is the upper bound (i.e., worst case) HQ of 0.4 associated with the consumption of contaminated insects. Given the linear relationship of HQs to application rate, the upper bound HQ for consumption of contaminated insects by a small mammal would reach a level of concern (HQ=1) at an application rate of 2.5 lb a.e./acre [1 lb a.e./acre ÷ 0.4]. As discussed in Section 2, the maximum labeled application rate is about 8 lb a.e./acre. At the maximum application rate, the upper bound of the HQ for consumption of contaminated insects by a small mammal would be about 3.2 [8 x 0.4].

These calculations are only approximations. As a convention, HQs are typically rounded to one significant decimal. For the example of the upper bound HQ of 0.4 for the consumption of contaminated insects by a small mammal, the actual numerical value of the HQ is about 0.3965. In most project-specific analyses, EXCEL workbooks are typically generated for the application rate being considered. Typically, the small rounding error in the above discussion and other similar discussions in the following subsections are inconsequential. In a few instances, rounding has an impact on the scaling of HQs, as noted below.

As discussed in Section 4.3.2.1, the HQs for mammals are based on a reproductive NOAEL of 175 mg/kg bw/day with a corresponding LOAEL of 375 mg/kg bw/day based on maternal toxicity including mortality in some dams (Rodwell et al. 1980b). The HQ of 0.4 (i.e., the upper bound HQ for the consumption of contaminated insects by a small mammal) is associated with a dose of about 69.4 mg/kg bw/day. The LOAEL of 375 mg/kg bw/day would be associated with an HQ of about 2.1 [375 mg/kg bw/day ÷ 175 mg/kg bw/day ≈ 2.143]. This HQ would, in turn, be associated with an application rate of about 5.25 lb a.e./acre [2.1 ÷ 0.4]. In other words, if an application rate of 1 lb a.e./acre is associated with a dose of 69.4 mg/kg bw/day, then a dose of 375 mg/kg bw/day would be associated with an application rate of about 5.4 lb a.e./acre [375 mg/kg bw/day ÷ (69.4 mg/kg bw/day/1lb a.e./acre) ≈ 5.4035] .

The highest central (i.e., most likely) estimate HQ is 0.1 at an application rate of 1 lb a.e./acre. This HQ is again associated with the consumption of contaminated insects by a small mammal. At the highest application rate of about 8 lb a.e./acre, this HQ would be 0.8, which is below the level of concern.

The above quantitative discussion has a reasonably simple interpretation. At application rates of 2.5 lb a.e./acre or less, worst-case exposure assessments indicate that mammals are not at risk. Based on the central and more likely estimates of exposure, no risks to mammals are apparent. As discussed in Section 4.1.2.1, this risk characterization is supported by well-documented field studies that failed to identify adverse effects in populations of small mammals following applications of Roundup (Sullivan 1990) as well as another unidentified formulation of glyphosate (Ritchie et al. 1987).

4.4.2.1.2. Less Toxic Formulations

The risk characterization for mammals and birds exposed to less toxic formulations is summarized in Worksheet G02 of the EXCEL workbooks that accompany this risk assessment (Attachments 2). At the unit application rate of 1 lb a.e./acre, the highest HQ for any mammalian receptor is 0.005, which is associated with the consumption of contaminated water

following an accidental spill. At the maximum aquatic application rate of 3.75 lb a.e./acre, the HQ for the accidental spill would be about 0.02 [(0.005/1 lb a.e. per acre) x 3.75 lb a.e./acre = 0.01875], which is below the level of concern by a factor of 50. According, less toxic formulations of glyphosate pose no apparent risks to mammals.

4.4.2.2. Birds

4.4.2.2.1. More Toxic Formulations

For terrestrial applications of the more toxic formulations of glyphosate, the risk characterization for birds is summarized in Worksheet G02 of the EXCEL workbooks that accompany this risk assessment (Attachments 1a-c). As summarized in Table 29, the risk characterization for birds is based on a somewhat higher acute NOAEL (540 mg/kg bw vs 175 mg/kg bw for mammals), but a somewhat lower longer-term NOAEL (43 mg/kg bw versus 175 mg/kg bw for mammals). These differences are reflected in lower acute but higher chronic HQs for birds, relative to mammals.

Nonetheless and as with mammals, none of the HQs for birds exceed the level of concern (HQ=1) at the unit application rate of 1 lb a.e./acre. For the unit application rate of 1 lb a.e./acre, the highest HQ for birds is the upper bound HQ of 0.3 associated with the longer-term consumption of contaminated grass by a large bird. Following the same general approach used above for mammals, the upper bound HQ for consumption of contaminated grass by a large bird would reach a level of concern (HQ=1) at an application rate of about 3.3 lb a.e./acre [1 lb a.e./acre ÷ 0.3]. At the maximum application rate of about 8 lb a.e./acre, the upper bound of this HQ would be about 2.4 [8 x 0.3]. In the study by Kubena et al. (1981) on which the NOAEC of 43 mg/kg bw/day is based, a 10-fold higher dietary exposure is associated with only mild signs of toxicity, including decreased body weight and changes in bone composition. Thus, there is no basis for asserting that severe adverse effects are likely to be observed in birds exposed to application rates greater than 3.3 lb a.e./acre or at the maximum labeled rate of about 8 lb a.e./acre.

The highest central estimate (i.e., most likely) HQ is 0.03 at an application rate of 1 lb a.e./acre. This HQ is again associated with the longer-term consumption of contaminated grass by a large bird feeding exclusively at the application site. At the highest labeled application rate of the 8 lb a.e./acre, this HQ would be 0.24, below the level of concern by a factor of about 4.

The qualitative interpretation of risks to birds is thus extremely simple. Application rates greater than about 3.3 lb a.e./acre will result in modest excursions above an HQ of 1 at the upper bounds for some longer-term exposures; however there is no direct evidence that these exposures would likely be associated with overt adverse effects.

4.4.2.2.2. Less Toxic Formulations

The risk characterization associated with the aquatic application of less toxic formulations of glyphosate is extremely simple for birds, as is the case for mammals. The highest HQ is 0.00005, associated with the longer-term consumption of contaminated water. At the maximum aquatic application rate of 3.75 lb a.e./acre, this HQ value would be about 0.0002, which is below the level of concern by a factor of 5000. This benign risk characterization for birds is

supported by several field studies indicating that aquatic applications of glyphosate are beneficial to waterfowl due to an improvement of habitat conditions (Section 4.1.2.2.3).

4.4.2.3. Reptiles and Amphibians (Terrestrial-Phase)

As discussed in Section 4.3.2.3, the available data on terrestrial-phase amphibians do not lend themselves to the types of dose-response assessments conducted for mammals and birds. Based on the approach used by U.S. EPA/OPPTS (2004), risks to terrestrial-phase amphibians would be characterized as the same as risks to birds.

4.4.2.4. Terrestrial Invertebrates

As discussed in Section 4.2.3, two sets of exposure scenarios are developed for terrestrial invertebrates following terrestrial foliar applications of more toxic formulations of glyphosate, direct spray and spray drift (Section 4.2.3.1) and the consumption of contaminated vegetation or prey (Section 4.2.3.1). For aquatic applications of less toxic formulations, risks to terrestrial invertebrates are not considered quantitatively because the exposure scenarios of greatest concern involve aquatic invertebrates (Section 4.4.3.4).

Risks associated with direct spray or spray drift are summarized in Worksheet G02b (Attachments 1b-c), based on the direct spray of a honeybee. At the unit application rate of 1 lb a.e./acre, the HQ for the direct spray of terrestrial invertebrates is 0.3. Thus, the HQ would reach the level of concern (HQ=1) at an application rate of about 3.3 lb a.e./acre. At the maximum application rate of about 8 lb a.e./acre, the HQ would be about 2.4. As discussed in Section 4.1.2.4.1, the study by Palmer and Krueger (2001a) reports marginally significant mortality (3/60 with a p-value of about 0.04) at a dose of 100 µg/bee, and this scenario corresponds to an HQ of 2. Thus, while risks to honeybees from a direct spray cannot be excluded at the highest application rate, the effects would not be substantial and probably would not be detectable. Regardless of the application rate, no exposures associated with spray drift exceed the level of concern at any application rate.

Risks to terrestrial invertebrates associated with the consumption of contaminated vegetation are summarized in Worksheet G08b (Attachments 1a-c). Exposures are assessed using the residue rates from Fletcher et al. (1994) for fruit/large insects, broadleaf vegetation/small insects, short grass, and long grass (Table 18). The risks associated with these exposures are assessed using the oral NOEC for honey bees of 50 µg/bee which corresponds to a dose of about 430 mg a.e./kg bw (Section 4.1.2.4.1). At the unit application rate of 1 lb a.e./acre, the upper bounds of the HQs modestly exceed the level of concern only for the consumption of short grass (HQ=1.2) but approach the level of concern for the consumption of broadleaf vegetation and small insects (HQ=0.7) and the consumption of long grass (HQ=0.6). The HQs for the consumption of broadleaf vegetation and small insects and the consumption of long grass would reach the level of concern at application rates of about 1.4 and 1.7 lb a.e./acre, respectively. The central estimates of exposure at the unit application rate of 1 lb a.e./acre yield HQs that are below the level of concern—i.e., 0.1 for the consumption of broadleaf vegetation and small insects as well as long grass and 0.3 for the consumption of short grass.

At the maximum application rate of about 8 lb a.e./acre, the upper bound HQs would exceed the level of concern for the consumption of short grass (HQ=10), broadleaf vegetation and small

insects (HQ=6), and long grass (HQ=5). Also at the maximum application rate, the central estimate of exposure would exceed the level of concern (HQ=2).

The use of toxicity data on honeybees as a surrogate for other terrestrial invertebrates consuming contaminated vegetation or prey adds uncertainty to this risk characterization. As discussed in Section 4.1.2.4.2., recent studies by Benamu et al. (2010) and Schneider et al. (2009) note adverse effects on longevity and fecundity in spiders and lacewings following the short-term consumption of prey contaminated with South American formulations of glyphosate. The extent to which these studies are relevant to U.S. formulations of glyphosate is uncertain. In addition, the exposure methods used in the studies by Benamu et al. (2010) and Schneider et al. (2009)—i.e., dipping prey in field solutions of the glyphosate formulations —does not closely correspond to the exposures modeled in the current Forest Service risk assessment. Nonetheless, the observations by Benamu et al. (2010) and Schneider et al. (2009) along with the risk quotients from Worksheet G08b raise concerns that moderate to high application rates of more toxic formulations of glyphosate could have an adverse impact on some terrestrial invertebrates.

As summarized in Appendix 4 (Table 3), the available field studies on terrestrial invertebrates do not, for the most part, reinforce a concern for terrestrial invertebrates. Most field studies suggest that effects on terrestrial invertebrates will be minimal and secondary to changes in vegetation. Nonetheless, none of the field studies directly contradicts the observations from the Benamu et al. (2010) and Schneider et al. (2009) studies.

4.4.2.5. Terrestrial Plants

As discussed in Section 4.3.2.5, no distinction is made between more and less toxic glyphosate formulations in the dose-response assessment for terrestrial plants. For terrestrial foliar applications, risks to nontarget terrestrial plants are characterized for accidental direct spray and spray drift (Worksheet G05), erosion of contaminated soil by wind (Worksheet G06), and offsite transport of glyphosate by runoff or sediment (Worksheet G04).

Glyphosate is not particularly effective as an herbicide when applied to soils. As indicated in Worksheet G04, the upper bound HQ associated with offsite transport in runoff is 0.02 at an application rate of 1 lb a.e./acre. At the maximum application of 8 lb a.e./acre, the corresponding HQ would be about 0.2, below the level of concern by a factor of 5. Consequently, the transport of glyphosate in runoff is not a concern. Similarly and as summarized in Worksheet G06, the movement of glyphosate in contaminated soil due to erosion by wind leads to an HQ of 0.1 for sensitive species at an application rate of 1 lb a.e./acre. Even at the highest labeled application rate of 8 lb a.e./acre, this exposure scenario does not lead to an HQ that exceeds the level of concern (HQ=0.8).

In foliar applications, however, glyphosate is an extremely effective herbicide. The HQs for sensitive and tolerant species of terrestrial plants associated with direct spray and spray drift are summarized in Table 31. This table, in turn, is based on the HQs in Worksheet G05 of the EXCEL workbooks for backpack applications (Attachment 1a), ground broadcast applications (Attachment 1b), and aerial applications (Attachment 1c). As discussed in Section 4.2.4.2, the estimates of drift at various distances downwind are based on AgDRIFT. No detailed studies are available on drift due to backpack applications. Drift estimates for backpack applications are based on an AgDRIFT Tier 1 run of a low boom ground application using Fine to

Medium/Coarse drop size distributions (rather than very fine to fine) as well as 50th percentile estimates of drift (rather than the 90th percentile used for ground broadcast applications). The estimates for backpack drift are intended to be conservative; however, the extent to which these estimates may overestimate (or in some cases underestimate) exposures cannot be determined.

As summarized in Worksheet G05, sensitive species of vegetation will be harmed and probably killed by a direct spray with glyphosate at an application rate of 1 lb a.e./acre, (HQ=769). As discussed in Section 4.3.2.5.1, the dose-response assessment for sensitive species of terrestrial vegetation is somewhat more conservative than the EPA assessment (U.S. EPA/OPP 2008a), based on the toxicity data for nontarget species of terrestrial vegetation from the study by Boutin et al. (2004). The HQ for direct spray, however, is substantially above the level of concern, and the more conservative dose-response assessment has little impact on the risk characterization for direct spray. Even for tolerant species of vegetation, the hazard quotient for direct spray (HQ=2) exceeds the level of concern. Thus, over the range of application rates that might be used in Forest Service programs, the unintended direct spray of nontarget terrestrial vegetation is likely to cause damage and may kill the vegetation that is sprayed accidentally. This risk characterization applies to virtually any effective herbicide.

The risk characterization for drift differs substantially for sensitive and tolerant species. For tolerant species, risks associated with drift appear to be minimal as a result of backpack and ground broadcast applications. For aerial applications, no HQs exceed the level of concern at distances of 25 feet or less at an application rate of 1 lb a.e./acre. At the maximum application rate of 8 lbs a.e./acre, risks could modestly exceed the level of concern (HQ=1.6) at a distance of 100 feet downwind.

At an application rate of 1 lb a.e./acre, risks to sensitive species from drift exceed the level of concern at distances of 100 feet for backpack applications, 500 feet for ground broadcast applications, and over 900 feet for aerial applications. For backpack applications, the HQ at 900 feet downwind would reach the level of concern (HQ=1) at an application rate of about 5 lb a.e./acre. For ground broadcast applications, the HQ at 900 feet downwind would reach the level of concern at an application rate of about 1.25 lb a.e./acre. Clearly, the risk characterization associated with drift to sensitive species of nontarget plants is impacted by use of the Boutin et al. (2004) study. As detailed in Section 4.3.2.5.1, this study is used to reduce the NOAEC of 0.02 lb a.e./acre from U.S. EPA/OPP (2008a) by a factor of 15.

4.4.2.6. Terrestrial Microorganisms

As discussed in Section 4.1.2.6 (Hazard Identification) and Section 4.3.2.6 (Dose-Response Assessment), glyphosate may be toxic to terrestrial microorganisms in laboratory cultures, and this toxicity is probably related to the inhibition of the shikimate pathway. Nonetheless, numerous field studies fail to demonstrate adverse effects on soil microorganism (Bromilow et al. 1996; Busse et al., 2001; Haney et al. 2002; Hart and Brookes 1996; Laatikainen and Heinonen-Tanski 2002; Nicholson and Hirsch 1998; Means et al. 2007; Sailaja and Satyapradad 2006; Stratton and Stewart 1992; Wardle and Parkinson 1991). The results of these studies are sufficient evidence that direct toxic effects on soil microorganism are not likely to occur due to glyphosate exposure. Glyphosate applications may cause changes in microbial populations due to effects on and changes in terrestrial vegetation.

4.4.3. Aquatic Organisms

The risk characterization for aquatic organisms is given Worksheet G03 of the EXCEL workbooks that accompany this risk assessment—i.e., Attachments 1a-c for terrestrial foliar applications of more toxic formulations and Attachment 2 for aquatic applications of less toxic formulations.

At the unit application rate of 1 lb a.e./acre, accidental spills of more toxic formulations of glyphosate lead to HQs that substantially exceed the level of concern for sensitive species of all groups of aquatic organisms—i.e., upper bound HQs of 379 for fish, 454 for amphibians, 242 for invertebrates, 1.8 for macrophytes, and 222 for algae. Even for presumably tolerant species, each of the upper bound HQs for an accidental spill exceeds the level of concern—i.e., 36 for fish, 7 for amphibians, 8 for invertebrates, 1.8 for macrophytes, and 5 for algae. This is not an unusual risk characterization. For many pesticides, large accidental spills into relatively small bodies of water lead to HQs that suggest adverse effects in most aquatic species.

The accidental spills associated with the aquatic application of a less toxic formulation of glyphosate lead to exceedances in the upper bound of the HQ for most but not all sensitive species—i.e., 36 for fish, 0.05 for amphibians, 7 for invertebrates, 14 for macrophytes, and 76 for algae. As discussed in Section 4.3.3.2.2.1, the relatively low HQ for amphibians is associated with several studies that clearly indicate that the acute toxicity of glyphosate IPA to amphibians is very low.

Because the risk characterization for non-accidental exposures is more relevant and in some ways more complex than that for accidental exposures, the risk characterization for accidental exposures is not discussed further in following subsections.

One added complexity for the less toxic formulations involves the use of surfactants with these formulations. This issue is addressed in Section 4.4.3.1.3.

4.4.3.1. Fish

4.4.3.1.1. More Toxic Formulations

For the more toxic formulations of glyphosate, all longer-term exposures lead to HQs that are below the level of concern. The upper bound of the longer-term HQ for sensitive species of fish is 0.1 at 1 lb a.e./acre. At the maximum application rate of 8 lb a.e./acre, the upper bound HQ would be 0.8, approaching but below the level of concern. For tolerant species of fish, the upper bound of the longer-term HQ at an application rate of 8 lb a.e./acre is 0.08, below the level of concern by a factor of about 12.

Peak exposures, however, do lead to HQs that exceed the level of concern. For sensitive species of fish, the HQs for an application rate of 1 lb a.e./acre are 0.2 (0.03 to 1.7). The central estimate of the HQ would reach a level of concern (HQ=1) at about 5 lbs a.e./acre. For the maximum application rate of 8 lb a.e./acre, the HQs for acute exposures would be about 2 (0.2 to 14). For tolerant species of fish, the upper bound of the acute HQ is 0.2 at an application rate of 1 lb a.e./acre, 1 at an application rate of 5 lb a.e./acre, and about 2 at an application rate of 8 lb a.e./acre.

