2,4-D
Human Health and Ecological Risk Assessment
FINAL REPORT

USDA, Forest Service

Forest Health Protection
USDA Forest Service
Rosslyn Plaza Building C, Room 7129C
1601 North Kent Street
Arlington, VA 22209

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ACRONYMS, ABBREVIATIONS, AND SYMBOLS

2,4-D	2,4-dichlorophenoxyacetic acid
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
ACGIH	American Conference of Governmental Industrial Hygienists
a.e.	acid equivalents
AEL	adverse-effect level
a.i.	active ingredient
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
BEE	butoxyethyl ester
bw	body weight
CBI	confidential business information
CI	confidence interval
cm	centimeter
CNS	central nervous system
DAA	days after application
DAT	days after treatment
d.f.	degrees of freedom
DMA	dimethylamine 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
EHE	2-ethylhexyl ester
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
ha	hectare
HQ	hazard quotient
IEE	Isooctyl (2-ethylhexyl) Ester
IPA	Isopropylamine salt
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
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
mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
mM	millimole
MOS	margin of safety
MRID	Master Record Identification Number
MSDS	material safety data sheet
MW	molecular weight
NAWQA	USGS National Water Quality Assessment
NCI	National Cancer Institute
NCOD	National Drinking Water Contaminant Occurrence Database
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
PCDD	polychlorinated dibenzo-dioxin
PCDF	polychlorinated dibenzo-furan
ppm	parts per million
RBC	red blood cells
RED	re-registration eligibility decision
RfD	reference dose
SERA	Syracuse Environmental Research Associates
TCDD	tetrachloro-dibenzo(p)dioxin
TEP	typical end-use product
TIPA	Triisopropanolamine salt
t.g.i.a.	Technical grade active ingredient

ACRONYMS, ABBREVIATIONS, AND SYMBOLS *(continued)*

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 (µg/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

OVERVIEW

2,4-D, the common name for 2,4-dichlorophenoxyacetic acid, is a selective systemic herbicide used to control broadleaf weeds. The USDA Forest Service uses 2,4-D in its vegetation management programs. This risk assessment addresses the human health and ecological effects of 2,4-D use in those programs. The herbicidal properties, environmental chemistry, and toxicology of 2,4-D are well studied. Given the immense amount of information available on 2,4-D and the numerous detailed and diverse reviews, no attempt is made to repeat all of the summaries provided in the reviews. Instead, these reviews are used to focus various aspects of both the hazard identification and dose response assessments. Nonetheless, key studies from the open literature were obtained and reviewed in the conduct of the current risk assessment. Potential exposures to 2,4-D are developed based on the anticipated use patterns and a number of relatively standard exposure scenarios used in most Forest Service risk assessments.

Estimates of risk are presented in terms of a hazard quotient. A hazard quotient is simply the quotient of an estimate of exposure divided by the appropriate toxicity value. Concern for the development of adverse effects increases as the value of the as hazard quotient increases. For 2,4-D, substantial concern is evident for workers, members of the general public, as well as several groups of organisms covered in the ecological risk assessment.

For many pesticides, including 2,4-D, accidental exposure scenarios, some of which are extremely conservative and perhaps implausible, lead to risk quotients that exceed the level of concern. 2,4-D is, however, somewhat atypical because many non-accidental exposure scenarios – i.e., exposures that are plausible under normal conditions of use – also exceed the level of concern and often by a very substantial margin.

Unless steps are taken to mitigate risks, workers involved in the application of 2,4-D and members of the general pubic who consume vegetation contaminated with 2,4-D could be exposed to 2,4-D levels greater than those which are generally regarded as acceptable. In some cases, the exceedances are substantial. Similarly, adverse effects in the normal use of 2,4-D salts or esters could occur in groups of nontarget organisms including terrestrial and aquatic plants, mammals, and possibly birds. Adverse effects on aquatic animals are not likely with formulations of 2,4-D salts except for accidental and extreme exposures at the upper ranges of application rates. The ester formulations of 2,4-D are much more toxic to aquatic animals and adverse effects are plausible in sensitive species and sometimes in relatively tolerant species.

The results of this risk assessment suggest that consideration should be given to alternate herbicides and that the use of 2,4-D should be limited to situations where other herbicides are ineffective or to situations in which the risks posed by 2,4-D can be mitigated.

PROGRAM DESCRIPTION

The Forest Service uses 22 herbicide formulations of 2,4-D in which the compound is available as salts, esters, or combinations of salts and esters, and all but one of the formulations are liquid. The Forest Service has used 13 other herbicide formulations in which 2,4-D is a component. Herbicide mixtures of 2,4-D combined with triclopyr, dicamba, picloram, or glyphosate are all used by the Forest Service. 2,4-D is registered for both ground and aerial applications. Also, several formulations of 2,4-D, including Aqua-Kleen, can be applied directly to water to control noxious weeds. Although 2,4-D is registered for aerial applications, the Forest Service does not use this method to apply 2,4-D. Nonetheless, aerial application methods are covered by this risk assessment in case the Forest Service should decide to use 2,4-D in aerial applications.

In Forest Service programs, herbicide formulations containing 2,4-D are most commonly used in wildlife opening, rights-of-way maintenance, and noxious weed control. Consequently, the most common application methods include backpack (selective foliar), hack-and-squirt, and roadside hydraulic spray applications. Many of the formulations are also registered for tree injection and stump removal. Aerial applications are considered in this risk assessment even though the Forest Service does not use or plan to use the method in its programs. The specific application rates used in ground or aerial programs vary according to local conditions and the nature of the target vegetation. For ground applications, the Forest Service typically applies between 0.5 and 4 lbs a.e./acre with an average typical application rate of 1 lb a.e./acre. The same rates are likely to be used for aerial applications, if conducted. The upper bound of 4 lbs a.e./acre is useful for site preparation or wildlife habitat improvement, which comprise relatively minor uses of 2,4-D. The direct application of 2,4-D formulations to water bodies may involve rates as high as 38 lbs a.e./acre.

HUMAN HEALTH RISK ASSESSMENT

Hazard Identification – The pharmacokinetics and toxicity of 2,4-D are well studied in laboratory animals, volunteers, epidemiology studies, and incidents involving attempted suicide. The enumeration of all these studies is well beyond the scope of this document. Because of the extensive literature available on 2,4-D, credible reviews conducted by U.S.EPA and WHO are used as sources in this hazard identification along with formation in the open literature that is not covered by existing reviews.

The WHO and FAO (Food and Agriculture Organization of the United Nations) have held ongoing joint meetings since the 1970's to discuss the use of pesticides and their impacts on health and the environment. The results of these meetings are published in a series of monographs. Concerning 2,4-D, the most recent comprehensive reviews and discussions on mammalian toxicology, ecological/environmental toxicity, and metabolism/environmental fate are published in WHO (1996), WHO (1997), and WHO (1998), respectively. In essence, WHO/FAO concluded that the toxicity of the salts and esters of 2,4-D in mammals are equivalent to that of the acid. WHO (1996) establishes an acceptable daily intake (ADI) for 2,4-D (sum of 2,4-D and its salts and esters, expressed as 2,4-D) in humans of 0 to 0.01 mg/kg/day. The basis for the WHO/FAO ADI is a NOAEL of 1 mg/kg/day derived from a 2-year carcinogenicity/

toxicity study with rats, and a 13-week study and and 1-year study with dogs. Based on the available human and animal evidence from subchronic, chronic, carcinogenicity, mutagenicity, developmental and reproductive toxicity studies in mice, rats, rabbits and dogs and with consideration of the available epidemiological database, WHO does not regard 2,4-D and its salts and esters as either genotoxic or carcinogenic.

U.S. EPA documents regarding the registration process for 2,4-D acid and 2,4-D salts and esters are available from the Federal Docket Management System under Docket Number EPA-HQ-OPP-2004-0167. The most current EPA/OPP documents relevant to the review of 2,4-D toxicity presented in this risk assessment are the Reregistration Eligibility Decision (RED) for 2,4-D (U.S. EPA/OPP 2005a), supported by risk assessments conducted by the Human Health Effects Division (HED) (U.S. EPA/OPP 2005a) and the Environmental Fate and Effects Division (EFED) (U.S. EPA/OPP 2004b). These documents have been revised and updated to reflect public comments; they are current as of this writing.

Based on numerous unpublished studies submitted by registrants as part of the pesticide registration process, U.S. EPA/OPP (2005a) concludes:

1. 2,4-D and its salts and esters are of low acute toxicity on the basis of oral, dermal, and inhalation routes of exposure, and are not skin sensitizers or primary skin irritants.
2. 2,4-D acid and salts are severe eye irritant.;
3. Renal clearance is key to 2,4-D toxicity. In laboratory animals, repeated oral exposure to amine salts and esters of 2,4-D at levels which saturate renal clearance is associated with toxic effects primarily manifest in eyes, blood, thyroid, liver, kidneys, adrenals, ovaries, and testes.
4. Systemic dermal toxicity is seen only following high-dose repeated exposures to DEA and DMA; no effects were observed following repeated exposures at the limit doses of 2,4-D, EHE and TIPA.
5. There are no repeat-dose inhalation studies for 2,4-D; a subchronic (28-day). Consequently, an inhalation study is required.
6. Developmental and reproductive effects are seen only in association with maternal toxicity above the threshold for renal clearance; however, a current 2-generation toxicity study is required for 2,4-D to address concerns about endocrine disruption (thyroid and gonads) and immune toxicity.
7. Neurotoxicity is seen in laboratory animals following high dose exposure. A developmental neurotoxicity study is required for 2,4-D.
8. 2,4-D is a Group D chemical (not classifiable) with regard to human carcinogenicity, and is not mutagenic; however, cytogenic effects were observed. 2,4-D is considered representative of the various forms (salts and esters) under consideration.

Based on recent studies published in the open literature, 2,4-D is toxic to the immune system and developing immune system, especially when used in combination with other herbicides. The mechanism of action of 2,4-D toxicity is cell membrane disruption and cellular metabolic processes. The molecular basis for 2,4-D toxicity to human lymphocytes and nerve tissue is likely the induction of programmed cellular death known as apoptosis.

Signs of neurological, cardiac, hepatic, and renal toxicity are evident in cases of 2,4-D poisoning following suicide attempts. A recent study conducted by Krieger et al. (2005) used measures of dosimetry as well as biomonitoring to determine the absorption and elimination of 2,4-D in volunteer workers spraying a mixture of 2,4-D and triclopyr for purposes of conifer release. This information is taken into account with other methods and information to estimate dermal absorption variables necessary to quantify potential risks (in this assessment) to forestry workers and the general public.

Exposure Assessment – All exposure assessments are summarized in Worksheet E01 for workers and Worksheet E02 for the general public of the EXCEL workbook for 2,4-D acid and salts (Attachment 1). Exposure assessments for workers and for members of the general public are given only in the worksheets on 2,4-D acid/salt. This approach is taken because exposures to 2,4-D in the use of both salt and ester formulations will be essentially identical in terms of acid equivalents of 2,4-D and because the salts and esters of 2,4-D are toxicologically equivalent in terms of potential effects in humans as well as other animals.

For workers applying 2,4-D, three types of application methods are modeled: directed ground spray, broadcast ground spray, and aerial spray. Based on well-documented data on worker exposures to 2,4-D, worker exposure rates typically used in Forest Service risk assessments are applicable to both salt and ester formulations of 2,4-D. Central estimates of exposure for workers are approximately 0.01 mg/kg/day for aerial and backpack workers and about 0.02 mg/kg/day for broadcast ground spray workers. Upper ranges of exposures are approximately 0.08 mg/kg/day for broadcast ground spray workers and 0.15 mg/kg/day for backpack and aerial workers. All of the accidental exposure scenarios for workers involve dermal exposures. Except for the scenario involving contact with contaminated gloves for 1 hour, the accidental exposures lead to estimated exposure levels below the general exposure levels estimated for workers.

For the general public, acute exposures range from about 0.0001 to about 2 mg/kg bw at an application rate of 1 lb a.e./acre. The upper bound of exposure, 2 mg/kg bw, is associated with a child's consumption of contaminated water after an accidental spill. The nature of this exposure scenario is highly arbitrary. The upper bound of exposure associated with the consumption of contaminated vegetation is 1.4 mg/kg bw. The other acute exposure scenarios lead to much lower dose estimates – i.e., ranging from 0.00009 to about 0.2 mg/kg bw.

For chronic or longer-term exposures, the modeled exposures are much lower than for acute exposures. Exposures to 2,4-D associated with the consumption of contaminated water or fish range from about 0.0000004 to about 0.0001 mg/kg/day. The upper bound of this range is

associated with the longer-term consumption of contaminated water. The longer-term consumption of contaminated vegetation leads to much higher estimated doses, ranging from 0.001 to about 0.2 mg/kg bw/day.

Dose-Response Assessment – Following standard practices for Forest Service risk assessments, the RfD values derived by U.S. EPA are adopted directly for the assessment of potential risks to humans exposed to 2,4-D. U.S. EPA/OPP (2005a) derived two acute RfDs and a chronic RfD for the protection of human health from exposures to 2,4-D and 2,4-D amine salts and esters: an acute RfD of 0.025 mg/kg/day for reproductive aged females and another RfD of 0.067 mg/kg/day for the general population. The former RfD is based on maternal toxicity, and the latter is based on acute neurotoxicity. In the current risk assessment, the lower acute RfD of 0.025 mg/kg/day is used to assess the consequences of acute exposures. This approach is taken because any of the acute exposure scenarios could involve a woman of child-bearing age.

A chronic RfD of 0.005 mg/kg/day is based on a rat NOAEL for chronic toxicity and a 2-generation reproduction study. These values are considered protective of potential developmental and reproductive effects, and are considered protective of children per the FQPA. There is an older chronic RfD of 0.01 mg/kg/day on U.S. EPA's IRIS database, but this value does not take into account the currently available information and is not used in the current risk assessment.

Risk Characterization – Based on upper bound hazard quotients, adverse health outcomes are possible for workers who could be exposed repeatedly over a longer-term period of exposure. Hazard quotients for workers spraying at the typical application rate of 1 lb a.e./acre are 16 for both backpack and aerial spray methods, and 30 for ground spray application. Short-term accidental exposures via contaminated gloves as well as some spill scenarios yield hazard quotients that are of concern, particularly for the scenario involving contaminated gloves that are worn for 1 hour which yields a hazard quotient of 94. For all of these hazard quotients, the magnitude of the hazard quotient is linearly related to the application rate.

As with hazard quotients for workers, hazard quotients for members of the general public are linearly related to application rate. Upper bound hazard quotients for accidental exposures associated with spills into a small body of water range from 0.8 (consumption of fish by non-subsistence populations at an application rate of 0.5 lb/acre) to 328 (a child consuming 1 liter of contaminated water at an application rate of 4 lbs a.e./acre). The amounts spilled are set at the amounts required to treat from 1 to100 acres. These assumptions are completely arbitrary and may be unrealistic. The scenario for an accidental spill into a small pond is intended only to illustrate the different consequences of spilling different amounts of 2,4-D.

Short-term consumption of contaminated fruits and vegetables could be of concern when either maternal toxicity or acute neurotoxicity are the endpoints of concern under assessment (i.e., using either of the existing acute RfDs for 2,4-D). Upper bound hazard quotients associated with the typical application rate of 1 lb a.e./acre are 7 for consumption of contaminated fruit and 54 for

consumption of contaminated vegetation. These estimates are based on an adult female. At the lowest anticipated application, hazard quotients are 4 and 27 for the consumption of fruits and vegetables, respectively. At the highest anticipated application rate, hazard quotients are 30 and 216 for the consumption of fruits and vegetables, respectively.

The only hazard quotients indicating that adverse health outcomes are plausible following longer-term exposure to 2,4-D are those associated with ingestion of contaminated fruits and vegetation by an adult female.   At the typical application rate of 1 lb a.e./acre, the central estimate of the hazard quotient for the consumption of contaminated vegetation is 5 with lower and upper bounds of 1 and 38. Because lower residues are anticipated on contaminated fruit, the hazard quotient associated with this scenario at an application rate of 1 lb a.e./acer is 0.3 with an upper bound of 5. Even at the lowest anticipated application rate, the upper bounds of the hazard quotients exceed a level of concern for both contaminated fruit (HQ = 3) and contaminated vegetation (HQ=19). At the highest anticipated application rate (4 lbs a.e./acre), upper bounds of the hazard quotient substantially exceed a level of concern for contaminated fruit (HQ = 21) and contaminated vegetation (HQ=152). Other longer-term exposure scenarios involving the consumption of either contaminated water or fish yield hazard quotients that are substantially below a level of concern even at the highest anticipated application rate.

ECOLOGICAL RISK ASSESSMENT
Hazard Identification – The toxicity of 2,4-D is fairly well characterized in plants and animals. As in the human health risk assessment, the toxicity of the various forms of 2,4-D – i.e., acid, salts, and esters – are all treated as equally toxic to birds and mammals. For terrestrial plants as well as aquatic plants and animals, the toxicity of 2,4-D acid and 2,4-D salts is considered separately from that of 2,4-D esters. Based on formulations used by the Forest Service, this assessment considers only data relevant to 2,4-D acid, the DMA and TIPA salts, and the BEE and the 2-ethylhexyl (also known as the iso-octyl ester) ester. This assessment relies heavily upon the analyses of the U.S. EPA/OPP for summaries of unpublished studies submitted to the U.S. EPA in support of the registration of 2,4-D. Nevertheless, this assessment also uses studies from the open literature, where appropriate, to identify hazards and quantify toxicity.

Based on classification schemes for acute toxicity developed by U.S. EPA, 2,4-D is slightly to moderately toxic to mammals; practically non-toxic to moderately toxic to birds; and practically non-toxic to honey bees. Among mammals, dogs are more sensitive than other species to the effects of 2,4-D due to their limited capacity to excrete organic acids. The U.S. EPA classifies the toxicity of 2,4-D to freshwater and marine fish as practically non-toxic for 2,4-D acid/salts and highly toxic for esters. A similar pattern of toxicity is observed for aquatic invertebrates and amphibians. 2,4-D does not cause effects on reproduction or fetal development in birds or mammals at exposures which do not cause toxic effects in maternal animals. The only available studies which address the potential for 2,4-D to have an adverse effect on the early growth and development of fish were conducted with fathead minnows. These studies demonstrate that the esters are more toxic than the acid or salts. 2,4-D causes phytotoxicity in nontarget plants at concentrations which are likely used under field conditions, if precautions are not taken to limit

spray drift. A limited number of studies suggest that the effects of 2,4-D on soil microorganisms and invertebrates are possible. While 2,4-D is not likely to cause mortality among honey bees at any of the application rates employed by the Forest Service, other species of insects, such as parasitic wasps may be affected, though the available studies do not lend themselves to defining dose-response relationships quantitatively.

Exposure Assessment – Terrestrial animals might be exposed to any applied herbicide from direct spray, the ingestion of contaminated media (vegetation, prey species, or water), grooming activities, or indirect contact with contaminated vegetation. The highest exposures for terrestrial vertebrates will occur after the consumption of contaminated vegetation or contaminated insects. In acute exposure scenarios, doses as high as 113 mg/kg are estimated (the consumption of contaminated insects by a small bird). Other routes of exposure, like the consumption of contaminated water or direct spray, lead to lower levels of exposure. In chronic exposure scenarios, the higher estimated daily doses range from about 7 to 10 mg/kg/day and are associated with highly conservative assumptions (e.g., 100% of the diet is contaminated) regarding the consumption of contaminated vegetation by a large mammal or bird. Less conservative but more plausible exposure assessments lead to much lower dose estimates – i.e., in the range of 0.01-0.2 mg/kg/day.

The primary hazards to non-target terrestrial plants are associated with unintended direct deposition or spray drift. Unintended direct spray will result in an exposure level equivalent to the application rate. At least some plants that are sprayed directly with 2,4-D at or near the recommended range of application rates will be damaged. Based on the AgDRIFT model, no more than 0.0058 of the application rate would be expected to drift 100 m offsite after low boom ground applications. In order to encompass a wide range of field conditions, GLEAMS simulations were conducted for clay, loam, and sand at annual rainfall rates from 5 to 250 inches. Under arid conditions (i.e., annual rainfall of about 10 inches or less), there is no or very little runoff. Under these conditions, degradation, not dispersion, accounts for the decrease of 2,4-D concentrations in soil. At higher rainfall rates, plausible offsite movement of 2,4-D results in runoff losses that range from about negligible up to about 0.5 of the application rate, depending on the amount of rainfall and soil type.

For 2,4-D acid and salts, the potential for effects on aquatic species is based on estimated concentrations of 2,4-D in water that are identical to those used in the human health risk assessment without additional elaboration. For 2,4-D esters, separate GLEAMS simulations were conducted to estimate peak concentrations of 2,4-D esters in water. Consistent with the approach taken by the U.S. EPA in the recent reregistration of 2,4-D, chronic exposures to aquatic organisms are not modeled for 2,4-D esters because the esters of 2,4-D will not persist in the environment. Although the peak concentrations of 2,4-D esters in water are likely to be lower than those of the salts, the separate estimates for 2,4-D esters are necessary for acute exposures because of the higher toxicity of 2,4-D esters to aquatic species.

Dose-Response Assessment – The available toxicity data on 2,4-D support separate dose-response assessments in eight classes of organisms: terrestrial mammals, birds, terrestrial invertebrates, terrestrial plants, fish, aquatic invertebrates, aquatic algae, and aquatic macrophytes. Different units of exposure are used for different groups of organisms depending on how exposures are likely to occur and how the available toxicity data are expressed. In Forest Service risk assessments, it is customary to derive a range of risks based on the most sensitive and most tolerant species within a given group of organisms (e.g. terrestrial mammals or aquatic plants). Given that the risk assessment for 2,4-D considers the salts and esters of 2,4-D as well as the acid, the most sensitive or tolerant species within a given group of organisms was selected on the basis of a combination of organism as well as the form of 2,4-D giving the most sensitive or tolerant result. Therefore, the toxicity values selected for ecological risk assessment, shown in Table 4-14, are based on the most sensitive or tolerant result obtained within the class of 2,4-D chemicals under consideration (e.g. salts or esters) as well as the species tested.

For terrestrial animals, the various salts and esters of 2,4-D are assumed equivalent in toxicity to that of the acid. Based on differences in solubility and toxicity, 2,4-D acid and salts are considered separately from 2,4-D esters for terrestrial plants and aquatic organisms. Based on both acute and chronic dietary toxicity values, mammals appear to be more sensitive than birds to 2,4-D exposure. Dogs are more sensitive than other mammals, including humans and rodents, due to their limited capacity to eliminate organic acids. On the basis of acute toxicity, mammals are approximately 14 to more than 300 times more sensitive than birds. On the basis of chronic toxicity, mammals are approximately 15-75 times more sensitive than birds.

For non-canine mammals, the dose-response assessment for acute toxicity is based on the same data as the human health risk assessment (i.e., the acute NOAEL of 25 mg a.e./kg for protection of reproductive age females). The acute NOEL of 1.1 mg a.e./kg from the study of Beasley et al. (1991) is used for assessing acute risks to canines and other sensitive carnivorous mammals. For non-canine mammals, the dose-response assessment for chronic toxicity is also based on the same data as the human health risk assessment (i.e., a chronic NOAEL of 5 mg a.e./kg/day). For canines and other sensitive carnivorous mammals, the dose-response assessment for chronic toxicity is based on the canine NOAEL of 1 mg a.e./kg/day.

An acute NOAEL of 415 mg a.e./kg is selected for birds on the basis of a gavage study with bobwhite quail. Based on a reproduction study, the chronic NOAEL for birds is set at 76 mg a.e./kg/day. Relatively little information is available on terrestrial insects. A contact toxicity value of 1075 mg/kg bw (for honey bees) is taken as a NOAEC for terrestrial invertebrates, although a study published in the open literature indicates that parasitic wasps may be more sensitive to 2,4-D at lower doses.

The toxicity of 2,4-D to terrestrial plants is well characterized. 2,4-D acid, salts, and esters are effective at inhibiting seed germination and are toxic after either direct spray or soil application. Based on the available toxicity studies submitted to the U.S. EPA, 2,4-D acid and 2,4-D salts appear to be equally toxic in both pre-emergent and direct spray applications (i.e., seedling

emergence versus vegetative vigor studies). In pre-emergent soil applications (i.e., seedling emergence studies), the NOAEC values for the most sensitive and tolerant combination of species and form of 2,4-D are 0.0093 and >4.2 lb a.e./acre, respectively. The corresponding values for direct spray (post-emergent bioassays) are 0.0075 and 2.1 lb a.e./acre.

Based on the available EPA-required toxicity studies, 2,4-D esters appear to be more toxic following direct spray than by pre-emergent application. In pre-emergent soil applications (i.e., seedling emergence studies), the NOAEC values for the most sensitive and tolerant combination of species and 2,4-D ester are 0.045 and >0.96 lb a.e./acre, respectively. The corresponding values for direct spray (post-emergent bioassays) are 0.0075and >0.96 l b a.e./acre.

2,4-D acid, salts, and esters are toxic to aquatic animals, with esters having greater toxicity than 2,4-D acid and salts. Because esters convert rapidly to the acid, only acute toxicity is considered for aquatic organisms exposed to esters. The chronic toxicity of esters is considered to be covered by assessments of chronic toxicity to 2,4-D acid. This approach is in agreement with that taken by U.S. EPA/OPP in their recent Reregistration Eligibility Decision document on 2,4-D. With regard to 2,4-D acid and salts, the acute LC_{50} values for sensitive and tolerant fish vary by a factor of about 9, with a range of 96.5 (carp, 2,4-D acid) to 830 mg a.e./L (rainbow trout, bluegill, DMA salt). For the esters of 2,4-D, the range of toxicity is a factor of 92.7, about an order of magnitude greater than with the acid/salts of 2,4-D, with 96-hour LC_{50} values ranging from 0.1564 (tidewater silverside) to 14.5 mg a.e./L (rainbow trout). For longer-term exposures to 2,4-D acid and salts, NOEC values range from 14.2 to 63.4 mg a.e./L based on bioassays in fathead minnow. These differences are relatively modest (i.e., a factor of about 4.5). Based on differences in acute toxicity and the assumption of similar differences in chronic toxicity, chronic NOAEC values ranging from 19 (approximated for carp) to 63.4 mg a.e./L (fathead minnow) are used to represent the most sensitive and tolerant fish species, respectively, exposed to 2,4-D acid and salts.

A similar pattern in the acute toxicity of 2,4-D to aquatic invertebrates is apparent for salts of 2,4-D (less toxic) and esters of 2,4-D (more toxic). For 2,4-D acid, LC_{50} values for aquatic invertebrates range from 25 mg/L for *Daphnia magna* to 1389 mg/L for crayfish.   This factor of about 55 is greater than the variability seen in fish – i.e., a factor of about 9. The increased variability in aquatic invertebrates is even more pronounced for 2,4-D esters with acute LC_{50} values ranging from 0.092 (grass shrimp, *Palaemonetes pugio*) to more than 66 mg a.e./L (scud, *Gammarus fasciatus*). The chronic data available on 2,4-D acid and salts are not concordant with the acute data on aquatic invertebrates, and the reason or reasons for the lack of concordance are not apparent.   As a conservative (protective) approach, this risk assessment uses a chronic NOEC value of 16.05 mg/L a daphnid study on the diethanolamine salt of 2,4-D for sensitive invertebrate species. For tolerant species, the NOEC of 75.7 mg/L from a daphnid study using the DMA salt of 2,4-D is used to characterize risk of longer-term exposures in tolerant species of aquatic invertebrates.

In amphibians, toads exposed to 2,4-D acid yielded the most sensitive result, with a 96-hour LC_{50} of 8.05 mg a.e./L; while leopard frogs exposed to 2,4-D acid yielded the most tolerant result, with a 96-hour LC_{50} of 359 mg/L. These values are used to evaluate acute exposures of amphibians to 2,4-D acid and salts. A single 96-hour LC_{50} of 0.505 mg a.e./L is used to evaluate acute exposures to 2,4-D esters. Data regarding chronic exposure of amphibians to 2,4-D are not available.

Aquatic algae are equal in sensitivity to fish and aquatic invertebrates. As with other species, 2,4-D acid and salts appear to be less toxic than 2,4-D esters, on the basis of limited testing. NOAEC values from EPA-required survival and growth studies are used to assess the toxicity of 2,4-D and its various salts, with values of 1.41 mg a.e./L (freshwater diatom, *Navicula pelliculosa*, DMA salt) and 56.32 mg a.e./L (Blue-green algae, *Anabaena flos aquae*, DMA salt), representing the most sensitive and most tolerant species, respectively. For esters of 2,4-D, the 2-ethylhexyl ester gave both the most sensitive and most tolerant result with the corresponding NOAEC values of 0.062 mg a.e./L (marine diatom, *Skeletonema costatum*) and 2.48 mg a.e./L (green algae, *Selanastrum capricornutum*), respectively. These values are used to assess acute exposures. As stated previously, chronic exposures are evaluated only for 2,4-D acid and salts. In the absence of long-term studies, the acute values are used to assess longer-term exposures to 2,4-D acid and salts.

Aquatic macrophytes are more sensitive than algae, as demonstrated by the EPA-required studies conducted with duckweed (*Lemna gibba*) as well as a published study on water milfoil. Based on studies submitted to the U.S. EPA, the range of sensitivities for duckweed obtained for 2,4-D acid and salts is represented by NOAEC values ranging from 0.0581 mg/L for 2,4-D acid to 0.128 mg a.e./L for the TIPA salt (most tolerant). The range of sensitivities for the 2,4-D esters are NOAEC values of 0.062 mg a.e./L for EHE and 0.141mg a.e./L for BEE. As noted in Section 4.1.3.4, the published study by Roshon et al. (1999) suggests that common water milfoil (*Myriophyllum sibiricum*) is substantially more sensitive than duckweed – i.e., the EC_{50} for the most sensitive endpoint in water milfoil is reported as 0.013 mg/L and the corresponding EC_{25} is 0.005 mg/L.

Risk Characterization – Because 2,4-D is an effective herbicide, unintended effects on nontarget vegetation are plausible. The effective use of 2,4-D is achieved by applying it to target vegetation at a time and in a manner that will minimize effects on nontarget plant species. If applied properly and with care, 2,4-D might have only minor effects on nontarget vegetation. Nonetheless, in the normal course of applying 2,4-D at rates effective for weed control, terrestrial plants may be adversely affected due to drift or runoff of the applied compound.

Damage to aquatic vegetation, particularly aquatic macrophytes, is likely in the event of an accidental spill or in the case of direct application of 2,4-D to control aquatic weeds. Longer-term exposure to 2,4-D concentrations associated with inadvertent contamination of water by runoff could affect sensitive species of macrophytes at the upper range of the application rates used in Forest Service programs.

Over the range of 2,4-D acid/salt application rates used in Forest Service programs (0.5-4 lb a.e./acre), adverse effects on fish, amphibians, and aquatic invertebrates are likely only in the event of an accidental spill. With regard to 2,4-D esters, however, adverse effects on aquatic animals (fish, invertebrates, amphibians) are plausible in association with runoff (all application rates) and would be expected in direct application for weed control and in cases of relatively large accidental spills.

Over the range of application rates used in Forest Service programs, adverse effects are plausible in mammals that consume contaminated vegetation or insects after 2,4-D is applied at the typical and maximum application rates. Adverse effects are unlikely, however, at the lower application rate. Similarly, adverse effects are plausible among carnivorous mammals that consume contaminated small mammals after 2,4-D is applied at the typical and maximum rates, but not the lowest anticipated application rate. Based on a comparison of NOAEL and LOAEL values, adverse effects are anticipated in some non-canid mammals, particularly at the highest application rate. There is no indication that substantial numbers of mammals would be subject to lethal exposure to 2,4-D. Consequently, adverse effects such as weight loss and reproductive impairment might occur but might not be readily apparent or easy to detect. Based on reproduction studies, birds appear to more tolerant than mammals to2,4-D. Furthermore, longer-term exposure to 2,4-D is not likely to cause adverse effects in birds. Nevertheless, adverse effects in birds after acute exposure to 2,4-D is a concern; however, the plausibility of adverse effects is much less compelling in birds than in mammals.

In addition to the direct effects mentioned above, secondary adverse effects in terrestrial and aquatic animals might result from adverse effects of 2,4-D on vegetation. These secondary effects associated with the depletion of vegetation are likely to vary over time and among different species of animals. Certain effects could be detrimental for some species – i.e., a reduction in the supply of preferred food or a degradation of habitat – but beneficial to other species – i.e., an increase in food or prey availability or an enhancement of habitat.

1. INTRODUCTION

The USDA Forest Service uses 2,4-D (2,4-dichlorophenoxyacetic acid) in its vegetation management programs. This document is an update to a risk assessment prepared in 1998 (SERA 1998a) and provides risk assessments for human-health effects and ecological effects to support an assessment of the environmental consequences of the using 2,4-D in these programs.

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 associated with 2,4-D and its commercial formulation, an assessment of potential exposure to the product, an assessment of the dose-response relationships, and a characterization of the risks associated with plausible levels of exposure. These are the basic steps recommended by the National Research Council of the National Academy of Sciences (NRC 1983) for conducting and organizing risk assessments.

Although this is a technical support document and addresses some specialized technical areas, 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 2001).

The series of human health and ecological risk assessments prepared for the USDA Forest Service are not, and are not intended to be, comprehensive summaries of all of the available information. This is particularly true with 2,4-D, for which numerous published studies are available. For instance, a cursory review of Toxline and PubMed (two commonly used databases) identified a total of more than 7600 citations relating to 2,4-D. In addition, numerous unpublished studies were submitted to the U.S. EPA/OPP to support the pesticide registration and re-registration of this compound. It is beyond the scope of and resources available for this risk assessment to cover all of the available literature in detail. Instead, the risk assessment is guided by and relies on the credible reviews in the open literature (e.g., Sassaman et al. 1984; USDA 1997; WHO 1997) as well as the very recent and comprehensive assessment by the U.S. EPA (e.g., U.S. EPA/OPP 2005a,b).

The open literature includes numerous reviews of available information on 2,4-D (e.g., Cox 2006; Garabrant and Philbert 2002; U.S. EPA/OPP 2005a,b and supporting documents; Munro et al. 1992; WHO 1996, 1997, 1998). Both the industrial perspective (e.g., Rowe and Hymas 1954; Munro et al. 1992) and the concerns of environmental activists regarding use (e.g., Cox 1999) are to be found in the reviews of 2,4-D. Regardless of perspective, all of these published reviews were consulted in the conduct of the current risk assessment. In light of the immense amount of information available on 2,4-D and the existence of detailed and diverse reviews, no attempt is made to repeat all of the summaries provided in these reviews; instead, these reviews are used to focus various aspects of both the hazard identification and dose response assessment as discussed

in Section 3 (Human Health Risk Assessment) and Section 4 (Ecological Risk Assessment). Nonetheless, key studies from the open literature were obtained and reviewed in the conduct of the current risk assessment. In addition to the open literature studies cited in SERA (1998a), approximately 100 additional open literature studies were obtained and are cited in the reference list (Section 5). Studies summarized in any of the previous reviews are so identified in the reference list – e.g, *as cited in ...*). In addition to reviews published in the open literature, there is an immense amount of information about 2,4-D on the Internet. For the most part, however, data derived from the Internet is not used unless the information is well-documented. The most useful database for the risk assessment of 2,4-D is the ECOTOX database compiled and reviewed by the U.S. EPA (U.S. EPA/ORD 2006). ECOTOX is also the main ecotoxicity database used by the Pesticide Action Network (PAN 2006).

Most of the studies submitted to the U.S. EPA in support of the reregistration of 2,4-D are summarized in various and detailed review documents provided by the U.S. EPA (e.g., U.S. EPA/OPP 2005a,b), and this Forest Service risk assessment relies heavily on those reviews. In addition, full copies of more than 100 submitted studies were requested from the U.S. EPA under the Freedom of Information Act (FOIA). Due to restrictions regarding the release of studies under FOIA, the requested studies were all originally submitted to the U.S. EPA after1986. Furthermore, the request was limited to studies relating to toxicity or environmental fate. Registrant submissions regarding specific formulations, including information on impurities, inerts, and manufacturing processes, cannot be released under FOIA and were not obtained for the current assessment. In response to the FOIA, the U.S. EPA kindly provided 123 cleared reviews of the requested studies as well as full copies of 47 studies. These are identified specifically in the reference list (Section 5).

The Forest Service will update this and other similar risk assessments on a periodic basis 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.

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 very 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 the calculations are relatively simple, and the very simple calculations are included in the body of the document.

Some of the calculations, however, are cumbersome. For those calculations, worksheets are included as attachments to the risk assessment. For 2,4-D, two sets of worksheets are given in two different EXCEL workbooks – i.e., collections of worksheets. One workbook covers standard terrestrial applications considered in this risk assessment (Attachment 1) and is applicable to all applications of 2,4-D salts as well as potential risks to most species, including humans, associated with the application of 2,4-D esters. The other workbook (Attachment 2)

covers risks to aquatic species as well as terrestrial plants in the application of ester formulations of 2,4-D. As detailed in Section 4.3, this approach is necessary because of the higher toxicity of 2,4-D esters relative to 2,4-D salts to aquatic species and terrestrial vegetation.

The worksheets provide the detail for the estimates cited in the body of this document. The worksheets are divided into the following sections: general data and assumptions, chemical specific data and assumptions, exposure assessments for workers, exposure assessments for the general public, and exposure assessments for effects on nontarget organisms. SERA (2004) contains documentation for the use of the EXCEL workbooks.

2. PROGRAM DESCRIPTION

2.1.   OVERVIEW

2,4-D, the common name for 2,4-dichlorophenoxyacetic acid, is a selective systemic herbicide used to control broadleaf weeds. The herbicidal properties, environmental chemistry, and toxicology of 2,4-D are well studied. The Forest Service uses 22 herbicide formulations of 2,4-D in which the compound is available as salts, esters, or combinations of salts and esters, and all but one of the formulations are liquid. The Forest Service has used 13 other herbicide formulations in which 2,4-D is a component. Herbicide mixtures of 2,4-D combined with triclopyr, dicamba, picloram, or glyphosate are all used by the Forest Service. 2,4-D is registered for both ground and aerial applications. Also, several formulations of 2,4-D, including Aqua-Kleen, can be applied directly to water to control noxious weeds. Although 2,4-D is registered for aerial applications, the Forest Service does not use this method to apply 2,4-D; nonetheless, aerial application methods are covered by this risk assessment in case the Forest Service should decide to use 2,4-D accordingly.

In Forest Service programs, herbicide formulations containing 2,4-D are most commonly used in wildlife opening, rights-of-way maintenance, and noxious weed control. Consequently, the most common application methods include backpack (selective foliar), hack-and-squirt, and roadside hydraulic spray applications. Many of the formulations are also registered for tree injection and stump removal. The specific application rates used in ground or aerial programs vary according to local conditions and the nature of the target vegetation. For ground applications, the Forest Service generally applies between 0.5 and 4 lbs a.e./acre with an average typical application rate of 1 lb a.e./acre. The same rates are likely to be used for aerial applications, if conducted. The upper bound of 4 lbs a.e./acre is useful for site preparation or wildlife habitat improvement, which comprise relatively minor uses of 2,4-D (i.e., about 4% of the acres treated with 2,4-D in 1995). The direct application of 2,4-D formulations to water bodies may involve rates as high as 38 lbs a.e./acre.

2.2.   CHEMICAL DESCRIPTION AND COMMERCIAL FORMULATIONS

2,4-D is a selective systemic herbicide used to control broadleaf weeds. The herbicidal properties, environmental chemistry, and toxicology of 2,4-D were investigated extensively, primarily because 2,4-D was used in combination with 2,4,5-T as the active ingredients in Agent Orange (Munro et al. 1992, USDA/FS 1989a,b,c, WHO 1988).

2,4-D is the common name for 2,4-dichlorophenoxyacetic acid:
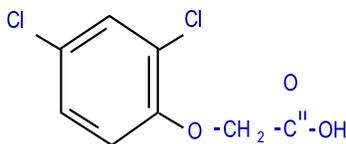
As summarized in Table 2-1, there are 22 herbicide formulations of 2,4-D in which the compound is present as salts, esters, or combinations of salts and esters (Kells 1997). All of the

2,4-D herbicides are formulated as liquids, except for Aqua-Kleen. Aqua-Kleen, which is a granular formulation of 2,4-D butoxyethyl ester in slow dissolving clay granules, is intended solely for the treatment of water. Most formulations of 2,4-D contain either the dimethyl amine salt or the isooctyl ester.

Commercial formulations of 2,4-D are specified in Table 2-1; herbicide formulations containing 2,4-D plus other herbicides are specified in Table 2-2. Herbicide mixtures containing 2,4-D plus triclopyr, dicamba, picloram, or glyphosate are used in Forest Service programs. Generally, this risk assessment is restricted to a quantitative consideration of the potential consequences of applying 2,4-D alone. Nonetheless, the consequences of using other herbicides with 2,4-D are considered as a connected action in the hazard characterization for human health and ecological effects.

Selected chemical and physical properties of 2,4-D, its salts, and commercially significant esters are summarized in Table 2-3. One potential concern associated with the use of 2,4-D is potential contamination with polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). The Environmental Fate and Effects Division (EFED) of the United States Environmental Protection Agency's Office of Pesticide Programs (U.S. EPA/OPP) reviewed available data on PCDD and PCDF concentrations in 2,4-D (Reg. No. 61272-3) and 2,4-D EHE (ethylhexyl ester) (Reg No. 61272-1) (U.S. EPA/OPP 2005c). The data are contained in a confidential memo cited by U.S. EPA/OPP (2005b) as: Dr. Stephan Funk, Memorandum, 11/26/91. Based on the memo, U.S. EPA/OPP (2005b) reports that 2,4-D and 2,4-D EHE contained two PCDDs at concentrations above the limit of quantification (LOQ), but does not state which two PCDDs were detected. EFED used this information to conduct a risk assessment for PCDD/PCDF contamination of 2,4-D, and concluded that risks associated with such contamination were likely inconsequential. U.S. EPA/OPP's assessment and findings are discussed in more detail in Sections 3.1.15 (Impurities and Metabolites) and in various subsections of Section 4 (Ecological Risk Assessment) as appropriate.

The publically available information on the inert ingredients contained in 2,4-D formulations is given in Table 2-4. This information is discussed further in Section 3.1.14 (Inerts and Adjuvants). As noted in Section 1 (Introduction), information submitted to the U.S. EPA on formulations – including information on impurities, inerts, and manufacturing processes – cannot be released under FOIA and was not obtained for the current risk assessment.

2.3.   APPLICATION METHODS
The general use and application of herbicides in silviculture are discussed both in the available literature (e.g., Cantrell and Hyland 1985) and in previously prepared risk assessments (USDA/FS 1989a,b,c). This risk assessment focuses on the aspects of herbicide application that are most germane to the exposure assessments for human health and ecological effects (sections 3.2 and 4.2). 2,4-D is registered for both ground and aerial applications. In addition, some formulations of 2,4-D may be applied directly to water for the control of noxious weeds.

In Forest Service programs, herbicide formulations containing 2,4-D are most commonly used in wildlife opening, rights-of-way maintenance, and noxious weed control. In these activities, the most common application methods include backpack (selective foliar), hack-and-squirt, and roadside hydraulic spray applications. Aerial applications are considered in this risk assessment but are not currently used in or planned for Forest Service programs.

The most commonly used ground application method for 2,4-D is backpack (selective) foliar applications. In selective foliar applications, the herbicide sprayer or container is carried by backpack and the herbicide is applied to selected target vegetation. Application crews may treat up to shoulder high brush, which means that chemical contact with the arms, hands, or face is plausible. To reduce the likelihood of significant exposures, application crews are directed not to walk through treated vegetation. Usually, a worker treats approximately 0.5 acre/hour with a plausible range from 0.25 to 1.0 acre/hour.

2,4-D may be used in hack and squirt applications, a form of cut surface treatment in which the bark of a standing tree is cut with a hatchet and the herbicide is applied with a squirt bottle. This treatment method 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 treats about 0.5 acre/hour with a plausible range from 0.25 to 1.0 acre/hour. Some formulations of 2,4-D are also labeled for injection bar applications in trees.

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

Although 2,4-D is registered for aerial applications, the Forest Service does not currently apply the compound aerially. Nonetheless, this risk assessment addresses the aerial application of 2,4-D to support its potential use if necessary (i.e., to control noxious weeds). In aerial applications, liquid formulations are applied through specially designed spray nozzles and booms. The nozzles are designed to reduce 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.

Several formulations (all dimethylamine salts) are labeled for direct application into bodies of water for the control of noxious weeds. This application method is quantitatively considered in this risk assessment.

2.4. MIXING AND APPLICATION RATES

The specific application rates used in a ground application vary according to local conditions and the nature of the target vegetation. As detailed in Table 2-5, the application rates for 2,4-D vary substantially with the purpose of the application (e.g., agricultural weed control, noxious weed control, right-of-way management, etc). The highest labeled rate for any terrestrial application is 9 lbs a.e./acre – the highest application rate for non-crop applications of Weedone LV6 EC. Much lower application rates – i.e., ranging from about 0.1 to 0.5 lbs a.e./acre – are recommended for various crop uses.

The uses of 2,4-D in Forest Service Programs for the years 2000 through 2004 are summarized in Table 2-5 in terms of vegetation management objective. The predominant use of 2,4-D by the Forest Service is in noxious weed control, which accounts for 86% of acres treated and 77% the pounds used by the Forest Service. The only other substantial use reported by the Forest Service is agricultural weed control, accounting for 12% of the acres treated and 22% of the pounds used in Forest Service programs. Other uses include rights-of-way management, facilities maintenance, and nursery weed control; however, the amount of 2,4-D used in these management objectives is insubstantial. Based on the total amount used and number of acres treated, the average application rate for 2,4-D in Forest Service programs is approximately 0.7 lbs a.e./acre. The application rate of 11.59 lbs a.e./acre reported for facilities management is atypical for ground applications and may be reporting error.

For this risk assessment, the typical application rate for 2,4-D will be taken as 1 lb a.e./acre, which is the average application rate for all applications conducted by the Forest Service from 2000 to 2004 rounded to one significant digit – i.e., 0.67 lb a.e./acre rounds to 1 lb a.e./acre. The range of application rates will be taken as: from 0.5 lbs to 4 lbs a.e./acre.

Much higher application rates could be used with the direct application of 2,4-D to water bodies (e.g., Madsen et al. 1998). Aqua-Kleen contains the butoxyethyl ester of 2,4-D in clay granules at a w/w concentration of 19% a.e. (i.e., 190 g 2,4-D a.e./kg formulated product). The product label for Aqua-Kleen (CRP 1997) recommends application rates of 100 lbs/acre or 19 lbs a.e./acre for the control of water milfoil or water stargrass. Rates of up to 200 lbs formulation/acre or 38 lbs a.e./acre are recommended for more resistant plants such as bladderwort, white water lily, very dense vegetation beds, or bodies of water that are more than 8 feet deep. Application rates of up to 38 lb a.e./acre are also recommended for other 2,4-D formulations that are labeled for direct application to water – i.e., 2,4-D Amine 4, 2,4-D 6 Amine. For Aqua-Kleen, a second application may be made 2-3 weeks after the initial application. The Forest Service does not typically employ 2,4-D in direct aquatic applications. Nonetheless, this treatment may occasionally be necessary and this type of application is included in this risk assessment to support this treatment option.

2.5. USE STATISTICS

The USDA Forest Service tracks and reports its use of pesticides by management use objectives (Table 2-5) and by geographical areas referred to as "*Regions*" (Table 2-6). As illustrated in Figure 2-2, 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.]

As illustrated in Figure 2-2 and detailed further by region in Table 2-6, the use of 2,4-D by the Forest Service between 2000 and 2004 occurred predominantly in the Rocky Mountain (36%), Northern (33%) and Inter-mountain (30%) regions, with considerably lesser amounts (<1%) used in the remaining regions. The total amount of 2,4-D used in all regions over the 4-year period was about 131,311.71 lbs (Table 2-6) for an average of about 32,800 lbs/year.

The use pattern by region given in Table 2-6 may not be predictive of uses in all Forest Service regions for the coming years. Onken (2006) noted that Region 3 (the Southwestern Region in the Forest Service system) may be using more 2,4-D as well as other herbicides in rights-of-way weed control with moderately expanded use in resource management within the National Forests of Region 3. This use will focus primarily on the protection of native plants against noxious weeds and invasive species.

2,4-D is used on a number of crops, and a summary of the agricultural uses of the compound is presented in Figure 2-3 (USGS 1998). These use statistics are for 1992, the most recent year for which data are available. As indicated in this figure, more than 37 million lbs of 2,4-D were applied primarily to pasture (43.25%), wheat and grains (21.29%), corn (12.22%), "other hay" (9.98%), soybeans (5.49%), barley (2.44%), rice (1.49%), sorghum (1.22%), oats (<1%) and sugar cane (<1%). The geographical distribution of the agricultural uses of 2,4-D are broader than those of the Forest Service, with substantial overlap between general agricultural and Forest Service uses occurring in Forest Service Regions 1 and 2 (Northern and Rocky Mountain Regions). The average use of 2,4-D by the Forest Service from 2000 to 2004 (32,800 lbs/year) is approximately 0.09% of the amount used in agriculture in 1992.

3. HUMAN HEALTH RISK ASSESSMENT

3.1. HAZARD IDENTIFICATION

3.1.1.   Overview

The pharmacokinetics and toxicity of 2,4-D are well studied in laboratory animals, volunteers, epidemiology studies, and incidents involving attempted suicide. The enumeration of all these studies is well beyond the scope of this document. Because of the extensive literature available on 2,4-D, credible reviews conducted by U.S.EPA and WHO are used as sources in this hazard identification, supplemented by information in the open literature that is not covered by existing reviews.

The WHO and FAO (Food and Agriculture Organization of the United Nations) have held ongoing joint meetings since the 1970's to discuss the use of pesticides and their impacts on health and the environment. The results of these meetings are published in a series of monographs. For 2,4-D, the most recent comprehensive reviews and discussions on mammalian toxicology, ecological/environmental toxicity, and metabolism/environmental fate are published in WHO (1996), WHO (1997) and WHO (1998), respectively. In essence, WHO/FAO concluded that the toxicity of the salts and esters of 2,4-D in mammals are equivalent to that of the acid. WHO (1996) establishes an acceptable daily intake (ADI) for 2,4-D (sum of 2,4-D and its salts and esters, expressed as 2,4-D) in humans of 0 to 0.01 mg/kg/day. The basis for the WHO/FAO ADI is a NOAEL of 1 mg/kg/day derived from a 2-year carcinogenicity/ toxicity study with rats, and a 13-week study and and 1-year study with dogs. Based on the available human and animal evidence from subchronic, chronic, carcinogenicity, mutagenicity, developmental and reproductive toxicity studies in mice, rats, rabbits and dogs and with consideration of the available epidemiological database, WHO does not regard 2,4-D and its salts and esters as either genotoxic or carcinogenic.

U.S. EPA documents regarding the registration process for 2,4-D acid and 2,4-D salts and esters are available from the Federal Docket Management System under Docket Number EPA-HQ-OPP-2004-0167. The most current EPA/OPP documents relevant to the review of 2,4-D toxicity presented in this risk assessment are the Reregistration Eligibility Decision (RED) for 2,4-D (U.S. EPA/OPP 2005a), supported by risk assessments conducted by the Human Health Effects Division (HED) (U.S. EPA/OPP 2005a) and the Environmental Fate and Effects Division (EFED) (U.S. EPA/OPP 2004b). These documents have been revised and updated to reflect public comments; they are current as of this writing

Based on numerous unpublished studies submitted by registrants as part of the pesticide registration process, U.S. EPA/OPP (2005a) concludes:

1.   2,4-D and its salts and esters are of low acute toxicity on the basis of oral, dermal and inhalation routes of exposure, and are not skin sensitizers or primary skin irritants;
2.   2,4-D acid and salts are severe eye irritants;

3.   Renal clearance is key to 2,4-D toxicity; in laboratory animals, repeated oral exposure to amine salts and esters of 2,4-D at levels which saturate renal clearance is associated with toxic effects primarily manifest in eyes, blood, thyroid, liver, kidneys, adrenals, ovaries and testes;

4.   Systemic dermal toxicity is seen only following high-dose repeated exposures to DEA and DMA but no effects are seen following repeated exposures at the limit doses of 2,4-D, EHE and TIPA;

5.   There are no repeat-dose inhalation studies for 2,4-D; a subchronic (28-day) inhalation study is required;

6.   Developmental and reproductive effects are seen only in association with maternal toxicity above the threshold for renal clearance; however, a current 2-generation toxicity study is required for 2,4-D to address concerns about endocrine disruption (thyroid and gonads) and immune toxicity;

7.   Neurotoxicity is seen in laboratory animals following high dose exposure. A developmental neurotoxicity study is required for 2,4-D;

8.   2,4-D is a Group D chemical (not classifiable) with regard to human carcinogenicity, and is not mutagenic; however, some cytogenic effects were observed. 2,4-D is considered representative of the various forms (salts and esters) under consideration.

Based on recent studies published in the open literature, 2,4-D is toxic to the immune system and developing immune system, especially when used in combination with other herbicides. The mechanism of action of 2,4-D toxicity is disruption of the cell membrane and cellular metabolic processes. The molecular basis for 2,4-D toxicity to human lymphocytes and nerve tissue is likely the induction of programmed cellular death known as apoptosis.

Signs of neurological, cardiac, hepatic, and renal toxicity are evident in cases of 2,4-D poisoning following suicide attempts. A recent study conducted by Krieger et al. (2005) used measures of dosimetry as well as biomonitoring to determine the absorption and elimination of 2,4-D in volunteer workers spraying a mixture of 2,4-D and triclopyr for purposes of conifer release. This information is taken into account with other methods and information to estimate dermal absorption variables necessary to quantify potential risks (in this assessment) to forestry workers and the general public.

3.1.2.   Mechanism of Action

Studies conducted within the last 10 years strongly suggest that the toxicity of chlorophenoxy herbicides, including 2,4-D, is due to a fundamental disruption of the basic structure and function of the cell, beginning at the level of the plasma membrane. Recent studies show that 2,4-D can induce a genetically programmed sequence of cellular death known as apoptosis (Lin and Garry 2000).

The mechanism of 2,4-D toxicity to humans and mammals is reviewed by Bradberry et al. (2000, 2004). 2,4-D and other chlorophenoxy herbicides were shown to disrupt the structure and

function of cellular membranes; interfere with cellular metabolism; and uncouple oxidative phosphorylation as a consequence of effects on the cell membrane and alteration of metabolism. These effects of 2,4-D on the cellular membrane are implicated in the observed toxicity of 2,4-D to hepatocytes; the dose-dependent disruption of 2,4-D to the cerebrovascular system and the developing brain; disruption of the neuromuscular junction (leading to muscle and nerve toxicity); and the observed adverse impacts of 2,4-D on blood cells and blood clotting (Bukowska et al. 1998; Duchnowicz and Koter 2003).

With regard to disruption of cellular metabolism, Bradberry et al. (2000) also note that chlorophenoxy acids such as 2,4-D are structurally similar to acetic acid and capable of forming analogues of acetyl Co-A. Acetyl-Co-A formation is fundamental to basic pathways involving glucose metabolism; and the production of cholesterol, steroid hormones, and the neurotransmitter acetylcholine. Disruption of these pathways is consistent with many of the observed 2,4-D-related toxic effects in studies with mammals, including alterations in cholesterol profiles, myotonia, cardiac arrhythmia, muscle twitching, immunotoxicity, and neurotoxicity.

Several recent studies investigating the molecular mechanism for cytotoxicity, show that 2,4-D induces apoptosis. Kaioumova et al. (2001a,b) demonstrate that the DMA salt of 2,4-D kills human lymphocytes through a direct damaging effect on mitochondria, which in turn causes the cascade of events associated with apoptosis. Similarly, De Moliner et al. (2002) demonstrate that 2,4-D acid is capable of inducing apoptosis in cultured rat cerebellar granule cells by initially damaging mitochondria. The results of Kaioumova et al. (2001a,b), however, are not consistent with those reported by Oakes and Pollack (1999), who associated mitochondrial damage with exposure to a commercial formulation of 2,4-D but attributed the damage to a proprietary surfactant rather than 2,4-D.

3.1.3.   Pharmacokinetics and Metabolism
3.1.3.1. General Considerations – The pharmacokinetics of 2,4-D are well studied in experimental mammals and humans. The available information is summarized in the open literature both in general reviews (e.g., Bradberry et al. 2004; U.S. EPA/OPP 2004a, 2005a; WHO 1984,1996) and in publications of physiologically based pharmacokinetic models for 2,4-D (Durkin et al. 2004; Kim et al. 1994, 1995, 1996). 2,4-D binds to serum albumin but is rapidly excreted, primarily as unchanged 2,4-D acid in the urine.

There are inter-species differences in the metabolism and elimination of 2,4-D. Following oral exposure, conjugated forms of 2,4-D were detected in the urine of dogs, humans, mice, rats, and hamsters (e.g., Aydin et al. 2005). The mechanism of renal clearance of 2,4-D is well known (e.g., Villalobos et al. 1996). It involves active secretion of the acid by the proximal tubules of the kidney, in a manner similar to excretion of paraminohippuric acid (PAH). The linear dose-dependent pharmacokinetics of 2,4-D are believed to result from saturation of the renal transport system. Dogs, which are more sensitive than other species to 2,4-D exposure (i.e., dogs experience adverse toxicity at lower doses), are more limited in their capacity to excrete organic acids, compared with other species such as rats (Bradberry et al. 2004; U.S. EPA/OPP 2005b).

After comparable doses, body burdens (mg 2,4-D/kg body weight) in dogs are higher than those in rats by a factor of more than 25 at a dose of 5 mg/kg. As the dose increases, the differences become more pronounced. At a dose of 50 mg/kg, the body burden in the dog is a factor of 200 greater than that in the rat (Van Ravenzwaay et al. 2003).

3.1.3.2. Absorption – For the current risk assessment, dermal exposures are considered quantitatively in a number of different exposure scenarios (Section 3.2.2.2). Two types of dermal exposure scenarios are considered: those involving direct contact with a solution of the herbicide (e.g., immersion) and those associated with accidental spills of the herbicide onto the surface of the skin. As detailed in SERA (2006), dermal exposure scenarios involving immersion or prolonged contact with chemical solutions use Fick's first law (zero-order absorption) and require an estimate of the permeability coefficient, K_p, expressed in cm/hour. For accidental spills, the assumption of first-order dermal absorption is used based on the first-order dermal absorption rate, k_a, in units of days^{-1}.

The key experimental study in the estimation of the first-order dermal absorption rate for 2,4-D in humans is that of Feldmann and Maibach (1974) who assayed the urinary excretion of ^{14}C-labeled 2,4-D acid in volunteers after both I.V. administration and dermal exposure. The radio-labeled compound in acetone was applied to the ventral surface of the forearm of volunteers or injected intravenously. Six subjects were used in each experiment. The publication does not specify whether the same individuals were used in both the intravenous and dermal studies. In the dermal application study, the acetone was evaporated from the skin surface over a period of fewer than15 seconds. The total applied dose of radioactivity ranged from 1 to 5 µCi. The 2,4-D was applied at a rate of 4 µg/cm^2 of skin surface over a skin area of 2.8-20 cm^2 (the area used for 2,4-D is not specified). The skin was not protected, and the subjects were asked not to wash the treated area for 24 hours. Data are not reported on precisely when and in what manner the treated areas of the skin were washed after the initial 24-hour post-application period. In both the dermal and intravenous studies, urinary elimination was quantified by measuring ^{14}C (i.e., parent compound and metabolites) in urine. All urine was collected for 5 days, with four collection periods on the first day and total daily urine collections for all subsequent days. Details of the excretion phase of this study are discussed further in Section 3.1.3.3.