As detailed in Section 4.3.3.1.1.2, all of the HQs are derived from surrogate NOAECs that are based on LC_{50} values. This approach represents concern for potential sublethal effects and to maintain consistency with the general approach to risk assessments for aquatic organisms used by the U.S. EPA/OPPTS (2004). The most literal use of the HQs would be to assert that HQs of 20 would be associated with substantial mortality. None of the anticipated HQs reaches a level of 20.

Another concern with the numerical expressions of risk is that all of the LC_{50} values used in the dose-response assessment involve fasted fish. As discussed in Section 4.3.3.1.1.2, the study by Holdway and Dixon (1988) suggests that the toxicity of glyphosate is reduced by about a factor of 10 in fed fish, relative to fasted fish. In other words, HQs for populations of fish in areas where the food supply is adequate could overestimate risk.

Another moderating consideration in the characterization of risk may involve sediment. While sediment may enhance the toxicity of glyphosate and/or some surfactants used with glyphosate to filter feeders (Hartman and Martin 1984; Tsui and Chu 2003), suspended sediments have been shown to reduce the toxicity of glyphosate to aquatic macrophytes. While somewhat speculative, it seems reasonable to assert that suspended sediments could reduce the bioavailability to fish of glyphosate and surfactants used with glyphosate.

Finally, the available field studies in fish (Section 4.1.3.1.5) suggest that applications of Roundup may be beneficial to fish. While these studies do not rule out potential toxicity, the field studies suggest that changes to aquatic habitats following applications of Roundup may be beneficial and may offset any toxic effects.

The most reasonable qualitative risk characterization is that risks to fish cannot be ruled out based on standard and conservative assumptions and methods for applications of more toxic formulations of glyphosate. Nonetheless, it is not clear that any effects would be evident in healthy populations of fish in habitats with adequate supplies of food. Adverse effects could be more likely, however, in stressed populations of fish.

4.4.3.1.2. Less Toxic Formulations

At a unit application rate of 1 lb a.e./acre, the upper bound HQ for tolerant species of fish is 0.009. At the maximum labeled rate for aquatic applications, 3.75 lb a.e./acre, the corresponding upper bound HQ is about 0.03, below the level of concern by a factor of about 30. Consequently, risks to tolerant species of fish are not evident and are not further considered.

For sensitive species of fish, the HQs at expected peak concentrations for an application rate of 1 lb a.e./acre are 0.1 (0.07 to 0.4). The corresponding HQs for longer-term exposures are 0.02 (0.005 to 0.2). The upper bounds of the HQs would reach at level of concern (HQ=1) at an application rate of 2.5 lb a.e./acre for acute exposures and 5 lb a.e./acre (i.e., higher than the maximum labeled rate) for longer-term exposures. For the maximum application rate of 3.75 lbs a.e./acre, the HQs for acute exposures would be about 0.4 (0.3 to 1.5) and the corresponding HQs for longer-term exposures would be 0.08 (0.02 to 0.8).

The most reasonable qualitative risk characterization is that risks to fish cannot be ruled out based on standard and conservative assumptions and methods for applications of less toxic

formulations of glyphosate at rates in excess of about 2.5 lb a.e./acre (acute effects). Reservations with this risk characterization are similar to those for the more toxic formulations. It seems more likely that adverse effects would be observed in stressed populations of fish and less likely that effects would be noted in otherwise healthy populations of fish.

Surfactants are a complicating factor in the risk characterization for less toxic formulations. A surfactant must be added to these formulations, and it is plausible that the surfactant could impact the toxicity of the formulations to fish and other aquatic organisms. As summarized in Appendix 6 (Table 5), some surfactants such as Agri-Dex (LC_{50} >1000 mg/L) are virtually nontoxic. For such surfactants, a quantitative consideration of the surfactant would not have an impact on the risk characterized above. Other surfactants, however, have LC_{50} values similar to that of POEA surfactants, and, to the extent possible, the toxicity of the surfactant should be considered, as discussed in the following subsection.

4.4.3.1.3. Toxic Surfactants and Less Toxic Formulations

As discussed in Section 3.1.4.3, the assumption of simple similar action can be used to estimate the toxicity of a mixture of two or more components. This subsection illustrates how toxicity data on a surfactant, X-77, may be used to modify the risk characterization for the use of a relatively toxic surfactant with a less toxic formulation of glyphosate. A concern with applying simple similar action to less toxic formulations of glyphosate and the surfactants listed in Appendix 6 (Table 5) is that the assumption of simple similar action is based on the premise that agents in the mixture do not interact. In other words, the agents are assumed to display dose additivity, as discussed in Section 3.1.4.3.2. X-77 is selected as an example because information is available on X-77 for evaluating the assumption of simple similar action.

As summarized in Appendix 6, U.S. EPA/OPP (2008a) lists several bioassays with glyphosate IPA and X-77 (MRIDs 78664, 78665, 40579303, 40579305, 40579306) as well as Rodeo and X-77 (MRIDs 40579301 and 40579302). The information from these studies cannot be used to assess the plausibility of simple similar action, however, because of the lack of information on the amount of surfactant used in the Rodeo studies and uncertainties in the glyphosate IPA solution used in the other studies.

The open literature study by Mitchell et al. (1987a), however, appears to be very similar to MRID 40579301 and may be a derivative of the MRID submission. In the open literature publication, Mitchell et al. (1987a) provide additional details which permit an assessment of the interaction of Rodeo and X-77. In this study, Mitchell et al. (1987a) assayed the toxicity of Rodeo as well as a Rodeo/X-77 mixture in rainbow trout. The 96-hour LC_{50} of the Rodeo formulation alone is reported as 580 mg a.i./L and 1100 mg formulation/L. The ratio of these two values is 0.527, suggesting that the IPA salt was present in the formulation at a concentration of 52.7%. This is very close to nominal concentration of 53.8% glyphosate IPA for Rodeo (Table 2), and the slight discrepancy may reflect rounding of the reported LC_{50} values.

Mitchell et al. (1987a) did not separately assay the X-77 surfactant. As summarized in Appendix 6 (Table 5), the reported LC_{50} of X-77 in rainbow trout is 4.3 mg/L. Thus, with respect to Rodeo expressed as a.i. equivalents, the relative potency of X-77 is about 135 [580 mg a.i./L ÷ 4.3 mg X-77/L ≈ $134.884_{ai/X77}$].

The Rodeo/X-77 mixture used in the Mitchell et al. (1987a) bioassay is characterized as 312 mL Rodeo, 699 mL water, and 4 mL X-77 surfactant—i.e., a total volume of 1015 mL. Thus, the proportion of the surfactant in the mixture is about 0.00394 [4 mL ÷ 1015 mL]. Taking 0.527 as the proportion of glyphosate IPA in the Rodeo formulation, the proportion of glyphosate IPA in the mixture was about 0.16 [0.527 x 312 mL ÷ 1015 mL ≈ 0.1620]. Based on the assumption of simple similar action, the expected LC_{50} of the formulation (i.e., the mixture of Rodeo, surfactant, and water that was tested) would be about 838 mg formulation/L:

$$\frac{580\ mg\ a.i/L}{0.16_{ai/form}\ +\ 135_{ai/X77}\ \times 0.00394_{X77/form}} \approx\ 838.27\ mg\ formulation/L$$

Adjusting for the proportion of glyphosate IPA in the mixture (≈16%), the predicted LC_{50} for the mixture in units of glyphosate IPA is about 134 mg a.i./L [838 mg formulation/L x 0.16 a.i./formulation = 134.08 mg a.i./L].

The actual LC_{50} for the Rodeo/X-77 mixture in the bioassay is reported as 130 mg a.i./L (Mitchell et al. 1987a, Table 3, p. 1034). The similarity between the predicted and observed LC_{50} values is striking, particularly because the LC_{50} for X-77 is taken from a study other than Mitchell et al. (1987a). Nonetheless, the above analysis indicates that the assumption of simple similar action may be appropriate for mixtures of Rodeo and X-77.

Under the assumption of simple similar action, the HQs for two or more components in a mixture can be added to generate a hazard index (HI), and the interpretation of the HI is identical to that of an HQ (e.g., U.S. EPA/ORD 2002). In the context of the current Forest Service risk assessment, the level of concern for the hazard index would be one.

Taking X-77 as an example, the LC_{50} for this surfactant in fish is 4.3 mg/L (Appendix 6, Table 5). Taking the same approach used in the dose response assessment for glyphosate, the surrogate NOAEC is 0.21 mg/L [4.3 mg/L x 0.05].

In addition to a toxicity value, the concentration of the surfactant needs to be estimated. As indicated in Table 4, the product labels for some of the less toxic formulations of glyphosate, such as Rodeo, Accord, and Foresters, indicate that surfactants may be used in the field solution at a concentration up to 10%. Mistretta (2010, personal communication) indicated that forestry applications will not involve concentrations of nonionic surfactants greater than 0.5%, equivalent to 0.5 g/100 mL or 5 mg/mL.

For acute exposures following an aquatic application, the peak concentration of the surfactant in surface water will be linearly related to the concentration of glyphosate in surface water. As indicated in Attachment 2 (Worksheet A01), the lower bound glyphosate concentration in a field solution at an application rate of 1 lb a.e./acre is 4.8 mg/mL. As summarized in Worksheet G03, the upper bound peak expected environmental concentration for glyphosate is about 0.1838 mg/L. Thus, the estimated peak concentration for the surfactant would be about 0.1915 mg/L [0.1838 mg a.e./L x 5 mg X-77/mL ÷ 4.8 mg a.e/mL].

An HQ of 0.9 [0.1915 mg/L ÷ 0.21 mg/L ≈ 0.9119] can be calculated for the surfactant, based on its concentration in water and its toxicity value. As indicated in Worksheet G03 (Attachment 2), the upper bound HQ for glyphosate is 0.4. Thus, under the assumption of simple similar action, the HI for glyphosate and X-77 combined is 1.3 [0.4 + 0.9 = 1.3]. In this particular example, the presence of the surfactant has an impact on the risk characterization, taking the HQ from below the level of concern (HQ=0.4) to an HI greater than the level of concern (HI=1.3).

Although not complex, these calculations are cumbersome. As a matter of convenience, the EXCEL workbook for the aquatic application of less toxic formulations (Attachment 2) contains two custom worksheets that implement the above computations using the toxicity data for X-77—i.e., Worksheet G03b which implements the derivation of the HQ for the surfactant and Worksheet G03c which combines the HQs for glyphosate and the surfactant. These worksheets could be used by Forest Service personnel to assess the consequences of using other surfactants. The custom worksheets contain only entries for fish and invertebrates because of limitations regarding the available toxicity data on the surfactants which might be used with the less toxic formulations of glyphosate.

The impact of the surfactant is directly proportional to its toxicity and its concentration in the mixture. In other words, as the toxicity of the surfactant or its concentration in the mixture increases, the impact of the surfactant increases. Application volume also affects the significance of the surfactant. For a fixed concentration of surfactant in a field solution, the impact of the surfactant will increase as the application volume increases. This is true because the functional application rate of the surfactant (at a fixed concentration in the field solution) will increase as the application volume increases. In the above example with X-77, a high application volume is used (i.e., 25 gallons/acre), and the combined impact of glyphosate and the surfactant qualitatively alter the risk characterization. At the minimum application volume of 5 gallons/acre, however, the HQ associated with the surfactant is 0.2 (because the amount of surfactant applied is five times lower) and the HI for the mixture is only 0.6—i.e., the HI is below the level of concern. Thus, using lower application volumes while keeping the concentration of the surfactant constant will decrease the impact of the surfactant because less surfactant is used as the application volume decreases.

4.4.3.2. Amphibians

4.4.3.2.1. More Toxic Formulations

As summarized in Table 29, the surrogate NOAEC for sensitive species of amphibians (0.04 mg a.e./L) is similar to that for fish (0.048 mg a.e./L), and the corresponding NOAEC for tolerant species of amphibians (2.6 mg a.e./L) is somewhat higher than the corresponding NOAEC in fish (0.5 mg a.e./L). Consequently, the quantitative risk characterization for amphibians is similar to that for fish.

As is the case for fish, none of the longer-term HQs for amphibians approach a level of concern at the unit application rate of 1 lb a.e./acre. As indicated in Worksheet G03, the upper bound of the longer-term HQ for sensitive species is 0.1. The underlying numerical value of the HQ (i.e., without rounding) is about 0.145. Consequently, the upper bound of the longer-term HQ for sensitive species of amphibians would reach a level of concern (HQ=1) at an application rate of about 6.9 lb a.e./acre. At the maximum application rate of about 8 lb a.e./acre, the upper bound

of the HQ for sensitive species would be about 1.2. For tolerant species of amphibians, the upper bound of the HQ at the maximum application rate of about 8 lb a.e./acre would be about 0.02, below the level of concern by a factor of 50.

For short-term peak exposures, the HQs for sensitive but not tolerant species of amphibians do exceed the level of concern (HQ=1). As indicated in Worksheet G03, the HQs are 0.3 (0.03 to 2) for sensitive species of amphibians. The underlying numerical value of the central estimate is 0.275. Thus, the central estimate of the HQ for sensitive species of amphibians would reach the level of concern (HQ=1) at an application rate of about 3.6 lb a.e./acre. At the maximum application rate of about 8 lb a.e./acre, the central estimate of the HQ for sensitive species of amphibians would be about 2.

The upper bound HQ of 2 for sensitive species of amphibians at the unit application rate of 1 lb a.e./acre indicates application rates of about 0.5 lb a.e./acre or less would be required to keep the HQ value below the level of concern (HQ=1). At the maximum application rate of 8 lb a.e./acre, the HQ (rounded to 1 significant place) would be about 17.

By definition, any HQ that exceeds 1 triggers concern. The nature of the concern for sensitive species of amphibians is best appreciated by examining the toxicity and exposure data on which the HQ is based. As discussed in Section 4.3.3.2.1.1, the lowest LC_{50} for amphibians is 0.8 mg a.e./L from the study by Relyea and Jones (2009) using American bullfrog larvae. This LC_{50} is not an outlier and is very similar to the LC_{50} of 1.1 mg a.e./L from Edginton et al. (2004) and 1.2 mg a.e./L from Bernal et al. (2009a). The other key toxicity study is that of Howe et al. (2004) in which concentrations of 0.6 and 1.8 mg a.e./L of two Roundup formulations were associated with decreases in growth and survival over a 42-day period of exposure. Both concentrations of Roundup Original were also associated with the development of intersex gonads. This effect, however, was not noted with glyphosate IPA, and the developmental effects appear to be most clearly associated with the surfactants rather than glyphosate.

As summarized at the top of Worksheet G03, the peak concentrations in water at an application rate of 1 lb a.e./acre are 0.011 (0.0013 to 0.083) mg a.e./L. For the maximum application rate of about 8 lb a.e./acre, the corresponding peak concentrations would be 0.088 (0.010 to 0.66) mg a.e./L.

At an application rate of 1 lb a.e./acre, the peak concentration of 0.083 mg/L is below the lowest LC_{50} (i.e., 0.8 mg/L) by a factor of about 10. As discussed in Section 4.3.3.2.1.1, the concentration-response relationship for acute lethality in amphibians appears to be very steep. Thus, while the upper bound HQ of 2 for acute exposure levels in amphibians exceeds the level of concern, it does not seem likely that an application rate of 1 lb a.e./acre would be associated with substantial or detectable mortality even in sensitive species of amphibians.

At an application rate of 8 lb a.e./acre, however, the upper bound concentration of about 0.7 mg a.e/L is remarkably close to the lowest acute LC_{50} of 0.8 mg a.e./L. At a peak concentration of 0.7 mg a.e./L, mortality, perhaps substantial mortality, would be expected in sensitive species of amphibians.

The peak concentration of 0.7 mg a.e./L is also somewhat higher than the LOAEL of 0.6 mg a.e./L for growth, survival, and the development of intersex gonads in the study by Howe et al. (2004). While Howe et al. (2004) is a longer-term study, adverse developmental effects—e.g., the development of intersex gonads—may be associated with very short-term events that occur during development. Thus, developmental effects in sensitive species of amphibians cannot be ruled out.

As noted in the risk characterization for fish (Section 4.4.3.1.1), there is a concern that the use of fasted fish during most acute toxicity studies may lead to a risk characterization that is overly conservative and perhaps grossly so. Bioassays on amphibians, however, are not as standardized as those in fish and it is not clear that most of the amphibian bioassays involve the use of fasted organisms. For example, Howe et al. (2004) specifically note that feeding had commenced by Gosner stage 25 tadpoles and that the acute bioassays by Howe et al. (2004) with Gosner stage 25 tadpoles did involve feeding. Howe et al. (2004) do not mention any fasting protocol prior to testing. In the study by Relyea and Jones (2009), no specific information on feeding is provided; however, other studies by Relyea (Relyea 2004, 2005a,c; Relyea et al. 2005) indicate that feeding was done during bioassays. Thus, the reservations about the use of fasted animals during fish bioassays do not appear to be relevant to most amphibian bioassays.

The verbal risk characterization for amphibians is somewhat more severe than that for fish. At an application rate of 1 lb a.e./acre, concerns for amphibians would be modest, and the likelihood of substantial or detectable effects appears to be low. As application rates increase toward the maximum labeled rate of 8 lb a.e./acre, the likelihood of observing adverse effects increases. At the maximum application rate, the upper bounds of potential exposure levels suggest that mortality and/or developmental effects would be expected. Thus, if more toxic formulations of glyphosate are applied at high rates near surface water that serves as a habitat for amphibians, efforts may be warranted to refine the exposure assessment based on site-specific considerations and to minimize the likelihood of the contamination of surface water.

4.4.3.2.2. Less Toxic Formulations

Unlike the case with more toxic formulations, the dose-response assessment for amphibians is very different from that for fish (Table 22). The acute NOAECs for amphibians range from 340 to 470 mg a.e./L, and there is no need to estimate NOAECs from LC_{50} values (Section 4.3.3.2.2.1). In addition, the longer-term NOAEC for developmental effects is 1.8 mg a.e./L from the study by Howe et al. (2004). As noted in Section 4.3.3.2.2.2, this chronic NOAEC is free-standing—i.e., no LOAEC is defined, and it seems likely that the actual NOAEC for less toxic formulations of glyphosate could be higher and perhaps much high than 1.8 mg a.e./L.

Given the above dose-response assessment, the risk characterization is simple and unambiguous. At an application rate of 1 lb a.e./acre, the highest HQ is 0.06—i.e., the upper bound HQ for longer-term exposures. At the maximum application rate of about 3.75 lb a.e./acre, the HQ would be 0.2, below the level of concern by a factor of 5. Thus, there is no basis for asserting that adverse effects in amphibians would be apparent even at the upper bound estimates of exposure at the maximum application rate.