The absorption factor of 0.058 reported by Feldmann and Maibach (1974) is often cited as if it were a first-order dermal absorption rate – i.e., 0.058 day^{-1}. A very similar value for dermal absorption 0.057 is reported by Ross et al. (2005) based on a review of several additional studies. These values, however, are not first-order dermal absorption rates because they are estimated from urinary excretion over a period of several days. Based on the kinetic analysis of the Feldmann and Maibach (1974) data, Durkin et al. (2004) estimated the first-order dermal absorption rate at 0.000011 min^{-1}, equivalent to 0.00066 hour^{-1} or 0.0158 day^{-1}. For this risk assessment, the first-order dermal absorption rate of 0.00066 hour^{-1} is used as the central estimate for exposure assessments involving first-order dermal absorption. This estimate of the first-order

dermal absorption rate is very close to the k_a of 0.000515 hour^{-1} derived in the previous Forest Service risk assessment (SERA 1998b) using the 'flip flop' principle (O'Flaherty 1981).

Feldmann and Maibach (1974) do not report absorption data for each volunteer, and formal estimates of the confidence interval on the dermal absorption rate cannot be derived. Nonetheless, Feldmann and Maibach (1974) do discuss the variability in dermal absorption among individuals, as follows:

> *Assuming a normal distribution, 1 person in 10 will absorb twice the mean value while 1 in 20 will absorb 3 times this amount* (Feldmann and Maibach 1974, p. 131).

Taking the factor of 3 as an approximate 95% estimated of variability (i.e., 1/20 = 0.05), the range for the first-order dermal absorption rate will be taken as 0.00022 hour^{-1} [0.00066 hour^{-1} ÷ 3] to 0.00198 hour^{-1} [0.00066 hour^{-1} × 3].

As noted above, scenarios that use Fick's first law require an estimate of the permeability coefficient, K_p, expressed in cm/hour. Based on the methods recommended by U.S. EPA (1992), estimates of the dermal permeability of 2,4-D acid with 95% confidence intervals are 0.0000242 (0.0000102-0.0000575) cm/hour (Worksheet B05).

The only experimental measure of the Kp for 2,4-D comes from the study by Sartorelli et al. (1998). In this study, the permeability coefficient of 2,4-D was determined *in vitro* using a static diffusion cell/monkey skin apparatus. Using this system, the K_p for 2,4-D was measured at 0.0052 ± 0.00296 cm/hr (Sartorelli et al. 1998, Table 2, p. 269). Sartorelli et al. (1998) do not specify the form of the 2,4-D (i.e., acid, salt or ester). The molecular weight and physical properties reported by Sartorelli et al. (1998, Table 1, p. 269), however, are consistent with 2,4-D acid. The central estimate of the *in vitro* K_p report by Sartorelli et al. (1998) is greater than the K_p estimated by the method of U.S. EPA (1992) by a factor of more than 200 [0.0052 cm/hr ÷ 0.0000242 cm/hr = 214]. It should be noted that the monkey skin used in the study by Sartorelli et al. (1998) was previously frozen and freezing the skin may have altered its permeability to 2,4-D. While there are uncertainties in the use of these *in vitro* measures of K_p and additional uncertainties in the use of data on monkeys to estimate dermal absorption in humans, the risk assessment uses the experimental data from Sartorelli et al. (1998) for exposure assessments requiring an estimate of dermal permeability (K_p). Thus, the Kp values entered into Worksheet B01 are 0.0052 (0.00224 to 0.00816) cm/hr. As discussed further in Section 3.4.2 (risk characterization for workers), the use of the K_p values from Sartorelli et al. (1998) have an impact on the risk characterization for worker exposure scenarios involving contaminated gloves.

Given differences in the chemical properties of various forms of 2,4-D (i.e., salts and ester), it is reasonable to expect differences, perhaps even substantial ones, in the dermal absorption rates for the various forms. Moody et al. (1990), however, make it very clear that there are no substantial differences regarding the rates of dermal absorption of 2,4-D acid, salts, or esters. In their study,

Moody et al. (1990) assayed the dermal absorption of several forms of 2,4-D in different vehicles, using volunteers and experimental mammals. These data are summarized in Table 3-1. For each of the 2,4-D compounds tested, this table gives the percent recovery of the compound in the urine and the excretion halftime reported by the investigators.

These investigators followed a protocol similar to that of Feldmann and Maibach (1974), except that they used a 14-day sampling period. For applications to the backs of rats and rabbits, the middorsal region was shaved. Similarly, the middorsal forearm and forehead regions of monkeys were shaved. In both studies, the treated area was washed after 24 hours. Unlike the Feldmann and Maibach (1974) study, the Moody et al. (1990) study does not provide data regarding the amounts of 2,4-D eliminated after various periods.

The Moody et al. (1990) study reveals substantial inconsistencies regarding the effects of exposure to the acid or amine formulations of 2,4-D, compared with the ester formulation. There is little difference among the acid, amine salt, and isooctyl ester, when applied to the backs of rabbits. When applied to the human forehead, the 2,4-D amine was absorbed to a much greater extent than the isooctyl ester, regardless of the vehicle (either acetone or the Esteron LV96 blank). In the monkey, the absorption of the amine and isooctyl forms are comparable, when applied to the forehead; however, the isooctyl form is absorbed far more readily than the amine salt, when applied to the forearm. There is, however, a less substantial difference between the absorption rate of 2,4-D acid and the isooctyl ester, applied to the monkey forearm. The highest cumulative absorption rate in the Moody et al. (1980) study is about 58% (2,4-D amine in water on the forehead of humans), which is extremely close to the 56% absorption rate for 2,4-D isooctyl ester in acetone applied to the forehead of monkeys.

As noted above, the relatively minor difference in the absorption rates for 2,4-D amine or salts, compared with the ester forms of 2,4-D is not consistent with anticipated differences based on skin permeation rates. Nonetheless, the study by Moody et al. (1990) is directly relevant to the salts and esters covered in this risk assessment. In addition and as noted in U.S. EPA (1992), solutions containing products that enhance the solubility of an agent, like 2,4-D esters in a carrier, are likely to impair the dermal absorption of the agent (i.e., decrease the partitioning of the lipophilic agent from the carrier into the skin). Conversely, various agents that enhance solubility also may enhance hydration of the epidermis and increase absorption. The available data are not sufficient for conducting a quantitative analysis of these competing processes. Consequently, the K_p for 2,4-D acid is applied to all exposure scenarios that require the use of Fick's first law.

In addition to uncertainties regarding the relative rates of 2,4-D dermal absorption among animal species, anatomical sites, and 2,4-D compounds (i.e., acid, salts, and esters), other factors influence the dermal absorption of 2,4-D. Although the Esteron formulation did not have a consistent effect on the dermal absorption of 2,4-D isooctyl ester in the Moody et al. (1990) study (Table 3-1), in another study by these investigators, the addition of DEET (N,N-diethyl-m-toluamide) to an aqueous solution of 2,4-D dimethylamine increased absorption

by a factor of about 2 (Moody et al. 1992). These investigators also found that washing the skin with soap and water removed about 35% of an applied dose of 2,4-D. Nonetheless, as documented in U.S. EPA (1992), washing also may result in a transient, if not longer lived, increase in the permeability of the skin to most compounds due to the increased hydration of the skin.

3.1.3.3. Excretion – The elimination of 2,4-D is dose-dependent and follows apparent first-order kinetics at low doses, as illustrated in the intravenous exposure study by Feldmann and Maibach (1974), in which the average whole-body halftime was estimated at about 13 hours. This halftime is virtually identical to the average halftime of 11.6 hours reported for humans in the oral pharmacokinetic study by Sauerhoff et al. (1977).

While excretion rates are not used directly in either the dose-response assessment or risk characterization, whole-body halftimes 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 time can be estimated based on the body burden immediately after a single dose, X_0 by the relationship:

$$X_{Inf}/X_0 = 1 / (1 - e^{-ke\ t^*})$$

where t* is the interval between dosing. Based on a halftime of 12 hours, the approximate average of the values reported by Feldmann and Maibach (1974) and Sauerhoff et al. (1977), the first-order elimination rate constant can be estimated at 0.058 hours^{-1} [ln(2)÷12 hours] or about 1.39 day^{-1}. Setting the interval between doses to 1 day – i.e., daily dosing – and using the above equation, the increase in body burden with exposure for an infinite period of time would be about 1.33. Thus, 2,4-D has a very low potential to accumulate in the body.

3.1.4. Acute Oral Toxicity

The database for the acute toxicity of 2,4-D and its various salts and esters is extensive and complete. The available relevant information, as reported by U.S. EPA/OPP (2004a, 2005a,b), is shown in Table 3-2. For acute oral toxicity, citations from both EFED (U.S.EPA/OPP 2004b) and HED (U.S.EPA/OPP 2005a) are presented because these two sources, which cite the same studies, report different values. The difference between the two sets of values is not addressed in the RED (U.S. EPA/OPP 2005a), and it is not apparent from consideration of molecular weights and acid equivalents. Based on the LD$_{50}$ values shown in Table 3-2, regardless of the exact value, U.S.EPA/OPP (2005a) considers 2,4-D acid, 2,4-D salts, and 2,4-D esters to be of low acute oral toxicity. Furthermore, with regard to acute oral toxicity and mammalian toxicity in general, U.S. EPA (2005a) concludes that the toxicity of 2,4-D salts and esters is equivalent to that of 2,4-D acid, and this conclusion is supported by the published study of Charles et al. (1996). Minor differences in toxicity between salt and esters are reported in the open literature (e.g., Schillinger 1980). Data shown in Table 3-2 substantiate the position that the salt and ester forms are toxicologically equivalent. Based on the available studies, the primary clinical effects

observed following acute oral exposure of laboratory animals are ataxia, myotonia, and decreased limb tone (U.S. EPA/OPP 2005a).

Important sub-lethal effects which occur at low doses following acute oral exposure of laboratory animals include maternal toxicity and neurotoxicity. The relevant studies that address these endpoints are presented in Appendices 1 (studies on reproductive and teratogenic effects) and 2 (studies addressing endpoints other than reproductive toxicity or teratogenicity), and are discussed in detail in the appropriate subsequent sections on neurotoxicity (Section 3.1.6), reproductive and teratogenic effects (Section 3.1.9) and dose-response assessment (Section 3.3). Of particular importance, is the maternal toxicity observed among pregnant rats and rabbits exposed orally to 2,4-D acid, salts, and esters for several days during organogenesis. The neurotoxic effects observed in dogs and rodents are also of concern, and are addressed further in the neurotoxicity section of this report. (3.1.6).

After acute lethal exposure, the signs of toxicity in humans include convulsions, vomiting, congestion of various organs, and degenerative changes in nerve cells (Mullison 1981). In non-lethal but toxic oral exposure to 2,4-D, the signs and symptoms of toxicity in humans include irritation to mouth, throat, and gastrointestinal tract, vomiting, chest and abdominal pain, diarrhea, muscle twitches, tenderness, and stiffness (Mullison 1981). Similar signs of acute toxicity were observed in monkeys (Hill and Carlisle 1947) and pigs (Bjorklund and Erne 1966) exposed to 2,4-D. Lethal overdoses of 2,4-D in humans, all of which involved suicides, are associated with serum levels of about 0.4-0.6 mg/L (Osterloch et al. 1983; Park et al. 1977; Singh et al. 2003). Total blood levels of about 7 mg/L are reported in other suicide cases (Smith and Lewis 1987).

3.1.5. Subchronic or Chronic Systemic Toxic Effects

Systemic toxicity encompasses virtually any effects that a chemical has after the chemical has been absorbed. Certain types of effects are of particular concern and are assessed with a specific subset of toxicity tests. Such effects are considered in the following subsections and include effects on the nervous system (Section 3.1.6), immune system (Section 3.1.7), endocrine function (Section 3.1.8), development or reproduction (Section 3.1.9), and carcinogenicity or mutagenicity (Section 3.1.10). This section summarizes the available information on other systemic effects and non-specific toxicity.

The subchronic and chronic oral toxicity of 2,4-D acid, salts, and esters was investigated in guideline-compliant studies submitted by registrants as part of EPA's pesticide registration process (U.S. EPA/OPP 2004a, 2005a,b). On the basis of these results, both U.S. EPA/OPP (2005a,b) and the WHO (1996) concluded that the toxicity of 2,4-D acid is representative of the toxicity of the class of 2,4-D acid, salts, and esters. Studies conducted with 2,4-D acid include subchronic studies with rats (Schulze and Dougherty 1998a) and dogs (Schulze 1990; Dalgard 1993a), and chronic toxicity/carcinogenicity studies conducted with rats (Jeffries et al. 1995), mice (Rowland 1996b; Scott et al. 1995) and dogs (Dalgard 1993d). These studies are shown in Table 3-3 along with other studies critical to the dose-response assessment (Section 3.3).

On the basis of the effect levels derived from the above studies, dogs are more sensitive than rats or mice to 2,4-D exposure, presumably due to the dog's inability to excrete organic acids. Nevertheless, at the lowest observed effect levels in both canines (3-3.75 mg/kg/day) and rodents (75-100 mg/kg/day), 2,4-D exposure was associated with decreases in body weight and food consumption, as well as adverse effects on the liver and kidneys. The latter effects were manifested as changes in clinical chemistry variables (e.g., liver and kidney enzyme alterations), organ weights, and microscopically identified kidney and liver pathology. In rats, adverse effects on the thyroid (increased thyroid weight), thyroid function (decreased T3 and T4), hematology (decreased red blood cells, hematocrit and hemoglobin in females, and platelets in both sexes), cholesterol and glucose metabolism (decreased blood glucose, cholesterol, triglycerides), sex organs (decreased ovary and testes weights), eyes (cataracts), adipose tissue (microscopic lesions), and lungs (microscopic lesions) were also observed. Decreased blood glucose, brain weights, and testes weights were also observed in dogs, along with hypo-spermatogenesis and prostate inactivity.

No substantial dose-duration relationship is apparent in the dog studies. The NOAEL values for acute (see Beasley et al. 1991, Appendix 2) subchronic and chronic exposures are all near 1 mg/kg/day. LOAEL values for dogs ranged from 3 to 3.75 mg/kg/day, following 90-day subchronic exposure, to 5 mg/kg/day following chronic exposure. These findings support the notion that the mechanism for renal clearance of 2,4-D is overwhelmed in dogs at a low dose following a single exposure. In rats, however, the dose-duration relationship is not pronounced and follows a more traditional inverse linear pattern. The NOAEL values for subchronic and chronic exposures are 15 and 5 mg/kg/day, respectively, and the corresponding LOAEL values are 100 and 75 mg/kg/day, respectively. Again, these values for rats do not suggest a pronounced dose-duration relationship, which is probably associated with the limited potential of 2,4-D to accumulate in the body (Section 3.1.3.3).

3.1.6. Effects on Nervous System

As discussed in Durkin and Diamond (2002), a neurotoxicant is a chemical that disrupts the function of nerves, either by interacting with nerves directly or by interacting with supporting cells in the nervous system. This definition of neurotoxicant distinguishes agents that act directly on the nervous system (direct neurotoxicants) from those agents that might produce neurological effects that are secondary to other forms of toxicity (indirect neurotoxicants). Virtually any chemical will cause signs of neurotoxicity in severely poisoned animals and can be classified as an indirect neurotoxicant.

Signs of neurotoxicity were observed in humans following acute lethal exposures to 2,4-D, as discussed in Section 3.1.4. Neurotoxic effects were observed in laboratory animals following acute, chronic, and developmental exposures to 2,4-D at higher doses that are likely to have saturated renal clearance (Bortolozzi et al. 1999a,b, 2002, 2003, 2004) or after direct intracerebral instillation (Bortolozzi et al. 2001).

Ttwo studies designed specifically to address the neurotoxicity of 2,4-D were submitted to EPA as part of the pesticide registration process. As with other effects in mammals, neurotoxicity studies conducted with 2,4-D acid are considered to be representative of the class of 2,4-D acid, esters, and salts. While EPA considers the submitted studies to be acceptable, the lack of a developmental neurotoxicity study conducted according to current EPA guidelines is considered to be a gap in the 2,4-D database (U.S.EPA/OPP 2005a,b).

The first study submitted to EPA is an acute neurotoxicity screening battery conducted with Fischer 344 rats (U.S. EPA/OPP 2005b; Mattsson et al. 1994a, summarized in Table 3-3). In this study, groups of rats were exposed orally to 2,4-D at doses 0, 13, 67, and 227 mg/kg/day. The NOAEL for the study was 67 mg/kg/day. The LOAEL was 227 mg/kg/day based on an increased incidence of in-coordination and slight gait abnormalities, described as forepaw flexing or knuckling, and decreased motor activity.   The NOAEL from this study serves as the basis for EPA's acute RfD for 2,4-D exposure, for the general population (U.S. EPA/OPP 2005a,b).

The second study submitted to EPA is a subchronic neurotoxicity screening battery conducted with rats (Mattsson et al. 1994b, summarized in Table 3-3). In this study, groups of rats were exposed to 2,4-D acid at doses of 0, 5, 75, and 150 mg/kg/day. The NOAEL for the study was 75 mg/kg/day. The LOAEL was 150 mg/kg/day, based on increased forelimb grip strength.

Notably, a recent study published in the open literature (Garcia et al. 2004) demonstrates a LOAEL of 70 mg/kg/day based on immuno-histochemical evidence of central nervous system damage in rat pups exposed to 2,4-D acid via mothers' milk for the first 25 days of their lives. This assessment is based on staining of various regions of the brain to indicate the presence of subsets of neurons as well as enzyme activity associated with healthy nerve function. At an exposure dose of 100 mg/kg/day, 25-day-old pups in the Garcia et al. (2004) study had significantly decreased body and brain weights, as well as a significant diminishment of neurons in the substantia nigra and ventral tegmental regions of the brain (See Table 3-3 for a summary of this study).

Other studies in the open literature that address the neurotoxicity of 2,4-D are summarized in Appendix 2. In general, these studies support the findings seen in the above studies submitted to EPA for rats, and support the general notion that dogs are more sensitive than other species following exposure to 2,4-D. Beasley et al. (1991) demonstrated a NOAEL of 1.3 mg/kg/day for dogs exposed to a single oral dose of DMA-4 (formulated dimethylamine salt of 2,4-D). Sub-clinical evidence of myotonia was observed in dogs exposed to doses of 8.8 mg/kg and higher. Steiss et al (1987) demonstrated similar results in a separate study with a NOEL of 25 mg/kg and a LOAEL of 50 mg/kg for myotonia (form of 2,4-D used was not identified in the study report). At higher doses (70 and 100 mg/kg administered subcutaneously every 48 hours on post-natal days 7 or 10 to day 25), signs of neurobehavioral toxicity and neuropathology were seen in neonatal rats (Rosso et al. 2000b).

There is discussion in the open literature of an association between 2,4-D exposure and a significant increase in the incidence of deaths due to amyotrophic lateral sclerosis in humans (Freedman 2001). This association was observed for a cohort of Dow Chemical Company employees potentially exposed to 2,4-D; however, there is neither direct evidence that the individuals in the study were actually exposed to 2,4-D, nor any direct measure of a dose-response relationship. As discussed further by Garabrant and Philbert (2002), 2,4-D is more likely to lead to adverse effects on the nervous system at doses that are above those associated with other signs of toxicity. In terms of the current risk assessment, it should be noted that the NOAEL used for the chronic RfD is a NOAEL of 5 mg/kg/day (Section 3.3). With the exception of dogs (which are discussed separately in the ecological risk assessment), the dose of 5 mg/kg/day is below any dose associated with signs of neurotoxicity.

3.1.7. Effects on Immune System

As discussed by Durkin and Diamond (2002), a variety of tests have been developed to assess the effects of chemical exposures on various types of 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. The impact of exposure to 2,4-D, alone or in combination with other herbicides, on the immune system is suggested in laboratory studies with animals and cultured human lymphocytes and in a case study with farmers exposed to chlorophenoxy herbicides.

In a study designed to assess the developmental immunotoxicity of the 2,4-D, Lee et al. (2001) exposed pregnant mice to the DMA salt of 2,4-D in the drinking water during days 6 through 16 of gestation (See Appendix 1 for details and Table 3-3 for a summary). Doses were equivalent to 0, 8.5, 37, and 370 mg a.e./kg bw/day. The maternal NOAEL for the study was 370 mg a.e./kg/day. Nonetheless, the NOAEL and LOAEL for offspring were 8.5 and 37 mg a.e./kg/day, respectively, based on decreased body weight gain and decreased kidney weights in females. At the highest dose of 370 mg a.e./kg/day, adverse effects were apparent on the immune systems of offspring evaluated 7 weeks after birth. These effects included suppression of lymphocyte stimulation by concanavalin A, and effects on counts and ratios of B-cells and cytotoxic T cells. The humoral immune response, measured as antibody production against sheep red blood cells and peritoneal phagocyte function were not affected in comparison with unexposed control pups. In addition, prenatal 2,4-D exposure at a dose of 370 mg a.e./kg/day had a slight anti-neoplastic effect. Pups exposed to 2,4-D *in utero* did not have significantly fewer urethane-induced lung adenomas, but the tumors they had were significantly smaller in diameter than those of controls. Lee et al. (2001) attributed this effect to the ability of 2,4-D to inhibit metabolic and/or enzyme pathways necessary for cellular growth and tissue development. The latter conclusion by Lee et al. (2001) is consistent with the mechanism of 2,4-D toxicity discussed in Section 3.1.2.

Faustini et al. (1996) evaluated short-term immunological changes in farmers who handled commercial formulations containing of 2,4-D and MCPA (4-chloro-2-methylphenoxyacetic acid) for 3 days. The average age of the farmers in this study was 44 years, and the mean of total

herbicide applied over the 3-day period was 39.1 kg (12- 55 kg). Blood samples were collected from farmers within 7 days prior to exposure, 1-12 days after exposure, and 50-70 days after exposure. Blood samples were used to determine lymphocyte subsets through use of monoclonal antibodies. The concentrations of 2,4-D and MCPA in the blood were not determined; however, statistically significant reductions in the following variables were determined within 1-12 days post-exposure: circulating helper and suppresor T cells, supressor T-cell diameter, cytotoxic T lymphocytes, natural killer cells, supressor T cells expressing the surface antigens HLA-DR, and lymphoproliferative responses to mitogen stimulation. All variables returned to pre-exposure levels 50-70 days after exposure. The immunosupression observed in this study is supported by previously cited results by Kaioumova et al. (2001a,b) which demonstrate that the DMA salt of 2,4-D kills human lymphocytes through induction of apoptosis.

Studies conducted by De La Rosa et al (2003, 2005) demonstrate that combining herbicides may have a synergistic effect in terms of adverse effects on immune function. De la Rosa et al. (2003) administered a single intraperitoneal dose of 2,4-D, propanil, or a combination of propanil and 2,4-D (equal portions) to groups of mice at doses ranging from 50 to 200 mg herbicide/kg body weight. Flow cytometry was used to assess B-cell populations in bone marrow on days 1, 2, 7, and 14 post-treatment. While neither 2,4-D nor propanil alone adversely affected B-cell populations, the mixture decreased the pre-B and IgM+ B-cell populations at all doses tested by day 2 post-treatment. In a subsequent study, De La Rosa et al. (2005) gave single intraperitoneal doses of 2,4-D, propanil, or a combination of propanil and 2,4-D to groups of mice to assess impacts on thymic atrophy and depletion. Thymic atrophy and depletion of thymocytes were observed only in mice treated with the combination of 2,4-D and propanil at a concentration of 150 mg each of 2,4-D and propanil/kg body weight.

3.1.8.   Effects on Endocrine System

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 availability, hormone receptor binding, or post-receptor processing).   The U.S. EPA has yet to adopt standardized screen tests for endocrine disruptors (Durkin and Diamond 2002).

U.S. EPA/OPP (2005a,b) required 2,4-D registrants to conduct a 2-generation reproduction study to address potential endocrine disruption immunotoxicity. The study is not completed and was not submitted to U.S. EPA. Rawlings et al. (1998) assessed the potential of 2,4-D as well as several other pesticides to cause endocrine disruption in sheep. In this study, a group of six ewes were administered 2,4-D in capsules at a dose of 10 mg/kg, 3 times per week, over a period of 43 days. No overt signs of toxicity were noted; however, the investigators observed a significant decrease in serum thyroxine (T_4) and suggest that the decrease might have been associated with competitive binding of serum proteins. As noted by Rosso et al. (1998), 2,4-D can be highly bound to serum albumin.   Rawlings et al. (1998) noted no significant effects, however, in terms of pathological changes to the ovary or on serum cortisol, insulin, estradiol, luteinizing hormone, or follicle-stimulating hormone.

Garry et al. (2001) conducted an occupational study on a group of male forestry workers involved in the application of 2,4-D. The study compares groups of male workers applying large amounts of 2,4-D with other groups of workers applying lesser amounts of 2,4-D. Exposures to 2,4-D were further quantified by concentrations of 2,4-D in the urine. The investigators report what seems to be a consistent increase in luteinizing hormone (LH) levels in the plasma that correlates to 2,4-D exposures. Garry et al. (2001, p. 497, column 3) note in their discussion that the statistical significance of these effects were marginal in backpack workers ($p = 0.053$) and boom spray applicators ($p = 0.089$) but was clearly significant when the responses in both groups were pooled ($p = 0.015$). Nevertheless, the data presented in Table 2 of the Gary et al. (2001, p. 498) study does not indicate any increase in LH in boom spray workers. Furthermore, Garry et al. (2001) note that the magnitude of the increase in LH levels does not appear to be clinically significant in the male workers.

Although female forestry workers were not included in this study, Garry et al. (2001) discuss a plausible speculation on potential effects in female workers:

> *From a different perspective and potentially of greater concern may be the effects of a minor increase in LH secretion on the menstrual cycle and ovulation. Whether small fluctuations of the level of LH can affect women's fertility is uncertain.* (Garry et al., 2001, p. 500, column 1)

3.1.9. Reproductive and Teratogenic Effects

3.1.9.1. Teratology Studies – Developmental studies are used to assess whether a compound has the potential to cause birth defects. These studies typically entail gavage administration to pregnant rats or rabbits on specific days of gestation. Teratology assays as well as studies on reproductive function (Section 3.1.9.2) are generally required for the registration of pesticides. Protocols for developmental studies are established by U.S. EPA/OPPTS (2005).

Studies conducted in response to U.S. EPA test requirements for pesticide registration comprise a relatively complete data set for the investigation of the potential for 2,4-D acid, salts, and esters to cause adverse effects in developing fetuses. The studies were conducted in rats and rabbits. In general, pregnant animals were exposed via gavage (corn oil) on days 6 through 15 of gestation (rats) or days 6 through 18 of gestation (rabbits). Pregnant females were evaluated for signs of toxicity, and fetuses were evaluated at termination of pregnancy. After extensive review, both the WHO (1996) and U.S. EPA/OPP (2005a, b) concluded that the developmental toxicity of 2,4-D salts and esters was no different from that of 2,4-D acid. Consequently,, U.S. EPA/OPP (2005a,b) concludes that 2,4-D acid is representative of the class of 2,4-D acid, salts, and esters. Appendix 1 summarizes the relevant U.S. EPA studies along with studies found in the open literature.

As detailed in Appendix 1 and discussed by U.S.EPA/OPP (2005a,b) and the WHO (1996), teratology studies on 2,4-D indicate that malformations are likely to occur only at doses that are

maternally toxic. Such doses are at or above the threshold for renal clearance (U.S. EPA/OPP, 2005a). In the rat, developmental toxicity was manifested primarily as an increased incidence of skeletal malformations. In rabbits, developmental toxicity was observed primarily as increased abortions (2,4-D acid) and increased incidences of seventh cervical ribs (DEA salt). Recent studies published in the open literature (Fofana et al. 2000, 2002) demonstrate that 2,4-D exposure during organogenesis can adversely affect kidney and urogenital formation in rats at doses that are maternally toxic (i.e., >50 mg/kg/day; see Appendix 1 for details).

The developmental immunotoxicity study conducted by Lee et al (2001) discussed in Section 3.1.7 (Effects on Immune System) suggests that *in utero* exposure to the DMA salt of 2,4-D causes adverse effects on the developing immune system in mice exposed to doses at or above the threshold for maternal toxicity.

A study investigating 2,4-D exposure in Vietnam veterans who handled Agent Orange found no association between exposure and the incidence of birth defects (Wolfe et al. 1995). There was, however, an increased incidence of nervous system defects in the offspring, which was associated with parental exposure to Agent Orange. Although the number of offspring is too small to allow for a formal statistical analysis, there appears to be an exposure-response relationship. In addition, there are weak exposure-response relationships for defects of the uro-genital system; however, none of the effects was statistically significant at p=0.05 (Wolfe et al. 1995). Since the veterans were exposed not only to 2,4-D but also to 2,4,5-T and TCDD, the relevance of these findings to the assessment of 2,4-D toxicity is questionable

On the basis of the existing animal studies, the NOAEL values for maternal toxicity for rats and rabbits exposed to 2,4-D acid during gestation are 25 mg/kg/day and 30 mg/kg/day, respectively. The corresponding maternal/developmental LOAEL values are 75 and 90 mg/kg/day for rats and rabbits, respectively. With regard to the salts and esters of 2,4-D, WHO (1996) cites an overall NOAEL of 10 mg a.e./kg/day for maternal toxicity in both rats and rabbits, and overall developmental LOAEL values of 50 mg a.e./kg/day for rats and 90 mg a.e./kg/day for rabbits. The developmental immunotoxicity study by Lee et al. (2001) suggests a lower threshold for developmental toxicity for the DMA salt in mice, with NOAEL and LOAEL values of 8.5 and 37 mg a.e./kg/day (decreased body weight gain; decreased kidney weight in females). With regard to the LOAEL for maternal toxicity in rabbits, WHO (1996) states: *"Unlike 2,4-D which produced maternal toxicity at the high dose (90 mg/kg/day), most of the amine salts and esters were maternally toxic at the middle (30 mg/kg/day) and high doses (60 - 90 mg/kg/day), as evidenced by mortality, clinical signs of neurotoxicity, abortions, and decreases in body weight gain. No gross visceral or skeletal malformations were seen in fetuses at any dose."* Thus, while 2,4-D acid may be representative of the class of 2,4-D acid, salts, and esters with regard to developmental toxicity, it is questionable that 2,4-D acid is representative of the class with regard to the threshold for maternal toxicity. This observation is discussed further in the dose-response section (Section 3.3). U.S. EPA/OPP (2005a) uses the NOAEL of 25 mg a.e./kg/day derived from the rat developmental toxicity study with 2,4-D as the basis for the acute RfD for reproductive-aged females.

3.1.9.2. Multigeneration Reproduction Studies – Reproduction studies involve exposing one or more generations of the test animal to the compound. Relatively standardized protocols for reproduction studies were established by U.S. EPA/OPPTS (2005). The general experimental method involves dosing the parental (P) generation (i.e., the male and female animals used at the start of the study) to the test substance prior to, during mating, after mating, and through weaning of the offspring (F1). In a 2-generation reproduction study, this procedure is repeated with male and female offspring from the F1 generation to produce another set of offspring (F2). 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 offspring.

There is a single 2-generation reproduction study in rats which meets U.S.EPA/OPP guidelines (U.S. EPA/OPP 2005a,b; WHO 1996). In the study, groups of Fischer rats were fed 2,4-D acid (97.5 % pure) in the diet at doses equivalent to 0, 5, 20, or 80 mg/kg body weight/day. The study is summarized both in Appendix 1 and Table 3-3. In summary, 2,4-D exposure at a dose (80 mg/kg/day) above the threshold for maternal toxicity, and hence, renal clearance, caused an increase in the length of gestation in parental generation (F_0) females.

As mentioned previously, U.S. EPA/OPP (2005a) required registrants to conduct and submit a current 2-generation reproduction study for 2,4-D acid that complies with more current guidelines and addresses issues pertaining to endocrine disruption and immune toxicity.

3.1.9.3. Target Organ Toxicity – As part of most standard acute and chronic toxicity studies, observations are often made on reproductive tissue – e.g., ovaries and testes.

Numerous studies suggest that 2,4-D exposure adversely affects reproductive organs. In chronic studies submitted in support of pesticide registration, rats exposed orally to 2,4-D at a dose of 75 mg/kg/day had lower testicular and ovarian weights than unexposed controls (U.S.EPA/OPP 2005a,b; Rowland 1996a; Jeffries et al. 1995). Dogs exposed orally to doses of 3 mg/kg/day had lower testicular weights, inactive prostates, and deficient sperm production in comparison with unexposed controls (U.S.EPA/OPP 2005a,b; Dalgard 1993a; Schulze 1990).

Lerda and Rizzi (1991) conducted sperm analyses on 32 men involved in the agricultural spraying of 2,4-D and compared those results with the results of sperm analyses on 25 men who were not exposed to 2,4-D. Exposure was characterized only by the average level of 2,4-D in the urine of the individual applicators, 9.02 mg/L. Furthermore, the study does not specify the sampling method for urine collections (i.e., intermittent or 24-hour collections). The frequency of morphological sperm abnormalities (asthenospermia, necrospermia, and teratospermia) was increased in exposed workers (72%), compared with controls (33%). In addition, there was evidence of decreased sperm mobility, increased sperm death, and decreased sperm counts in the exposed workers. The differences were statistically significant at $p < 0.01$ (Lerda and Rizzi 1991). The authors do not specify the 2,4-D formulation, crop, application method, or application rate.

Munro et al. (1992) reviewed the worker study by Lerda and Rizzi (1991) and considered it to be flawed due to the nature of the matched control group and possible problems during handling of the sperm. Another, and perhaps more substantial criticism, is that exposure to 2,4-D occurred in March-July of 1989, and the sperm samples were not taken until 6 months later (P-2 data in the study) or about 1 year later (P-3 data in the study). According to Lerda and Rizzi (1991):

> *It can be concluded that exposure to 2,4-D at the above concentrations produces a harmful effect on the germinal epithelium, causing alterations of spermatogenesis.* (p. 49)

This statement is not supported by the data presented in the study. At best, the study shows that the incidence of sperm anomalies was higher in a group of pesticide applicators than it was in a group of individuals who did not apply pesticides.

Studies on experimental animals, nonetheless, support a concern for the effects of 2,4-D on the testes. Without specifically addressing the rat and dog studies cited above, which may not have been available at the time of the review, Munro et al. (1992) suggest that in animal studies, effects on sperm *"can easily be accounted for on the basis of systemic toxicity, stress, and changes in thyroid hormone status induced secondarily to 2,4-D toxicity."* In both the dog and rat studies, other signs of toxicity (including decreased body weight gain, altered clinical chemistry variables, and effects on the thyroid and other organ systems in rats) were manifest at the doses associated with decreased sex organ weights and, in the case of dogs, decreased sperm maturity, production ,and prostate function

Other studies support a concern for the potential effects of 2,4-D on testicular function, including two studies not cited in the Munro review (Nicolau 1983; Lutz-Ostertag and Lutz 1970). Nicolau (1983) reported that 2,4-D (100 ppm in the diet of rats or 15 mg/kg/day) slightly alters the diurnal patterns of RNA, DNA, and protein synthesis in the testes with more pronounced effects observed in the thyroid and adrenals. The testicular effects of the amine salt of 2,4-D in fowl are reported by Lutz-Ostertag and Lutz (1970). The inhibition of testicular DNA synthesis was also noted in mice after single oral doses of 200 mg/kg (Seiler 1979). In addition, de Duffard et al. (1995) demonstrated that the butyl ester of 2,4-D blocks the action of testosterone in the behavioral performance of castrated rats. Adverse effects in rat embryo cultures were also observed (Sameshima et al. 2002, 2004).

3.1.10. Carcinogenicity and Mutagenicity

Three kinds of data are commonly used to assess potential carcinogenic hazard. These data include epidemiology studies, bioassays on mammals, and tests for genetic toxicity, including mutagenicity.

The carcinogenicity and mutagenicity of 2,4-D are well studied and extensively reviewed and debated. The three important long-term chronic oral toxicity/carcinogenicity studies in rats, mice, and dogs are summarized in Table 3-3. Neither the Science Advisory Board of the U.S.

EPA (U.S. EPA 1994) nor WHO (1996) concluded that 2,4-D, and its salts and esters are carcinogenic or mutagenic. In fact, previously mentioned studies which demonstrate that 2,4-D has apoptic (Section 3.1.2) and anti-neoplastic activity (Section 3.1.7; Lee et al. 2001) suggest that 2,4-D inhibits rather than promotes cell growth. A structurally similar herbicide, 2,4-dichlorophenoxybutyric acid, also yielded negative results in a carcinogenicity study in rodents (Charles and Leeming 1998).

EPA/OPP (2005a) states:

*"2,4-D is classified as a Group D chemical [not classifiable as to human carcinogenicity]. Based on the overall pattern of responses observed in both **in vitro** and **in vivo** genotoxicity tests, 2,4-D was not mutagenic, although some cytogenic effects were observed. 2,4-D acid is currently considered to be representative of all nine member chemicals of the 2,4-D case."*

"In the past, there were concerns that the diethanolamine salt of 2,4-D might be a carcinogen. The HED Hazard Science Policy Council [HASPC] recently reviewed the available toxicology data on diethanolamine (DEA) and related compounds. The HASPC concluded that it was not likely that exposure to the DEA salt of 2,4-D resulting from occupational use would pose a carcinogenic risk to humans. While liver tumors were observed in mice following dermal exposure to DEA, there was no evidence of carcinogenicity in rats following dermal exposure, and there was no evidence of a genotoxic or mutagenic concern. Although no formal assessment has been performed on the proposed mode of action [choline deficiency], this mode of action was considered plausible for the mouse hepatocellular tumors observed following dermal exposure to DEA, as were other confounding factors (e.g., use of ethanol as vehicle), and humans are generally refractive to choline deficiency. Additionally, the low use pattern indicates that there is no potential long-term dermal exposure to the diethanolamine salt of 2,4-D in agricultural uses. The HASPC also determined that, at this time, no carcinogenicity studies are required for the DEA salt of 2,4-D."

It is the general policy and position of the USDA/Forest Service to defer to the U.S. EPA on issues relating to quantitative risk assessment for potential carcinogenic effects in humans. Nonetheless, it is recognized that concern exists with the potential carcinogenic effects of 2,4-D in humans based on epidemiology studies (e.g., Ballester et al. 1993; McDuffie et al. 2001). As detailed by WHO (1996), other reports of this nature are published but the results are most often marginal:

> *Epidemiological studies have suggested an association*
> *between exposure to chlorophenoxyacetic acid herbicides,*
> *including 2,4-D, and two forms of cancer in humans:*
> *soft-tissue sarcomas and non-Hodgkin's lymphoma. The results*
> *of these studies are not consistent, however, the associations*
> *found are weak, and conflicting conclusions have been reached*
> *by the investigators. In addition, most of these studies did not*

*provide information on exposure specifically to 2,4-D, and the
risk was related to the general category of phenoxy herbicides,
which might include 2,4,5-trichlorophenoxyacetic acid
(2,4,5-T) and substances contaminated with dioxins,
specifically 2,3,7,8-TCDD. While some of the studies have
shown a relationship between exposure to 2,4-D and
non-Hodgkin's lymphoma, others (including those with positive
results) have produced inconsistent findings, raising doubts
about whether the relationship is causal.*

Notwithstanding the above assessments, studies are available in the open literature that report
positive dose-response relationships for some assays of genotoxicity (e.g., Tripathy et al. 1993).
Some of these studies are reviewed by Cox (2006); however, a number of other studies noting no
or very little increased risk also have been published (e.g., Asp 1994; Burns et al. 2001).
Nonetheless, reports of mutagenic activity and reports of increased risk for some forms of cancer
in populations exposed to 2,4-D clearly and understandably enhance concern with the use of
2,4-D in vegetation management programs. This is acknowledged. Nonetheless, as noted above,
the USDA Forest Service will defer to the evaluation of the U.S. EPA (U.S. EPA/OPP 2005a)
which is supported by the evaluation of WHO (1996), and this risk assessment will not attempt to
quantify the potential risk of carcinogenicity. As detailed further in Section 3.4, the risk
characterization for 2,4-D based on well-documented systemic toxic effects is sufficiently severe
to warrant the careful use of this product to reduce exposure apart from any potential concern for
carcinogenicity or mutagenicity.

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

Studies on effects of pesticides and pesticide formulations are relatively standardized and include
assays for acute eye irritation (OPPTS 870.2400), acute dermal irritation (OPPTS 870.2500), and
skin sensitization (OPPTS 870.2600). The acute irritation studies typically involve rabbits. The
test material is applied either to one eye of the animal or to an area of the skin (intact or abraded).
In the eye irritation studies, the untreated eye of the animal typically serves as the control. In the
dermal studies, an untreated area of the skin typically serves as a control. Both eye and skin
irritation studies are used to classify pesticides (corrosive to non-irritant), and these
classifications reflect how the pesticide or pesticide formulations must be labeled.

3.1.11.1. Skin Irritation – Studies which assess the dermal irritation potential of 2,4-D and the
salts and esters of 2,4-D are summarized in Table 3-2. On the basis of these studies,
U.S.EPA/OPP (2005a,b) classifies 2,4-D salts and esters as very mild to slight (Class III and IV)
dermal irritants. The dermal irritation test for 2,4-D acid was unacceptable.

3.1.11.2. Skin Sensitization – Studies that assess the potential for 2,4-D and its salts and esters to
cause allergic skin reactions are summarized in Table 3-2. Based on the negative results seen in
these studies, U.S. EPA/OPP (2004a) does not consider 2,4-D and its salts and esters to be
dermal sensitizers.

3.1.11.3. Ocular Effects – Studies that assess the potential for 2,4-D and its salts and esters to irritate the eyes are summarized in Table 3-2. Based on these studies, U.S. EPA/OPP (2005a) classifies2,4-D acid and salts as severe eye irritants, and 2,4-D esters as not irritating to the eyes.

3.1.12.　　　Systemic Toxic Effects from Dermal Exposure

Studies that address the acute systemic dermal toxicity of 2,4-D acid and 2,4-D salts and esters are summarized in Table 3-2. The LD_{50} for each of these studies was greater than the test limits of the study, indicating that 2,4-D acid, salts, and esters are practically non-toxic following dermal exposure (U.S. EPA/OPP 2005a,b)..

U.S. EPA/OPP (2005a,b) reports that subchronic (21-day) dermal exposure studies in rabbits were conducted with 2,4-D acid, salts, and esters. Groups of rabbits were exposed to doses of 10, 100, and 1000 mg a.e./kg/day. No systemic toxicity was observed at the highest dose tested (1000 mg/kg/day) in studies with 2,4-D acid, BEE, EHE, IPA, and TIPA. Liver toxicity and one death were observed following "high-dose" exposure (not specified by EPA, but presumably 1000 mg/kg) to the DEA salt, and one death was observed following "high-dose" exposure to the DMA salt.

3.1.13.　　　Inhalation Exposure

Acute inhalation studies for 2,4-D acid, salts, and esters are summarized in Table 3-2. Based on these studies, U.S. EPA/OPP (2005a,b), considers 2,4-D and its salts and esters to be of low toxicity with regard to acute inhalation exposure. ACGIH (2006) adopted a TLV of 10 mg/m^3. The documentation for this TLV involves a general review of toxicity and pharmacokinetics data on 2,4-D. The quantitative assessment, however, appears to be largely judgmental.

There are currently no repeat-exposure inhalation studies for 2,4-D acid, salts, or esters. U.S. EPA/OPP (2005a) indicates that the systemic toxicity of 2,4-D is likely to be similar following oral and inhalation exposures, due to the rapid absorption and limited metabolism of 2,4-D observed following oral exposure. Nevertheless, portal-of-entry effects can only be assessed reliably from an inhalation study; therefore, U.S. EPA/OPP (2005a) is requiring registrants to conduct and submit a 28-day inhalation toxicity study for 2,4-D.

A recent study conducted by Batelle Memorial Institute in conjunction with U.S. EPA National Exposure Research Laboratory, Research Triangle Park, North Carolina, suggests that exposure to 2,4-D through contact with dust may be a significant source of residential exposure to 2,4-D following lawn treatment. In order to estimate exposure for young children, Nishioka et al. (2001) studied the distribution of 2,4-D in air and on surfaces inside homes following lawn application of 2,4-D. Based on samples of indoor air and surface wipes taken from floors, table tops and window sills, Nishioka et al. (2001) concluded that these sources of 2,4-D could result in an exposure dose in young children of 1-10 ug/day from floors, and 0.2-30 ug/day from table tops. These exposure estimates are approximately 10 times higher than the estimated daily exposure to 2,4-D from dietary sources (approximately 1.3 ug/day).

3.1.14.   Inerts and Adjuvants

As summarized in Table 2-4, several inert ingredients are listed for certain formulations of 2,4-D acid, esters, and salts. The known toxicity information for these inerts, as well as U.S. EPA's pesticide inert list classification are summarized in Table 3-4.

3.1.15.   Impurities and Metabolites

Metabolites - As discussed in Section 3.1.3, 2,4-D is rapidly absorbed and eliminated, primarily as unchanged compound in the urine. Hence, there is little concern about the toxicity of the few conjugated metabolites identified in the urine of humans, dogs ,and mice, but not rats (excrete unchanged 2,4-D).

Although 2,4-D is not metabolized extensively in mammals, it degrades in the environment to form the metabolite, 2,4-dichlorophenol. Although 2,4-dichlorophenol was not detected in vegetation or water samples after the application of 2,4-D, it was detected in aqueous sediments at approximately the same concentrations as 2,4-D (Hoeppel and Westerdahl 1983).

2,4-Dichlorophenol is a toxic metabolite. The RfD for 2,4-dichlorophenol is 0.003 mg/kg/day, based on impaired immunological function (U.S. EPA 1997). The RfD for 2,4-dichlorophenol is only slightly lower than the current U.S. EPA/OPP (2005a,b) RfD for 2,4-D (0.005 mg/kg/day). Because there is no indication that workers or the general public will be exposed to substantial amounts of 2,4-dichlorophenol, the formation of this compound in sediment as part of the environmental degradation process does not contribute substantially to the risks associated with the use of 2,4-D.

Impurities - There is little published information on the impurities in commercial formulations of 2,4-D. Hansen et al. (1971) reported that a commercial sample of 2,4-D contained low concentrations of monochlorophenoxyacetic acid (0.1%), 2,6-dichlorophenoxy-acetic acid (2.3%), 2,4,6-trichlorophenoxyacetic acid (0.2%), and bis(2,4-dichlorophenoxy)-acetic acid (0.7%). Because the toxicity studies on 2,4-D used in this risk assessment were conducted with technical grade 2,4-D, it is likely that the toxicity of the minor impurities is encompassed by the studies used as the basis for this risk assessment.

One potential concern associated with the use of 2,4-D is contamination with polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). EFED has reviewed available data on PCDD and PCDF concentrations in 2,4-D (Reg. No. 61272-3) and 2,4-D EHE (ethylhexyl ester) (Reg No. 61272-1) (U.S. EPA/OPP 2005c). The data are contained in a confidential memo cited by U.S. EPA/OPP (2005c) as: Dr. Stephan Funk, Memorandum, 11/26/91. Based on the memo, U.S. EPA/OPP (2005b) reports that 2,4-D and 2,4-D EHE contained two PCDDs at concentrations above the limit of quantification (LOQ), but does not state which two PCDDs were detected. SERA obtained a version of this memo (cleared by U.S. EPA), dated March 2, 1993 (Funk 1993). In the memo, Dr. Funk states:

"Only 2,3,7,8-tetrachlorodibenzo-p-dioxin and 1,2,3,7,8-pentachlorodibenzo-p-dioxin were found at or above the EPA loq's [limits of quantification]. Two of eight technical 2,4-D's contained 2,3,7,8-TCDD slightly above the 0.1 ppb loq. Three of eight technical 2,4-D's contained 1,2,3,7-8-PCDD at concentrations greater than the 0.5 ppb loq. None of the remaining thirteen chlorinated dibenzo-p-dioxins and dibenzofurans were found at or above the EPA loq's in the technical 2,4-D. Data on the 2,4-DB and 2,4-DP acids and derivatives of all three acids are too limited at this time to be useful to the 2,4-D panel."

EFED used this information to conduct a detailed risk assessment for PCDD/PCDF contamination of 2,4-D and concluded that risks associated with such contamination were likely inconsequential.

Some early samples of commercial formulations of 2,4-D were shown to contain N-nitrosodimethylamine at levels less than1-5 ppm (Hindle et al. 1987). As reviewed by Munro et al. (1992), the formation of nitrosamines in 2,4-D formulations was associated with the use of nitrates in preserving metal containers used for shipping 2,4-D formulations. Currently, metal containers are not used to ship 2,4-D. The U.S. EPA no longer requires that 2,4-D samples be assayed for nitrosamines unless nitrates, nitrites, or other nitrosating agents are used in the formulation.

N-nitrosodimethylamine also was found in mixtures of 2,4-D amine and fertilizers at levels less than 0.05-0.25 ppm and attributed to the contamination of the 2,4-D amine salt with N-nitrosodimethylamine (Wigfield and McLenaghan 1990). Nitrosamines were not found in water or sediment samples taken after the application of commercial formulations of 2,4-D dimethylamine (Hoeppel and Westerdahl 1983).

3.1.16.   Toxicologic Interactions

As discussed in Section 3.1.2 (Mechanism of Action) 2,4-D disrupts the cell at a fundamental level. Consequently, interactions between 2,4-D and the many other chemicals that interact with cell membranes and cell metabolism are likely to occur. As discussed in Section 3.1.7 (Immunotoxicity), there is evidence that 2,4-D used in combination with other herbicides such as propanil has a synergistic effect on immunotoxicity in mice. As discussed in section 3.1.2, 2,4-D can induce programmed cell death (apoptosis). This suggests a potential for additive, synergistic, or inhibitory effects on other apoptic agents, depending upon the nature of the agent and its mechanism for induction of the apoptic cascade of events. Sunscreens were shown to enhance the dermal absorption of 2,4-D (Brand et al. 2002, 2003; Pont et al. 2003a,b, 2004).

Cavieres et al. (2002, 2003) noted an apparently U-shaped dose-response relationship (i.e., hormesis) in the effect of a commercial mixtures of 2,4-D, dicamba, and mecoprop on reproductive parameters in mice. The published data are not sufficient to determine whether the observed effects are due to synergism.

3.2. EXPOSURE ASSESSMENT
3.2.1. Overview
All exposure assessments 2,4-D acid and salts are summarized in Worksheet E01 for workers and Worksheet E02 for the general public (Attachment 1: SERA EXWS 06-43-29-01c). For both workers and members of the general public, exposure assessments are given only in the worksheets on 2,4-D acid and 2,4-D salts. This approach is taken because exposures to 2,4-D in the use of both salt and ester formulations are essentially identical in terms of acid equivalents of 2,4-D and because the salts and esters of 2,4-D are toxicologically equivalent in terms of potential effects in humans as well as other animals.

For workers applying 2,4-D, three types of application methods are modeled: directed ground spray, broadcast ground spray, and aerial spray. Based on well-documented data on worker exposures to 2,4-D, worker exposure rates typically used in Forest Service risk assessments are applicable to both salt and ester formulations of 2,4-D. Central estimates of exposure for workers are approximately 0.01 mg/kg/day for aerial and backpack workers and about 0.02 mg/kg/day for broadcast ground spray workers. Upper ranges of exposures are approximately 0.08 mg/kg/day for broadcast ground spray workers and 0.15 mg/kg/day for backpack and aerial workers. All of the accidental exposure scenarios for workers involve dermal exposures. Except for the scenario involving contact with contaminated gloves for 1 hour, the accidental exposures lead to dose estimates that are lower than the general exposure levels estimated for workers.

For the general public, acute levels of exposures range from about 0.0001 to about 2 mg/kg bw at an application rate of 1 lb a.e./acre. The upper bound of exposure, 2 mg/kg bw, is associated with a child's consumption of contaminated water from an accidental spill. This exposure scenario is highly arbitrary. The upper bound of exposure associated with the consumption of contaminated vegetation is 1.4 mg/kg bw. The other acute exposure scenarios lead to much lower dose estimates – i.e., ranging from 0.00009 to about 0.2 mg/kg bw.

Modeled chronic or longer-term exposures are much lower, compared with acute exposures. 2,4-D exposures associated with the consumption of contaminated water or fish range from about 0.0000004 to about 0.0001 mg/kg/day. The upper bound of this range is associated with the longer-term consumption of contaminated water. The longer-term consumption of contaminated vegetation leads to much higher estimated doses, ranging from 0.001 to about 0.2 mg/kg bw/day.

3.2.2. Workers
The Forest Service uses a standard set of exposure assessments in all risk assessment documents. While these exposure assessments vary depending on the characteristics as well as the relevant data on the specific chemical, the organization and assumptions used in the exposure assessments are standard and consistent. All of the exposure assessments for workers as well as members of the general public are detailed in the worksheets on 2,4-D acid and 2,4-D salts that accompany this risk assessment (Attachment 1: SERA EXWS 06-43-29-01c). Detailed documentation for these worksheets is presented in SERA (2005). This section on workers and the following

section on the general public provide a plain verbal description of the worksheets and discuss the 2,4-D specific data used in the worksheets.

It should be noted that worker exposure assessments are given only in the worksheets on 2,4-D acid and 2,4-D salts. The worksheets for 2,4-D esters (Attachment 2: SERA EXWS 06-43-29-02c) include only exposure assessments involving terrestrial vegetation and aquatic organisms. This approach is taken for several reasons. First, the pharmacokinetics of 2,4-D acid, and its salts and esters appear to be similar (Section 3.1.3). Second, absorbed dose rates for 2,4-D esters are consistent with absorbed dose rates for salts of other herbicides that are used to estimate occupational exposure rates. This finding is discussed further in Section 3.2.2.1 with particular emphasis on the recent occupational exposure study by Krieger et al. (2005). Last, as discussed in the dose-response assessment (Section 3.3), there is no basis for asserting that the toxicity of 2,4-D salts differ from the toxicity of 2,4-D esters with respect to human exposure.

Worksheet E01 summarizes the exposure assessments for workers. Two types of exposure assessments are considered: general and accidental/incidental. The term *general* exposure assessment is used to designate exposures involving absorbed dose estimates based one 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. The exposure assessments developed in this section as well as other similar assessments for the general public (Section 3.2.3) are based on the typical application rate of 1 lb a.e./acre (Section 2). The consequences of using different application rates in the range considered by the Forest Service are discussed further in the risk characterization (Section 3.4), and these risks are detailed in Worksheets E02a (central application rate), E02b (lower bound of application rate), and E02c (upper bound of application rate).

As discussed in Section 2, some formulations of 2,4-D are registered for direct application to water, and this method of application is considered in this risk assessment. There are no estimates for the amount of 2,4-D that might be applied in 1 day; therefore, the exposure assessment is based on the assumption that 1 acre is treated at application rates ranging from 19 to 38 lbs a.e./acre. Variations from these assumptions also are considered in the risk characterization (Section 3.4.).

3.2.2.1. General Exposures
3.2.2.1.1. Terrestrial Applications – As described in SERA (2006), worker exposure rates are expressed in units of mg of absorbed dose per kilogram of body weight per pound of chemical handled. These estimates are derived from biomonitoring studies – i.e., studies in which the estimates of absorbed dose are based on measurements of the amount of pesticides excreted by workers. Based on analyses of several different pesticides using a variety of application methods, default exposure rates are estimated for three different types of applications of liquid formulations: directed foliar (backpack), boom spray (hydraulic ground spray), and aerial. The general exposure rates used for each group of workers are:

directed foliar	0.003	(0.0003 - 0.01)	mg/kg per lb a.i. handled/day
boom spray	0.0002	(0.00001 - 0.0009)	mg/kg per lb a.i. handled/day
aerial	0.00003	(0.000001 - 0.0001)	mg/kg per lb a.i. handled/day.

These worker exposure rates are based on numerous studies on 2,4-D as well as several other herbicides. The worksheets associated with the exposure assessments for each group are: Worksheets C01a (directed foliar), C01b (boom spray), and C01c (aerial).

As noted in SERA (2006), the estimated exposure rates for backpack applications exclude the backpack applicators from the study by Lavy et al. (1987). In that study, the exposure rates of approximately 0.03 (0.01-0.1) mg/kg/lb a.e. handled resulted from backpack application scenarios in which the workers often walked through vegetation recently sprayed with 2,4-D and received heavy dermal exposure from contact with treated vegetation. The exposure conditions described in this study, which involved near saturation of the workers' clothing with 2,4-D, are considered atypical of Forest Service sponsored applications. Consequently, the exposure rates from the study by Lavy et al. (1987) are not used in the derivation of exposure rates for backpack applications.

Krieger et al. (2005) conducted a study on backpack applications of 2,4-D under conditions typical of those employed in Forest Service programs. The study is useful for further assessing the worker exposure rates discussed above.

Krieger et al. (2005) monitored the exposure of individuals using backpack sprayers to apply a commercial formulation of triclopyr and 2,4-D (Garlon*4™ and 2,4-D LV6™) for purposes of conifer release and regeneration in Klamath National Forest in Northern California. Dermal exposure was assumed to be the primary route of exposure due to the low volatility of the pesticides under study (i.e., low potential for inhalation) and the practice of directing the spray downward. As such, a combination of monitoring techniques was used to assess dermal exposure for the individual mixing the herbicide and filling tanks, for the field supervisor, and for the eight-member spray crew.

The crew worked 7-8.5 hours per day for a 6-day period. Sprayers refilled their tanks approximately every 30 minutes, and treated from 1.1 to 10 acres each day. At the end of 6 days, workers had treated 55 acres of forest with 24 gallons each of the above triclopyr and 2,4-D formulations. Based on the total gallons used and the total number of acres treated, the average 2,4-D application rate during the study was 2.4 lb a.e./acre.

2,4-D and triclopyr exposure and absorption was assessed in three ways: by monitoring outer clothing, face, and neck (potential dermal exposure), by monitoring deposition on the skin via internal clothing (whole-body dosimetry), and by monitoring urine output for active ingredients and metabolites. The results of the study are used to evaluate the general relationship between exposure estimates using passive dosimetry and urine biomonitoring, and to provide estimates of exposure under typical conditions encountered in a forestry application of 2,4-D and triclopyr.

Based on the average total amount of 2,4-D and triclopyr applied by each backpack worker – i.e., 16.5 lbs 2,4-D per worker and 12 lbs triclopyr per worker – and the average estimates of absorbed dose based on urinary excretion – i.e., 0.23 mg/kg/day for 2,4-D and 0.043 mg/kg/day for triclopyr – the estimated occupational exposure rates were 0.0014 mg/kg/day per lb 2,4-D applied and 0.0036 mg/kg/day per lb triclopyr. This range of worker rates is very close to the central estimate of 0.003 (0.0003 - 0.01) mg/kg per lb a.i. handled/day typically used in Forest Service risk assessments for backpack applications. Thus, for the current risk assessment, the backpack worker exposure 0.003 (0.0003 - 0.01) mg/kg per lb a.i. handled/day are maintained.