For amphibians, there is no information regarding the toxicity of surfactants that may be used with the less toxic formulations of glyphosate. As discussed in Section 4.4.3.1.3, the use of a

relatively nontoxic surfactant would probably have no impact on the risk characterization. If a toxic surfactant is used, the toxicity of the surfactant could dominate the risk characterization. In general and assuming a fixed concentration of the surfactant in the field solution, the use of low application volumes will reduce the impact of a toxic surfactant, relative to high application volumes.

4.4.3.3. Aquatic Invertebrates

4.4.3.3.1. More Toxic Formulations

As noted in Section 4.4.3.2.1, the dose-response assessment for fish and amphibians do not differ remarkably, and the HQs for these two groups are similar. As also summarized in Table 29, the dose-response assessment for aquatic invertebrates does not differ substantially from the corresponding assessments for fish and amphibians. For sensitive species, the surrogate NOAEC for aquatic invertebrates (0.075 mg a.e./L) is modestly higher than that for fish (0.048 mg a.e./L) or amphibians (0.04 mg a.e./L). For tolerant species, the surrogate NOAEC for aquatic invertebrates (2.3 mg a.e./L) is virtually identical to that for amphibians (2.6 mg a.e./L).

As with fish, all longer-term exposures for aquatic invertebrates lead to HQs that are below the level of concern. The upper bound of the longer-term HQ for sensitive species of aquatic invertebrates is 0.08 at 1 lb a.e./acre. At the maximum application rate of 8 lb a.e./acre, the upper bound HQ would be 0.6, below the level of concern by a factor of about 1.7. For tolerant species of aquatic invertebrates, the upper bound of the longer-term HQ at an application rate of 8 lb a.e./acre is 0.02, below the level of concern by a factor of about 50.

For acute exposures, the HQs are below the level of concern for tolerant species of aquatic invertebrates. At the unit application rate of 1 lb a.e./acre, the upper bound of the HQ is 0.04. At the maximum application rate of about 8 lb a.e./acre, the corresponding HQ would be 0.3, below the level of concern by a factor of about 3.

For acute exposures of sensitive species of aquatic invertebrates, the HQs are 0.1 (0.02 to 1.1) at a unit application rate of 1 lb a.e./acre. The underlying numerical value of the central estimate of the HQ is about 0.1467. The HQ would reach the level of concern at an application rate of about 6.8 lb a.e./acre. At the maximum application rate of 8 lb a.e./acre, the central estimate of the HQ would be about 1.2.

The upper bound HQ for acute exposures of sensitive species of aquatic invertebrates are substantially higher than the central estimate—i.e., an upper bound HQ of about 1.1 at an application rate of 1 a.e./acre. At an application rate of 8 lb a.e./acre, the upper bound HQ would be about 9.

The only HQ values that require elaboration are the upper bound HQs for sensitive species of aquatic invertebrates. As discussed in Section 4.3.3.3.1.1, the lowest reported EC_{50} is 1.5 mg a.e./L for amphipods from the study by Tsui and Chu (2004) using an unspecified Roundup formulation from Monsanto. The toxicity value from Tsui and Chu (2004) is only a factor of two lower than the 3 mg a.e./L EC_{50} from the study by Folmar et al. (1979) in *Daphnia magna* with the original Roundup formulation. Thus, the EC_{50} from Tsui and Chu (2004) does not appear to be an outlier.

As discussed in the risk characterization for amphibians (Section 4.4.3.2.1), the estimated upper bound of the peak concentration of glyphosate in surface water is about 0.083 mg a.e./L at an application rate of 1 lb a.e./acre. At an application rate of 8 lb a.e./acre, the upper bound of the peak concentration in surface water is estimated at about 0.7 mg a.e./L.

At an application rate of 1 lb a.e./acre, the estimated concentration of 0.083 mg a.e./L is below the lowest EC_{50} by about a factor of 18 [1.5 mg a.e./L ÷ 0.083 mg a.e./L ≈ 18.07]. At the maximum application rate of 8 lb a.e./acre, the estimated concentration of 0.07 mg a.e./L is below the lowest EC_{50} by about a factor of about 2 [1.5 mg a.e./L ÷ 0.7 mg a.e./L ≈ 2.143]. As with all other groups of aquatic animals, the concentration-response relationships for the more toxic formulations of glyphosate appear to be very steep (Section 4.3.3.3.1.1). Thus, at an application rate of 1 lb a.e./acre, there is no basis for asserting that substantial mortality in sensitive species of aquatic invertebrates would be seen. Even at an application rate of 8 lb a.e./acre, some of the studies in Appendix 8 (Table 2) suggest that mortality at about one-half of the EC_{50} would be quite modest and might be undetectable. This risk characterization is supported by several field studies in which very little impact was observed on aquatic invertebrates following applications of Roundup or other similar formulations (Appendix 8, Table 8).

4.4.3.3.2. Less Toxic Formulations

As with fish and amphibians, the risks associated with the less toxic formulations of glyphosate are minimal. At the unit application rate of 1 lb a.e./acre, the highest HQ is 0.1, the upper bound of the HQ associated with longer-term exposures in sensitive species of aquatic invertebrates. At the maximum application rate of 3.75 lb a.e./acre, the HQ is about 0.4.

For acute exposures, the upper bound HQ for sensitive species is 0.07 at an application rate of 1 lb a.e./acre and 0.3 at an application rate of 3.75 lb a.e./acre. As with longer-term exposures, the worst-case HQ at the maximum application rate for aquatic applications approaches but does not exceed the level of concern.

As with fish, some information is available on the toxicity of surfactants, and this information may be used to assess the consequences of using a surfactant with a less toxic formulation of glyphosate. An example of how a surfactant may be considered using the data on aquatic invertebrates is given in the following subsection.

4.4.3.3.3. Toxic Surfactants and Less Toxic Formulations

Details of the method used to assess the impact of using a toxic surfactant, X-77, with a less toxic formulation, as it relates to fish, are provided in Section 4.4.3.1.3. Given the available data on the toxicity of X-77 to aquatic invertebrates (Appendix 8, Table 6), the same method for assessing the impact of a toxic surfactant on a less toxic formulation of glyphosate can be applied to aquatic invertebrate exposure. The only reservation in doing so relates to the general lack of data from which to assess the nature of the interaction between X-77 and less toxic formulations of glyphosate. By analogy to the data on fish, the assumption (for the sake of illustration) is made that the toxicological interaction is additive. The computations are implemented in Attachment 2, Worksheet G03b (the impact of X-77) and Worksheet G03c (the combined effect of X-77 and the glyphosate formulation).

As noted in the previous subsection, none of the HQs for the effect of the less toxic glyphosate formulation exceed the level of concern. At an application rate of 1 lb a.e./acre, the upper bound of the HQ for sensitive species and peak exposures is 0.07. The lowest LC_{50} for X-77 in an aquatic invertebrate is 2 mg/L in *Daphnia magna* (Henry et al. 1994). Using the same approach taken with the less toxic glyphosate formulations, the LC_{50} of 2 mg/L is multiplied by a factor of 0.05 to approximate a NOAEC of 0.1 mg/L. The NOAEC for the less toxic formulation of glyphosate is taken as 2.7 mg a.e./L for sensitive species (Table 30). Thus, for this example, the surfactant is more toxic than the formulation by a factor of 27 [2.7 mg a.e./L ÷ 0.1 mg X-77/L].

As detailed in Worksheet G03b, the upper bound of the HQ for X-77 for sensitive species of aquatic invertebrates for expected peak exposures is 1.9. In Worksheet G03c, the HQ of 0.07 for the less toxic formulation is added to the HQ of 1.9 for X-77, and the resulting HI is then rounded to one significant place (HI=2). These calculations are all based on an application volume of 25 gallons/acre and a surfactant concentration of 0.5%.

As noted in Section 4.4.3.1.3, the application volume (assuming a fixed concentration of the surfactant) has a substantial influence on the HI, and the impact of the surfactant can be reduced by lowering the application volume. If the application volume is reduced to 5 gallons/acre and all other factors are kept the same, the HQ for X-77 is reduced to 0.4 and the resulting HI for X-77 plus the formulation is reduced to 0.5. Thus, in this example, decreasing the application volume results in the surfactant having no substantial impact on the qualitative risk characterization—i.e., the HI is below the level of concern. In general, decreasing the application volume at a fixed concentration of the surfactant in the field solution will diminish the impact of the surfactant because less surfactant is being used.

4.4.3.4. Aquatic Plants

4.4.3.4.1. More Toxic Formulation

As discussed in Section 4.3.3.4.2, the dose-response assessment for sensitive species of aquatic macrophytes is based on the dose-response assessment for sensitive species of algae. Thus, the risk characterizations for sensitive species of aquatic plants, both algae and macrophytes, are identical. For non-accidental exposures, the more toxic formulations of glyphosate may pose a risk to sensitive species of aquatic plants with an upper bound HQ of 1 at the unit application rate of 1 lb a.e./acre and an HQ of 8 at an application rate of 8 lb a.e./acre.

For tolerant species of aquatic plants, the NOAECs differ for algae (NOAEC=3.8 mg a.e./L) and macrophytes (NOAEC=170 mg a.e./L). While these differences are substantial, they have no impact on the risk characterization for non-accidental exposures. For non-accidental exposures, the upper bound HQs are below the level of concern (HQ=1) by a factor of 50 for algae and a factor of 2000 for aquatic macrophytes.

Several field studies note that the more toxic formulations of glyphosate at application rates of up to 2 lb a.e./acre did not have a substantial impact on algae (Appendix 9, Table 4). Assuming that the species of algae encompassed in the field studies were not the most sensitive, the results of these field studies are consistent with the risk characterization, based on HQs. At sub-toxic

concentrations, glyphosate may stimulate cell growth; moreover, increases in primary productivity are noted in some field studies (i.e., Perez et al. 2007; Schaffer and Sebetich 2004).

Based on the accidental spill scenarios, the HQs for sensitive species of algae and aquatic macrophytes are 55 (4 to 222), suggesting that sensitive species of aquatic plants would be damaged or killed in the event of an accidental spill. HQs for tolerant species of aquatic macrophytes – i.e., 0.03 (0.002 to 0.1) – do not exceed the level of concern and HQs for tolerant species of algae – i.e., 1.2 (0.1 to 5 – bracket the level of concern.

4.4.3.4.2. Less Toxic Formulation

Aquatic applications of the formulations of glyphosate that are classified as less toxic in the current Forest Service risk assessment are designed to control macrophytes. At the unit application rate of 1 lb a.e./acre, the HQs are 0.9 (0.4 to 2) for sensitive species of aquatic macrophytes. At the maximum aquatic application rate of 3.75 lb a.e./acre, the corresponding HQs for sensitive species of aquatic macrophytes are 3 (1.6 to 8). For tolerant species of aquatic macrophytes, however, the HQs are far below the level of concern – i.e., 0.002 (0.0008 to 0.004). While efficacy is not a focus of the current risk assessment, these very low HQs suggest that glyphosate may not be effective in controlling tolerant species of aquatic macrophytes.

For sensitive species of algae, the maximum HQ at an application rate of 1 lb a.e./acre is 0.8, the upper bound of the HQ for peak exposures. This HQ would reach the level of concern (HQ=1) at an application rate of about 1.25 lb a.e./acre. At the maximum aquatic application rate of 3.75 lb a.e./acre, the corresponding HQ is 3. This HQ corresponds to a concentration in surface water of about 0.7 mg a.e./L. As summarized in Table 30, the EC_{50} for sensitive species of algae is 2.3 mg a.e./L. While some growth inhibition might be observed at the concentration of 0.7 mg a.e./L, the extent of inhibition could be minor.

5. REFERENCES

NOTE: The initial entry for each reference in braces {} simply specifies how the reference is cited in the text. The final entry for each reference in brackets [] indicates the source for identifying the reference.

DER01	**DERs of registrant studies obtained from http://www.epa.gov/pesticides/chemical/foia/cleared-reviews**
E-Docket01	**EPA-HQ-OPP-2006-0323 at http://www.regulations.gov. This is associated with the 2007 tolerances for glyphosate DMA in U.S. EPA/OPP 2007a.**
Internet	**References obtained from various sites on the Internet.**
Gly03	**Open literature studies taken from the 2003 Forest Service risk assessment on glyphosate.**
GlyArch1	**Archived papers from previous Forest Service risk assessments.**
MRID03	**CBI studies reviewed for an summarized in the 2003 Forest Service risk assessment (SERA 2003).**
MCS	**Papers on Multiple Chemical Sensitivity**
Reg	**Information from registrants**
SET00	**Papers from preliminary scoping and other communications.**
SET01	**Brown and burn.**
SET02	**NAL Papers rescreened from previous RAs.**
SET03	**Initial update literature search.**
SET04	**Initial screen of EPA documents.**
SET05	**Sundry papers based on secondary screening.**
SET06	**Additional papers based on secondary screening.**
SET07	**More non-Hodgkin lymphoma papers.**
SET08	**Additional studies from Dave Bakke, USDA/FS/R5.**
SET09	**Studies added after peer review.**
SET10	**Additional studies added after peer review, primarily studies relating to testosterone in rats.**
Sec	**Summary of citation from a secondary source.**
Std	**Standard references used in most Forest Service risk assessments.**

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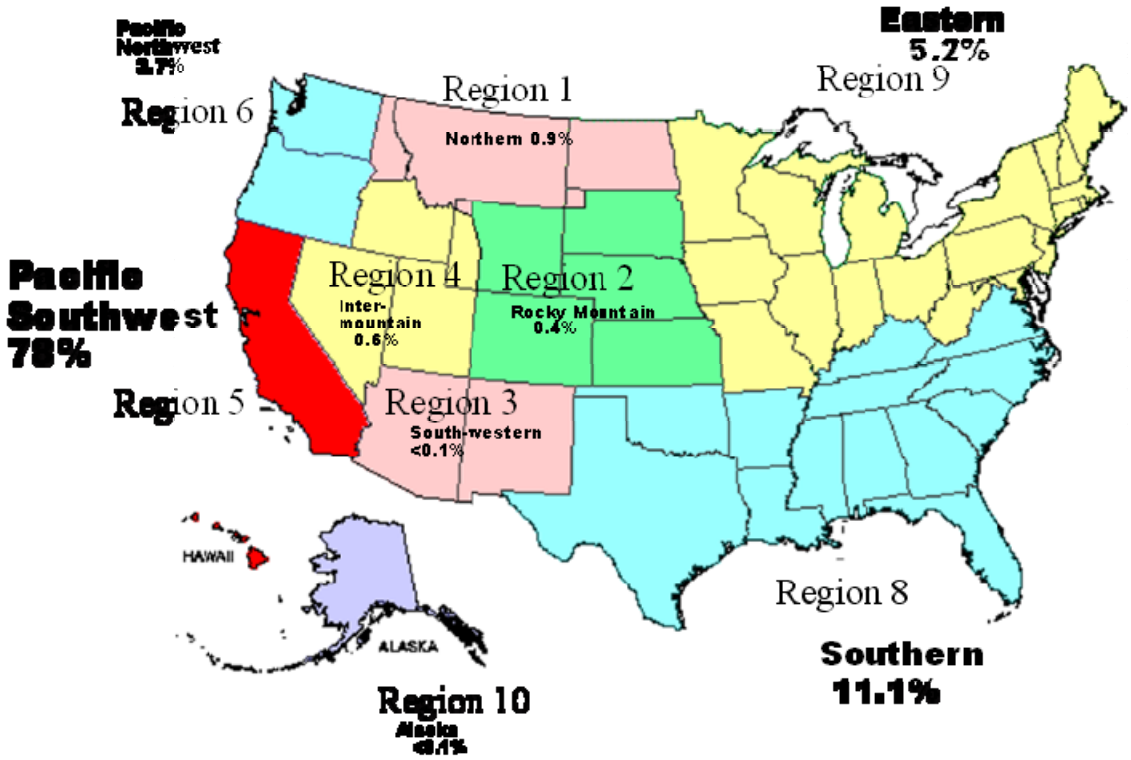
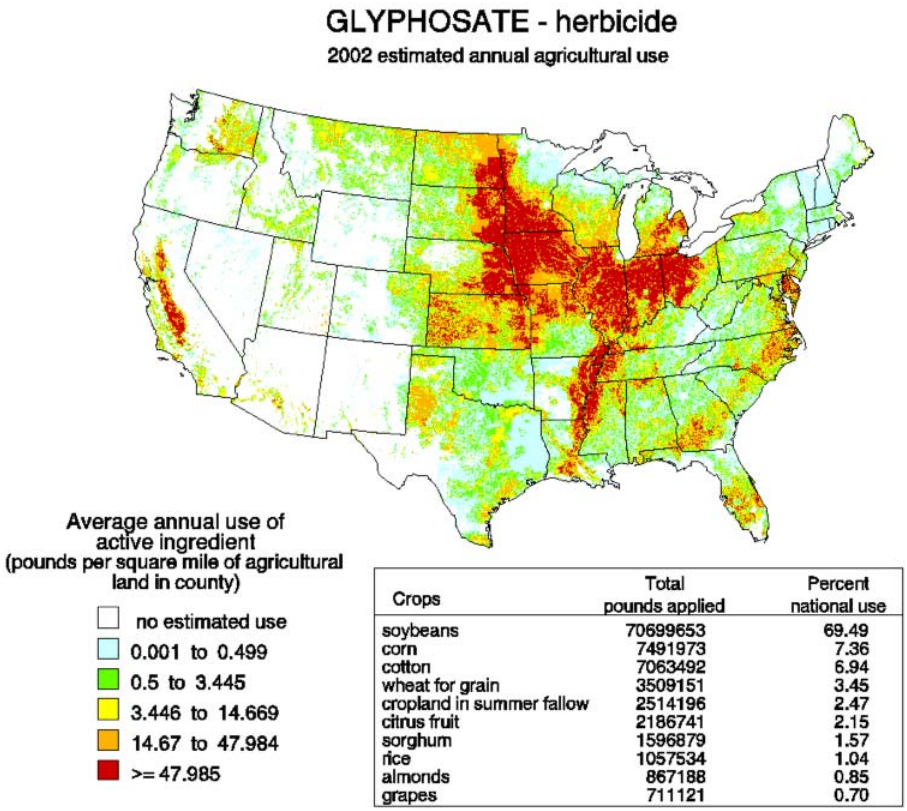


Figure 1: Use of Glyphosate by FS Region (2000 to 2004)