Coble et al. (2005) developed a pesticide exposure algorithm using data on concentrations of 2,4-D and a related herbicide, MCPA (4-chloro-2-methylphenoxyacetic acid) in the urine of pesticide applicators. This general algorithm, however, did not correlate well with 2,4-D. Harris et al. (2001) developed a kinetic-based algorithm that appears to correlate well with the excretion of 2,4-D in the urine of pesticide applicators based on the amount of 2,4-D handled by the workers. Nonetheless, given the more directly relevant studies summarized above, the approaches discussed by Coble et al. (2005) and Harris et al. (2001) are not further considered in this risk assessment.

3.2.2.1.2. Aquatic Applications – As discussed in Section 2, Aqua-Kleen is a granular formulation of 2,4-D that can be applied at relatively high rates (i.e., 19-38 lbs a.e./acre) directly to bodies of water. This is not a common practice in Forest Service; consequently, the number of acres that might be treated per hour or per day cannot be generalized. Furthermore, the literature does not include worker exposure studies associated with the application of granular 2,4-D formulations directly to bodies of water.

Harris et al. (1992) is the only available study regarding worker exposure to liquid and granular formulations of 2,4-D to turf. In this study, average levels of 2,4-D in the urine of applicators applying a liquid formulation were about 200 μg/person with an average amount handled of 300 g (Table IV in Harris et al. 1992), including workers with undetectable levels of 2,4-D in the urine. In workers applying an average of 550 g of a granular formulation, the average urine level was about 20 μg/person. Harris et al. (1992) report that the detectable levels of 2,4-D in the urine of workers applying liquid formulations were all associated with accidental spills. Only one of nine workers applying the granular formulation had detectable levels of 2,4-D in the urine (169 μg/person per 1200 g or 141 μg/person·kg a.i. handled). Ignoring non-detectable or trace quantities in workers handling the liquid formulations (Table IV in Harris et al. 1992), the average exposure rate was about 250 μg/kg a.i. handled for workers using a liquid formulation. Thus, while the use of a granular formulation of 2,4-D leads to lower average exposures when all workers were considered, the exposure levels were comparable between the formulations for individuals in which 2,4-D could be detected.

The similarities between exposure rates from the applications of granular and liquid formulations of 2,4-D increase confidence in the relevance of a study regarding the application of a liquid formulation of 2,4-D to water (Nigg and Stamper 1983). In this study regarding the use of 2,4-D

amine to control water hyacinths, absorbed doses were assayed as total urinary elimination of the compound over a 24-hour period in four workers who applied the liquid formulation by airboat handguns. Occupational exposure rates for these workers can be estimated at 0.0009 (0.0004 - 0.002) mg/kg bw per lb a.e. handled (SERA 1998a). These rates are between those given above for backpack workers and workers involved in hydraulic ground broadcast applications. Given the similarities between the occupational exposure rates for workers involved in the liquid and granular formulations of 2,4-D to turf (Harris et al. 1992), the data from Nigg and Stamper (1983) on workers involved in the aquatic application of a liquid 2,4-D formulation are used to estimate exposure rates for workers involved in the aquatic application of granular 2,4-D (i.e., Aqua-Kleen).

Because of the atypical nature of the exposure scenario involving aquatic applications and because aquatic applications typically would not be conducted together with standard terrestrial applications, both the exposure assessment for this scenario as well as risk characterization are presented in Worksheet C01d. The potential risks to workers involved in this activity are discussed further in Section 3.4.

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 herbicides into the eyes or to involve various dermal exposure scenarios.

As summarized in Section 3.1.11.1, 2,4-D 2,4-D salts and esters are not strong skin irritants and have been classified by U.S.EPA/OPP (2005a,b) as a very mild (Class III) to slight (Class IV) dermal irritants. The available literature does not include quantitative methods for characterizing exposure or responses associated with splashing a solution of a chemical into the eyes or the effects of dust from 2,4-D granules getting into the eyes. Consequently, accidental exposure scenarios of this type are considered only qualitatively in the risk characterization (Section 3.4).

There are various methods for estimating absorbed doses associated with accidental dermal exposure (SERA 2006). Two general types of exposure are modeled: those involving direct contact with a solution of the herbicide and those associated with accidental spills of the herbicide onto the surface of the skin. Any number of specific 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 surface of the skin and by varying the surface area of the skin that is contaminated. 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 in which the specific calculations are detailed.

Exposure scenarios involving direct contact with solutions of the chemical are characterized by immersion of the hands for 1 minute or wearing contaminated gloves for 1 hour. Generally, it is not reasonable to assume or postulate that the hands or any other part of a worker will be immersed in a solution of a herbicide for any period of time. Properly designed gloves can provide effective protection from 2,4-D over the course of a work day (Lin and Hee 1999; Moody and Nadeau 1994). On the other hand, accidental contamination of gloves or other clothing is quite plausible. For these exposure scenarios, the key element is the assumption that wearing gloves grossly contaminated with a chemical solution is equivalent to immersing the hands in a solution. In either case, the concentration of the chemical in solution that is in contact with the surface of the skin and the resulting dermal absorption rate are essentially constant. For these scenarios, absorbed dose is estimated using Fick's first law (zero-order absorption) based on an estimate of the permeability coefficient, K_p, expressed in cm/hour.

Exposure scenarios involving chemical spills onto the skin are characterized by a spill onto the lower legs as well as a spill onto the hands. In these scenarios, it is assumed that a solution of the chemical is spilled onto a given surface area of skin and that a certain amount of the chemical adheres to the skin. The absorbed dose is then calculated as the product of the amount of the 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 concentration of the chemical in the liquid), the first-order absorption rate (k_a), and the duration of exposure.

The estimates of the permeability coefficient (K_p) and first-order absorption rate (k_a) are given in Section 3.1.3.2.

3.2.3. General Public
3.2.3.1. General Considerations – Under normal conditions, members of the general public should not be exposed to substantial levels of 2,4-D as a result its use in Forest Service programs. Nonetheless, any number of exposure scenarios can be constructed for the general public, depending on various assumptions regarding application rates, dispersion, canopy interception, and human activity. Several standard and highly conservative scenarios are developed for this risk assessment.

The exposure scenarios developed for this risk assessment include both acute and longer-term or chronic exposure durations. All of the acute exposure scenarios are primarily accidental. They assume that an individual is exposed to the compound either during or shortly after its application. Specific scenarios are developed for direct spray, dermal contact with contaminated vegetation, as well as the consumption of contaminated fruit, water, and fish. Most of these scenarios should be regarded as extreme, some approaching the limit of plausibility. The longer-term or chronic exposure scenarios parallel the acute exposure scenarios for the consumption of contaminated fruit, water, and fish but are based on estimated levels of exposure for longer periods after application. As with the exposure assessments for workers, exposure assessments for members of the general public are limited to 2,4-D acid. The rationale for this approach is given in Section 3.2.2.

All of the exposure scenarios developed for the general public are summarized in Worksheet E02 of the workbook for 2,4-D acid (Attachment 1). As with the worker exposure scenarios, details of the assumptions and calculations involved in these exposure assessments are given in the worksheets that accompany this risk assessment (Worksheets D01a–D10b). The remainder of this section focuses on a qualitative description of the rationale for and quality of the data supporting each of the assessments.

3.2.3.2. Direct Spray – Direct sprays involving ground applications are modeled in a manner similar to accidental spills for workers (Section 3.2.2.2). In other words, it is assumed that the individual is sprayed with a solution containing the compound and that an amount of the compound remains on the skin and is absorbed by first-order kinetics. These exposure scenarios, assume that during a ground application, a naked child is sprayed directly with a liquid formulation of 2,4-D. This scenario also assumes that the child is completely covered (that is, 100% of the surface area of the body is exposed) (Worksheet D01a). These are extremely conservative exposure scenarios and are likely to represent upper limits of plausible exposure. An additional set of scenarios is included involving a young woman who is accidentally sprayed over the feet and legs (Worksheet D01b). For each of these scenarios, specific assumptions are made regarding the surface area of the skin and body weight as detailed in Worksheets D01a and D01b along with the sources used for making the assumptions.

3.2.3.3. Dermal Exposure from Contaminated Vegetation – In this exposure scenario, it is assumed that the herbicide is applied at a given rate and that an individual comes in contact with sprayed vegetation or other contaminated surfaces at some period after the spray operation. For these exposure scenarios, some estimates of dislodgeable residue and the rate of transfer from the contaminated vegetation to the surface of the skin must be available. Dermal exposure is estimated using the published method by Durkin et al. (1995) for assessing the absorbed dose of 2,4-D from dermal contact with treated turf. Details regarding the implementation of this method are provided in Worksheet D02 (Attachment 1). The exposure scenario assumes a contact period of 1 hour and further assumes that the chemical is not effectively removed by washing until 24 hours after exposure. 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.

3.2.3.4. Contaminated Water – The ways in which water can become contaminated with herbicides include, runoff, leaching from contaminated soil, a direct spill, or unintentional contamination from drift during an application. The three exposure scenarios considered in this risk assessment for acute exposure to 2,4-D from consumption of contaminated water include an accidental spill into a small pond, accidental direct spray of or incidental drift into a pond or stream, and the contamination of a small stream or pond by runoff, sediment loss, or percolation. In addition, longer-term estimates of concentrations in water are based on a combination of modeling and monitoring data. Each of these scenarios is considered in the following subsections.

3.2.3.4.1. Accidental Spill – The accidental spill scenario assumes that a young child consumes contaminated water shortly after an accidental spill into a small pond. The specifics of this scenarios are given in Worksheet D05 of the workbooks for liquid and granular formulations. Because this scenario is based on the assumption that exposure occurs shortly after the spill, no dissipation or degradation of the pesticide is considered. This scenario is dominated by arbitrary variability, and the specific assumptions used generally overestimate exposure. The actual concentrations in the water would depend heavily on 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 that is consumed.

For liquid formulations, Forest Service risk assessments use a standard scenario – the spill of 200 gallons of a *field solution* – i.e., the pesticide diluted with water to the concentration that is anticipated in Forest Service programs (Section 2). Based on the spill scenario for a liquid formulation at an application rate of 1 lbs/acre, the concentration of 2,4-D in a small pond is estimated to range from about 0.9 to 18.0 mg/L with a central estimate of about 4.5 mg/L (Worksheet D05). These concentrations are linearly related to application rate. The consequences of using the full range of applications considered in this risk assessment are discussed in Section 3.4 (Risk Characterization), and the hazard quotients are given in Worksheets E04a, E04b, and E04c.

As discussed in Section 2, some formulations of 2,4-D esters may be applied directly to water. As discussed below (Section 3.2.3.4.6), the concentrations in water from direct application range from about 1 to 2 mg/L. Because these concentrations are lower than both the central estimate and upper bound of concentrations associated with an accidental spill, a separate exposure assessment and risk characterization for the consumption of contaminated water after the direct application of 2,4-D to water are not developed.

3.2.3.4.2. Accidental Direct Spray/drift for a Pond or Stream – These scenarios are less severe but more plausible than the accidental spill scenario described above. The U.S. EPA typically uses a 2-m deep pond to develop exposure assessments (SERA 2004). If such a pond is directly sprayed with 2,4-D at the nominal application rate of 1 lbs/acre, the peak concentration in the pond would be about 0.05 mg/L, equivalent to 50 µg/L or 50 ppb (Worksheet D10a). This concentration is a factor of about 360 below the upper bound of the peak concentration of 18 mg/L after the accidental spill of a liquid formulation (Worksheets D05). Worksheet D10a also models concentrations at distances of 25-900 feet down wind based on standard values adapted from AgDrift (SERA 2006). Based on these estimates, 2,4-D concentrations in a small pond contaminated by drift would range from about 0.00005 mg/L (50 ppt) to 0.056 mg/L (56 ppb).

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. Much lower concentrations, ranging from about 0.00008 mg/L (80 ppt) to 0.01 mg/L (10 ppb), are estimated based on drift at distances of 25-900 feet (Worksheet 10b).

3.2.3.4.3. GLEAMS Modeling – For compounds like 2,4-D, which may be applied over a large proportion of a watershed, drift and even direct spray are not the only and may not be the greatest source of contamination of surface water. Water contamination may also occur from soil runoff, sediment, or percolation. Depending on local conditions, these losses can lead to substantial contamination of ponds or streams. Estimated concentrations of 2,4-D in surface waters can be based both on modeling and monitoring data. This section describes the relatively standardized modeling approach used in Forest Service risk assessments and is followed by subsections on both other modeling efforts and the available monitoring data.

Modeling of 2,4-D concentrations in stream water conducted for this risk assessment are based on GLEAMS (Groundwater Loading Effects of Agricultural Management Systems) modeling. GLEAMS is a root zone model that can be used to examine the fate of chemicals in various types of soils under different meteorological and hydrogeological conditions (Knisel and Davis 2000). As with many environmental fate and transport models, the input and output files for GLEAMS can be complex. The general application of the GLEAMS model and the use of the output from this model to estimate concentrations in ambient water are detailed in SERA (2004). When used to model the runoff of 2,4-D from turf, GLEAMS performed somewhat better than PRZM (Ma et al. 1999). As discussed further below, PRZM is another root zone model which is generally used by U.S. EPA.

For the current risk assessment, the application site was assumed to consist of a 10-hectare square area that drained directly into a small pond or stream. The chemical specific values as well as the details of the pond and stream scenarios used in the GLEAMS modeling are summarized in Table 3-5.

For this human health risk assessment, GLEAMS modeling is conducted only for 2,4-D acid/salts, which is consistent with the approach taken by U.S. EPA/OPP (2005a). As discussed in Section 3.2.2 of this risk assessment and as explained in U.S. EPA/OPP (2005a), all forms of 2,4-D are considered to be toxicologically equivalent to 2,4-D acid. Moreover, concentrations of 2,4-D acid in ambient water will be somewhat higher for 2,4-D salts than for any of its esters. This conclusion is discussed further in Section 4.2, the exposure assessment for ecological effects. A distinction between 2,4-D acid or salts and 2,4-D esters is maintained in the ecological risk assessment because of well-documented and sometimes substantial differences in the toxicity of 2,4-D acid/salts and 2,4-D esters to some aquatic organisms.

Estimates of runoff, sediment, and percolation concentrations in a stream adjacent to a treated plot were determined by running the GLEAMS model, as discussed in Section 6.4 of SERA (2004). The results of the GLEAMS modeling for the small stream are summarized in Table 3-6 and the corresponding values for the small pond are summarized in Table 3-7. These estimates

are expressed both as average and peak concentrations in water. All of these GLEAMS runs were conducted at an application rate of 1 lb a.e./acre, the typical application rate anticipated in Forest Service programs (Section 2). Consequently, the concentrations given in these tables are equivalent to water contamination rates (WCR) – i.e., the concentration of the compound in water in units of ppb (µg/L) normalized for an application rate of 1 lb a.e./acre.

Surface water contamination is not estimated for very arid regions – i.e., annual rainfall of 10 inches or less. It should be noted, however, that this result may be an artifact of the way the GLEAMS modeling is conducted. As summarized in Table 3-6 and discussed in SERA (2004), the generic GLEAMS modeling used in this risk assessment is based on a rainfall pattern in which rainfall occurs every 10th day and the amount of rainfall is uniform each day. Thus, for an annual rainfall of 10 inches per year, the amount of rainfall in each event is about 0.25 inches – i.e., 10 inches per year divided by 37 rainfall events per year. In applying these results to a specific region, the actual rainfall pattern in the region should be considered. For extreme rainfall events, actual concentrations that could occur may be better estimated by using the current GLEAMS modeling based on higher annual rainfall rates.

At higher rainfall rates and the application rate of 1 lb a.e./acre, the modeled peak concentrations in streams range from about 0.06 ppb (sand at an annual rainfall rate of 50 inches) or less to about 440 ppb (clay soil at an annual rainfall rates of 100 inches per year) (Table 3-6).

Modeled peak concentrations in a small pond (Table 3-7) are only somewhat lower than those modeled in the stream. As with the stream modeling, no surface water contamination is expected in very arid regions. For regions with annual rainfall rates of 15 inches or more, the modeled peak concentrations in ponds at an application rate of 1 lb a.e./acre range from less than 0.02 ppb (sand at an annual rainfall rate of 50 inches) to about 135 ppb (clay at annual rainfalls rates of 150 inches per year or more).

The GLEAMS scenarios do not specifically consider the effects of accidental direct spray. As discussed in Section 3.2.3.4.2, direct spray of a standard pond could result in peak concentrations of about 50 ppb, about a factor of about 3 less than the 135 ppb peak concentration modeled in ponds as a result of contamination associated with relatively higher rainfall rates – i.e., 100 inches per year or more. Thus, while accidental direct sprays may be worst-case scenarios in areas that are relatively arid, accidental direct sprays may not be worst-case in areas with extreme rainfall.

3.2.3.4.4. Other Modeling Efforts – A summary of the GLEAMS modeling discussed above as well as modeling of 2,4-D conducted by the U.S. EPA/OPP (2004b, 2005a) is given in Table 3-8. U.S. EPA/OPP (2004b) used two water contamination models: PRZM/EXAMS and SCI-GROW. As discussed in SERA (2004), PRZM/EXAMS is a model, or more accurately a system of linked models, that the U.S. EPA uses to assess plausible concentrations of pesticides in water after agricultural applications. SCI-GROW is a Tier 1 screening model developed by the U.S. EPA to provide very conservative upper bound estimates of concentrations of a

compound in groundwater based on a given application rate, number of applications, the interval between applications, and standard environmental fate parameters for a specific compound (i.e., a subset of those summarized in Table 3-5).

The U.S. EPA/OPP (2004b) modeled concentrations of 2,4-D in water at several different application rates (U.S. EPA/OPP 2004b, Table 5, p. 47 and Table 6, p. 205). In Table 3-8, the reported concentrations are normalized to 1 application at 1 lb a.e./acre by dividing by the product of the application rate and number of applications used by U.S. EPA/OPP (2004b). The estimate of the peak concentration from PRZM/EXAMS is 30 ppb at an application rate of 1 lb a.e./acre. This concentration is associated with the application of 2,4-D in apple orchards in North Carolina. The GLEAMS estimates of peak concentrations are substantially higher, 140 ppb for a pond and 440 ppb for streams. This pattern is typical and is the result of extremely conservative assumptions built into the GLEAMS modeling (SERA 2004). The central estimates of concentrations in water as well as the estimates of longer-term averages from the GLEAMS modeling are comparable (i.e., within factors of about 2 to 5) of the estimates reported by the U.S. EPA/OPP (2004b). This again is a very typical pattern. As noted in SERA (2004), PRZM and GLEAMS, both of which are root zone models, tend to yield comparable results when similar input values are used. For the GLEAMS modeling, the input parameters associated with central estimates of exposure (e.g., more typical rainfall rates) are more closely related to the implementation of PRZM/EXAMS used in the modeling by the U.S. EPA.

The estimated concentrations of 2,4-D in groundwater based on SCI-GROW in U.S. EPA/OPP (2004b) are much lower than any of the central estimates based either on GLEAMS or PRZM/EXAMS (Table 3-8), which is unusual. As noted by the U.S. EPA/OPP (2004b) and discussed further in the following subsection, the estimate of 0.03 ppb is not consistent with the monitoring data. Although SCI-GROW is a Tier 1 model that generally provides highly protective estimates of pesticide concentrations in groundwater, such is not the case for 2,4-D. Because these estimates are not consistent with monitoring data, the concentrations estimated using SCI-GROW are not used in the current risk assessment. This is the same approach taken by the U.S. EPA. The consistency of the modeling estimates with other monitoring data is discussed further in the following subsection.

All of the models used to simulate the environmental fate of 2,4-D rely on estimates of soil halftimes. While most studies suggest soil halftimes ranging from about 2 to 10 days for 2,4-D acid and esters (Table 2-3), the rate of degradation can be affected by many site specific factors. For example, Entry (1999) noted that high nitrogen concentrations in soil will inhibit the degradation of 2,4-D as well as other herbicides.

The modeling efforts discussed in this section and the previous section are based on the assumption that runoff and sediment losses are the major route of transport. As discussed by Rawn et al. (1999), volatilization of 2,4-D as well as other herbicides with subsequent redeposition in precipitation may account for a significant proportion of water contamination under some circumstances. This process is not considered quantitatively in either the GLEAMS

modeling or the PRZM/EXAMS modeling. As discussed further below, however, the modeling estimates are reasonably consistent with the available monitoring data.

3.2.3.4.5. Monitoring Data – Extensive monitoring data are available on 2,4-D. The most relevant studies are summarized in Table 3-9. The upper section of the table summarizes studies in which 2,4-D concentrations in water are similar to what can be expected from application rates generally used in Forest Service programs. These studies are directly useful in assessing the plausibility of the water contamination rates based on modeling (Table 3-8). The lower section of Table 3-9 summarizes more general monitoring surveys. While these studies are not as useful in evaluating the plausibility of the modeled concentrations, they can help to characterize background or general levels of the exposure, as discussed further in Section 3.2.3.7.

The most detailed and relevant study on 2,4-D concentrations in water characterizes applications to a watershed (Waite et al. 1992). The study involves monitoring 2,4-D in surface water (streams and ponds) in a 2800-hectare (≈6900 acres) watershed in Saskatchewan, Canada. Over a 3-year period, known amounts (i.e., 347, 410, and 209 kg) of 2,4-D were applied, and water samples were collected weekly in 1985 and 1986 and four times in 1987. As indicated in Table 3-9, water contamination rates ranged from about 0.6 to 8 ppb per lb/acre in ponds and 1.5 to 4.3 ppb per lb/acre in streams. These concentrations are encompassed by peak concentrations in ponds and streams based on GLEAMS modeling (Table 3-8). The somewhat higher concentrations in pond water relative to stream water are also consistent with the pattern seen in the GLEAMS modeling. The estimated water contamination rate in a more recent study in stream water by Kreuger et al. (1999) is also consistent with both the GLEAMS modeling and the earlier study by Waite et al. (1992).

As noted in the previous section, the very low estimate in the concentration of 2,4-D in groundwater from SCI-GROW – i.e., 0.03 ppb in Table 3-8 – is inconsistent with the much higher concentrations of 2,4-D monitored in groundwater – i.e., 1.7-4.5 ppb in Table 3-9. In general, concentrations monitored in groundwater appear to be comparable to or somewhat less than concentrations monitored in surface water. This is determination is illustrated in Table 3-9 with general monitoring data from the USGS National Water Quality Assessment in which groundwater concentrations of up to about 15 ppb are reported as well as in the study by Waite et al. (1992) from which water contamination rates of up to 4.5 ppb per lb a.e./acre can be derived. Again, both concentrations in groundwater as well as water contamination rates for groundwater tend to be somewhat lower than the corresponding values for surface water.

3.2.3.4.6. Aquatic Weed Control – As noted in Section 2, some formulations of 2,4-D (e.g., Aqua-Kleen) may be applied directly to standing bodies of water for aquatic weed control. The product label notes application rates of up to 200 lbs formulation/acre. The BEE formulation in Aqua-Kleen is present at a proportion of 0.276, this corresponds to 55.2 lb 2,4-D BEE per acre. Since the conversion factor for this ester to the acid is 0.688 (Table 2-3), the application rate for the ester corresponds to 38 lb a.e./acre. As noted in Section 2, the typical application rate for 2,4-D in Forest Service programs may range from 19 to 38 lbs a.e./acre.

The resulting concentration in water depends on the depth of the treated body of water. The product label recommends higher application rates in water that is more than 8 feet deep. Consistent with the pond modeling using GLEAMS (Table 3-5), the assumption is made that Aqua-Kleen is applied to a pond that is 2 meters deep. As detailed in Worksheet 17c of Attachment 2 (workbook for 2,4-D esters), the peak estimates of the concentration of 2,4-D in pond water are 1.06 mg a.e./L at an application rate of 19 lbs a.e./acre and 2.12 mg a.e./L at an application rate of 38 lbs a.e./acre. This range of concentrations is somewhat higher than peak concentrations monitored by Hoeppel and Westerdahl (1983), which ranged from about 0.6 to 0.7 mg/L 1 day after 20-40 lbs a.e./acre of Aqua-Kleen were applied to a lake 1- to 2-meters deep. The lower 2,4-D concentrations monitored by Hoeppel and Westerdahl (1983) may be due to the slow release of 2,4-D from the granular formulation.

Nonetheless, from the perspective of the human health risk assessment, the concentrations of 2,4-D in water after direct application for weed control are within the range of concentrations encompassed by the accidental spill scenarios, GLEAMS modeling, and available monitoring data. Hence, this exposure scenario is not considered specifically in the risk characterization; however, because 2,4-D BEE is more acutely toxic to aquatic organisms, this application method is given further consideration in the ecological risk assessment for those species (Section 4.2.5).

   3.2.3.4.7. Concentrations in Water Used for Risk Assessment – Table 3-10 summarizes the concentrations of 2,4-D in water used for the current risk assessment. The upper part of this table gives the concentrations expected at the typical application rate of 1 lb a.e./acre in units of micrograms per liter or ppb. The lower part of this table gives the water contamination rates, the normalized concentrations in water converted to units of ppm or mg/L per lb a.e./acre. The conversion from ppb to ppm is made because these latter units – i.e., ppm or mg/L – are used in the worksheets in the various exposure scenarios involving contaminated water in both the human health and ecological risk assessments. Because the typical application rate is 1 lb a.e./acre and this application is used in the GLEAMS modeling, the estimated concentrations in the top and bottom parts of this table are identical except for the conversion from ppb to ppm.

The upper range of the expected peak concentration of 2,4-D in surface water is taken as 440 ppb/L per lb a.e./acre. This estimate is based on peak 2,4-D concentrations in streams modeled in GLEAMS. As indicated in Table 3-6, this estimate is comparable to the peak concentration in streams modeled at an annual rainfall rate of 100 inches per year in areas with predominantly clay soils. The upper bound encompasses estimates used by U.S. EPA in the recent reregistration eligibility decision document on 2,4-D – i.e., 70-118 ppb (U.S. EPA/OPP 2005a, Table 8, p. 27). The central estimate and lower bound for peak concentrations are based on the GLEAMS modeling of a small pond (Table 3-7 of this risk assessment). The central estimate of 0.02 mg/L is comparable to the peak concentration modeled in a small pond at an annual rainfall rate of 20 inches, again in areas with predominantly clay soils. For the lower bound of the peak concentration, an argument may be made that concentrations of 2,4-D are likely to be essentially zero – i.e., applications at sites that are distant from open bodies of water and in areas in which runoff or percolation are not likely to occur. For this risk assessment, the lower range of the peak

water contamination rate will be set at 2 ppb or 0.002 mg/L per lb/acre. This is in the lower range of non-zero concentrations modeled in ponds at an annual rainfall rate of 50 inches in regions with predominantly sandy soils.

As noted in Table 3-8, these peak water contamination rates are likely to encompass some accidental exposures, such as direct spray. In other words, while accidental direct spray or inadvertent contamination due to drift might be considered extreme or at least atypical exposures, higher concentrations in water could be associated with normal use of 2,4-D in some areas.

Longer-term concentrations of 2,4-D in surface water will be much lower than peak concentrations. At an application rate of 1 lb/acre, the highest longer-term concentration is taken as 3.3 ppb or 0.0033 mg/L. This is the maximum longer-term concentration modeled using GLEAMS (Table 3-7, clay soil, annual rainfall rates of 100 inches or more). This estimate is also somewhat above the highest longer-term average concentration modeled by U.S. EPA/OPP (2004b) – i.e., 2.2 ppb per lb a.e./acre, as summarized in Table 3-8 of this risk assessment. The central estimate of the longer-term water contamination rate is taken as 0.4 ppb or 0.0004 mg/L, which is the 2,4-D concentration modeled in ponds at an annual rainfall rate of 15 inches per year in areas with predominantly clay soils. As with the estimates of peak concentrations, the lower bound of the longer-term concentration could be taken as zero. For the current risk assessment, this lower bound is taken as 0.02 ppb or 0.00002 mg/L, which coincides approximately with the longer-term concentration of 2,4-D in streams modeled using GLEAMS at an annual rainfall rate of 100 inches per year in regions with predominantly loamy soils.

3.2.3.5. Oral Exposure from Contaminated Fish – U.S. EPA/OPP (2004a) waived the requirement for bioconcentration studies in fish because there are data indicating that 2,4-D will not bioconcentrate. This approach is consistent with the information on biokinetics in mammals (Section 3.1.3) indicating that 2,4-D appears to reach steady-state rapidly and seems also to have a limited capacity to bioconcentrate or bioaccumulate. In addition, the biokinetics of 2,4-D in catfish after oral administration indicate rapid excretion of 2,4-D and a low potential for bioaccumulation (Plakas et al. 1992). Moreover, at least one field study indicates that applications at a very high rate of 2,4-D amine or ester to a lake did not cause the compound to bioaccumulate in game fish (Hoeppel and Westerdahl 1983).