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

Figure 2: Agricultural Use of Glyphosate in 2002

Source: USGS 2003a

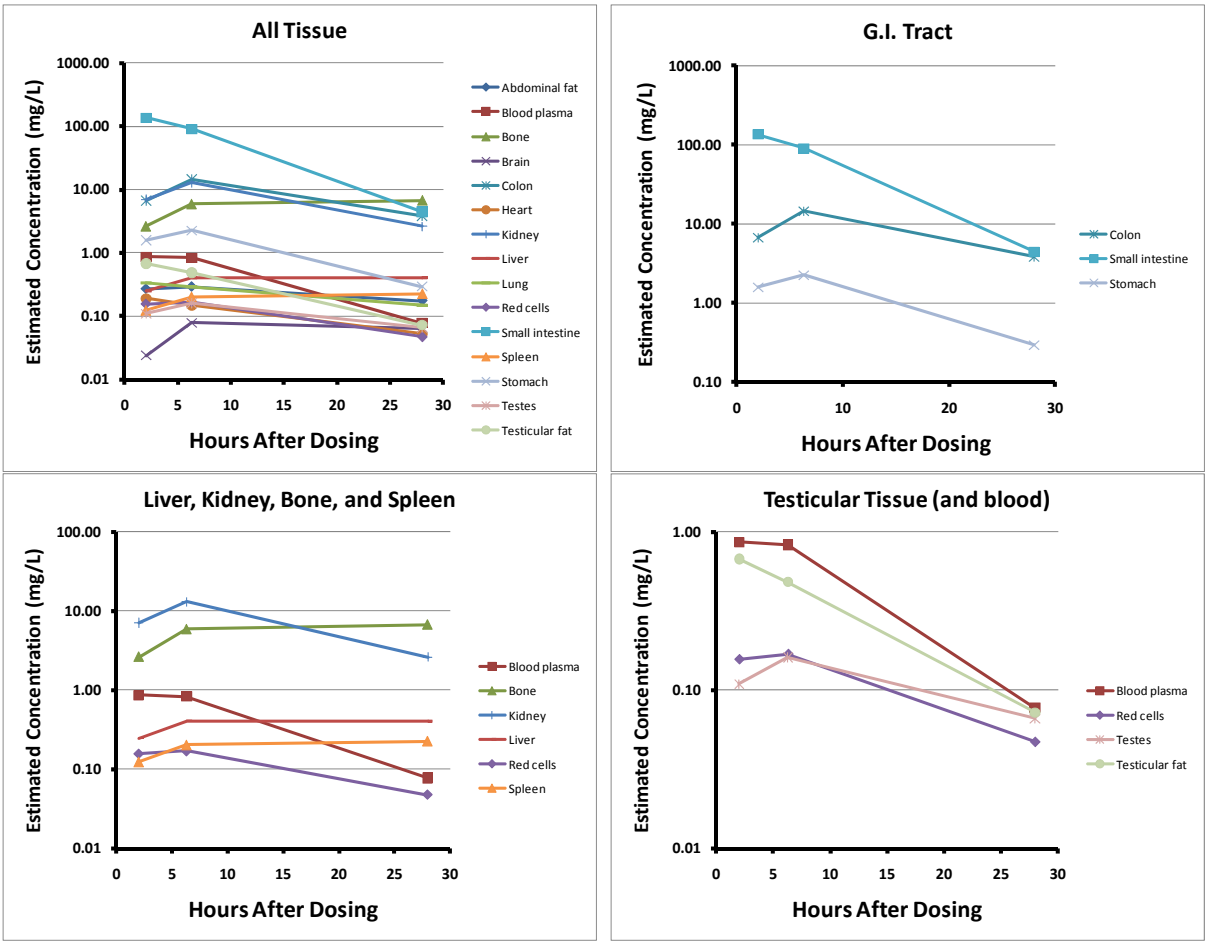


Figure 3: Glyphosate in Tissues After Oral Dosing

Data from Brewster et al. 1991
See Section 3.1.3.1. for discussion.

Note: The upper left figure includes all of the data from Brewster et al. 1991. The other three figures are subsets for various groups of tissues. Note the differences in scale in the Y-axes.

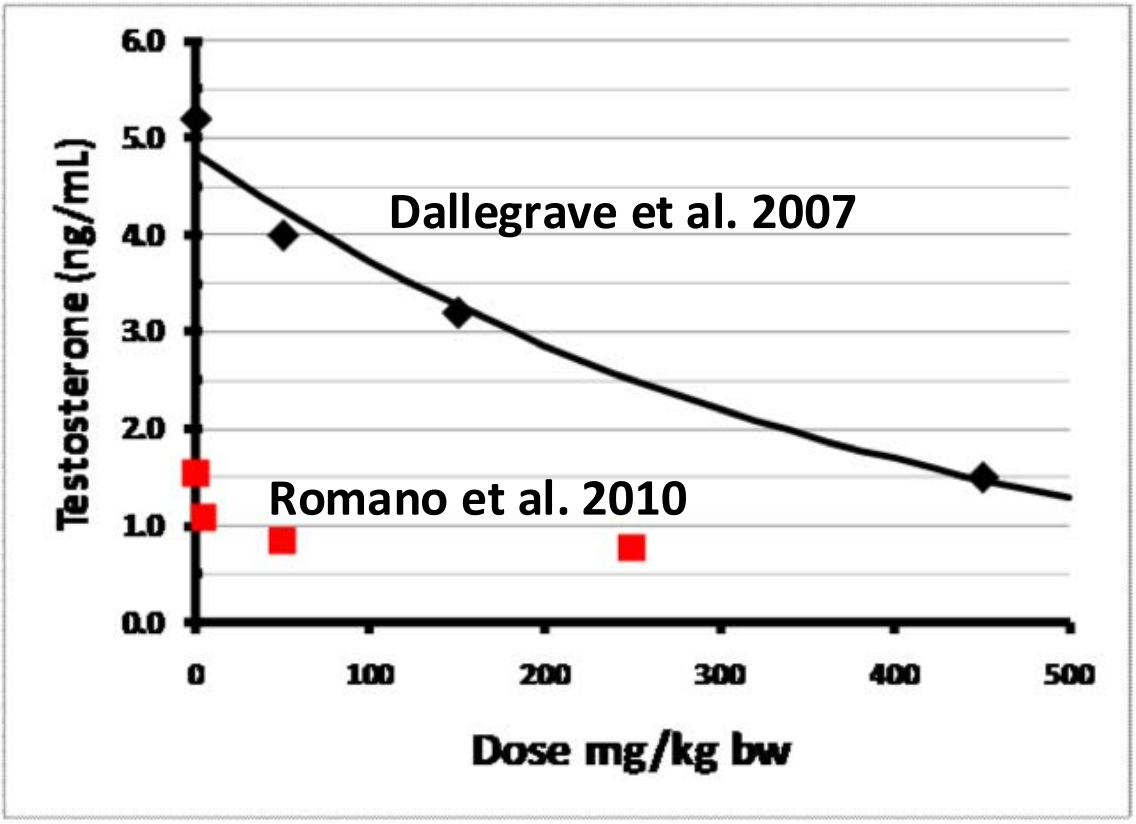


Figure 4: Dose-Response Relationship for Serum Testosterone

See Section 3.1.9.1.2 for discussion

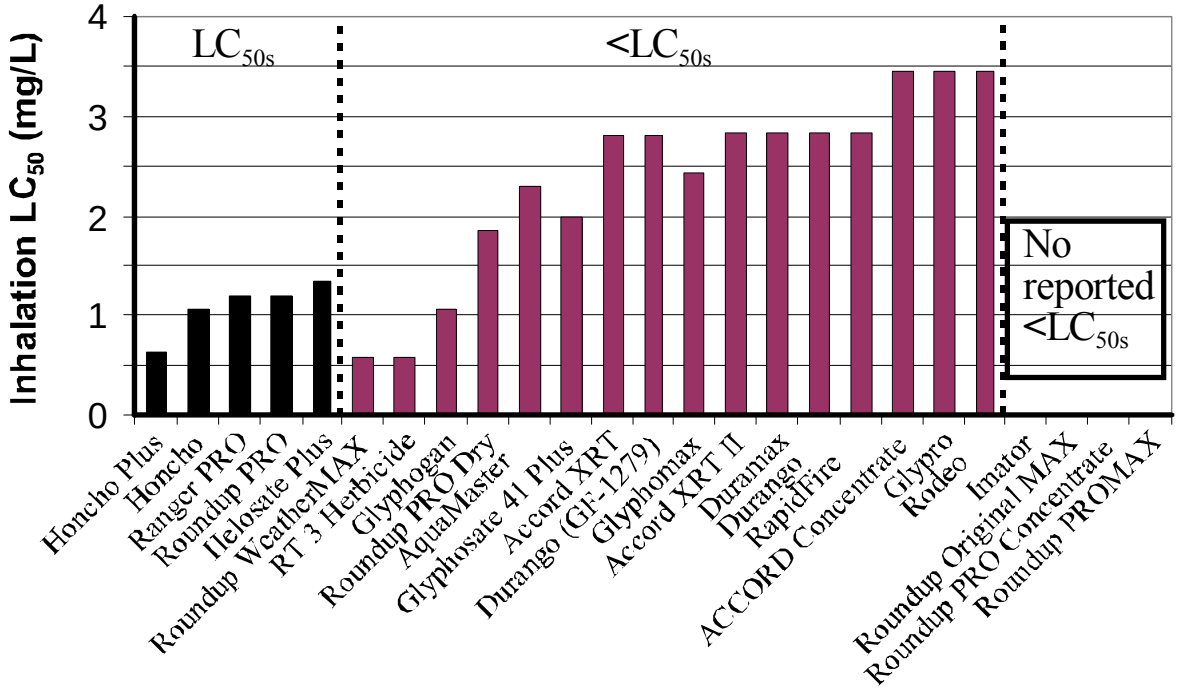


Figure 5: Inhalation toxicity of glyphosate formulations

See Appendix 1, Table 1 for data.
See Section 3.1.13.1 for discussion.

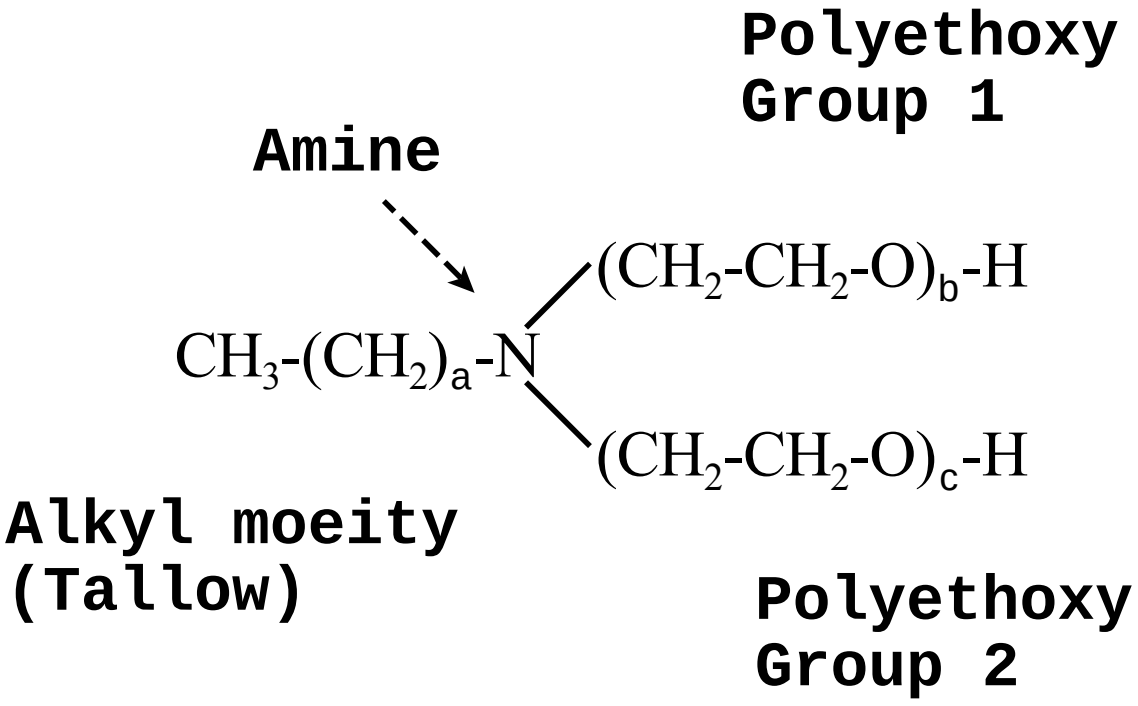
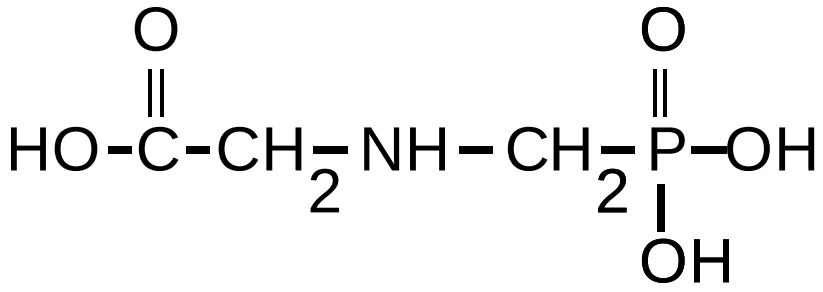
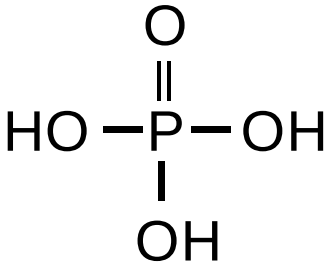


Figure 6: General Structure of POEA Surfactant

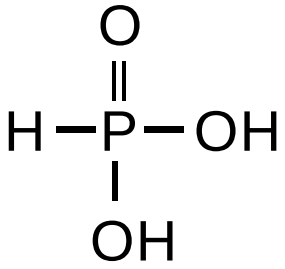
Modified and elaborated from Brausch and Smith (2007)
See Section 3.1.14 for discussion



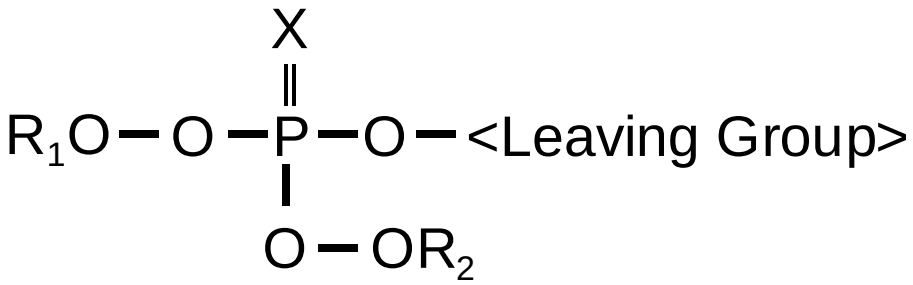
Glyphosate



Phosphoric Acid



Phosphorous Acid



Organophosphate

X = oxygen or sulfur

R_n = organic groups, ethyl or methyl

Leaving group is specific to OP compound

Figure 7: Structure of Glyphosate and Organophosphates

Modified and elaborated from Budavari (1989) and NPIC (2010)
See Section 3.1.6. for discussion

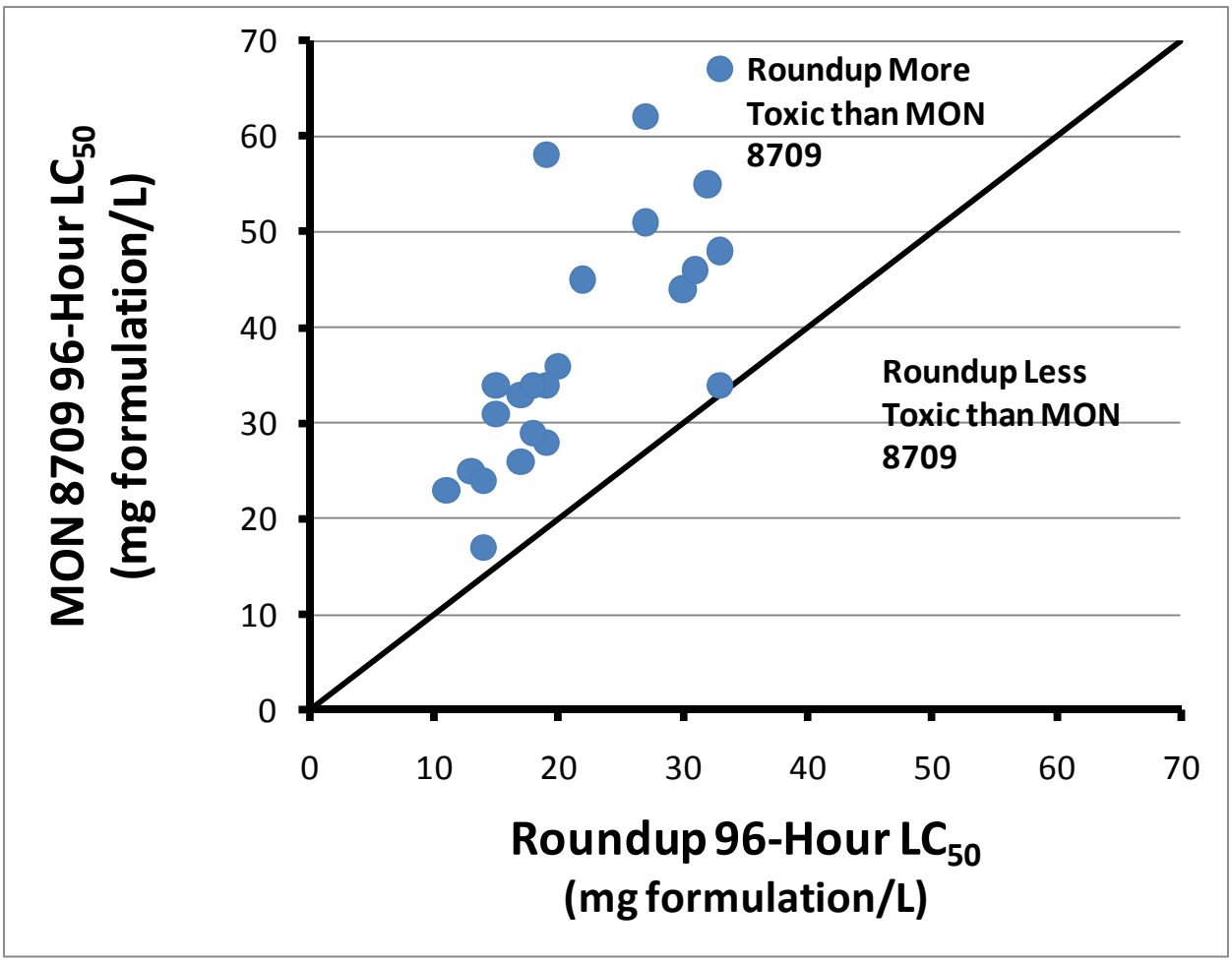


Figure 8: Comparative Toxicity of Roundup and MON 8709

Note: This figure plots comparable LC_{50} values for salmonids from the study by Wan et al. (1989) for Roundup and MON 8709 formulations. The species and LC_{50} values are summarized in Table 23. The solid diagonal line designates the line of equal toxicity. All of the points are above this line, indicating that the Roundup formulation, which contains 15% of the MON 0818 surfactant, is more toxic than the MON 8709 formulation which contains on 10% of the MON 0818 surfactant.