The approach taken by U.S. EPA/OPP (2004a) has experimental support and is consistent with some field studies, however, other data indicate that 2,4-D and/or 2,4-D metabolites may bioaccumulate. Wang et al. (1994) determined that BCF values, based on total residues, ranged from about 10 to 40 in carp and *Tilapia* after 0.5-14 days of exposure to concentrations of 0.05-0.5 ppm of ^{14}C-labeled 2,4-D. It must be emphasized, however, that this study defined bioconcentration as radioactivity in fish divided by the radioactivity in water. *[The footnote in Table 3, p. 400, of this paper appears to be an error. It indicates that bioconcentration was defined as water/fish. The correct definition, however, is given in the text of the paper.]* Because only total radioactivity was measured, it is likely that some of the apparent bioconcentration was associated with metabolites of 2,4-D. In addition, the Wang et al. (1994b)

study involved the use of 2,4-D labeled on the acetate moiety. As discussed in WHO (1998, p.217), that acetate moiety is easily lost during aerobic degradation in a water/sediment system. Thus, the ^{14}C label can be incorporated into fish tissue as residues that have no structural relationship to 2,4-D. Thus, it is likely that the residues measured in the fish tissue in the study by Wang et al. (1994b) represent incorporated ^{14}C from the metabolized (i.e., mineralized) acetate moiety of 2,4-D. Because of these considerations, the approach taken by U.S. EPA/OPP (2004a) will be used and the BCF used in this risk assessment will be set at one, indicating that no bioconcentration of 2,4-D is expected and that the concentration of 2,4-D in fish will be the same as the concentration of 2,4-D in water. Even this assumption is likely to be somewhat conservative (i.e., overestimate exposure) because of the metabolism of 2,4-D by fish.

3.2.3.6. Oral Exposure from Contaminated Vegetation – Although none of the Forest Service applications of 2,4-D 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.

In most Forest Service risk assessments, 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). This is identical to the approach used by U.S. EPA/OPP (2005a).

For chronic exposures, both initial concentrations and a halftime on vegetation are required to estimate the time-weighted average exposure (Worksheet D04a and D04b). Consistent with the approach taken in GLEAMS modeling which is in turn consistent with values used by U.S. EPA/OPP (2004b), a halftime of 8.8 days is taken from the review by Willis and McDowell (1987). This estimate is comparable to the range of 5 to 9 days recommended by Knisel and Davis (2000) in the documentation for GLEAMS.

These values are also consistent with the field study by Morton et al. (1967), which noted a 5- to 10-fold decrease in 2,4-D concentrations on range grass over a 28-day observation period. Assuming simple first order elimination, the elimination coefficient, k_e, can be estimated from the fraction of the original residue, *f*, remaining after time *t* as:

$$k_e = -\ln(f)/t.$$

Thus, the apparent dissipation/degradation rate ranges from 0.05 to 0.082 days^{-1}, corresponding to half-times of about 8-14 days. Similar halftimes for 2,4-D can be estimated from a field study in triticale, a hybrid of wheat and rye (Cessna 1990). In this study, residues on 2,4-D treated triticale ranged from 0.001 to 0.15 of initial values after 21 days, corresponding to decay rates of 0.09-0.33 days^{-1} or half-times of approximately 1-7.7 days. Over the next 21 days, the proportion

remaining—relative to the value on day 21—ranged from 0.136 to 0.34 of initial values, corresponding to decay rates of 0.051-0.095 days^{-1} or half-times of approximately 7.3-13.5 days (Cessna 1990).

3.3. DOSE-RESPONSE ASSESSMENT
3.3.1. Overview
Following standard practices for Forest Service risk assessments, the RfD values derived by U.S. EPA are adopted directly for the assessment of potential risks to humans. U.S. EPA/OPP (2005a) derives two acute RfDs and a chronic RfD for the protection of human health from exposures to 2,4-D and 2,4-D amine salts and esters. The acute RfDs include an RfD of 0.025 mg/kg/day for reproductive aged females, based on maternal toxicity and another RfD of 0.067 mg/kg/day for the general population, based on neurotoxicity. In the current risk assessment, the lower acute RfD of 0.025 mg/kg/day is used to assess the consequences of acute exposures. This approach is taken because any of the acute exposure scenarios could involve a woman of child-bearing age. The chronic RfD of 0.005 mg/kg/day is based on a rat NOAEL for chronic toxicity and a 2-generation reproduction study. These values are considered protective of potential developmental and reproductive effects, and are considered protective of children per the FQPA. There is an older chronic RfD of 0.01 mg/kg/day on EPA's IRIS database, but this value does not take into account the currently available information and is not used in the current risk assessment.

3.3.2. Chronic RfD
As stated previously, the toxicity of 2,4-D acid, esters, and salts is well-represented by 2,4-D acid. Consequently, regulatory agencies like the U.S. EPA and WHO (1996) derive doses for "acceptable" human intake on the basis of 2,4-D acid. The WHO (1996) acceptable daily intake for 2,4-D acid, salts, and esters, expressed as 2,4-D acid, is 0-0.01 mg/kg/day. The U.S. EPA derived two different chronic RfD values for 2,4-D. An older value appears on the IRIS database(U.S. EPA 1987), and the more recent value was derived by HED (U.S. EPA/OPP 2005b) and appears in the Registration Eligibility Document for 2,4-D (U.S. EPA/OPP 2005a).

U.S. EPA's IRIS database lists an RfD of 0.01 mg/kg/day for 2,4-D (U.S. EPA 1987). The RfD is based on a purported NOAEL of 1 mg/kg/day from a 90-day oral bioassay and a 1-year interim report from a 2-year rat feeding study by Dow Chemical Company (1983). U.S. EPA applied an uncertainty factor of 100. The dose of 5 mg/kg/day was considered a LOAEL based on hematological changes. This is also the basis for the WHO (1996) acceptable daily intake of 0-0.01 mg/kg/day mentioned above. IRIS (U.S. EPA 1987) describes the study as follows:

"Hematologic, hepatic, and renal toxicity were demonstrated in a study in Fischer rats (strain 344) during subchronic feeding performed at the Hazleton Laboratories (1983). 2,4-D (97.5% pure) was added to the diet chow and fed to the rats for 91 days at doses calculated to be 0.0 (controls), 1.0, 5.0, 15.0, or 45.0 mg/kg/day. In each of the five groups there were 20 animals/sex and 40 animals/treatment group, for a total of 200 animals. Criteria examined to determine toxicity were survival, daily examination for clinical symptomatology, weekly change in body weights, growth rates, food intake, ophthalmologic changes, changes in organ weights, and clinical, gross and histopathologic alterations. The results of the study demonstrated statistically significant reductions in mean hemoglobin (both sexes), mean hematocrit and red blood cell levels (both sexes), and mean reticulocyte levels (males only) at the 5.0 mg/kg/day dose or higher

after 7 weeks. There were also statistically significant reductions in liver enzymes LDH, SGOT, SGPT, and alkaline phosphatase at week 14 in animals treated at the 15.0 mg/kg/day or higher doses. Kidney weights (absolute and relative) showed statistically significant increases in all animals at the 15.0 mg/kg/day dose or higher at the end of the experimental protocol. Histopathologic examinations correlated well with kidney organ weight changes showing cortical and subcortical pathology. Increases in ovarian weights, T-4 levels, and a decrease in BUN were reported, but were not considered to be treatment- related. "

Using more recent studies (i.e., the MRID studies shown in Table 3-3), U.S. EPA/OPP (2005a,b) derived a chronic RfD of 0.005 mg/kg/day for 2,4-D. This value is based on a NOAEL of 5 mg/kg/day observed in the chronic toxicity/carcinogenicity study for rats (Jeffries et al. 1995). It is important to note that this study used the same form of 2,4-D (acid) and strain of rats employed in the above Dow Chemical Company (1983) study cited as the basis for the IRIS RfD. The LOAEL for the more recent (Jeffries et al. 1995) study is 75 mg/kg/day, on the basis of adverse effects on the eyes, thyroid, kidney, liver, blood cells, testes, ovaries, lungs, and adipose tissue (summarized in Table 3-3, first page).

In deriving the RfD of 0.005 mg/kg/day, U.S. EPA (2005a,b), divided the NOAEL of 5 mg/kg/day by an uncertainty factor of 1000: factors of 10 each for differences in sensitivity between and within species; and an additional factor of 10 for uncertainty in the database. U.S. EPA uses the additional uncertainty factor of 10 to account for the lack of a current 2-generation reproduction study using the *current* protocol, which would address endocrine disruption and immunotoxicity, and the lack of a developmental neurotoxicity study.

U.S. EPA/OPP's choice of the NOAEL of 5 mg/kg/day as the basis for a chronic RfD for 2,4-D is well supported by other studies, some of which were conducted subsequent to the Dow Chemical Company (1983) study and IRIS RfD derivation. Studies that support the NOAEL of 5 mg/kg/day include the 2-generation reproduction study (NOAEL = 5; LOAEL = 20 mg/kg/day; Table 3-3, Appendix 1), the mouse carcinogenicity/chronic toxicity study (NOAEL = 5 mg/kg/day; LOAEL = 62/150 mg/kg/day; Table 3-3), and all of the developmental toxicity studies conducted with rats and rabbits (Appendix 1).   The NOAEL of 5 mg/kg/day is also supported by results of more recently conducted studies published in the open literature (Table 3-3): the developmental immunotoxicity study of Lee et al. (2001), and the developmental neurotoxicity of Garcia et al. (2004).   Lee et al. (2001) is particularly important because of its well-defined effect levels. The study defines a NOAEL of 8.5 mg a.e./kg/day for mouse pups evaluated 7 weeks after *in utero* exposure to 2,4-D DMA salt on days 6-16 of gestation and a LOAEL of 37 mg a.e./kg/day was defined for pups, based on decreased body weight gain and kidney weights (females only). The maternal NOAEL and pup immune LOAEL in this study is 370 mg a.e./kg/day, based on adverse effects on lymphocytes.

 The LOAEL values for dogs in the subchronic and chronic studies are less than or equal to the NOAEL of 5 mg/kg/day (Table 3-3), however, dogs are unusually sensitive to 2,4-D toxicity due to their limited ability to eliminate organic acids. Furthermore, with regard to 2,4-metabolism

and elimination, dogs are less similar than rats and mice to humans. Accordingly, the dog studies are not considered in the derivation of a chronic RfD for human exposure, and this assessment adopts the U.S. EPA/OPP (2005a,b) RfD of 0.005 mg/kg/day for the assessment of chronic human exposure to 2,4-D acid, esters, and salts.

3.3.3. Acute RfD

U.S. EPA/OPP (2005a,b) derives two acute RfDs to protection human health from exposures to 2,4-D and 2,4-D amine salts and esters. The acute RfDs include a value of 0.025 mg/kg/day to protect reproductive-age females and a value of 0.067 mg/kg/day to protect the general population (Table 3-11).

The acute dietary RfD for reproductive-age females is based on a maternal NOAEL of 25 mg/kg/day is derived from the developmental toxicity study on 2,4-D acid in rats (Nemec et al. 1983; Rodwell et al. 1983) (Table 3-3, Appendix 1). The RED and the HED supporting science chapter for the RED (U.S. EPA 2005b) state that 2,4-D acid is representative of the class of 2,4-D acid, salts, and esters under consideration, and, accordingly, serves as the basis for deriving the dietary RfD values. Nevertheless, examination of the study results reported in detail by EFED (U.S. EPA/OPP 2004b) and WHO (1996), but not HED (U.S. EPA/OPP 2005b), calls into question whether the threshold for maternal toxicity is represented by 2,4-D acid.

Some results suggest that maternal toxicity is seen in rats and rabbits exposed to salts and esters at acid equivalent doses lower than 25 mg a.e./kg/day. The relevant studies are summarized in Table 3-12. Specifically, the studies conducted with rabbits and the DEA and IPA salts (Rodwell 1991b; Breslin et al. 1991b), and with rats and the TIPA salts yielded LOAEL values for maternal toxicity less than 25 mg a.e./kg. Neither HED nor the RED explain further why 25 mg/kg was chosen as a NOAEL for maternal toxicity in spite of these findings. A review of the cleared toxicity reviews of these studies released by U.S. EPA (Whitby 1990; Rowland 1992) is not illuminating. However, as the following paragraph illustrates, EPA's use of an additional uncertainty factor of 10 along with the NOAEL of 25 mg/kg places the resulting acute RfD mid-way between the range of values which could be derived using the lower LOAEL values from the above-mentioned studies.

The studies in Table 3-12 suggest that the LOAEL for maternal toxicity lies somewhere between 10 and 30 mg a.e./kg/day. Using an uncertainty factor of 1000 (factors of 10 each for inter- and intra-species variability, and 10 for both using a LOAEL and uncertainty in the database) would then yield an acute RfD between 0.010 and 0.030. EPA's RfD of 0.025, which is based on a NOAEL of 25 but includes an additional uncertainty factor of 10 for uncertainty in the database, lies mid-way between these values.

U.S. EPA/OPP (2005a,b) derives a second acute RfD of 0.067 mg/kg/day for the "general population" (Table 3-11). This value is based on the acute neurotoxicity screening study in rats, which yielded a NOAEL of 67 mg/kg/day and a LOAEL of 227 mg/kg/day on the basis of gait abnormalities.

For the current risk assessment, all acute occupational exposures could involve female workers. Consequently, this assessment adopts U.S. EPA/OPP's acute RfD of 0.025 mg/kg/day for the assessment of acute human exposure to 2,4-D acid, salts, and esters.

3.4. RISK CHARACTERIZATION
3.4.1. Overview
This section combines estimates of exposure, as discussed in the exposure assessment (Section 3.2) with estimates of toxicity discussed in the dose-response assessment (Section 3.3) to estimate potential risks for Forest Service workers and members of the general public. Estimates of risk are presented in terms of a hazard quotient. A hazard quotient is simply the quotient of an estimate of exposure divided by the appropriate toxicity value. Hazard quotients which are less than 1 indicate that an adverse health outcome would not be expected. Hazard quotients greater than 1 indicate that adverse health outcomes may be plausible. Concern for the development of adverse effects increases as the value of the as hazard quotient increases.

For workers, longer-term exposures associated with ground spray applications of 2,4-D yield risks twice those associated with either backpack or aerial application methods. Based on upper bound hazard quotients which exceed 1, adverse health outcomes are possible for workers exposed repeatedly over a longer period of time. Hazard quotients for workers spraying at the typical application rate of 1 lb a.e./acre are 16 for both backpack and aerial spray methods, and 30 for ground spray application. Short-term accidental exposures via contaminated gloves as well as some spill scenarios yield hazard quotients that are of concern, particularly for the scenario involving contaminated gloves that are worn for 1 hour, which yields a hazard quotient of 94. For all of these hazard quotients, the magnitude of the hazard quotient is linearly related to the application rate.

As with hazard quotients for workers, hazard quotients for members of the general public are linearly related to application rate. Upper bound hazard quotients for accidental exposures associated with spills into a small body of water range from 0.8 (consumption of fish by non-subsistence populations at an application rate of 0.5 lb a.e./acre) to 328 (a child consuming 1 liter of contaminated water at an application rate of 4 lb a.e./acre). The amounts spilled are set at the amounts required to treat from 1 to100 acres. These assumptions are completely arbitrary and may be unrealistic. The scenario for an accidental spill into a small pond is intended simply to illustrate the different consequences of spilling different amounts of 2,4-D.

Short-term consumption of contaminated fruits and vegetables could be of concern with respect to assessing the risk of maternal toxicity or acute neurotoxicity (i.e., using either of the existing acute RfDs for 2,4-D). Upper bound hazard quotients associated with the typical application rate of 1 lb a.e./acre are 7 for consumption of contaminated fruit and 54 for consumption of contaminated vegetation. These estimates are based on contaminated fruit or vegetation consumption by an adult female. At the lowest anticipated application, hazard quotients are 4 and 27 for the consumption of contaminated fruits and vegetables, respectively. At the highest anticipated application, hazard quotients are 30 and 216 for the consumption of contaminated fruits and vegetables, respectively.

The only hazard quotients indicating that adverse health outcomes are plausible following longer-term exposure to 2,4-D are those associated with ingestion of contaminated fruits and vegetation

by an adult female. At the typical application rate of 1 lb a.e./acre, the central estimate of the hazard quotient for the consumption of contaminated vegetation is 5 with lower and upper bounds of 1 and 38. Because lower residues are anticipated on contaminated fruit, the hazard quotient associated with this scenario at an application rate of 1 lb a.e./acre is 0.3 with an upper bound of 5. Even at the lowest anticipated application rate, the upper bounds of the hazard quotients exceed a level of concern for both contaminated fruit (HQ = 3) and contaminated vegetation (HQ=19). At the highest anticipated application rate (4 lb a.e./acre), upper bounds of the hazard quotient substantially exceed a level of concern for contaminated fruit (HQ = 21) and contaminated vegetation (HQ=152). Other longer-term exposure scenarios involving the consumption of either contaminated water or fish yield hazard quotients that are substantially below a level of concern even at the highest anticipated application rate.

3.4.2. Workers

A quantitative summary of the risk characterization for workers is presented in Worksheet E02 of the Workbook for 2,4-D acid and salts (Attachment 1). There is a total of three worksheets in this series, with each worksheet covering the typical (E02a), lowest anticipated (E02b), and highest anticipated application rates (E02c). The worksheet for each application rate is divided into two sections. The first section covers acute accidental or incidental exposures involving accidental spills on the hands or lower legs, and contaminated gloves. The second portion covers more general longer-term exposures involving backpack, ground, and aerial spray applications.

For workers as well as members of the general public, the quantitative risk characterization is expressed as the hazard quotient, which is the ratio of the estimated exposure from Worksheet E01 to the RfD. For acute accidental/incidental exposures, the acute RfD of 0.025 mg/kg derived by U.S. EPA/OPP (2005a,b) is used (Section 3.3.3). For longer-term general exposures – i.e., exposures that could occur over the course of several days, weeks, or months during an application season – the chronic RfD of 0.005 mg/kg/day, also derived by U.S. EPA/OPP (2005a,b), is used (Section 3.3.2).

As discussed previously, U.S. EPA/OPP (2005a,b) derives two acute RfD values; one for protection of reproductive-age females, and one for the general population. This assessment uses the former RfD (0.025 mg/kg) derived on the basis of maternal toxicity. Using this RfD ensures that the risk estimates provided in this assessment are protective of the most sensitive workers, females of child-bearing age. Risks to male workers could be more than 2 times lower, given that an acute RfD of 0.067 mg/kg has been derived for the general population (i.e., anyone other than reproductive-age females) on the basis of neurotoxic effects (U.S. EPA/OPP 2005a,b). The chronic RfD (0.005 mg/kg/day), which is based on general chronic toxicity, is equally applicable to male and female workers.

As shown in worksheets E02a through E02c, the hazard quotients for accidental/incidental exposures of workers are greater than the level of concern – i.e., an HQ of 1 – for all exposure scenarios involving contaminated gloves worn for 1 hour and for the upper range of exposures for workers wearing contaminated gloves for 1 minute. These are more or less arbitrary exposure

scenarios used in all Forest Service risk assessments to characterize the importance of good work practices. For 2,4-D, aggressive measures are warranted to provide workers with adequate protective clothing and the need to keep the protective clothing free of gross contamination. The only other hazard quotient for the accidental scenarios that is greater than 1 involves the upper bound estimate for a spill on the lower legs involving the highest likely application rate of 4 lb a.e./acre, with a value of 1.8.

Based on the hazard quotients derived in this risk assessment, adverse health effects associated with longer-term worker exposures are of concern (i.e., the HQ exceeds 1). As shown in worksheets E02a through E02c, the central and upper bound estimates of hazard quotients for general longer-term exposures are all greater than 1 across the range of application rates. When considering upper-bound estimates, the hazard quotients for ground spray application are approximately twice that of either backpack or aerial spray methods. For the typical application rate (1 lb a.e./acre), upper bounds of the hazard quotients are 16 for both backpack and aerial spray application and 30 for ground spray. The upper bound hazard quotients for the lowest application rate (0.5 lb a.e./acre) and highest application rate (4 lb a.e./acre) are linearly proportional.

The quantitative risk characterization for workers given in the U.S. EPA/OPP (2005a,b) reregistration eligibility decision (RED) document is generally comparable to that given in this risk assessment. For short-term scenarios (U.S. EPA/OPP 2005a, Table 22, p. 48), the RED gives "base-line" margins of safety (MOEs) ranging from about 1 to 3.2. The margin of exposure, as used by U.S. EPA/OPP (2005a) is calculated as some toxicity benchmark – i.e., a NOAEL or LOAEL – divided by an estimate of exposure. The target MOE used by U.S. EPA/OPP (2005a) is 100. In this respect, the MOE is analogous to the reciprocal of the hazard quotient (as defined in this Forest Service risk assessment) without the uncertainty factor. Thus, the MOE values of 1-3.2 derived by the U.S. EPA correspond to hazard quotients (as defined in this Forest Service risk assessment) of about 30-100. The base-line exposures defined by U.S. EPA are those associated with no use of special protective equipment. As noted in Worksheets E02a to E02c of this risk assessment, the central and upper range of hazard quotients for wearing contaminated gloves for 1 hour are 15 to 94, comparable to the levels of concern noted by U.S. EPA/OPP (2005a) – i.e., about 30-100.

For intermediate exposure scenarios (U.S. EPA/OPP 2005a, Table 23, pp. 49-50), some margins of safety range from 1.1 to 78. These values correspond to hazard quotients (again as defined in this Forest Service risk assessment) of about 1.3-90. As noted in Worksheets E02a to E02c of this risk assessment, the hazard quotients that exceed a level of concern (i.e., an HQ of 1) for the longer-term exposure of workers range from 1.3 to 120, again very similar to the range from U.S. EPA/OPP (2005a).

While it is beyond the scope to this risk assessment to provide a detailed discussion of the differences in methodology between the U.S. EPA/OPP (2005a) RED and the current Forest Service risk assessment, the differences are substantial. As discussed in Section 3.2.2, this Forest

Service risk assessment uses estimates of absorbed dose combined with the acute or chronic oral RfD. U.S. EPA/OPP (2005a), on the other hand, uses estimates of deposited dose combined with toxicity studies that reflect the anticipated route of exposure – i.e., dermal or inhalation. Thus, the similarities in the quantitative expressions of risk are striking. While these similarities may be serendipitous, expressions of risk for workers involved in the application of 2,4-D appear to be similar based on estimates of absorbed dose, deposited dose, or internal doses based on a PBPK model (Durkin et al. 2004).

Concern for potential adverse effects in workers involved in forestry applications of 2,4-D is enhanced and reenforced by the recent study by Garry et al. (2001) which reports elevated levels of serum luteinizing hormone in forestry workers involved in backpack applications of 2,4-D (see discussion in Section 3.1.8).

3.4.3. General Public
A quantitative summary of the risk characterization for the general public is presented in the workbook for 2,4-D acid and salts (Attachment 1). Risks involving exposures associated with the typical (1 lb a.e./acre), lowest anticipated (0.5 lb a.e./acre), and highest anticipated (4 lb a.e./acre) application rates are shown in worksheets E04a, E04b, and E04c, respectively. As with the risk characterization for workers, risk is expressed quantitatively as the hazard quotient using the RfD values derived by U.S. EPA/OPP (2005a,b) – i.e., the acute RfD of 0.025 mg/kg for the protection of reproductive-age females (Section 3.3.3) or the chronic RfD of 0.005 mg/kg/day (Section 3.3.2). As before, the lower of the two available acute RfD values is chosen to account for possible exposure of reproductive-age females.

Upper bound hazard quotients for direct spray of a whole naked child with 2,4-D acid or salts are greater than 1 for all application rates, ranging from a value of 3 for 0.5 lb a.e./acre, to a value of 28 for 4 lb a.e./acre. Use of the acute RfD of 0.067 mg/kg (general population; basis is acute neurotoxicity in rats) instead of the acute RfD of 0.025 mg/kg (basis is maternal toxicity) would still result in upper bound hazard quotients greater than the level of concern , which is 1. While this scenario is highly unlikely, it is a standard extreme scenario that is used in all Forest Service risk assessments as an indicator of the most serious exposures which could result from accidental spraying of members of the general public. All pesticide applications are conducted in a manner to avoid accidental spraying of members of the general public; however, this scenario suggests that such caution is particularly warranted with the use of 2,4-D.

Based on central and upper-bound hazard quotients, adverse health outcomes are plausible following an accidental spill of 2,4-D into a small body of water. Upper bound hazard quotients for a young child consuming contaminated water following an accidental spill are 82, 41, and 328 for the typical, lowest, and highest anticipated application rates, respectively. Estimates of exposure via consumption of contaminated fish following an accidental spill result in hazard quotients of concern (i.e., greater than 1) for both subsistence and typical fish consumption scenarios. For subsistence populations (i.e., those who may eat wild caught fish as a necessity rather than a sport), upper bound hazard quotients for fish consumption range from a low value

of 4 for the lowest anticipated application rate to a high of 32 for the greatest anticipated application rate. Comparable hazard quotients for consumption by the general population range from 0.8 at the lowest application rate to 7 at the highest application rate.

The plausibility of these exposure scenarios following an accidental spill, however, is uncertain. This scenario assumes that the amount spilled ranges from the amount required to treat 1 acre to the amount required to treat 100 acres, with a central estimate based on the amount required to treat 10 acres. These assumptions are completely arbitrary and are intended only to illustrate potential risks over a broad range of conditions.

The only other acute exposure scenario which yields hazard quotients greater than 1, are the scenarios involving an adult female ingesting contaminated fruit or vegetation. Upper bound estimates of hazard quotients for an adult female ingesting contaminated fruit are 7, 4, and 30, in association with the typical, lowest, and highest anticipated application rates. The comparable upper bound hazard quotients for an adult female ingesting contaminated vegetation are 54, 27, and 216, respectively. These values indicate substantial concern for the inadvertent consumption of contaminated vegetation based on the RfD for maternal toxicity. Using the higher acute RfD of 0.067 would still result in hazard quotients much greater than 1 in these scenarios, indicating that effects other than maternal toxicity, such as neurotoxicity, are plausible for these acute exposure scenarios.

On the basis of hazard quotients presented in worksheets E04a through E04c, the only longer-term exposures which could plausibly result in adverse health effects are those associated with consumption of fruit and vegetation. The upper bound hazard quotients for ingestion of contaminated vegetation are higher than those for ingestion of fruits, with values of 38, 19, and 152, for application rates of 1, 0.5, and 4 lb a.e./acre, respectively. These results suggest that adverse health effects are plausible should such exposures occur. On the basis of the animal studies discussed in Section 3.1, these adverse effects could target the developing fetus as well as the blood, kidney, liver, thyroid, eyes, reproductive system, immune system, and nervous systems of adults.

To the extent that comparisons are possible, this Forest Service risk assessment is consistent with the recent risk assessment by U.S. EPA (U.S. EPA/OPP 2004a, 2005a). U.S. EPA/OPP (2004a, 2005a) estimates risks to the general population due to 2,4-D exposures for residential turf application and post-application scenarios. In the scenarios involving turf, EPA assumes an average application rate of 1.5 lb/acre, and that spot treatments would be repeated over a 2- to 3-week period (i.e., short-term exposure). None of the risks calculated for any turf scenario exceeded EPA levels of concern. The conclusion is consistent with this Forest Service risk assessment in which none of the scenarios involving contact with contaminated vegetation reach a level of concern. The scenarios in this Forest Service assessment involving consumption of contaminated fruits and vegetables have no comparable counterparts in EPA's analysis.

3.4.4. Sensitive Subgroups

As with exposures to almost any chemical, there is particular concern for children, women who are pregnant or may become pregnant, the elderly, or individuals with any number of diseases. As discussed previously, reproductive-age females are sensitive to 2,4-D exposure. Developing fetuses are also sensitive to 2,4-D exposure at doses that are toxic to the mother. These issues were taken into account in the derivation of the acute and chronic RfD values for 2,4-D.

As discussed in the hazard identification section (3.1), sunscreens increase the dermal permeability of 2,4-D. Consequently, individuals using sunscreens may absorb a greater dose of the compound, making them more likely than others to have adverse effects associated with dermal to 2,4-D.

Studies with animals and humans suggest that 2,4-D is capable of causing adverse effects to the immune system. Accordingly, individuals who are immuno-compromised (e.g. the very young, the elderly, individuals with chronic illness) may be unusually sensitive to 2,4-D.

The mechanism of action of 2,4-D involves disruption of the cell at the level of the membrane and basic metabolic functions. Individuals who have sdiseases involving the integrity of the cell membrane (e.g. sickle cell anemia) may be more sensitive than others to 2,4-D exposure.

As with many chemicals, there is some evidence that individuals, particularly children, who are malnourished may be at increased risk when exposed to 2,4-D (e.g., Ferri et al. 2003).

3.4.5. Connected Actions

The Council on Environmental Quality (CEQ, which provides the framework for implementing NEPA, defines connected actions (40 CFR 1508.25) as actions which occur in close association with the action of concern; in this case, the use of a pesticide. Actions are considered to be connected if they: (i) Automatically trigger other actions which may require environmental impact statements; (ii) Cannot or will not proceed unless other actions are taken previously or simultaneously, and (iii) Are interdependent parts of a larger action and depend on the larger action for their justification. Within the context of this assessment of 2,4-D, "connected actions" include actions or the use of other chemicals which are necessary and occur *in close association* with use of 2,4-D.

As discussed in Section 2, and shown in Table 2-2, 2,4-D is often formulated with other pesticides such as picloram, MCPA, triclopyr, dicamba and glyphosate. These pesticides are well studied, and their actions are discussed in other published and unpublished documents. Further discussion of these effects is outside the scope of this risk assessment; however, it is important to note that some studies suggest that 2,4-D in combination with other pesticides, such as propanil, may cause an adverse synergistic effect on immune function. As discussed in Section 3.1.7 (Immunotoxicity), there is evidence that 2,4-D used in combination with propanil, synergises immunotoxic effects in mice. In addition, some studies have implicated synergistic effects with mixtures of 2,4-D and picloram in terms of reproductive impairment (Oakes et al.

2002a,b). Mixtures of 2,4-D and 2,4,5-T have been used in past, but are no longer used in Forest Service programs. The risks of developmental neurotoxicity from exposure to 2,4-D and 2,4,5-T are documented in Razzaghi and Kodell (2004).