See Section 4.1.3.1.2.4. for a fuller discussion.

Table 1: Chemical and Physical Properties of Glyphosate

Item	Value	Reference
	Identifiers[1]	
Common name:	Glyphosate	Tomlin 2004
CAS and IUPAC Name	N-(phosphonomethyl)glycine	
CAS No.	Acid/Salt (Abbrev) — CAS No. Acid — 1071-83-6 Monoammonium (Am) — 114370-14-8 Dimethyl amine (DMA) — 34494-04-7 Potassium (K) — 70901-12-1 Isopropyl amine (IPA) — 038641-94-0	Tomlin 2004
Molecular formula	$C_3H_8NO_5P$	Tomlin 2004
	Chemical Properties(1)	
Henry's Law Constant	<2.1 x 10^{-7} Pa m^3 mol^{-1}	Tomlin 2004
Hydrolysis	Stable at pH 3, 6, and 9	Tomlin 2004
	Stable	U.S. EPA/OPP 2008a, Table 2.4
Kow	<0.00063 [Log = < -3.2]	Tomlin 2004
	0.00032 [Log = < -3.5]	Schuette 1998
	0.000407 [pH 1.77, Log Kow = -3.39] 0.0000417 [pH 4.61, Log Kow = -4.38] 0.0000141 [pH 6.86, Log Kow = -4.85] 0.0000724 [pH 9.00, Log Kow = -4.14]	Chamberlain et al. 1996
Molecular weight (g/mole)	Acid/Salt — MW — a.i. to a.e. Acid — 169.07 — N/A Monoammonium — 186 — 0.91 Dimethyl amine — 214.16 — 0.79 Potassium — 208.17 — 0.81 Isopropyl amine — 228.2 — 0.74 a.i. to a.e. calculated as MW of acid ÷ MW of salt.	Tomlin 2004, Budavari 1989, and Honegger (2010) (for monoammonium salt only). U.S. EPA/OPP 2008a, Table 2.2[2].
Melting point	189.5±0.5 °C	Tomlin 2004
pKa	2.34 (20 °C), 5.73 (20 °C), 10.2 (25 °C)	Tomlin 2004
	0.8, 2.35, 5.84, 10.48	U.S. EPA/OPP 2008a, Table 2.3
	0.8 (first phosphonic), 2.34 (carboxylate), 5.73 (second phosphonic), 10.2 (amine)	Mose et al. 2008
Photolysis (aqueous)	Stable	U.S. EPA/OPP 2008a, Table 2.4
Specific gravity	1.705	Tomlin 2004
Thermal decomposition	>200 °C	Tomlin 2004
Vapor pressure	1.31 x 10^{-2} mPa (25 °C)	Tomlin 2004
	< 7 x 10^{-9} mm Hg (25 °C)	Weber 1991
Water solubility	10,500 mg/L (pH 1.9, 20 °C)	Tomlin 2004
	12,000 mg/L (25 °C)	USDA/ARS 1995
	900,000 mg/L (IPA salt)	Knissel and Davis 2000
	11,600 mg/L (25 °C)	Schuette 1998

Item	Value	Reference
	Environmental Fate Properties	
Foliar washoff fraction	0.4 (IPA)	Knissel and Davis 2000
Foliar half-life	2.5 days (IPA)	Knissel and Davis 2000
	8 to 10 days	Feng and Thompson 1990
	10.6 to 26.6 days	Newton et al. 1984
	9.5-14.3 days	USDA/ARS 1995
	4 to 7 days	U.S. EPA/OPP 2008a, MRID 45646001, p. 70
Kd	5.3 to 900 (9 soils)	European Commission 2002
	5.86 (mL/g)	Magga et al. 2008
	61 g/m^3	Schuette 1998
	271 to 1140 L/kg	Sorensen et al. 2006
	33 324	USDA/ARS 1995
	227.8 (94.5 to 461.5) in Ap horizon 762 (44 4690) in Bs horizon	Vinther et al. 2008
	152.6 to 251.9 L/kg	Xu et al. 2009
	Soil / Average Kd / Average Koc Sand / 170 / 58,000 Sandy loam / 18 / 3,100 Sandy loam / 230 / 13,000 Silty clay loam / 680 / 33,000 Silty clay loam / 1,000 / 47,000	U.S. EPA/OPP 2008a, Table 2.4 MRID 4432646
Koc	884 to 60,000 (9 soils)	European Commission 2002
	24,000 [IPA]	Knissel and Davis 2000
	2640 2100 [Recommended value] 500	USDA/ARS 1995
	554 to 34,000	Piccolo et al; 1994
	See Kd entry above	U.S. EPA/OPP 2008a, Table 2.4
	253 to 987 (volcanic ash derived soils)	Caceres-Jensen et al. 2009
Soil half-life (NOS)	45 to 60 days	Feng and Thompson 1990
	85.6 to 103.5 days (mineralization)	Getenga and Kengara 2004
	47 days (IPA)	Knissel and Davis 2000
	29 to 40 days	Newton et al. 1984
	30 to 40 days	Smith and Aubin 1993
	37 (2-174) [Recommended values]	USDA/ARS 1995
	18 to 41 days (mineralization)	Reimer et al. 2005
	20 to 40 days	Weber 1991
Soil half-life, aerobic	96.4 days	Schuette 1998
	4 to 180 days (20°C, laboratory)	European Commission 2002
	1.8 and 5.4 days (sandy loam)	U.S. EPA/OPP 2008a, Table 2.4
	2.6 days (silt loam)	U.S. EPA/OPP 2008a, Table 2.4
Soil half-life, anaerobic	22.1 days	Schuette 1998
	0.9 (0.6-1.1) days	USDA/ARS 1995
	100 to 1000 days (for mineralization)	Borggaard and Gimsing 2008
Field dissipation half-life, terrestrial	2.8 to 30 days	Hatfield 1996
	1 to 130 days (13 sites)	European Commission 2002
	21 to 180 days	Laitinen et al. 2006

<table>
<tr><th>Item</th><th>Value</th><th>Reference</th></tr>
<tr><td></td><td>44 days</td><td>Schuette 1998</td></tr>
<tr><td>Field dissipation half-life, terrestrial (continued)</td><td>
<table>
<tr><td colspan="2">Field dissipation half-times</td><td></td></tr>
<tr><td>Glyphosate</td><td>AMPA</td><td>State</td></tr>
<tr><td>1.7</td><td>131</td><td>TX</td></tr>
<tr><td>7.3</td><td>119</td><td>OH</td></tr>
<tr><td>8.3</td><td>958</td><td>GA</td></tr>
<tr><td>13</td><td>896</td><td>CA</td></tr>
<tr><td>17</td><td>142</td><td>AZ</td></tr>
<tr><td>25</td><td>302</td><td>MN</td></tr>
<tr><td>114</td><td>240</td><td>NY</td></tr>
<tr><td>142</td><td>No data</td><td>IA</td></tr>
<tr><td colspan="3">All halftimes are in days.</td></tr>
</table>
</td><td>U.S. EPA/OPP 2008a, Table 2.4
MRIDs 42607501 and 42765001</td></tr>
<tr><td>Forestry dissipation</td><td>Foliar $t_{½}$ < 1 day
Ecosystem:
Glyphosate: 100 days
AMPA 118 days</td><td>U.S. EPA/OPP 2008a, Table 2.4
MRID 41552801</td></tr>
<tr><td></td><td>Prolonged dissipation (>300 days to 50% soil residues) with complex kinetics.</td><td>Newton et al. 2008</td></tr>
<tr><td>Water, photolysis half-time</td><td>33 days (pH 5)
69 days (pH 7)
77 days (pH 9)</td><td>European Commission 2002</td></tr>
<tr><td>Water half-times</td><td>14 day (minimum rate)
42 to 70 days (typical range)</td><td>Reinert and Rodgers 1987</td></tr>
<tr><td></td><td>5.8 to 7.4 days (aquatic mesocosm)</td><td>Perez et al. 2007</td></tr>
<tr><td></td><td>> 35 days</td><td>Schuette 1998</td></tr>
<tr><td></td><td><1 day (pond)</td><td>Trumbo 2005</td></tr>
<tr><td></td><td>50 to 70 days</td><td>U.S. EPA/ODW 1992</td></tr>
<tr><td>Water, aerobic metabolic half-times</td><td>14.1 days (water-silty clay loam sediment)</td><td>U.S. EPA/OPP 2008a, Table 2.4
MRIDs 41723601 and 42372503</td></tr>
<tr><td>Water, anaerobic metabolic half-times</td><td>8 to 199 days</td><td>U.S. EPA/OPP 2008a, p. 25</td></tr>
<tr><td></td><td>208 days (silty clay loam sediment</td><td>U.S. EPA/OPP 2008a, Table 2.4,
MRIDs 41723701 and 42372502</td></tr>
<tr><td></td><td>203 day</td><td>Dix 1998, MRID 44621801</td></tr>
<tr><td>Water, field dissipation half-time</td><td>7.5 days</td><td>U.S. EPA/OPP 2008a, Table 2.4
MRID 41552801</td></tr>
<tr><td></td><td>Rapid dissipation in water. Sediment concentration > 1 ppm at 1 year</td><td>U.S. EPA/OPP 2008a, Table 2.4
MRID 41552801</td></tr>
</table>

[1] All values apply to glyphosate acid unless otherwise specified.

[2] The conversion factor for the monoammonium salt of glyphosate is given by U.S. EPA/OPP 2008a, Table 2.2, as 0.94. The conversion factor of 0.77 given on p. 75 of U.S. EPA/OPP 2008a appears to apply to the tri-ammonium salt. Note also that U.S. EPA/OPP 2008a uses a molecular weight of 170.8 for glyphosate (Table 2.3, p. 25). This appears to reflect a more fully protonated species. The more conventional MW of 169.07 is used in the current Forest Service risk assessment. This minor difference is inconsequential.

Table 2: Glyphosate Formulations Identified by the Forest Service

Formulation Name	Supplier	EPA Reg. No.	Form	Salt	% a.i.	Surfac-tant	Other
Accord	Monsanto	524-326	L	IPA	41.5%		Aq
Accord Concentrate	DowAgro Sciences	62719-324	L	IPA	53.8%		
Accord SP	DowAgro Sciences	62719-322	L	IPA	41%	X	No longer available
Accord XRT	DowAgro Sciences	62719-517	L	IPA	53.6%	X-POEA[10]	
Accord XRT II	DowAgro Sciences	62719-556	L	DMA	50.2%	Inferred	
Aqua Star	Albaugh, Inc.	42750-59	L	IPA	53.8%	?[7]	
AquaMaster (a.k.a. Export and Rodeo)	Monsanto	524-343	L	IPA	53.8%		Aq
AquaNeat	Riverdale	228-365	L	IPA	53.8%		Aq
Buccaneer	Tenkoz Inc	55467-10	L	IPA	41.0%	X	
Buccaneer Plus	Tenkoz Inc	55467-9	L	IPA	41.0%	X	
Cornerstone	Winfield Solutions Agrisolutions	1381-191 71368-20-1381	L	IPA	41.0%	X	
Cornerstone Plus	Winfield Solutions	1381-192	L	IPA	41.0%	?	
Credit Extra	Nufarm	71368-65	L	Am K	17.86% 16.26%	X POEA?	
Credit Systemic Extra	Nufarm	71368-20	L	IPA	41.0%	X POEA?	
Diamondback	EZ-Ject	83220-1	Sh	IPA	83.5%		Injection
DuraMax	DowAgro Sciences	62719-556	L	DMA	50.2%	Inferred	
Durango (GF-1279)	DowAgro Sciences	62719-517	L	IPA	53.6%	X-POEA[10]	
Durango DMA (GF-1280)	DowAgro Sciences	62719-556	L	DMA	50.2%	Inferred	
Eliminator [4,6]	Gro Tec, Inc	71995-27	L	IPA	41.0%	X	
Foresters' Non Selective	Riverdale	228-381	L	IPA	53.8%	None[8]	
Glyphogan	Makhteshim Agan	66222-105	L	IPA	41.0%	Inferred	
Glyphomax 41 Plus [4]	DowAgro Sciences	62719-322	L	IPA	41.0%	Inferred	
Glyphomax XRT	DowAgro Sciences	62719-517	L	IPA	53.6%	X-POEA[10]	
Gly Star Plus	Albaugh Inc	42750-61	L	IPA	41.0%	X	
Glyphosate VMF	DuPont	352-609	L	IPA	53.8%		Cancelled ?
Glyphosate 41 Plus	CropSmart	42750-61-72693	L	IPA	41.0%	?	
GlyphoMate 41 or Pronto	PBI/Gordon Corporation	2217-847	L	IPA	41.0%	X	
Glyfos Aquatic	Cheminova A/S	4787-34	L	IPA	53.8%		Aq
Glyfos X-TRA	Cheminova A/S	4787-23	L	IPA	41.0%	X 15%[6]	
Glypro	DowAgro Sciences	62719-324	L	IPA	53.8%		
Gly-4 Plus	Universal Crop Protection Alliance	72693-1	L	IPA	41.0%	X	
Helosate Plus	Helm Agro US, Inc	74530-4	L	IPA	41.0%	Inferred	
Hi-yield Killzall	Voluntary Purchasing Groups Inc	67760-49-7401		IPA	53.8%		Aq
Honcho (a.k.a. Roundup Original)	Monsanto	524-445	L	IPA	41.0%	X	
Honcho Plus	Monsanto	524-454	L	IPA	41.0%	X	
Imitator Plus	Drexel Chemical	19713-526	L	IPA	41.0%	?	

Formulation Name	Supplier	EPA Reg. No.	Form	Salt	% a.i.	Surfac-tant	Other
KGro Grass and Weed Killer[5]	Swiss Farms Products Inc,	71995-27-73327	L	IPA	1.92%		
Mirage	Loveland Products	34704-866	L	IPA	41.0%	Inferred	
Ranger Pro	Monsanto	524-517	L	IPA	41.0%	X	
RapidFire	DowAgro Sciences	62719-556	L	DMA	50.2%	Inferred	
Rattler	Monsanto	524-445-ZE-5905	L	IPA	41.0%		
Razor	Nufarm	228-366 [1]	L	IPA	41.0%	X 8%[8]	
Razor Pro	Nufarm	228-366 [1]	L	IPA	41.0%	X 14%[8]	
Rodeo	DowAgro Sciences	62719-324	L	IPA	53.8%		
Roundup Original Max	Monsanto	524-539 [3]	L	K	48.7%	X	
Roundup Pro	Monsanto	524-475 [2]	L	IPA	41.0%	X 14.5%	
Roundup Pro Concentrate	Monsanto	524-539 [3]	L	IPA	50.2%	X 13%	
Roundup ProDry	Monsanto	524-505	G	Am	71.4%	X	
Roundup ProMax	Monsanto	524-579	L	K	48.7%	X	
Roundup UltraMax	Monsanto	524-512	L	IPA	50.2%	X	
Roundup UltraDry	Monsanto	524-504	G	Am	71.4%	X 25%	
Roundup WeatherMax	Monsanto	524-537	L	K	48.8%	X	
RT 3	Monsanto	524-544	L	K	48.8%	X	

[1] Razor and Razor Pro appear to have the same EPA Registration number but the formulations are different.

[2] Based on the EPA master product label, this registration number applies to the following brand names: Roundup Ultra Herbicide; Roundup Ultra RT Herbicide; Roundup Pro Herbicide; Roundup Original II CA; MON 77360 Herbicide; Roundup W Herbicide; Gly 41 Herbicide.

[3] Based on the Product Labels and MSDSs, Roundup Original Max and Roundup Pro Concentrate have the same EPA registration number but contain different salts of glyphosate.

[4] Need specimen label. The EPA labels are not clear (are ambiguous) in terms of the formulation(s) covered.

[5] MSDS cannot be located, including searches of http://www.msdsonline.com and http://www.cdms.net.

[6] From Lajmanovich et al. 2003 but not specifically identified as Glyphos Plus.

[7] Bringolf et al. (2007) state that Aqua Star does not contain the MON 0808 POEA surfactant. It is not clear whether or not this formulation contains a less toxic surfactant.

[8] Information confirmed by Nufarm (Ehresman 2010a).

[9] Dow (Fonseca 2010a) has indicated that Accord SP (EPA Reg. No. 62719-322) is not longer commercialized.

[10] Based on information provided by Dow AgroSciences (Fonseca 2010a)

Key:

Form: L=Liquid; G=Granular; Sh=Shells

Salt: Am=Ammonium salt: DMA=Dimethylamine salt; IPA=Isopropylamine salt; K=Potassium salt;

Other: Aq=Aquatic application; Inj=Injection.

Formulations containing herbicides other than glyphosate as the a.e. are not included.

Table 3: Company Product Codes for Some Glyphosate Formulations

Company	Product Code	EPA Reg. No.	Formulations
Dow AgroSciences[1]	NAF-552	62719-324	Accord Concentrate, Glypro and Rodeo
Dow AgroSciences[1]	NAF-545	62719-322	Glyphomax Plus, Accord SP
Dow AgroSciences[1]	GF-1279	62719-517	Accord XRT; Durango; Glyphomax XRT.
Dow AgroSciences[1]	GF-1280	62719-556	Accord XRT II; Duramax; Durango DMA; RapidFire
Monsanto[3]	MON 02139		Roundup (NOS), Hildebrand et al. 1982
Monsanto[3]	MON 20033	524-435	EZ-Ject Capsules
Monsanto[3]	MON 20047	524-400	Roundup Rainfast
Monsanto[3]	MON 02139		Roundup (NOS)
Monsanto[3]	MON 77360	524-475	Roundup Ultra Herbicide; Roundup Ultra RT Herbicide; Roundup Pro Herbicide; Roundup Original II CA; MON 77360 Herbicide; Roundup W Herbicide; Gly 41 Herbicide
Monsanto[3]	MON 77063	524-504	Roundup Ultradry
Monsanto[3]	MON 65005	524-475	An older formulation of Roundup PRO, Monsanto MSDS from Nov 1995
Nufarm	NUP3b99	228-381, 71368-21	Foresters' and Aquaneat

[1] Information from Dow AgroSciences (Fonseca 2010a,b).
[2] Information from Nufarm (Ehresman 2010a).
[3] Information from U.S. EPA/OPP (2008a), EPA generic labels, publications, and information from various web sites as indicated in column 4.

See Section 2.2.2 for discussion.

Table 4: Label Summary of Glyphosate Formulations

Formulation(s)	Known Components	Application Rates and Volumes [1]	Adjuvants
Ammonium Salt			
Roundup ProDry Roundup UltraDry	Am 71.4% a.i. 64.9% a.e. Surfactant	Max. rate: 12.2 lbs/acre (7.92 lb a.e./acre) 2-25 gallons/acre	No additional surfactants recommended. Drift control additives may be used.
Dimethylamine Salt			
Accord XRT II DuraMax Durango DMA RapidFire	DMA 50.2% a.i. 5.4 lb a.i./gallon 4 lbs a.e./gallon	Max. rate: 2 gal/acre (8 lb a.e./acre) 3-60 gallons/acre	Nonionic surfactants not generally recommended but may be use at 0.125 to 0.25 percent. In California, helicopter application may be made for forestry site preparation.
Isopropylamine Salt			
Glyphomax Plus [2] Glyphosate 41 Plus Helosate Plus Imitator Plus Mirage Rattler	IPA 41% a.i. (30.4% a.e.) 4 lbs a.i./gallon 3 lbs a.e./gallon	Max. rate: 10.6 qts/acre (7.95 lb a.e./acre) 3-40 gallons/acre	Nonionic surfactants may be used at 0.5% surfactant (for 70% a.i. surfactants) or 1% surfactant (for <70% a.i. surfactants). Ammonium sulfate [3], colorants, dyes, or drift control additives may be used.
GlyphoMate 41 or Pronto	IPA 41% a.i. 3.8 lbs a.i./gallon 2.8 lbs a.e./gallon	Max. rate: 10.6 qts/acre (7.42 lb a.e./acre) 3-30 gallons/acre	Nonionic surfactants with at least 70% a.i. surfactants. Ammonium sulfate [3], colorants, dyes, or drift control additives may be used.
Buccaneer Buccaneer Plus Glyphogan Honcho Honcho Plus Ranger Pro	IPA 41% a.i. 4 lbs a.i./gallon 3 lbs a.e./gallon 480 g a.i./L 356 g a.e./L Surfactant	Max. rate: 10.6 qts/acre (7.95 lb a.e./acre) 3-40 gallons/acre	Nonionic surfactants with at least 70% a.i. surfactants. Ammonium sulfate [3], colorants, dyes, or drift control additives may be used.
Accord SP Glyfos X-TRA Gly Star Plus	IPA 41% a.i. 4 lbs a.i./gallon 3 lbs a.e./gallon Surfactant	Max. rate: 10.6 qts/acre (7.95 lb a.e./acre) 3-40 gallons/acre	No additional surfactants recommended. Ammonium sulfate [4], colorants, dyes, or drift control additives may be used.
Gly-4 Plus Roundup Pro	IPA 41% a.i. 4 lbs a.i./gallon 3 lbs a.e./gallon 480 g a.i./L 356 g a.e./L Surfactant	Max. rate: 10.6 qts/acre (7.95 lb a.e./acre) 3-40 gallons/acre	No additional surfactants recommended. Colorants, dyes, or drift control additives may be used.
Razor	IPA 41% a.i. 3 lbs a.e./gallon Surfactant (8%)	Max. rate: 10.6 qts/acre (7.95 lb a.e./acre)	Nonionic surfactants with at least 70% a.i. surfactants. Ammonium sulfate [3], colorants, dyes, or drift control additives may be used.
Razor Pro	IPA 41% a.i. 3 lbs a.e./gallon Surfactant (14%)	Max. rate: 10.6 qts/acre (7.95 lb a.e./acre) 3 to 100 gallons/acre	No additional surfactants are recommended Ammonium sulfate [3], colorants, dyes, or drift control additives may be used.
Accord Cornerstone	IPA 41.5% 3 lbs a.e./gallon	Max. rate: 10.6 qts/acre (7.95 lb a.e./acre) 10 to 460 gallons per acre.	Nonionic surfactants with at least 80% a.i. surfactants, 2 quarts per 100 gallons of spray. Drift control additives may be used.

Formulation(s)	Known Components	Application Rates and Volumes [1]	Adjuvants
Roundup Pro Conc. Roundup UltraMax	IPA 50.2% 3.7 lbs a.e./gallon Surfactant	Max. rate: 8.5 qts/acre (7.9 lb a.e./acre) 3-40 gallons/acre	Additional surfactants not generally required but nonionic surfactants may be used. Ammonium sulfate [3], colorants, dyes, or drift control additives may be used.
Accord XRT Durango Glyphomax XRT	IPA 53.6% 5.4 lbs a.i./gallon 4 lbs a.e./gallon	Max. rate: 8 qts/acre (8 lbs a.e./acre) 3-80 gallons/acre	Additional surfactants not generally required but nonionic surfactants (0.125-0.25%) may be used. Ammonium sulfate [3], colorants, dyes, or drift control additives may be used.
Accord Concentrate Foresters' Non Selective Herbicide Rodeo	IPA 53.8% 5.4 lbs a.i./gallon 4 lbs a.e./gallon 648 g a.i./L 480 g a.e./L	Terrestrial Max. rate: 7.5 qts/acre (7.5 lbs a.e./acre) Aquatic Max. rate: 7.5 pts/acre (≈3.9 lbs a.e./acre) 3-60 gallons/acre	Nonionic surfactants with at least 70-80% a.i. surfactants at 10% v/v. Colorants, dyes, or drift control additives may be used.
AquaMaster Aqua Star Glyphosate VMF Glypro	IPA 53.8% 5.4 lbs a.i./gallon 4 lbs a.e./gallon 648 g a.i./L 480 g a.e./L	Terrestrial Max. rate: 7.5 qts/acre (7.5 lbs a.e./acre) Aquatic Max. rate: 7.5 pts/acre (≈3.9 lbs a.e./acre) 3-60 gallons/acre	Nonionic surfactants with at least 70% a.i. surfactants at 0.5 to 2.5 % v/v or greater depending on formulation. Colorants, dyes, or drift control additives may be used.
AquaNeat Glyfos Aquatic Hi-yield Killzall	IPA 53.8% 4 lbs a.e./gallon	Aquatic Max. rate: 7.5 pts/acre (≈3.9 lbs a.e./acre) 3-20 gallons/acre	Labeled only for aquatic applications. Nonionic surfactants with at least 50-70% a.i. surfactants at 2% v/v or greater. Colorants, dyes, or drift control additives may be used.
Potassium Salt			
RT 3	K Salt 48.8% 5.5 lbs a.i./gallon 4.5 lbs a.e./gallon 660 g a.i./L 540 g a.e./L Surfactant	Max. Rate: 5.3 qts/acre (6.0 lbs a.e./acre) 3-40 gallons/acre	No additional surfactant may be added. Ammonium sulfate [3], colorants, dyes, or drift control additives may be used.
Roundup Original Max	K Salt 48.7% 5.5 lbs a.i./gallon 4.5 lbs a.e./gallon 660 g a.i./L 540 g a.e./L Surfactant	Max. Rate: 5.3 qts/acre (6.0 lbs a.e./acre) 10-64 gallons/acre	Surfactant recommended at application volumes of >30 gallons/acre or application rates of <≈0.6 lb a.e./acre. Ammonium sulfate [3], colorants, dyes, or drift control additives may be used.
Roundup ProMax	K Salt 48.7% 5.5 lbs a.i./gallon 4.5 lbs a.e./gallon 660 g a.i./L 540 g a.e./L Surfactant	Max. Rate: 7 qts/acre (8.0 lbs a.e./acre)	No additional surfactant recommended. Colorants, dyes, or drift control additives may be used.

Formulation(s)	Known Components	Application Rates and Volumes [1]	Adjuvants
Roundup WeatherMax	K Salt 48.8% 5.5 lbs a.i./gallon 4.5 lbs a.e./gallon 660 g a.i./L 540 g a.e./L Surfactant	Max. Rate: 7 qts/acre (8.0 lbs a.e./acre) 3-60 gallons/acre	No additional surfactants may be added. Ammonium sulfate [3], colorants, dyes, or drift control additives may be used.

[1] Some maximum application rates apply only to non-crop (forestry) uses. The application volumes are given as gallons of field solution applied per acre.
[2] Only have supplemental labels.
[3] Ammonium sulfate: 1 to 2 % dry ammonium sulfate by weight or 8.5 to 17 pounds per 100 gallons may be used.
[4] Label and MSDS indicate that Glyphomate 41 and Pronto contain 41% IPA and 2.8 lb a.e./gallon. This is not consistent with other 41% formulations which indicate 3 lb a.e./gallon.

Table 5: Classification of formulations

	Apparent Toxicity					
Confidence	Low Toxicity		Medium Toxicity		High Toxicity	
High Confidence	**Accord**	Glyphosate VMF			Buccaneer	**Roundup Orig.**
	Accord Conc	Glypro			Cornerstone	Roundup Pro
	AquaMaster	**Rodeo**			Eliminator	Roundup Pro Conc.
	AquaNeat				Gly Star Plus	Roundup ProDry
	Foresters				**Honcho**	Roundup ProMax
	Glyfos Aquatic				Ranger Pro	Roundup UltraMax
Medium Confidence	Diamondback		Accord SP	Glyphomax Plus	Glyphogan	
			Buccaneer Plus	Gly-4 Plus	Glyphos X-TRA	
			Cornerstone Plus	Honcho Plus	Roundup Orig. Max	
Low Confidence	Aqua Star		Accord XRT		Accord XRT II	RapidFire
			Durango		DuraMax	Roundup WeatherMax
			Glyphomax XRT		Durango DMA	
			Mirage		Helosate Plus	RT 3

Note: Table 6 lists other formulations from Table 2 that are not classified.

Table 6: Formulations not classified

Formulation Name	Supplier	Rationale
Credit Extra	Nufarm	While the toxicity values can be tracked, they are for the a.e. The MSDS states that the formulations *may contain* a tallow amine surfactant.
Credit Systemic Extra	Nufarm	While the toxicity values can be tracked, they are for the a.e. The MSDS states that the formulations *may contain* a tallow amine surfactant.
GlyphoMate 41 or Pronto	PBI/Gordon Corporation	No aquatic data on MSDS.
Glyphosate 41 Plus or GLY- 4 Plus	CropSmart	Cannot associate MSDS entries with toxicity values. Cannot determine if the formulation contains a surfactant.
Hi-yield Killzall	Voluntary Purchasing Groups Inc	No useful mammalian or aquatic information on MSDS to assess the toxicity of this formulation.
Imitator Plus	Drexel Chemical	No useful mammalian or aquatic information on MSDS to assess the toxicity of this formulation.
KGro Grass and Weed Killer	Swiss Farms	Cannot locate label or MSDS.
Rattler	Monsanto	No aquatic data on MSDS.
Razor	Nufarm	While the toxicity values can be tracked, they are for the a.e. This formulation contains a surfactant at a concentration of 8% but the toxicity of the surfactant cannot be determined.
Razor Pro	Nufarm	While the toxicity values can be tracked, they are for the a.e. This formulation contains a surfactant at a concentration of 14% but the toxicity of the surfactant cannot be determined.
Roundup UltraDry	Monsanto	The MSDS states that: *Monsanto has not conducted environmental toxicity studies with this product*. NCAP (2010) has identified inerts as: polyethoxylated tallow amine, polyethylene glycol, sodium sulfite and three inerts that are not disclosed.

Table 7: Uses of Glyphosate by the Forest Service from 2000 to 2004

Management Objective	Pounds	Acres	Lbs/ acre	Fractional Use by Lbs
Release, Conifer	101,174	31,521	3.21	0.585
Site preparation	33,976	16,099	2.11	0.197
Weeds, Noxious	17,114	17,502	0.98	0.099
Release, Hardwood or Other	6,150	3,736	1.65	0.036
Weeds, Agricultural	3,294	3,553	0.93	0.019
Aquatic Weed Control	2,415	388	6.23	0.014
Weeds, Nursery	2,413	1,152	2.09	0.014
Right-of-Way Management	1,820	1,644	1.11	0.011
Wildlife Habitat Improvement	1,326	4,614	0.29	0.008
Other *	979	801	1.22	0.006
Weed Control, Seed Orchard	914	1,322	0.69	0.005
Grassland Restoration	516	291	1.77	0.003
Recreation Improvement	336	609	0.55	0.002
Housekeeping/Facilities Maintenance	221	556	0.40	0.001
Weeds, Other	202	161	1.25	0.001
Grand Total for lbs and acres/ Weighted Average lbs/acre	172,849	83,949	2.06	1

*Other: Fuels reduction, hardwood control, research, and understory/midstory treatment.

Table 8: Glyphosate Use by Forest Service Regions, 2000 to 2004

Forest Service Region	Pounds	Acres	Proportion of Total Lbs.	Proportion of Total Acres	Average Application Rate (lbs/acre)
Region 1: Northern	1,478.46	2,002.22	0.009	0.024	0.74
Region 2: Rocky Mountain	637.11	1,207.98	0.004	0.014	0.53
Region 3: Southwestern	6.0	6.0	<0.001	<0.001	1
Region 4: Intermountain	1,065.14	1,622.93	0.006	0.019	0.66
Region 5: Pacific Southwest	135,653.70	35,628.32	0.782	0.424	3.81
Region 6: Pacific Northwest	6,396.87	4,755.06	0.037	0.057	1.35
Region 8: Southern	19,338.41	18,546.58	0.111	0.221	1.04
Region 9: Eastern	8,961.12	20,247.35	0.052	0.241	0.44
Region 10: Alaska	0.33	1	<0.001	<0.001	0.33
Total	**173,537.14**	**84,017.45**	**Average:**		**2.07**

Table 9: Definitive LD_{50} values for glyphosate formulations

Formulation (EPA Reg. No.)	Components	LD_{50} (mg formulation/kg bw)	LD_{50} (mg a.e./kg bw)	Reference/Comment
From U.S. EPA/OPP (2008a)				
Roundup Rainfast (524-440)	25.1% IPA salt	3750	696	From Table 5.5 of U.S. EPA/OPP. MRID 41305404. Not identified as a formulation used by the Forest Service.
Glyphomax (62719-323)	41% IPA salt, 30.4% a.e.	3803	1156	From Table 5.5 of U.S. EPA/OPP. MRID 44918601. Not identified as a formulation used by the Forest Service. U.S. EPA/OPP 2008a reports the LD_{50} as 724 mg a.e./kg bw.
Roundup UltraDry (524-504)	71.4% monoammonium salt, 25% surfactant	5827	3204	From Table 5.5 of U.S. EPA/OPP (2008a). MRID 44615502. This formulation is used by the Forest Service (Table 2). Note: The MSDS reports an LD_{50} of 3700 mg/kg bw – i.e., a conversion from formulation to a.e. U.S. EPA/OPP 2008a reports the LD_{50} as 2599 mg a.e./kg bw.
EZ-Ject Capsules (524-435)	83.5% IPA salt	5000	3089	From Table 5.5 of U.S. EPA/OPP. MRID 41142304. Not identified as a formulation used by the Forest Service.
MON-14420, Water Soluble Granules (524-424)	65.8% a.e, mono-ammonium salt	2686	1767	From Table 5.5 of U.S. EPA/OPP. MRID 40853903. Not identified as a formulation used by the Forest Service. Not included in Appendix 2 – i.e., data not summarized in U.S. EPA/OPP 2008a or other sources.
HM-2028	11.4% a.i. NOS		357	From U.S. EPA/OPP 2008a, Appendix J, Table J-26. The commercial name of the formulation cannot be identified.
From MSDS's				
Ranger Pro and Roundup Pro	41% IPA salt, surfactant ?	5108	1549	Definitive LD_{50} of 5108 mg/kg reported on MSDS, assumed to be reported in units of formulation .
Helosate Plus	41% IPA salt, surfactant?	5000	1517	Definitive LD_{50} of 5108 mg/kg reported on MSDS, assumed to be reported in units of formulation .
Roundup ProDry	71.4% monoammonium salt, 64.9% a.e. with surfactant	3794?	3794?	Definitive LD_{50} of 3794 mg/kg reported on MSDS. The MSDS states: *Data obtained on product and components*. It is not clear if the LC50 is in units of formulation, a.i., or a.e.
Open Literature				
Roundup	(41% a.i., 15% surfactant)	5337	1619	Baba et al. 1989 (Japanese open literature)
Roundup (Brazil)	(360 g a.e./L, 18% surfactant)	2300	828	Dellegrave et al. 2007

Table 10: Concentration of glyphosate in rat tissues after oral dosing

Tissue	Estimated Concentrations (mg/L) in Tissues After Dosing at 10 mg/kg bw		
	Hours After Dosing		
	2	6.3	28
Abdominal fat	0.27	0.29	0.18
Blood plasma	0.86	0.83	0.08
Bone	2.62	5.86	6.71
Brain	0.02	0.08	0.06
Colon	6.70	14.52	3.82
Heart	0.19	0.15	0.05
Kidney	7.03	12.95	2.60
Liver	0.25	0.41	0.40
Lung	0.33	0.29	0.15
Red cells	0.16	0.17	0.05
Small intestine	135.38	90.75	4.46
Spleen	0.12	0.20	0.23
Stomach	1.58	2.26	0.29
Testes	0.11	0.16	0.07
Testicular fat	0.67	0.48	0.07

Data from Brewster et al. 1991
See Figure 3 for illustration.
See Section 3.1.3.1. for discussion.

Table 11: Summary of Suicidal Ingestions of Glyphosate Formulations

Formulation	Amount Consumed (mL)	Body Weight	Estimated Dose mg formulation/kg bw)	Outcome	Reference
Formulation with surfactant	100	70	1,714	Survived	Hsiao et al. 2008
Roundup (41% a.i.)	200	70	3,429	Survived	Moon et al. 2006
Roundup NOS	240	70	4,114	Survived	Sampogna and Cunard 2007
Roundup concentrate NOS	225	60	4,500	Died	Temple and Smith 1992
Roundup (41% a.i.)	300	70	5,143	Survived	Moon et al. 2006
Chinese formulation, 41% a.i.	400	60	8,000	Died	Chang and Chang 2009
Glyphosate, 41% a.i., surfactant 15%	500	70	8,571	Died	Stella and Ryan 2004
Glyphosate, 41% a.i., surfactant 15%	1000	70	17,143	Died	Stella and Ryan 2004
		Geometric Mean:	5,337		

[a] The publications do not specify body weights. Default body weights of 60 kg for females and 70 kg for males is assumed. Dose is estimated assuming a formulation density of 1.2 g/mL (1,200 mg/mL) for the formulations. This density is typical of many formulations that contain surfactants.

See Section 3.1.4.4 for discussion.