As discussed in detail in Sections 3.1.14 (Inerts and Adjuvants) and 3.1.15 (Impurities and Metabolites), 2,4-D formulations contain inert components and impurities that may have an impact on risks to human health and the environment. There is no evidence from the available studies discussed throughout this document that the presence of the impurities and metabolites in 2,4-D formulations have a significant impact on health risk. After conducting a risk assessment on the basis of limited sampling, U.S.EPA/OPP (2005a, b) concluded that the concentration of dioxins potentially present in 2,4-D formulations is likely to be low (based on limited sampling where only detectable concentrations of 2 PCDDs were above limits of quantification) and that human health and ecological risks associated with such contamination are inconsequential.

3.4.6. Cumulative Effects
This assessment considers known chemical interactions or actions, which taken in consideration with the proposed pesticide use, would affect the quality of human health and the environment (i.e., modify risks to human health and ecological receptors within the context of the risk assessment).

It is beyond the scope of the current risk assessment to identify and consider all agents that might interact with 2,4-D or cause cumulative effects with 2,4-D. To do so quantitatively would require a complete set of risk assessments on each of the other agents to be considered. Cumulative effects, within the context of the Food Quality Protection Act (requires assessment of chemicals with a similar mode of action), are addressed by the U.S. EPA/OPP (2005b) in the HED science chapter which supports the RED for 2,4-D (U.S. EPA/OPP 2005a):

> *2,4-D is a member of the alkylphenoxy herbicide class of pesticides. This class also includes MCPA, 2,4-DB, and 2,4-DP. A cumulative risk assessment has not been performed as part of this human health risk assessment because the Agency has not yet made a determination as to which compounds to which humans may be exposed, if any, have a common mechanism of toxicity.*

That 2,4-D can induce programmed cell death (apoptosis), as discussed in section 3.1.2, suggests a potential for additive, synergistic, or inhibitory effects on other apoptic agents, depending upon the nature of the agent and it's mechanism for induction of the apoptic cascade of events. As discussed in Section 3.1.2 (Mechanism of Action) 2,4-D disrupts the cell at a fundamental level; therefore, interactions are likely to occur between 2,4-D and any of the many other chemicals that affect cell membranes and cell metabolism.

The current Forest Service risk assessment specifically considers the effect of repeated exposures to 2,4-D for both workers and members of the general public. The chronic RfD is used as an index of acceptable longer-term exposures. Consequently, the risk characterizations presented in

this risk assessment for longer-term exposures specifically addresses and encompasses the potential impact of the cumulative effects of 2,4-D. As discussed in Sections 3.4.2 and 3.4.3, the risk of adverse health effects is increased for workers engaged in spray applications of 2,4-D as well as nearby residents who might consume fruit or vegetation contaminated with spray drift from forestry applications (i.e., hazard quotients exceed 1).

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION
4.1.1. Overview
The toxicity of 2,4-D is fairly well characterized in plants and animals. As in the human health risk assessment, the toxicity of the various forms of 2,4-D – i.e., acid, salts, and esters – are all treated as equally toxic to birds and mammals. For terrestrial plants as well as aquatic plants and animals, the toxicity of 2,4-D acid and 2,4-D salts is considered separately from that of 2,4-D esters. Based on formulations used by the Forest Service, this assessment considers only data relevant to 2,4-D acid, the DMA and TIPA salts, and the BEE and the 2-ethylhexyl (also known as the iso-octyl ester) ester. The critical toxicity information for ecological assessment is summarized in Table 4-1 (Terrestrial Animals), Table 4-2 (Terrestrial Plants), Tables 4-3, 4-4, 4-5 (Aquatic Animals), and Table 4-6 (Aquatic Plants). This assessment relies heavily upon the analyses of the U.S. EPA/OPP (2004b, 2005a) for summaries of unpublished studies submitted to the U.S. EPA in support of the registration of 2,4-D. In addition, this Forest Service risk assessment uses studies from the open literature, where appropriate, to identify hazards and quantify toxicity.

Based on classification schemes for acute toxicity developed by U.S. EPA, 2,4-D is slightly to moderately toxic to mammals; practically non-toxic to moderately toxic to birds; and practically non-toxic to honey bees. Among mammals, dogs are more sensitive than other species to the effects of 2,4-D, due to their limited capacity to excrete organic acids. The U.S. EPA classifies the toxicity of 2,4-D to freshwater and marine fish as practically non-toxic for 2,4-D acid/salts and highly toxic for esters. A similar pattern of toxicity is observed for aquatic invertebrates and amphibians. 2,4-D does not cause effects on reproduction or fetal development in birds or mammals at exposures which do not cause toxic effects in maternal animals. The only available studies that address the potential for 2,4-D to adversely affect early growth and development in fish were conducted with fathead minnows. These studies demonstrate that the esters are more toxic than the acid or salts. 2,4-D causes phytotoxicity in nontarget plants at concentrations which are likely used under field conditions, if precautions are not taken to limit spray drift. A limited number of studies suggest that the effects of 2,4-D on soil microorganisms and invertebrates are possible. While 2,4-D is not likely to cause mortality among honey bees at any of the application rates employed by the Forest Service, other species of insects, such as parasitic wasps may be affected, though the available studies do not lend themselves to quantitatively defining dose-response relationships.

4.1.2. Toxicity to Terrestrial Organisms
4.1.2.1. Mammals – Most of the information on the toxicity of 2,4-D in mammals as well as other species comes from unpublished bioassays submitted to the U.S. EPA for the registration of 2,4-D. While some studies are conducted directly by the registrant, most toxicity studies are performed by commercial testing laboratories. The critical studies for assessing ecological toxicity of 2,4-D acid, ester, and salts in mammals are summarized in Table 4-1.

As discussed in the human health risk assessment (Section 3.1) and detailed in Appendices 1 and 2, the toxicity of 2,4-D acid, salts, and esters to mammals is relatively well characterized in a several standard studies on a variety of animals. In general, the toxicity of 2,4-D salts and esters is regarded equivalent to that of 2,4-D acid. Hence, studies conducted with 2,4-D acid are regarded by U.S. EPA/OPP (2005a) as representative of the class for mammals. EFED (U.S. EPA/OPP 2004b) notes that the toxicity database for acids, salts, and esters is pooled for assessment of birds and mammals in the ecological assessment because of the tendency for salts and esters to convert rapidly to the acid form in the terrestrial environment, and because of a limited toxicity database for birds.

The mode of action of 2,4-D is fairly well characterized in mammals. 2,4-D physically disrupts the cell membrane, interferes with cellular metabolism, and uncouples oxidative phosphorylation. More recently, 2,4-D was shown to induce the cascade of events leading to programmed cell death known as apoptosis (Section 3.1.2). These mechanisms can account for many of the toxic effects of 2,4-D observed at doses which exceed the organism's capacity for renal clearance of 2,4-D acid. Due to their limited capacity to excrete organic acids, dogs are more sensitive than rats, mice, or humans to the toxic effects of 2,4-D (Van Ravenzwaay et al. 2003).

Based on the LD_{50} values shown in Table 3-1, U.S. EPA/OPP (2005a) considers 2,4-D acid, and its salts and esters to be slightly to moderately toxic. Furthermore, with regard to acute oral toxicity, and mammalian toxicity in general, U.S. EPA (2005a) concludes that the toxicity of 2,4-D salts and esters is equivalent to that of 2,4-D acid. Data shown in Table 3-1 substantiate this position. Based on the available studies, the primary clinical effects observed following acute oral exposure of laboratory animals are ataxia, myotonia, and decreased limb tone (U.S. EPA/OPP 2005a).

As noted in Section 3.1, sublethal effects, including maternal toxicity and neurotoxicity are seen following acute exposure to doses well below the rat LD_{50} values (579-1300 mg a.e./kg bw) summarized in Table 3-1. Maternal toxicity is seen at doses between 10 and 30 mg a.e./kg/day in pregnant rats and rabbits exposed to various salts and esters of 2,4-D (Table 3-5). In addition, neurotoxicity is observed in dogs exposed via gavage to a single oral doses greater than 8.8 mg a.e./kg bw (Beasley et al.1991; Steiss et al. 1987, summarized in Appendix 2, first page).

Subchronic and chronic toxicity studies validate the observation that dogs are more sensitive than rats or mice to 2,4-D exposure (Tables 3-2, 4-1). In both rodents and dogs, exposure to 2,4-D caused decreases in body weight and food consumption, as well as adverse effects on the liver and kidneys. The latter effects were manifest as changes in clinical chemistry variables (e.g., liver and kidney enzyme alterations), organ weights, and microscopically identified kidney and liver pathology. In rats, adverse effects on the thyroid (increased thyroid weight), thyroid function (decreased T3 and T4), hematology (decreased red blood cells, hematocrit and hemoglobin in females, and platelets in both sexes), cholesterol and glucose metabolism (decreased blood glucose, cholesterol, triglycerides), sex organs (decreased ovary and testes

weights), eyes (cataracts), adipose tissue (microscopic lesions), and lungs (microscopic lesions) were also observed. Decreased blood glucose, brain weights, and testes weights were also observed in dogs, along with hypospermatogenesis and prostate inactivity. While concerns have been expressed over the association of 2,4-D exposures in dogs and the development of canine malignant lymphoma (Sternberg 1992), a causal relationship is not evident (Hayes et al. 1992; Kaneene and Miller 1999).

As discussed in Section 3.1.9, studies conducted in response to U.S. EPA test requirements for pesticide registration comprise a relatively complete data set for the investigation of the potential for 2,4-D acid, salts, and esters to adversely affect developing fetuses. After extensive review, both the WHO (1996) and U.S. EPA/OPP (2005a, b) concluded that the developmental toxicity of 2,4-D salts, and esters was no different from that of 2,4-D acid. As such, U.S. EPA/OPP (2005a,b) concluded that 2,4-D acid is representative of the class of 2,4-D acid, salts, and esters. As detailed in Appendix 1 and discussed by U.S.EPA/OPP (2005a,b) and the WHO (1996), teratology studies on 2,4-D indicate that malformations are likely to occur only at doses that are maternally toxic. Such doses are at or above the threshold for renal clearance (U.S. EPA/OPP, 2005a). In the rat, developmental toxicity was manifest primarily as an increased incidence of skeletal malformations. In rabbits, developmental toxicity was observed primarily as increased abortions (2,4-D acid) and increased incidence of seventh cervical ribs (DEA salt). Recent studies published in the open literature (Fofana et al. 2000, 2002) demonstrate that 2,4-D exposure during organogenesis can adversely impact kidney and urogentital formation in rats at doses which are maternally toxic (i.e., >50 mg/kg/day; see Appendix 1 for details).

The developmental immunotoxicity study of Lee et al (2001) discussed previously in Section 3.1.7 (Effects on Immune System) suggests that *in utero* exposure to the DMA salt of 2,4-D can cause adverse effects on the developing immune system in mice at doses at or above the threshold for maternal toxicity.

As discussed in Section 3.1.9, there is a single 2-generation reproduction study in rats which meets U.S. EPA/OPP guidelines (U.S. EPA/OPP 2005a,b; Rodwell 1985). In the study, groups of Fischer rats were fed 2,4-D acid (97.5 % pure) in the diet at doses equivalent to 0, 5, 20, or 80 mg/kg body weight/day. The study is summarized both in Appendix 1 and Table 3-2. In summary, 2,4-D exposure at a dose (80 mg/kg/day) above the threshold for maternal toxicity, and hence, renal clearance, caused an increase in the length of gestation in parental generation (F_0) females.

As mentioned previously, U.S. EPA/OPP (2005a) has required registrants to conduct and submit a current 2-generation reproduction study for 2,4-D acid which complies with more current guidelines, and addresses issues pertaining to endocrine disruption and immune toxicity.

4.1.2.2. Birds – The critical toxicity studies on birds, which are reported by U.S. EPA/OPP (2004a,) are summarized in Table 4-1.   Acute oral gavage studies were conducted in mallard ducks and bobwhite quail with 2,4-D acid, DEA, DMA, IPA, and TIPA salts, and the 2-ethylhexyl, BEE, and IPA esters. This risk assessment is concerned only with 2,4-D acid, DMA, TIPA, and BEE. LD_{50} values from these studies range from 404.6 mg a.e./kg bw for the DEA salt in quail, to greater than 3851.2 mg a.e./kg bw for the DMA salt in mallard ducks. 5-Day dietary studies were conducted in mallard ducks and bobwhite quail with 2,4-D acid, DEA, DMA, IPA, and TIPA salts, and BEE, 2-ethylhexyl, and isoppropyl esters. The LC_{50} values from these studies were all greater than the highest concentrations tested. Dietary LC_{50} values range from greater than3035 ppm for the TIPA salt in mallard ducks, to greater than 6000 ppm for the 2-ethylhexyl ester in mallard duck and bobwhite quail.   Based on the results of these studies, U.S. EPA classifies 2,4-D as practically non-toxic to moderately toxic to birds.

A single avian reproduction study was conducted with bobwhite quail and 2,4-D acid (Mitchell et al. 1999). The study yielded a free-standing NOAEC of 962 mg a.e./kg diet on the basis of eggs cracked per eggs laid. No other details are reported by U.S. EPA/OPP (2004b) for this study.

Studies reported in the open literature generally support the notion that birds are less sensitive than mammals with regard to maternal toxicity, developmental, and reproductive effects associated with 2,4-D exposure. No effects were noted on hens' eggs at a dose of 50 ppm injected directly into the egg (Mullison 1981). No reproductive differences were noted between Japanese quail hatched from eggs sprayed with 2,4-D and those hatched from untreated eggs (Hilbig et al. 1976). Laying capacity, fertility, and hatching rate of eggs were measured in this study. Exposure to the butoxyethyl ester of 2,4-D (Esteron 99) at approximately 10 lb a.i./acre caused no adverse effects on White Leghorn chicken eggs (Somers et al. 1978). No effects were noted on laying rates, egg shell thickness, egg or yolk weight, hatching success or viability of offspring when hens were fed 2,4-D at rates of 50 and 150 mg/kg/day (Whitehead and Pettigrew 1972).

Arias (1994) injected a commercial formulation containing 37% of 2,4-D isooctyl ester diluted in acetone into the air spaces of chicken eggs. The chemical identity of the other 63% of the ingredients of the commercial preparation was unknown. The 15 day LD_{50} was 8.6 mg/egg expressed as 2,4-D or 2,4-D ester, the specific identity was not stated. The study also observed changes in the livers of the chicken embryos in the form of vacuoles in the hepatocytes and changes in the bile canaliculi. The changes, however, were not dose related.

4.1.2.3. Terrestrial Invertebrates – As is the case with most herbicides, relatively little information is available on the toxicity of 2,4-D to terrestrial invertebrates. U.S. EPA/OPP (2005a) reports only two direct contact bioassays using the honey bee. One was conducted with the DMA salt (Palmer and Krueger 1997e), and the other was conducted with the 2-ethylhexyl ester (Palmer and Krueger 1997a). There was no mortality and no signs of toxicity at the limit of

the test (100 ug/bee) in either study. On the basis of these results, U.S. EPA/OPP (2004a, 2005a) classifies 2,4-D as practically non-toxic to bees.

There is a study in the open literature which suggests that 2,4-D may be toxic to parasitic wasps. Ozkan and Yanikolu (1999) conducted studies (Table 4-1) with the parasitic hymenopteran *Pimpla turionellae*, which lays its eggs in the pupae of a host moth, where the eggs hatch and continue to live until they transform and emerge as adults.   The first series of studies entailed direct of treatment of host pupae with 2,4-D, and measuring egg hatching rate. 2,4-D at concentrations of 50 ppm and higher resulted in significantly reduced hatchability of eggs in comparison with controls. No eggs survived when pupae were treated with concentrations of 100 ppm and higher. Subsequent studies demonstrated a concentration-dependent decline in egg glycogen content in concert with reduced egg survival. Studies in which parental wasps were treated via exposure to 2,4-D in the diet (honey) demonstrated that 300 ppm 2,4-D in the diet also inhibited survival and hatching of eggs laid in untreated host pupae. No other concentrations were tested; therefore, 300 ppm is a free-standing LOAEC from the dietary exposure study.

2,4-D induced cytochrome P450 in the southern armyworm (*Spodoptera eridania*) and caused synergistic effects on insecticide toxicity (Kao et al. 1995). Exposure to 2,4-D caused decreased carbaryl and permethrin toxicity. Larvae of the wheat sawfly are susceptible to 2,4-D, though it has little effect on either adults or eggs (Gall and Dogger 1967). The 2,4-D product used in this experiment was a combination of the isopropyl ester and butyl ester.

The mortality rate of adult millipedes, *Scytonotus simplex*, exposed to a dose of 0.34 mg a.i./cm^2 (30 lb a.e./acre) of the butoxyethanol ester of 2,4-D [Esteron 99] was much higher than that of the control group which was not exposed to herbicides (Hoy 1985). Mortality (45%) was greatest among millipedes exposed to 2,4-D by contact and through consumption of treated food items. Mortality was also observed at a much lower application rate of 0.034 mg a.i./cm^2 (3 lbsa.e./acre). Slugs took up 2,4-D not only through ingestion of contaminated food, but also through contact with contaminated soil (Haque and Ebing 1983).

The response of earthworms to 2,4-D is variable. Some studies report no measurable effect on earthworm numbers in the field (Potter et al. 1990) or earthworm growth in a microcosm (Gile 1983). On the other hand, Martin (1982) reports that earthworm growth may decrease in response to the presence of 2,4-D in soil.

Mortality of coccinellid (i.e., Lady Beetle) larvae increased 4 fold in all age groups, and the time to pupation increased for all but 1-day-old larvae following exposure to 2,4-D amine (Adams et al. 1986). Larval deformity was more prevalent when larvae were sprayed during later stages of development.   A commercial formulation of 2,4-D and picloram has been implicated in impaired reproductive function in dung beetles (*Canthon cyanellus*) (Martinez et al. 2001).

4.1.2.4. Terrestrial Plants (Macrophytes) – The mechanism of action of 2,4-D in plants is well studied. 2,4-D is an effective herbicide that kills both target and nontarget species. Information relevant to nontarget species is discussed in the following sections.

4.1.2.4.1. Mechanism of Action – 2,4-D is a plant growth regulator and acts as a synthetic auxin or hormone (U.S. EPA/OPP 2005a,b; WHO 1997). 2,4-D alters the plant metabolism and growth characteristics, often causing a proliferation of abnormal growth that interferes with the transport of nutrients throughout the plant.   Plants readily absorb 2,4-D amine through their roots and leaves. The ester forms of 2,4-D are readily absorbed through the leaves. Both the ester and the amine forms of 2,4-D are translocated, usually via the phloem, to the meristematic regions of the plant. Plants rapidly metabolize both the amine and ester forms of 2,4-D to 2,4-D acid by monooxygenases (Benveniste et al. 2005).

Following application, 2,4-D concentrations in foliage decrease initially due to translocation and metabolism. Further decreases of 2,4-D from foliage are slight after the initial period of loss. Detectable traces of 2,4-D remain in evergreen foliage for almost 1 year after application (Newton et al. 1990).   Re-sprouting of vegetation treated the previous year suggests that 2,4-D does not remain at phytotoxic levels for even 1 year (Ghassemi et al. 1981).

As discussed in several reviews and risk assessments (e.g., Sassaman et al. 1984; USDA 1997; U.S. EPA/OPP 2004b, 2005a; WHO 1997), dicotyledonous (broadleaf) plants are more susceptible than monocotyledonous plants (grasses) to 2,4-D. Tolerant plants can metabolize, inactivate, or excrete 2,4-D from their roots. 2,4-D can damage plants on contact, cause abnormalities to existing plant parts, affect new growth, or affect future growth and development. 2,4-D is absorbed through the cuticles of leaves and shoots and is translocated throughout the plant. High application rates cause burning around the site of application due to rapid death of tissue. This effect reduces the movement of 2,4-D from the exposed area to other parts of the plant resulting in only minor injury away from the burned area. 2,4-D slows the growth of some tissues while increasing the growth of other tissues, resulting in twisting or bending of stems, leaves, and petioles. It also causes etiolation or elongation of stems and petioles. New growth is affected when abnormal tissues proliferate at stem and root tips and cambium layers. Leaves that were developing at the time of application appear thickened with prominent veins and distorted margins. Dormant flower buds at the time of application produce abnormal flowers. In some species, 2,4-D can cause flower induction and cause parthenogenetic or seedless fruit to develop from unfertilized flowers. Roots are more sensitive than shoots to 2,4-D; however, few signs of toxicity are reported because they are less obvious. 2,4-D increases the permeability of root membranes that can lead to a loss of nutrients and possibly increase risk of invasion by pathogens.

4.1.2.4.2. Toxicity – The U.S. EPA typically relies on standardized bioassays for seed germination, seedling emergence (pre-emergence applications), and vegetative vigor (post-emergence applications) to assess the potential effects of herbicides on nontarget plants. Such studies were conducted for 2,4-D acid and the various 2,4-D salts and esters.   On the basis of

differences in solubility and toxicity, 2,4-D acid and salts are considered separately from the esters. The studies relevant to ecological risk assessment are summarized in Table 4-2, as reported by U.S. EPA/OPP (2004a.).

On the basis of the existing seedling emergence and vegetative vigor studies, 2,4-D esters are more toxic than 2,4-D acid and salts to terrestrial plants. With regard to seedling emergence and the salts and esters with which the Forest Service is concerned, the greatest toxicity was observed with mustard exposed to the DMA salt of 2,4-D (NOAEC = 0.0093 lb a.e./acre) (Backus and Crosby 1992) and radishes exposed to the 2-ethylhexyl ester (NOAEC = not reported; EC_{25} = 0.045 lb a.e./acre (U.S. EPA/OPP 2004a, MRID 435269-01). With regard to vegetative vigor, the greatest toxicity was observed with onions exposed to 2,4-D acid (NOAEC < 0.0075 lb a.e./acre) (Backus 1992b), and with soybean exposed to the 2-ethylhexyl ester of 2,4-D (NOAEC = 0.0075 lb a.e./acre (U.S. EPA/OPP 2004a, MRID 423439-02). As noted by Fagliari et al. (2005), early stage tomato plants are more sensitive than mature plants to 2,4-D.

4.1.2.5. Terrestrial Microorganisms –Unicellular heterotrophic algae (*Polytoma uvella* and *Polytomella papillata*) respond to increasing concentrations of 2,4-D with decreases in cell numbers, fresh weight, dry weight, and starch content (Pelekis et al. 1987). Changes were observed at 2,4-D concentrations ranging from 10^{-4} to $2 \cdot 10^{-3}$ M. *Prototheca chlorelloides,* another unicellular heterotrophic alga, was not sensitive to 2,4-D and did not exhibit the types of changes observed for the other species.

Algae living in the soil respond to 2,4-D in a similar manner as do the aquatic algae; low concentrations of 2,4-D can be stimulatory; however, high concentrations retard growth and increase mortality. This initial stimulatory effect followed by inhibition is shown by *Chlorella pyrenoidosa*, a green alga found in soil and water. The alga had increased net oxygen uptake and production at 2,4-D concentrations of $1 \cdot 10^{-4}$M; however, at higher concentrations ($1 \cdot 10^{-3}$M) oxygen uptake and production decreased (Bertagnolli and Nadakavukaren 1974).

Concentrations of 2,4-D greater than 1000 ppm significantly reduced the radial growth of three species of ectomycorrhizal fungi (*Cenococcum geophilum, Pisolithus tinctorius,* and *Hebeloma longicaudum*) (Estok et al.1989). 2,4-D at concentrations of 10 ppm had little effect on the growth of *Tricholoma saponaceum, T. pessundatum,* and *Amanita citrina*, three other ectomycorrhizal species; however, it was inhibitory at higher concentrations and completely suppressed growth at concentrations more than 1000 ppm (Ibola1978). As would be expected of a weak acid, the toxicity of 2,4-D to fungi increases with decreasing pH (Cabral et al. 2003).

4.1.3. Aquatic Organisms
4.1.3.1. Fish – Standard toxicity bioassays to assess the effects of 2,4-D on fish are summarized in Tables 4-3 (acute studies from open literature) and Table 4-4 (unpublished studies submitted to the U.S. EPA to support registration). The data in these tables include studies that address only the 2,4-D acid, salts, and esters used by the Forest Service. As with plants and other aquatic organisms, the esters are more toxic than the acid and salts of 2,4-D.

U.S. EPA/OPP (2004a, 2005a) classifies 2,4-D acid and salts as practically non-toxic to fish on the basis of the available acute studies. U.S. EPA/OPP (2004a, 2005a) classifies 2,4-D esters as slightly toxic to highly toxic. Both freshwater and saltwater studies are considered together, as the range of observed toxicity appears to apply equally to species in both freshwater and saltwater environments. On the basis of the EPA-required studies, the most sensitive results were obtained for the rainbow trout, *Oncorhynchus mykiss,* exposed to the TIPA salt (96-hour LC_{50} = 162 mg a.e./L) and the tidewater silverside, *Menidia berrylima*, exposed to the 2-ethylhexyl ester (96-hour LC_{50} = >0.1564 mg a.e./L). The comparable most tolerant results were obtained with the rainbow trout exposed to the DMA salt (96-hour LC_{50} = 830 mg a.e./L) and the 2-ethylhexyl ester (96-hour LC_{50} = 14.5 mg a.e./L). These toxicity values from studies submitted to EPA are comparable to published LC_{50} values for trout (e.g., Martinez-Tabche et al. 2004).

Studies from the open literature (Table 4-3) consistently demonstrate acute toxicity for 2,4-D acid at concentrations lower than those observed in the most sensitive species in the EPA-required studies (Table 4-4). LC_{50} values for 2,4-D acid which are less than the 80.24 mg a.e./L value reported by EPA are also found in the open literature for carp (63.24 mg/L, Sarikaya and Yilmaz 2003), white perch (40 mg/L, Rehwoldt et al. 1977), striped bass (70.1 mg/L, Rehwoldt et al. 1977), lake trout (45 mg/L, Johnson and Finley 1980), carp (5.1 mg/L, Vardia and Durve 1981), cutthroat trout (64 mg/L, Johnson and Finley 1980), and banded killfish (26.7 mg/L, Rehwoldt et al. 1977).

In addition, Vardia and Durve (1981) report six 96-hour LC_{50} values for carp ranging from 5.1 to 35 mg/L. The variability in the Vardia and Durve (1981) study reflects variations in temperature, with toxicity increasing as temperature increases. The 96-hour. LC_{50} at 39°C (102°F) was 5.1 mg/L. At 20°C (68°F) the 96-hour. LC_{50} was 31.25 mg/L. When Rehwoldt et al. (1977) tested carp at 20°C they reported a 96-hour. LC_{50} of 96.5 mg/L. Clearly, the 96-hour. LC_{50} of 5.1 mg/L reported by Vardia and Durve (1981) also involved temperature stress and/or an increasing toxicity of 2,4-D with increasing temperature. As noted in Section 4.1.3.3, similar patterns were observed in studies with aquatic invertebrates (i.e., Sarkar 1991).

Several acute 96-hour NOEL values for the 2-ethylhexyl ester of 2,4-D (also known as the isooctyl ester) are reported in the open literature. These values range from 1 mg/L for Coho and Chum salmon fry (Meehan et al. 1974) to 10 mg/L for rainbow trout (Meehan et al. 1974) and pink salmon fingerlings (Wan et al. 1991).

2,4-D is reported to cause behavioral effects in some fish species. Swimming behavior of green sunfish was affected by the butoxyethanol ester after 60 minutes of exposure to 100 ppm (Sargent et al. 1970). In carp, effects on swimming behavior were pronounced at concentrations not associated with lethality (24 ppm in the study by Sarikaya and Yilmaz 2003). Rainbow trout exposed to a butoxyethanol ester of 2,4-D (Aqua-Kleen) became lethargic and could not avoid capture (Dodson and Mayfield 1979). The rheotropic response of rainbow trout was also modified such that they no longer oriented themselves into the water current. Smaller fish were

the least affected, while larger fish were the first to die. Behavioral effects were noted in bleak larvae exposed to concentrations of the sodium salt of 2,4-D (Biró 1979).

No changes in the reproductive behavior (nest guarding) were observed in red ear sunfish (*Lepomis microlophus*) or bluegills (*Lepomis macrochirus*) exposed to concentrations of up to 11 mg/L of the DMA salt (Bettoli and Clark 1992). 2,4-D did not affect movement patterns of largemouth bass in Guntersville Reservoir, Alabama (Bettoli and Clark 1992).

2,4-D inhibited the secretion of p-aminohippuric acid (PAH) by cell cultures of winter flounder proximal tubules (Dawson and Renfro 1993). This effect is of interest because many potentially toxic anions are secreted into urine in the proximal tubule of the vertebrate kidney. Inhibition of the secretion of PAH, an exogenous anion, indicates that the transport system used in the secretion of toxic anions was also inhibited. 2,4-D was also shown to inhibit glutathione *S*-transferase enzymes, a group of enzymes important in the biotransformation and detoxification of compounds having an electrophilic center (Dierickx 1985). Subsequent studies by Gomez et al (1998, 1999, 2002) demonstrate pathological changes in kidney tissue among tench (*Tinca tinca*) exposed to 2,4-D for 12 days at one-half the experimentally determined LD_{50} concentration for this species (400 mg/L).

U.S. EPA/OPP (2004b) reports the results of early life-stage tests conducted with 2,4-D acid, DEA, DMA, BEE, and EHE. These studies were conducted with the fathead minnow, *Pimphales promelas*. In these studies, the esters were more toxic than 2,4-D acid and salts. For the acid and salts, the NOAEC for survival and reproduction ranged from 14.2 mg a.e./L (DMA salt) to 63.4 mg a.e./L (2,4-D acid). The LOAEC values associated with these results are 23.6 mg a.e./L (length) and >102 mg a.e./L (larval survival), respectively. For the esters, the NOAEC for survival and reproduction ranged from 0.0555 mg a.e./L (BEE) to 0.0792 mg a.e./L (ethylhexyl ester). The LOAEC values associated with these results are 0.0791 mg a.e./L (survival) and >0.1452 (larval survival), respectively.