Table 12: Summary of Developmental and Reproduction Studies

Species	Doses (mg/kg bw/day)[1]		Endpoint	Reference
	NOAEL	LOAEL		
Developmental/Teratology Studies				
Glyphosate acid				
Rat	≈1,000	≈1,000	Maternal: Liver Offspring: No effects	Beuret et al. 2005
Rat	ND 455	455	Maternal: Body weight ↓ Offspring: No effects	Daruich et al. 2001
Rat	1000	ND	Maternal and Fetal	Moxon 1996a
Rat	1000 1000	3500 3500	Maternal: Mortality Fetal: Delayed ossification	Rodwell et al. 1980a
Rabbit	100 175	175 300	Maternal: GI toxicity Fetal: Delayed ossification	Moxon 1996b
Rabbit	**175**[2] 350	350 ND	Maternal: Mortality Fetal: No effects	Rodwell 1980b
Roundup (Brazilian Formulation, 360 a.e./L and 18% (w/v) surfactant)				
Rat	750 ND	1000 500	Maternal: Mortality Fetal: Delayed ossification	Dallegrave et al. 2003
Rat	450 450 150	ND ND 450	Maternal: No effects Fetal at birth: No effects. Fetal: Decrease in testosterone in male offspring at puberty.	Dallegrave et al. 2007
POEA Surfactant (Monsanto)				
Rat–POEA	15 300	100 ND	Maternal: Signs of toxicity Fetal: No effects	Farmer et al. 2000b
Rat–Neutralized POEA	50 150	150 ND	Maternal: Mortality Fetal: No effects	Farmer et al. 2000b
Multigeneration Reproduction Studies (Glyphosate Only)				
Glyphosate acid				
Rat	500	1500	Parental and fetal toxicity	Reyna 1985, MRID 41621501
Rat	740 740	2268 2268	Parental: Body weight ↓ Offspring: Body weight ↓ and litter size.	Farmer et al. 2000a
Rat	30 30 **10**[2]	ND ND 30?	Parental: No effects Reproduction: No effects Fetal systemic: focal tubular dilation of the kidney in F_{3b} pups.	Schroeder and Hogan 1981, MRID 81674 and 105995
MON 0818 Surfactant				
Rat	≈53 ≈15	ND ≈53	No parental toxicity. Decrease litter sizes and other reproductive endpoints.	Knapp 2006, MRID 47097401

[1]Doses in units of mg a.e./kg bw for glyphosate and glyphosate formulations. Units of surfactant for the studies on surfactants.

[2]The NOAELs used by U.S. EPA/OPP (1993b) and U.S. EPA/ORD (1990) for the EPA chronic RfDs for glyphosate are given in bold in the NOAEL column.

See Section 3.1.9. for discussion.

For study details, see Appendix 2, Table 3 (Glyphosate and Formulations) and Table 5 (POEA surfactants)

Table 13: General Site Conditions used in Gleams-Driver runs

Field Characteristics	Description		
Type of site	Mixed pine-hardwood		
Treated and total field areas	10 acres		
Field width	660 feet		
Slope	0.1		
Depth of root zone	60 inches		
Cover factor	0.15		
Type of clay	Mixed		
Surface cover	No surface depressions		
Pond Characteristics	**Description**		
Surface area	1 acre		
Drainage area:	10 acres		
Initial Depth	2 meters		
Minimum Depth	1 meter		
Maximum Depth	3 meters		
Sediment Depth	2 centimeters		
Stream Characteristics	**Description**		
Width	2 meters		
Flow Velocity	6900 meters/day		
Flow Rate	710,000 liters/day		
Soil Specific Factors [a]	**Clay**	**Loam**	**Sand**
Runoff potential	High	Moderate	Low
Surface type	Road	Woods	Meadow
Surface condition	Hard surface	Fair	Dirt
[a] Detailed input values for the soil types are given in SERA (2007b, Tables 2 and 3) .			

Table 14: Location and Weather Classification for Standard Gleams-Driver Sites

Location	Precipitation	Temperature	Average Annual Rainfall (inches)	Average Annual Temperature (◦F)
HI, Hilo	Wet	Warm	126.06	73.68
WA, Quillayute [1]	Wet	Temperate	95.01	49.14
NH, Mt. Washington	Wet	Cool	98.49	27.12
FL, Key West	Average	Warm	37.68	77.81
IL, Springfield	Average	Temperate	34.09	52.79
MI, Sault Ste. Marie	Average	Cool	32.94	40.07
AR, Yuma Test Station	Dry	Warm	3.83	73.58
CA, Bishop	Dry	Temperate	5.34	56.02
AK, Barrow	Dry	Cool	4.49	11.81

[1] Based on composite estimation in WEPP using a latitude of 47.94 N and a longitude of -124.54 W. See SERA (2006c) for details.

Table 15: Chemical parameters used in GLEAMS modeling

Parameter	Clay	Loam	Sand	Note/Reference
Halftimes (days)				
Aquatic Sediment		208		Note 1
Foliar		10		Note 2
Soil		5.4		Note 3
Water		21		Note 4
Soil $K_{o/c}$, mL/g	3100 (2000 to 24,000)			Note 5
Sediment K_d, mL/g	420 (18 to 1000)			Note 6
Water Solubility, mg/L	12,000			Note 7
Foliar wash-off fraction	0.6			Note 8
Fraction applied to foliage	0.5			Note 9

Note 1	U.S. EPA/OPP 2008a, Table 2.4, MRIDs 41723701 and 42372502
Note 2	Central value from Feng and Thompson 1990 and Newton et al. 1984. This value is modestly higher (more conservative) than the foliar half-time of 7 days used by U.S. EPA/OPP 2008a, p. 70, from MRID 45646001.
Note 3	Central value from U.S. EPA/OPP 2008a, Table 3.1, 90% upper bound of the mean value from MRIDs 42372501 and 44320642.
Note 4	Central value from U.S. EPA/OPP 2008a, Table 3.1. As somewhat lower values (14.1 days) are reported in U.S. EPA/OPP 2008a, Table 2.4 MRIDs 41723601 and 42372503.
Note 5	Central value from U.S. EPA/OPP 2008a, Table 3.1, lowest non-sand value. The upper bound is from Knissel and Davis (2000). The lower bound is taken as the geometric mean from Gerritse et al. (1996). Lower values in some soils are plausible. A triangular distribution was used in the Gleams-Driver runs.
Note 6	Mean and range of Kd values for sand to silty clay loam soils from U.S. EPA/OPP 2008a, Table 2.4 MRID 4432646. A triangular distribution was used in the Gleams-Driver runs.
Note 7	Central value from U.S. EPA/OPP 2008a, Table 3.1.
Note 8	From Knissel and Davis (2000). The use of surfactants could lower foliar washoff which would in turn lower concentrations in water.
Note 9	This is a standard assumption in Gleams-Driver modeling in Forest Service risk assessments.

Note to Forest Service personnel: The Gleams-Driver data for glyphosate has been updated to reflect the above values. For Soil $K_{o/c}$ and Sediment K_d only the central values are entered into the Gleams-Driver data file. If you want to use ranges/distributions, you will need to use the Full Run facility in Gleams-Driver.

Table 16: Summary of modeled and monitored concentrations in surface water

Scenario	Concentrations (ppb or µg/L)	
	Peak	**Long-Term Average**
MODELING FOR THIS RISK ASSESSMENT (1 lb a.e./acre)		
Direct Spray and Spray Drift		
Pond, Direct Spray (Section 3.2.3.4.2) [a]	112	N/A
Pond, drift at 25 feet (Section 3.2.3.4.2) [a]	1	N/A
Stream, Direct Spray (Section 3.2.3.4.2) [a]	91	N/A
Stream, drift at 25 feet (Section 3.2.3.4.2) [a]	0.76	N/A
Gleams-Driver		
Ground Broadcast Applications, 1 lb a.e./acre		
Pond (Section 3.2.3.4.4) [b]	1.3 (0 to 29)	0.19 (0 -4.5)
Stream (Section 3.2.3.4.4) [c]	2.9 (0 - 83)	0.088 (0 - 2.6)
Aquatic Application	74 (37-184)	11 (2.4 – 105)
Other Modeling		
U.S. EPA		
GENEEC [d]	11.0	5.8 [60 day ave.]
Aquatic Application [e]	56	
Monitoring [f]		
Over-sprayed stream, British Columbia (Kreutzweiser et al. 1989)	90 (WCR)	5 (WCR)
Over-sprayed streams in Oregon (Newton et al. 1984)	93 (WCR)	
Over-sprayed streams in Oregon, Michigan, and Georgia (Newton et al. 1994)	28 to 280 (WCR)	
Streams, southeast U.S. (Neary and Michael 1996)	0.003 to 0.007	
Surface water, agric. area in Portugal (Abrantes et al. 2009)	3.87	
Ephemeral pools in U.S. (Battaglin et al. 2009)	328 (max)	
Surface water in agric. areas in France (Botta et al. 2009)	75 to 90	
Surface water in agric. areas in Argentina (Peruzzo et al. 2008)	100 to 700	
Concentration in U.S. streams (USGS, Scribner et al. 2003)	<0.1 (median) to 8.7 (max)	
Concentration in U.S. surface water (USGS, Scribner et al. 2008)	< 0.1 to 427	
Ontario surface water (Stuger et al. 2008)	40.8	
Well water (Smith et al. 1996)	5.7 (WCR)	

[a] Section 3.2.3.4.2 discusses expected concentrations in terms of the unit application rate of 1.0 lb a.e./acre. The values for direct spray and drift are taken from Worksheet 10a (direct spray and drift as 25 feet for a pond) and Worksheet 10b (direct spray and drift as 25 feet for a stream).
[b] See Appendix 10, Tables 7 and 8, for more detailed site-specific summary of pond modeling.
[c] See Appendix 10, Tables 5 and 6, for more detailed site-specific summary of stream modeling.
[d] U.S. EPA/OPP 2008a, Table 3.2 adjusted to a WRC (an application rate of 1 lb/acre) from an application rate of 7.95 lb a.e/acre.
[e] U.S. EPA/OPP 2008a, Table 3.2 adjusted to a WRC (an application rate of 1 lb/acre) from an application rate of 3.75 lb a.e./acre.
[f] Unless specifically noted with a *(WCR)* following the values, monitored concentrations are not associated with an application rate.

Table 17: Surface water concentrations used in this risk assessment

(see Section 3.2.3.4.6 for discussion)

	Water contamination rate in mg/L per lb/acre applied [a]	
	Peak	**Longer-term**
Terrestrial Applications		
Central	0.011 [b]	0.00019 [e]
Lower	0.0013 [c]	0.000088 [f]
Upper	0.083 [d]	0.0058 [g]
Aquatic Applications [h]		
Central	0.074	0.011
Lower	0.037	0.0024
Upper	0.18	0.11

[a] Water contamination rates – concentrations in units of mg a.e./L expected at an application rate of 1 lb a.e./acre. Units of mg a.e./L are used in the EXCEL workbook that accompanies this risk assessment.

[b] Based on U.S. EPA/OPP (2008a).

[c] Based on central estimate from Gleams-Driver modeling for ponds.

[d] Based on upper bound of Gleams-Driver modeling for streams.

[e] Based on central estimate from Gleams-Driver modeling of ponds.

[f] Based on central estimate from Gleams-Driver modeling of streams.

[g] Based on GENEEC modeling by U.S. EPA/OPP (2008a).

[h] Based on dilution model. See Worksheet B04a in Attachment 2 (EXCEL workbook for aquatic applications.

See Table 16 for additional data.
See Section 3.2.3.4.6 for discussion.

Table 18: Estimated residues in food items per lb a.i. applied

Food Item	Concentration in Food Item (ppm per lb a.i./acre)		
	Central [a]	Lower [b]	Upper [a]
Broadcast Foliar Applications			
Short grass	85	30	240
Tall grass	36	12	110
Broadleaf/forage plants and small insects	45	15	135
Fruits, pods, seeds, and large insects	7	3.2	15

[a] From Fletcher et al. (1997) and U.S. EPA/EFED 2001, p. 44.
[b] Central values × (Central Value ÷ Upper Value).

Table 19: Summary of Risk Characterization for Workers

Scenario	Hazard Quotients		
	Central	Lower	Upper
Accidental Exposures			
Contaminated Gloves, 1 min.	2E-06	2E-07	2E-05
Contaminated Gloves, 1 hour	1E-04	1E-05	9E-04
Spill on Hands, 1 hour	2E-04	3E-05	1E-03
Spill on lower legs, 1 hour	6E-04	7E-05	3E-03
General Exposures			
Aquatic Applications	0.005	0.002	0.01
Aerial Applications	0.007	0.0001	0.04
Ground Broadcast Applications	0.01	0.0003	0.08
Backpack Applications	0.007	0.0002	0.04

See Attachments 1a-c and Attachment 2 for details.
All of the above HQs are based on an application rate of 1 lb a.e./acre.
See Section 3.4.2. for discussion and consideration of other application rates.

Table 20: Risk Characterization for the General Public, Terrestrial Applications

Scenario	Receptor	Hazard Quotients		
		Central	Lower	Upper
Accidental Acute Exposures (dose in mg/kg/event)				
Direct Spray of Child, whole body	Child	9E-03	1E-03	4E-02
Direct Spray of Woman, feet and lower legs	Adult Female	9E-04	1E-04	4E-03
Water consumption (spill)	Child	0.2	8E-03	**1.0**
Fish consumption (spill)	Adult Male	2E-03	2E-04	8E-03
Fish consumption (spill)	Subsistence Populations	9E-03	8E-04	4E-02
Non-Accidental Acute Exposures (dose in mg/kg/event)				
Vegetation Contact, shorts and T-shirt	Adult Female	5E-04	2E-04	1E-03
Contaminated Fruit	Adult Female	6E-03	3E-03	9E-02
Contaminated Vegetation	Adult Female	8E-02	6E-03	0.7
Swimming, one hour	Adult Female	2E-09	6E-11	7E-08
Water consumption	Child	4E-04	3E-05	5E-03
Fish consumption	Adult Male	5E-06	6E-07	4E-05
Fish consumption	Subsistence Populations	2E-05	3E-06	2E-04
Chronic/Longer Term Exposures (dose in mg/kg/day)				
Contaminated Fruit	Adult Female	9E-04	4E-04	1E-02
Contaminated Vegetation	Adult Female	1E-02	9E-04	0.1
Water consumption	Adult Male	3E-06	9E-07	1E-04
Fish consumption	Adult Male	5E-09	2E-09	2E-07
Fish consumption	Subsistence Populations	4E-08	2E-08	1E-06

See Attachments 1a-c for details.

All of the above HQs are based on an application rate of 1 lb a.e./acre.
See Section 3.4.3. for discussion and consideration of other application rates.

Table 21: Risk Characterization for the General Public, Aquatic Applications

Scenario	Receptor	Hazard Quotients		
		Central	Lower	Upper
Accidental Acute Exposures (dose in mg/kg/event)				
Direct Spray of Child, whole body	Child	No exposure assessment.		
Direct Spray of Woman, feet and lower legs	Adult Female	No exposure assessment.		
Water consumption (spill)	Child	0.2	8E-03	**1.0**
Fish consumption (spill)	Adult Male	2E-03	2E-04	8E-03
Fish consumption (spill)	Subsistence Populations	9E-03	8E-04	4E-02
Non-Accidental Acute Exposures (dose in mg/kg/event)				
Vegetation Contact, shorts and T-shirt	Adult Female	No exposure assessment.		
Contaminated Fruit	Adult Female	No exposure assessment.		
Contaminated Vegetation	Adult Female	No exposure assessment.		
Swimming, one hour	Adult Female	2E-07	5E-08	8E-07
Water consumption	Child	3E-03	8E-04	1E-02
Fish consumption	Adult Male	3E-05	2E-05	8E-05
Fish consumption	Subsistence Populations	2E-04	8E-05	4E-04
Chronic/Longer Term Exposures (dose in mg/kg/day)				
Contaminated Fruit	Adult Female	No exposure assessment.		
Contaminated Vegetation	Adult Female	No exposure assessment.		
Water consumption	Adult Male	2E-04	2E-05	2E-03
Fish consumption	Adult Male	3E-07	6E-08	3E-06
Fish consumption	Subsistence Populations	3E-06	5E-07	2E-05

See Attachment 2 for details.

All of the above HQs are based on an application rate of 1 lb a.e./acre.
See Section 3.4.3. for discussion and a consideration of other application rates.

Table 22: Toxicity of Glyphosate Formulations to Fish

Formulation	96-hour LC_{50} mg a.e./L	
	Lower Bound	**Upper Bound**
Roundup, all studies	0.96 mg a.e./L Rainbow trout, pH 7.2 Folmar et al. 1979	10 mg a.e./L Several species, pH 6.3 Wan et al. 1989
MON 77360 (e.g., Roundup Ultra)	1.6 mg a.e./L Rainbow trout MRID 45365003	
MON65005 (e.g., older Roundup Pro)	2.4 mg a.e./L Bluegill sunfish MRID 44538203	
GF-1280 (e.g., Accord XRT II)	4.3 mg a.e./L Rainbow trout Hughes 2006a	
GF-1279 (e.g., Accord XRT)	11.26 mg a.e./L Zebra fish Bidinotto 2005a	
Roundup with 15% "W" Data from 1980s	>30 mg a.e./L Bluegill sunfish and trout MRID s 78656 and 78655	
Roundup with Geronol CF/AR surfactant	NOAEL: 450 mg a.e./L Rainbow trout MRID 44738201 /1996	
Roundup Biactive (Australian formulation)	NOAEC: 800 mg a.e./L Rainbow trout MRID 44738201	
Rodeo (no surfactant)	429 mg a.e./L Rainbow trout MRID 40579301 (Mitchell et al. 1987a)	
Rodeo (X-77 surfactant)	96.4 mg a.e./L Rainbow trout MRID 40579301 (Mitchell et al. 1987a)	180.2 mg a.e./L Chinook salmon MRID 40579305

See Section 4.1.3.1.2.2. for discussion.

Table 23: Joint Action of Glyphosate and POEA in Fish from Wan et al. (1989)

pH	LC_{50} mg/L		Potency (Gly ÷ POEA)	Roundup LC_{50} (mg formulation/L)			MON 8709 LC_{50} (mg formulation/L)		
	Glyphosate (a.e.)	POEA		Obser-ved	Predic-ted	Pred./ Obs.	Obser-ved	Predic-ted	Pred./ Obs.
Coho salmon									
6.3	27	4.6	5.9	32	27.9	0.87	55	36.1	0.66
7.2	36	3.2	11.3	27	22.8	0.84	51	31.2	0.61
7.8	112	2.8	40	33	23.3	0.71	34	33.9	1.00
7.8	111	2.9	38.3	30	24.1	0.8	44	34.9	0.79
8.2	174	1.8	96.7	13	15.6	1.2	25	23	0.92
Chum salmon									
6.3	10	2.7	3.7	20	13.9	0.7	36	17.2	0.48
7.2	22	2.4	9.2	19	16.4	0.86	58	22.1	0.38
7.8	99	2.6	38.1	15	21.6	1.44	34	31.3	0.92
8.2	148	1.4	105.7	11	12.1	1.1	23	18	0.78
Chinook salmon									
6.3	19	2.8	6.8	33	17.8	0.54	67	23.3	0.35
7.2	30	2.8	10.7	27	19.9	0.74	62	27.1	0.44
7.8	102	2.7	37.8	19	22.4	1.18	28	32.5	1.16
7.8	108	2.6	41.5	22	21.7	0.99	45	31.6	0.7
8.2	211	1.7	124.1	17	14.8	0.87	33	22	0.67
Pink salmon									
6.3	14	4.5	3.1	33	21.4	0.65	48	26	0.54
7.2	23	2.8	8.2	31	18.7	0.6	46	25	0.54
7.8	94	1.5	62.7	17	12.8	0.75	26	18.8	0.72
7.8	102	2.6	39.2	19	21.6	1.14	34	31.4	0.92
8.2	190	1.4	135.7	14	12.2	0.87	24	18.1	0.75
Rainbow trout									
6.3	10	2	5.0	33	11.5	0.35	48	14.7	0.31
7.2	22	2.5	8.8	15	17	1.13	31	22.8	0.74
7.8	99	1.6	61.9	18	13.6	0.76	34	20	0.59
7.8	93	2.6	35.8	18	21.5	1.19	29	31.1	1.07
8.2	197	1.7	115.9	14	14.8	1.06	17	21.9	1.29

See Section 4.1.3.1.2.4 for discussion.

See Attachment 3, Worksheets **"Wan et al. 1989 Roundup"** and **"Wan et al. 1989 MON 8709"** for calculations.

Table 24: Joint Action of Glyphosate and POEA in Fish from Folmar et al. (1979)

	pH	Glyphosate LC_{50} (mg a.e./L)	MON 0818 (mg surf./L	Potency (Gly-a.e to MON 0818)	Observed LC_{50} Formulation (mg a.e./L)	Observed Formulation LC_{50} (mg form/L)[1]	Predicted Formulation LC_{50} (mg form/L)	Predicted LC_{50} ÷ Observed LC_{50}
Trout	6.5	140	7.4	18.9	7.6	24.7	44.6	1.8
	9.5	240	0.65	369.2	1.4	4.5	4.3	0.96
Bluegills	6.5	140	1.3	107.7	4.2	13.6	8.5	0.63
	9.5	220	1.0	220	1.8	5.8	6.6	1.14

[1] Folmar et al. (1979, Table 6, p. 276) reports the observed LC_{50} values for Roundup in units of mg a.e./L. These are converted to units of mg formulation/L by dividing the reported LC_{50} by the 0.308, the approximate proportion of glyphosate a.e. in the Roundup formulation.

See Section 4.1.3.1.2.4 for discussion.
See Attachment 3, Worksheet "Folmar et al. 1979 fish" for calculations.

Table 25: Toxicity of Glyphosate and Glyphosate Formulations to Amphibians

Formulation	96-hour LC_{50} Values	
	Lower Bound	**Upper Bound**
Glyphosate acid	75.2 mg a.e./L Australian tree frog, adult MRID 43839601	121 mg a.e./L *Litoria moorei*, tadpoles Mann and Bidwell 1999
Glyphosate IPA	>17 mg a.e./L to >466 mg a.e./L Several species, tadpoles Howe et al. 2004; Mann and Bidwell 1999	
Rodeo (no surfactant)	604.2 [pH 8] to 6870 [pH 7.6 to 7.9] mg a.e./L *Xenopus laevis*, embryos (blastula) Edginton et al. 2004b; Perkins 1997; Perkins et al. 2000	
Glyphosate IPA with 10-45% Geronol CF/AR	>100 mg a.e./L to >450 mg a.e./L [NOAECs] *Ranidella signifera*, tadpole MRID 44738201 in U.S. EPA/OPP 2008a	
Roundup Biactive	>17.9 mg a.e./L *Rana clamitans* Howe et al. 2004	>494 mg a.e./L *Crinia insignifera*, tadpole Mann and Bidwell 1999
Glyfos BIO with 3-7% POEA	>17.9 mg a.e./L Green Frog Howe et al. 2004	
Glyfos with 15% POEA	0.93 mg a.e./L *Scinax nasicus*, GS 18-24 Lajmaovich et al. 2003	
Glyphos with Cosmo-Flux (South American formulation)	1.2 mg a.e./L *D. microcephalus*, GS 10-11 Bernal et al. 2009a	2.7 mg a.e./L *R. marilla*, GS 10-11 Bernal et al. 2009a
Roundup Original Max	0.8 mg a.e./L American bullfrog, larvae Relyea and Jones 2009	3.2 mg a.e./L Spotted salamander, larvae Relyea and Jones 2009
Roundup Original (15% POEA)	2.2 mg a.e./L Green Frog Howe et al. 2004	>8.0 mg a.e./L Wood frog Howe et al. 2004
Roundup (MON 2139)	2.9 mg a.e./L *Litoria moorei*, tadpole Mann and Bidwell 1999	51.8 mg a.e./L *Crinia insignifera*, metamorph Mann and Bidwell 1999
Vision (with 15% MON 0818)	2.7 mg a.e./L *Rana clamitans*, GS 21-24 Wojtaszek et al. 2004	11.47 mg a.e./L *Rana pipiens*, GS 21-24 Wojtaszek et al. 2004
Vision (with 15% POEA)	1.1 mg a.e./L *Rana pipiens*, Larvae, pH 7.5 Edginton et al. 2004a	15.6 mg a.e./L *Xenopus laevis*, Larvae, pH 6 Edginton et al. 2004a

GS=Gosner Stage.

See Appendix 7 for details.
See Section 4.1.3.2 for discussion.

Table 26: Toxicity of Glyphosate Formulations to Aquatic Invertebrates

Formulation	48-Hour[2] EC_{50}/LC_{50} mg a.e./L	
	Lower Bound	**Upper Bound**
Roundup [1]	1.5 mg a.e./L amphipod Tsui and Chu 2004	62 mg a.e./L amphipod Folmar et al. 1979
Roundup Ultramax	2.9 mg a.e./L Mussel, larvae Bringolf et al. 2007	5.9 mg a.e./L Mussel, juvenile Bringolf et al. 2007
MON 77360 (e.g., Roundup Ultra)	3.2 mg a.e./L *Daphnia magna*, Drottar and Krueger 2000c	
MON 65005 (e.g., older Roundup Pro)	2.7 mg a.e./L *Daphnia magna*, MRID 44538201	
Roundup, 18% IPA salt (home use product?)	>13.5 mg a.e./L (24 hours) Mussel, larvae, Conners and Black 2004	
GF-1280 (e.g., Accord XRT II)	≈25 mg a.e./L *Daphnia magna*, Hughes 2006c	
GF-1279 (e.g., Accord XRT)	≈ 19 mg a.e./L *Daphnia magna*, Sesso 2005a	
Roundup with 15% "W" Data from 1980s	21.7 mg a.e./L *Daphnia magna*, MRID 78657	
Glyphosate monoammonium salt (MON 14420), 68.5%	28.8 mg a.e./L *Daphnia magna*, MRID 45777401	
Roundup with X-77 surfactant	>39 mg a.e./L *Daphnia magna*, MRID 78666	
Ron-Do (coco-amide surfactant) Argentinean formulation	≈46 mg a.e./L *Daphnia magna*, Alberdi et al. 1996	
Roundup with "AA" surfactant), (MON 2139 NF-80-AA)	68.3 mg a.e./L *Daphnia magna* MRID 108109	94.5 mg a.e./L *Daphnia magna* MRID 78660
Roundup with Geronol CF/AR surfactant	220 mg a.e./L *Daphnia magna* MRID 44738201	810 mg a.e./L *Daphnia magna* MRID 44738201
Roundup Biactive (Australian formulation)	81.5 mg a.e./L *Ceriodaphnia dubia* Tsui and Chu 2004	150 mg a.e./L *Daphnia magna* MRID 44738201
Aqua Star (no surfactant?)	>148 mg a.e./L Mussel, Bringolf et al. 2007	
Rodeo (no surfactant)	218 mg a.e./L *Daphnia magna* Henry et al. 1994	4140 mg a.e./L Midge (*Chironomus riparius*) larvae Buhl and Faerber (1989)
Spasor (Portuguese formulation)	≈227 mg a.e./L *Daphnia magna*, Pereira et al. 2009	
Glyphosate IPA, 62.4%, no surfactant	401 mg a.e./L *Daphnia magna*, MRID 78663	
MON 77945 (IPA concentrate)	833 mg a.e./L *Daphnia magna*, MRID 44715410	

[1] Does not include LC_{50} of 21,633 mg a.e./L for Roundup in crayfish reported by Abdelghani et al. 1997 or LC50 of 0.377 mg a.e./L reported by Brausch et al. 2006. See text for discussion.

[2] Unless otherwise specified.

See 4.1.3.3.2.2 for discussion.

Table 27: Toxicity of Glyphosate and Glyphosate Formulations to Algae

Formulation	EC_{50} mg a.e./L	
	Lower Bound	**Upper Bound**
Glyphos (IPA)	0.12 mg a.e./L *Navicula pelliculosa* MRID 45666701	0.68 mg a.e./L *Selenastrum capricornutum*[1] MRID 45666702
GF-1280 (DMA e.g., Accord XRT II)	0.40 mg a.e./L *Pseudokirchneriella subcapitata*[1] Hughes 2006b	
Glyphosate monoammonium salt (MON 14420), 68.5% [granular designed for repackaging]	1.85 mg a.e./L *Selenastrum capricornutum*[1] MRID 45777403	
Roundup, NOS	1.85 mg a.e./L *Selenastrum capricornutum*[1] Tsui and Chu 2003	19 mg a.e./L *Selenastrum capricornutum*[1] Cedergreen and Streibig 2005
GF-1279 (IPA e.g., Accord XRT)	5.2 mg a.e./L *Pseudokirchneriella subcapitata*[1] Sesso 2005b	
Ron-Do (coco-amide surfactant) Argentinean formulation	9.1 mg a.e./L *Scenedesmus acutus* and *Scenedesmus quadricauda* Saenz et al. 1997	
MOS 78568 (monoammonium)	11.2 a.e./L *Selenastrum capricornutum*[1] MRID 45767102	
Roundup with Geronol CF/AR surfactant	39 mg a.e./L *Selenastrum capricornutum*[1] MRID 44738201	97 mg a.e./L *Selenastrum capricornutum*[1] MRID 44738201
Rodeo (no surfactant)	29 mg a.e./L *Ankistrodesmus* sp. Gardner et al. 1997	
Glyphosate acid	2.27 mg a.e./L *Skeletonema costatum* Tsui and Chu 2003	590 mg a.e./L *Chlorella pyrenoidosa* Maul and Wright 1984

[1] *Pseudokirchneriella subcapitata* is the newer designation for *Selenastrum capricornutum*. In the above table, the designation used in the study is reported.

See Section 4.1.3.4.3.1. for discussion.

Table 28: Toxicity of Glyphosate and Glyphosate Formulations to Macrophytes

Formulation, Duration	EC_{50} mg a.e./L (unless otherwise specified)		
	Lower Bound		Upper Bound
Glyphosate acid	1.56 mg a.e./L Watermilfoil Perkins 1997	10 to 47 mg a.e./L *Lemna* sp.	170 mg a.e./L NOAEC Eelgrass Nielsen and Dahllof (2007)
Roundup, 2-days	>16.91 mg a.e./L *Lemna minor* Lockhart et al. 1989	No data on potentially tolerant species	
Roundup Max, 2-days	6.5 mg a.e./L *Lemna gibba* Sobrero et al. 2007		
Glyphos, 7-days	7.7 mg a.e./L *Lemna gibba* MRID 45666704		
Roundup, 7-days	3.4 mg a.e./L *Lemna minor* Cedergreen and Streibig 2005		
Roundup Max, 10-days	8.2 mg a.e./L *Lemna gibba* Sobrero et al. 2007		
Roundup, 14-days	1.5 mg a.e./L *Lemna minor* Hartman and Martin 1984 NOEC of ≈7.4 mg a.e./L with suspended clay and ≈1 mg a.e./L without clay		
Rodeo, 14 days	0.84 mg a.e./L Watermilfoil Perkins 1997	7.60 mg a.e./L *Lemna gibba* Perkins 1997	
Roundup, 14-days	1.22 mg a.e./L Watermilfoil Perkins 1997	4.58 mg a.e./L *Lemna gibba* Perkins 1997	

See Section 4.1.3.4.3.2. for discussion.

Table 29: Ecological toxicity values for more toxic formulations

Group/Duration Organism	Endpoint	Toxicity Values (a.e.)	Reference
Terrestrial Animals			
Acute			
Non-canine Mammals	Developmental NOAEL	175 mg/kg bw	Section 4.3.2.1.
Canine Mammals	Developmental NOAEL	175 mg/kg bw	Section 4.3.2.1.
Birds	Acute dietary NOAEL	540 mg/kg bw	Section 4.3.2.2
Honey Bee (oral)	Acute dietary NOAEL	430 mg/kg bw	Section 4.3.2.4.1
Honey Bee (contact)	Acute contact NOAEL	260 mg/kg bw	Section 4.3.2.4.2
Longer-term			
Small Mammal	Developmental NOAEL	175 mg/kg bw/day	Section 4.3.2.1
Large Mammal	Developmental NOAEL	175 mg/kg bw/day	Section 4.3.2.1
Bird	Subchronic NOAEL	43 mg/kg bw/day	Section 4.3.2.2.
Terrestrial Plants			
Soil	Sensitive	NOAEC, seedling emergence	3.6 lb/acre	Section 4.3.2.5.2
	Tolerant	NOAEC, seedling emergence	5.0 lb/acre	
Foliar	Sensitive	Estimated NOAEC, foliar	0.0013 lb/acre	Section 4.3.2.5.1
	Tolerant	NOAEC, foliar	0.445 lb/acre	
Aquatic Animals			
Acute			
Amphibians	Sensitive	LC_{50} of 0.8 mg a.e./L x 0.05	0.04 mg/L	Section 4.3.3.2.1.1
	Tolerant	LC_{50} of 51.9 mg a.e./L x 0.05	2.6 mg/L	
Fish	Sensitive	LC_{50} of 0.96 mg a.e./L x 0.05	0.048 mg/L	Section 4.3.3.1.1.1
	Tolerant	LC_{50} of 10 mg a.e./L x 0.05	0.50 mg/L	
Invertebrates	Sensitive	LC_{50} of 1.5 mg a.e./L x 0.05	0.075 mg/L	Section 4.3.3.3.1.1
	Tolerant	LC_{50} of 46 mg a.e./L x 0.05	2.3 mg/L	
Longer-term			
Amphibians	Sensitive	Use acute value	0.04 mg/L	Section 4.3.3.2.1.2
	Tolerant	Use acute value	2.6 mg/L	
Fish	Sensitive	Use acute value	0.048 mg/L	Section 4.3.3.1.1.2
	Tolerant	Use acute value	0.5 mg/L	
Invertebrates	Sensitive	Use acute value	0.075 mg/L	Section 4.3.3.3.1.2
	Tolerant	Use acute value	2.3 mg/L	
Aquatic Plants			
Algae	Sensitive	NOAEC	0.082 mg a.e./L	Section 4.3.3.4.1.1.
	Tolerant	EC_{10}	3.8 mg a.e./L	
Macrophytes	Sensitive	NOAEC*	0.082 mg a.e./L	Section 4.3.3.4.2.
	Tolerant	NOAEC	170 mg a.e./L	

Table 30: Ecological toxicity values for less toxic formulations

Group/Duration Organism		Endpoint	Toxicity Values (a.e.)	Reference
Terrestrial Animals				
Acute				
Non-canine Mammals		Reproductive NOAEL	500 mg/kg bw	Section 4.3.2.1.
Canine Mammals		Reproductive NOAEL	500 mg/kg bw	Section 4.3.2.1.
Birds		Acute dietary NOAEL	1500 mg/kg bw	Section 4.3.2.2
Honey Bee (oral)		Acute dietary NOAEL	860 mg/kg bw	Section 4.3.2.4
Honey Bee (contact)		Acute contact NOAEL	860 mg/kg bw	Section 4.3.2.4
Longer-term				
Small Mammal		Reproductive NOAEL	500 mg/kg bw/day	Section 4.3.2.1
Large Mammal		Reproductive NOAEL	500 mg/kg bw/day	Section 4.3.2.1
Bird		Reproductive NOAEL	58 mg/kg bw/day	Section 4.3.2.2.
Terrestrial Plants				
Soil	Sensitive	NOAEC, seedling emergence	3.6 lb/acre	Section 4.3.2.5.2
	Tolerant	NOAEC, seedling emergence	5.0 lb/acre	
Foliar	Sensitive	Estimated NOAEC, foliar	0.0013 lb/acre	Section 4.3.2.5.1
	Tolerant	NOAEC, foliar	0.445 lb/acre	
Aquatic Animals				
Acute				
Amphibians	Sensitive	Acute NOAEC	340 mg a.e./L	Section 4.3.3.2.2.1
	Tolerant	Acute NOAEC	470 mg a.e./L	
Fish	Sensitive	LC_{50} of 10 mg a.e./L x 0.05	0.50 mg a.e/L	Section 4.3.3.1.2.1
	Tolerant	LC_{50} of 429 mg a.e./L x 0.05	21. mg a.e./L	
Invertebrates	Sensitive	LC_{50} of 53.2 mg a.e./L x 0.05	2.7 mg a.e./L	Section 4.3.3.3.2.
	Tolerant	LC_{50} of 4140 mg a.e./L x 0.05	210 mg a.e./L	
Longer-term				
Amphibians	Sensitive	Developmental NOAEC	1.8 mg a.e./L	Section 4.3.3.2.2.2
	Tolerant	Developmental NOAEC	1.8 mg a.e./L	
Fish	Sensitive	Use acute values	0.50 mg a.e/L	Section 4.3.3.1.2.2
	Tolerant	Use acute values	21 mg a.e./L	
Invertebrates	Sensitive	Developmental NOAEC	1 mg a.e./L	Section 4.3.3.3.2.2
	Tolerant	Use acute values	210 mg a.e./L	
Aquatic Plants				
Algae	Sensitive	EC_{50} of 2.3 mg/L÷ 10	0.23 mg a.e./L	Section 4.3.3.4.1.2.
	Tolerant	EC_{50} of 590 ÷ 10	59 mg a.e./L	
Macrophytes	Sensitive	NOAEC	0.082 mg a.e./L	Section 4.3.3.4.2.
	Tolerant	NOAEC	170 mg a.e./L	

Table 31: Risk characterization for terrestrial plants from direct spray or drift

	HQ Values		
	Backpack	**Ground Broadcast**	**Aerial**
Distance Downwind (Feet)	**Sensitive Species**		
0	**769**	**769**	**769**
25	**6**	**27**	**172**
50	**3**	**14**	**132**
100	**1.9**	**7**	**75**
300	0.7	**3**	**24**
500	0.4	**1.6**	**15**
900	0.2	0.8	**10**
	Tolerant Species		
0	**2**	**2**	**2**
25	2E-02	8E-02	0.5
50	1E-02	4E-02	0.4
100	5E-03	2E-02	0.2
300	2E-03	8E-03	7E-02
500	1E-03	5E-03	4E-02
900	7E-04	2E-03	3E-02

HQs based on 1 lb a.e./acre.
See Section 4.4.2.5 for discussion.