4.1.3.2. Amphibians and Reptiles – Mortality increased among adult crested newts (*Triturus cristatus carnifex*) exposed to EHE in water (Zaffaroni et al. 1986). Within 3 hours at a concentration of 200 mg/L, all of the test animals were dead. After 72 hours of exposure at concentrations of 100, 125, and 150 mg/L, all animals were dead. Males may be more sensitive than females. All of the males died following 31 days of exposure to 50 mg/L, while none of the females died. Only one animal (male) died following 21 days of exposure to 25 mg/L. Vacuolar degeneration of the liver parenchyma and necrosis of the kidney tubules were observed in organisms that died. Newts at the highest concentrations tested died after a period of paralysis. 2,4-D may inhibit hepatic glutathione-S-transferase, which could impair the detoxification mechanisms for other chemicals; however, more data are needed to test this effect (Zaffaroni et al. 1986).

Toad tadpoles (*Bufo melanostictus*) exhibited behavioral abnormalities and later death following exposure to 2,4-D acid (Vardia et al. 1984). The 96–hour LC_{50} for 2,4-D acid was 8.05 mg/L. In

this species, amphibian eggs were more resistant than larve to pesticides and herbicides. The median survival time was 10.5 hours for the highest 2,4-D concentration tested (11 mg/L). In *Xenopus laevis*, however, a much lower concentration – i.e., 1 µM or 0.221 mg/L – was associated with the disruption of oocyte maturation (Stebbins-Boaz et al. 2004).

U.S. EPA/OPP required registrants to conduct a series of acute toxicity tests on leopard frogs (*Rana pipiens*) with 2,4-D acid, salts, and esters (most and least sensitive results summarized in Table 4-4). The most sensitive result was obtained with the 2-ethylhexyl ester (96-hour LC_{50} = 0.505 mg a.e./L), and the least sensitive result was obtained with 2,4-D acid (96-hour LC_{50} = 359 mg a.e./L).

The mode of action of 2,4-D in amphibians is unclear. As in mammals, 2,4-D does appear to be neurotoxic. Using skin preparations from *Caudiverbera caudiverbera* (the Chilean Wide Mouth Frog), exposures to 2,4-D concentrations ranging from 0.01 to 1 mM (about 2.21 to 221 mg/L) were associated with a decrease in neuroepithelial synapse stimulatory response after electrical stimulation (Suwalsky et al. 1999).

Very little information is available on the toxicity of 2,4-D to reptiles. Crain et al. (1999) report that 2,4-D had no effect on developing alligator embryos. In this study, alligator eggs were treated at stage 21 (i.e., just before the onset of gonadal differentiation) at doses of up to 14 mg/kg by applying 2,4-D acid in 95% ethanol to the surface of the egg. No effects were noted on sexual development, gonadal morphology, or gonadal aromatase activity.

4.1.3.3. Aquatic Invertebrates – The toxicity of various forms of 2,4-D are relatively well characterized in several species of aquatic invertebrates. The U.S. EPA (U.S. EPA/OPP 2004b, p. 232ff) classifies the toxicity 2,4-D acid and salts to aquatic invertebrates as *slightly toxic* to *practically non-toxic*. The esters of 2,4-D are classified as *slightly toxic* to *moderately toxic*.

As with other groups of organisms covered in this risk assessment, the U.S. EPA bases its assessment of 2,4-D on studies submitted in support of the registration of 2,4-D. The critical studies used by U.S. EPA are summarized in Table 4-4.   The most sensitive species in the studies used by U.S. EPA is *Daphnia magna*, a very small aquatic crustacean. Larger species of crustaceans as well as molluscs appear to be less sensitive to 2,4-D.

The most sensitive result reported by U.S.EPA/OPP (2004b) is the 96-hour LC_{50} of 25 mg/L for the water flea, *Daphnia magna,* exposed to 2,4-D acid; while the least sensitive result is a 96-hour LC_{50} of 830 mg a.e./L for the fiddler crab (*Uca pugilator*) exposed to the DMA. With regard to the acute toxicity associated with exposure to esters of 2,4-D, the most sensitive result was obtained with the grass shrimp, *Palaemonetes pagio*, exposed to the 2-ethylhexyl ester (96-hour LC_{50} = 0.092 mg a.e./L) (Ward and Boeri. 1991f). The least sensitive result was obtained with the eastern oyster, *Crassostrea virginica,* exposed to the 2-ethylhexyl ester (96-hour LC_{50} > 66 mg a.e./L) (Ward and Boeri 1991d). Based on a comparison of the most

sensitive species, the esters of 2,4-D are more toxic than the salts by a factor of about 271 [25 mg/L ÷ 0.092 mg a.e./L].

Studies required by U.S. EPA to address the chronic toxicity of 2,4-D to aquatic invertebrates were conducted with the water flea, *Daphnia magna*, and various forms of 2,4-D. These 21-day studies are designed to assess survival and reproduction. The study involving exposure to 2,4-D acid yielded NOAEC and LOAEC values of 79 and 151 mg/L, respectively (Ward and Boeri. 1991c). The LOAEC is based on the number of young. The study involving exposure to the DMA salt of 2,4-D yielded a 21-day LC_{50} of 75.7 mg a.e./L (Ward 1991a). A NOAEC value was not established for this study. As with acute toxicity, the chronic toxicity of 2,4-D esters appears to be greater than that of either 2,4-D acid or 2,4-D salts. As summarized in Table 4-4, the chronic NOAEC for daphnids is 0.2 mg/L (Gerishe et al. 1989), a factor of nearly 400 less than the NOAEC for 2,4-D acid (i.e., 79 mg/L) and the DMA salt of 2,4-D (75.7 mg/L).

Studies in the published literature corroborate the conclusion that the toxicity of 2,4-D esters is greater than that of either 2,4-D acid or salts to aquatic invertebrates. Table 4-5 summarizes published LC_{50} values for the forms of 2,4-D considered in this risk assessment: 2,4-D acid, the DMA salt of 2,4-D, and the BEE and EHE esters of 2,4-D. The studies were identified from the general literature as well as literature summarized in U.S. EPA's ECOTOX database (U.S. EPA/ORD 2006). Some published studies (e.g., George et al. 1982) do not clearly identify the form of 2,4-D, and these studies are not included in Table 4-5. In selecting data for inclusion into Table 4-5, preference was given to 48-hour LC_{50} values, and LC_{50} values for less than 1 day or greater than 4 days were generally excluded. The one exception is a 14-day LC_{50} value for a species of oyster – i.e., 64.2 mg/L from the study by Davis and Hidu (1969). This value was included because it is the only available LC_{50} value in mollusks. Notably, some of the open literature studies summarized in Table 4-5 (e.g., Alexander et al. 1985) contain data that were also submitted to the U.S. EPA. This slight overlap has only a slight impact on the general interpretation of the concordance of the open literature with the studies used by U.S. EPA.

The data in Table 4-5 are illustrated in Figure 4-1 as cumulative probability plots for 2,4-D and the DMA salt of 2,4-D as well as for the BEE and EHE esters of 2,4-D combined. As detailed in Posthuma et al. (2002), cumulative probability plots, also referred to as species sensitivity distributions, are a method of visualizing and analyzing variability in species sensitivity. The plots are constructed by ordering values of equally effective doses (e.g., LC_{50} values) and calculating the cumulative frequencies of these doses. In the current analysis, the cumulative probability plot is used only to visually illustrate the differences between the toxicity of 2,4-D acid and salts, relative to 2,4-D esters.

As illustrated in Figure 4-1, the LC_{50} values available in the open literature are consistent with the assessment by U.S.EPA/OPP (2004b) that 2,4-D acid and salts are less toxic than the esters of 2,4-D. The plots for the acid/salt versus the esters are approximately parallel and indicate that the esters are more toxic than the acid/salts by a factors of about 50-200, somewhat less of a difference than noted above in the studies used by U.S. EPA. While the differences in the

toxicity of the acid/salts and esters of 2,4-D are reasonably consistent, it should be noted that there are no consistent patterns within each group in terms of clear groupings of organisms, and there is substantial variability in reported LC_{50} values within the same species.

Similar to the toxicity of many other pesticides, the toxicity of 2,4-D increases with increasing temperature. In tests on several groups of aquatic invertebrates including zooplankton, snails, oligochaetes, and insect larvae, Sarkar (1991) noted lower LC_{50} values (i.e., higher toxicity) as water temperature increased. As discussed in Section 4.1.3.1, Vardia and Durve (1981) observed a similar pattern in the toxicity of 2,4-D exposure in carp.

4.1.3.4. *Aquatic Plants* – Table 4-6 summarizes the studies required by U.S. EPA regarding the effects of 2,4-D on aquatic plants. 2,4-D is an effective herbicide that adversely affects aquatic and terrestrial plants. Consequently, the U.S. EPA requires a relatively standard group of studies on both unicellular aquatic algae as well as aquatic macrophytes. These studies are typically conducted over a 5-day period under controlled laboratory conditions.

As in studies conducted with aquatic animals, the toxicity of 2,4-D acid and salts is considered separately from that of the esters. Based on the standard bioassays of algal cell growth conducted with 2,4-D acid and salts, the DMA salt produced both the most sensitive and least sensitive result. The most sensitive species appears to be *Navicula pelliculosa* (a freshwater diatom; 5-day EC_{50}: 3.88 mg/L and a corresponding NOAEC of 1.41 mg/L) (Hughes 1990a). The least sensitive species appears to be a freshwater blue-green alga, *Anabaena flos-aquae*, with a 5-Day EC_{50} of 156 mg/L and a corresponding NOAEC of 56.32 mg/L (Hughes 1989). With regard to esters, the most sensitive results were obtained with the 2-ethylhexyl ester. The most sensitive species appears to be the marine diatom, *Skeletonema costatum*, with a 5-day EC_{50} of 0.066 mg a.e./L and a corresponding NOAEC of 0.062 mg a.e./L (Hughes 1990b). The least sensitive species appears to be a freshwater green alga, *Selanastrum capricornutum*, with a 5-Day EC_{50} greater than 19.8 mg a.e./L and a corresponding NOAEC of 2.48 mg a.e./L (Hughes 1990c).

Studies submitted to U.S. EPA regarding the toxicity of 2,4-D to aquatic macrophytes include only duckweed (*Lemna gibba*), a common test species on which the U.S. EPA requires toxicity studies. The relevant studies are summarized in Table 4-6. For 2,4-D acid and salts, the most sensitive result was obtained with 2,4-D acid (EC_{50} of 0.695 mg a.e./L and a NOAEC of 0.0581 mg a.e./L) (Hughes et al. 1997). The least sensitive result was obtained with the TIPA salt (EC_{50} of 1.28 mg a.e./L and a NOAEC of 0.128 mg a.e./L) (Hughes et al. 1994). For esters of 2,4-D, the most sensitive result was obtained with the 2-ethylhexyl ester (EC_{50} of 0.33 mg a.e./L and a NOAEC of 0.062 mg a.e./L) (Hughes 1990d). The least sensitive result was obtained with BEE (EC_{50} of 0.3974 mg a.e./L and a NOAEC of 0.141 mg a.e./L) (Borges et al. 2004).

A greater range of toxicity values for aquatic macrophytes are reported in the open literature. Lower toxicity values are reported for a target species, common water milfoil (*Myriophyllum sibiricum*) in the published study by Roshon et al. (1999) – i.e., 14-day EC_{50} values of 0.018 mg/L based on shoot growth and 0.013 mg/L based on root length. At least some nontarget

species may be much less susceptible to 2,4-D. Sprecher et al. (1998) report no effects on sago pondweed (*Potamogeton pectinatus*) at concentrations of up to 2 mg/L as WEEDAR 64, a formulation of the DMA salt of 2,4-D. Despite the common name of this aquatic macrophyte, sago pondweed is a native species that is important in many aquatic ecosystems (e.g., Kantrud 1990). As discussed by Sprecher et al. (1998), the lesser sensitivity of sago pondweed is probably attributable to its structure – i.e., a narrow leafed monocot. As noted above (Section 4.1.2.4.1), broadleaf plants are more sensitive than narrow leaf plants, such as grasses, to 2,4-D.

4.2. EXPOSURE ASSESSMENT
4.2.1. Overview
Terrestrial animals might be exposed to any applied herbicide from direct spray, the ingestion of contaminated media (vegetation, prey species, or water), grooming activities, or indirect contact with contaminated vegetation. The highest exposures for terrestrial vertebrates will occur after the consumption of contaminated vegetation or contaminated insects. In acute exposure scenarios, doses as high as 113 mg/kg are estimated (the consumption of contaminated insects by a small bird). Other routes of exposure, like the consumption of contaminated water or direct spray, lead to lower levels of exposure. In chronic exposure scenarios, the higher estimated daily doses range from about 7 to 10 mg/kg/day and are associated with highly conservative assumptions (e.g., 100% of the diet is contaminated) regarding the consumption of contaminated vegetation by a large mammal or bird. Less conservative but more plausible exposure assessments lead to much lower dose estimates – i.e., in the range of 0.01-0.2 mg/kg/day.

The primary hazards to non-target terrestrial plants are associated with unintended direct deposition or spray drift. Unintended direct spray will result in an exposure level equivalent to the application rate. At least some plants sprayed directly with 2,4-D at or near the recommended range of application rates will be damaged. Based on the AgDRIFT model, no more than 0.0058 of the application rate is expected to drift 100 meters offsite after low boom ground applications. In order to encompass a wide range of field conditions, GLEAMS simulations were conducted for clay, loam, and sand at annual rainfall rates ranging from 5 to 250 inches. Under arid conditions (i.e., annual rainfall of approximately 10 or fewer inches), there is no or very little runoff. Under these conditions, degradation, not dispersion, accounts for the decrease of 2,4-D concentrations in soil. At higher rainfall rates, plausible offsite movement of 2,4-D results in runoff losses that range from nearly negligible to about 0.5 of the application rate, depending on the amount of rainfall and soil type.

For 2,4-D acid and salts, the potential for effects on aquatic species is based on estimated concentrations of 2,4-D in water that are identical to those used in the human health risk assessment without additional elaboration. For 2,4-D esters, separate GLEAMS simulations were conducted to estimate peak concentrations of 2,4-D esters in water. Consistent with the approach taken by the U.S. EPA in the recent reregistration of 2,4-D, chronic exposures to aquatic organisms are not modeled for 2,4-D esters because the esters of 2,4-D will not persist in the environment. Although the peak concentrations of 2,4-D esters in water are likely to be lower than those of the salts, the separate estimates for 2,4-D esters are necessary for acute exposures because of the higher toxicity of 2,4-D esters to aquatic species.

4.2.2. Terrestrial Animals
Terrestrial animals might be exposed to any applied pesticide from direct spray, the ingestion of contaminated media (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 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.   For dermal exposures to terrestrial animals, the units of measure 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. As in the human health risk assessment, all exposure scenarios for mammals are covered in the Workbook for 2,4-D acid and salts. The rationale for this approach is discussed in Section 3.2.2. Briefly, the salts and esters of 2,4-D are considered to be toxicologically equivalent; therefore exposures to the salts and esters will be comparable at comparable application rates.

The exposure assessments for terrestrial animals are summarized in Worksheet G01 (Attachment 1). The computational details for each exposure assessment presented in this section are provided as scenario-specific worksheets (Worksheets F01 through F16b). Given the large number of species at risk of exposure to pesticides and the varied diets for each of these species, a numerous exposure scenarios could be generated; however, for this generic risk assessment, an attempt was made to limit the number of exposure scenarios.

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, than large animals will receive for a given type of exposure. Consequently, most general exposure scenarios for mammals and birds are based on a small mammal or bird. For mammals, the body weight is taken as 20 grams, typical of mice, and exposure assessments are conducted for direct spray (F01 and F02a), consumption of contaminated fruit (F03a, F04a, F04b), and contaminated water (F05, F06, F07). Generally, herbicide concentrations on grasses will be higher than concentrations on fruits and other types of vegetation (Fletcher et al. 1994). Although, generally, small mammals do not 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 foracute exposures (Worksheet F10) and longer-term exposures (Worksheets F11a and F11b). Other exposure scenarios for mammals involve the consumption of contaminated insects by a small mammal (Worksheet F14a) and the consumption of small mammals contaminated by direct spray by a large mammalian carnivore (Worksheet F16a). Exposure scenarios for birds involve the consumption of contaminated insects by a small bird (Worksheet F14b), the consumption of contaminated fish by a predatory bird (Worksheets F08 and F09), the consumption by a predatory bird of small mammals contaminated by direct spray, and the consumption contaminated grasses by a large bird (F12, F13a, and F13b).

Clearly, the number of exposure assessments that might be generated seems almost limitless; however, the specific, highly conservative, exposure scenarios outlined in this section are designed to identify the groups of organisms and routes of exposure of greatest concern and to serve as guides to more detailed site-specific assessments.

4.2.2.1. Direct Spray – The unintentional direct spray of wildlife during broadcast applications of herbicides is a plausible 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 absorbed depends on the application rate, the surface area of the organism, and the rate of absorption.

For this risk assessment, three groups of direct spray or broadcast exposure assessments are conducted (Worksheets F01, F02a, and F02b). The first spray scenario, which is defined in Worksheet F01, involves a 20 g mammal that is sprayed directly over one half of the body surface as the chemical is being applied. This exposure assessment assumes first-order dermal absorption. The second exposure assessment (detailed in Worksheet F02a), which is highly conservative and likely to overestimate exposure, assumes complete absorption over day 1 of exposure. The assessment is included in an effort to encompass the plausibility of increased exposure due to grooming. The third exposure assessment is developed using the typical body weight of a honey bee, again assuming complete absorption of the compound. There are no exposure assessments for the direct spray of large mammals, principally because allometric relationships dictate that the amounts of a compound to which large mammal will be exposed on the basis of body weight as a result of direct spray is less than amount to which smaller mammals will be exposed.

4.2.2.2. Indirect Contact – As in the human health risk assessment (see Section 3.2.3.3), the only approach for estimating the potential significance of indirect dermal contact is to assume a relationship between the application rate and dislodgeable foliar residue.   Unlike the human health risk assessment in which transfer rates for humans are available, there are no transfer rates available for wildlife species. Wildlife, compared with humans, are likely to spend longer periods of time in contact with contaminated vegetation. It is reasonable to assume that for prolonged exposures an equilibrium may be reached between levels on the skin, rates of absorption, and levels on contaminated vegetation. No data regarding the kinetics of such a process, however, are available. In the absence of such data, no quantitative assessments are made for this scenario in the ecological risk assessment.

4.2.2.3. Ingestion of Contaminated Vegetation or Prey – Since 2,4-D will be applied to vegetation, 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 bird (Worksheet 14a) and a small mammal (Worksheet 14b). As with residues on vegetation and consistent with the approach taken by U.S.

EPA/OPP (2004b), the empirical relationships recommended by Fletcher et al. (1994) are used to estimate residues in contaminated insects (Worksheets F14a and F14b).

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 16a). In addition to the consumption of contaminated vegetation, insects, and other terrestrial prey, 2,4-D may reach ambient water and fish. Thus, a separate exposure scenario is developed for the consumption of contaminated fish by a predatory bird in both acute (Worksheet F08) and chronic (Worksheet F09) exposures. Details of each scenario are given in the cited worksheets.

Since multi-route exposures (e.g., the consumption of contaminated vegetation and contaminated water) are likely, numerous exposure assessments could be developed to account for the various combinations. In the current risk assessment, such assessments are not included because, as illustrated in Worksheet G01, the predominant route of plausible exposure is the consumption of contaminated vegetation by herbivores or the consumption of prey by predators; therefore, explicit considerations of multiple routes of exposure would have no impact on the characterization of risk.

4.2.2.4. Ingestion of Contaminated Water – The methods for estimating 2,4-D concentrations n water are identical to those used in the human health risk assessment (Worksheet B04). The only major differences in the estimates are in regard to the weight of the animal and the amount of water consumed. These differences are detailed and documented in the worksheets regarding the consumption of contaminated water (F05, F06, F07).

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 amount of the spilled solution is taken as 200 gallons for liquid formulations.

In the exposure scenario involving contaminated ponds or streams due to contamination by runoff or percolation, the factors that affect the variability are the water contamination rate, (see Section 3.2.3.4.2) and the application rate.

4.2.3. Terrestrial Plants
In general, 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.

4.2.3.1. Direct Spray – Unintended direct spray will result in an exposure level equivalent to the application rate. For many types of herbicide applications, it is plausible that some 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 (see below).

4.2.3.2. Off-Site Drift – Because off-site drift is more or less a physical process that depends on droplet size and meteorological conditions rather than the specific properties of the herbicide, estimates of off-site drift can be modeled using AgDrift (Teske et al. 2001). AgDrift is a model developed as a joint effort by the U.S. EPA, the Forest Service, and the Spray Drift Task Force, a coalition of pesticide registrants.

For aerial applications, AgDrift permits very detailed modeling of drift based on the chemical and physical properties of the applied product, the configuration of the aircraft, as well as wind speed and temperature. For ground applications, AgDrift provides estimates of drift based solely on distance downwind as well as the types of ground application: low boom spray, high boom spray, and orchard airblast. Representative estimates based on AgDrift (Version 1.16) are given in Worksheets G05a-c for low boom applications and Worksheets G06a-c for aerial applications. For the current risk assessment, the AgDrift estimates are used for consistency with comparable exposure assessments conducted by the U.S. EPA. In addition, AgDrift represents a detailed evaluation of numerous field studies and is likely to provide more reliable estimates of drift (Teske et al. 2001).

While drift of droplets during backpack applications is likely to be less than any form of broadcast application, comparable methods of quantifying drift after backpack applications are not available.

4.2.3.3. Runoff – 2,4-D or any other herbicide may be transported to off-site soil by runoff, sediment loss, or percolation. All of these processes are considered in estimating contamination of ambient water. For assessing off-site soil contamination, however, only runoff and sediment losses are considered. This approach is reasonable because off-site runoff and sediment loss could contaminate the off-site soil surface and could impact nontarget 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 impact off-site terrestrial vegetation.

Based on the results of the GLEAMS modeling (Section 3.2.3.4.3), the proportion of the applied 2,4-D lost by runoff and sediment loss is estimated for clay, loam, and sand at rainfall rates ranging from 5 to 250 inches per year. These values are summarized in Table 4-7 and are used in Worksheets G04a-c to estimate functional off-site exposure rates to nontarget plants that are associated with runoff and sediment losses.

The amount of herbicide 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. GLEAMS outputs 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 2,4-D in clay, loam, and sand modeled using GLEAMS is summarized in Table 4-8. Based on the GLEAMS modeling, 2,4-D may penetrate to about 18 inches in clay. In loam or sand, detectable residues are modeled to occur at 60 inches. Because the GLEAMS modeling used a 60-inch root zone, the actual penetration in loam or sand could be greater than 60 inches.

It should be noted that the actual depth to which 2,4-D could penetrated will depend on a number of site specific conditions that may not be characterized well by the generic GLEAMS modeling used in this risk assessment. Thus, greater or lesser soil penetration could occur in actual field applications of 2,4-D.

4.2.3.4. Contaminated Irrigation Water – Unintended direct exposures of nontarget plant species may occur through the use of contaminated ambient water for irrigation. Effects on nontarget vegetation were observed with irrigation water contaminated by other herbicides (e.g., Bhandary et al. 1997; Gomez de Barreda et al. 1993). The levels of exposure associated with this scenario depend on the concentration of 2,4-D in the ambient water used for irrigation and the amount of irrigation water applied. As detailed in Section 3.2.3.4, concentrations of 2,4-D in ambient water can be quantified.

The amount of irrigation water that may be applied is highly dependent on climate, soil type, topography, and plant species under cultivation. Thus, the selection of an irrigation rate is somewhat arbitrary. Typically, plants require 0.1-0.3 inches of water per day (Delaware Cooperative Extension Service 1999). In the absence of any general approach of determining and expressing the variability of irrigation rates, the application of 1inch of irrigation water is used in this risk assessment. This amount is somewhat higher than the maximum daily irrigation rate for sandy soil (0.75 inches/day) and substantially higher than the maximum daily irrigation rate for clay (0.15 inches/day) (Delaware Cooperative Extension Service 1999).

As discussed by NAS (1989), a major determinant of the amount of irrigation water used is the type of irrigation employed. Thus, flood irrigation uses the highest volume of water per irrigated acre, sprinkler irrigation uses an intermediate volume (further influenced by the type of equipment employed), and drip irrigation uses the least volume of water per acre. Associated with these irrigation methods, flood irrigation generates the greatest volume of irrigation return water (which is likely to be contaminated by salts, fertilizers and pesticides), sprinkler irrigation returns an intermediate volume, and drip irrigation may return very little, if any. Concomitant with the volumes released are also differences in the concentrations of contaminants that enter groundwater and/or surface water. These types of site and condition specific variations in irrigation rates, however, cannot be generally modeled. If irrigation may play a major role at a specific site, other more specific modeling approaches may need to be considered.

Based on the estimated concentrations of 2,4-D in ambient water and an irrigation rate of 1 inch per day, the estimated functional application rate of 2,4-D to the irrigated area is about 4.5×10^{-4} (1×10^{-5} to 2×10^{-2}) lb/acre (Worksheet F15). This level of exposure is comparable to

contamination associated with offsite drift after low boom ground applications [Worksheets G05a-c]. Thus, specific worksheets characterizing risk for this exposure scenario are not developed.

*4.2.3.5. **Wind Erosion*** – Wind erosion is a major transport mechanism for soil (e.g., Winegardner 1996). Although no specific incidents of nontarget damage from wind erosion are reported in the literature for 2,4-D, this mechanism is associated with the environmental transport of other herbicides (Buser 1990).

Wind erosion leading to off-site contamination of pesticides is highly site specific. The amount of 2,4-D that might be transported by wind erosion depends on several factors, including the application, the depth of incorporation into the soil, the persistence in the soil, the wind speed, and the topographical and surface conditions of the soil. Under desirable conditions, like relatively deep (10 cm) soil incorporation, low wind speed, and surface conditions that inhibit wind erosion, it is likely that wind transport of 2,4-D would be neither substantial nor significant.

For this risk assessment, the potential effects of wind erosion are estimated in Worksheets G07a-c. In these worksheets, it is assumed that 2,4-D is incorporated into the top 1 cm of soil. This 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 field studies conducted on agricultural sites that 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 Worksheets G07a-c, the offsite losses are estimated to reach up to about 0.014% of the nominal application rate. Larney et al. (1999), however, report that wind erosion of 2,4-D and other herbicides could be associated with losses up to 1.5% 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 Worksheets G07a-c may be somewhat more realistic for forestry applications, which will not generally be 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 50-100 feet from the application site (G07a-c). All of these estimates, both for wind erosion and offsite drift, are likely to be highly variable based on site and weather conditions.

4.2.4. Soil Organisms
As discussed in Section 3.2.3.4.3, GLEAMS models 2,4-D concentrations in soil as well as estimates off-site movement (runoff, sediment, and percolation). Based on the GLEAMS modeling, concentrations in clay, loam, and sand over a wide range of rainfall rates are summarized in Table 4-9 for the top 60 inches of soil and Table 4-10 for the top 1 foot of soil. Peak soil concentrations in the top 1 foot of soil range from about 0.11 to 0.17 ppm at an application rate of 1 lb/acre.   As rainfall rate increases, maximum and average soil concentrations are substantially reduced in sand and, to a lesser extent, in loam because of losses

from soil through percolation. The potential consequences of such exposures for soil invertebrates and soil microorganisms are discussed in Section 4.4 (Risk Characterization).

4.2.5. Aquatic Organisms

For the application of 2,4-D salts, the plausibility of effects on aquatic species is based on estimated concentrations of 2,4-D in water that are identical to those used in the human health risk assessment. These values are summarized in Table 3-10 and discussed in Section 3.2.3.4.7.

The modeling for 2,4-D acid and salts, however, is not sufficient for assessing exposure to 2,4-D esters. As discussed by U.S. EPA/OPP (2004b), 2,4-D esters are not persistent in surface soils or surface water. Consequently, longer-term exposures to 2,4-D esters will not occur and are not quantitatively considered. Some very important physical and chemical properties of 2,4-D esters, however, are much different from those of 2,4-D acid or the salts of 2,4-D (Table 2-3).   For example, 2,4-D esters have much higher Kow and Koc values, compared with 2,4-D acid or salts. Consequently, 2,4-D esters sorb to soils at a much greater extent, compared with 2,4-D salts, at least for a short period of time until the esters are hydrolyzed to 2,4-D acid and the corresponding alcohol.   This is an important issue in this risk assessment because 2,4-D esters are much more toxic than 2,4-D salts to aquatic organisms. This fact is considered further in Section 4.3.3 (dose-response assessment for aquatic species).

In order to consider the potential effects of 2,4-D esters to aquatic species, GLEAMS modeling was conducted using chemical and physical properties for 2,4-D esters adapted from both the U.S. EPA/OPP (2004b) exposure assessment for 2,4-D esters as well as parameters recommended for the modeling of 2,4-D esters in the documentation for GLEAMS (Knisel and Davis 2000). The specific parameters used in the GLEAMS modeling for 2,4-D esters are summarized in Table 4-11. By comparison to Table 3-5, the corresponding table for 2,4-D acid, the most significant differences are in Koc (100 for esters vs 1.1 for the acid), Kd (0.3 to 3 for esters vs 0.08 to 1 for acid), water solubility (12 mg/L for ester and 569 mg/L for acid), and halftime in water (1 day for the ester and 45 days for the acid).

A summary of the peak concentrations of 2,4-D esters in streams is given in Table 4-12 and a similar summary for ponds is given in Table 4-13. These tables correspond to the tables for 2,4-D acid (Tables 3-6 and 3-7) except that the longer-term average concentrations are omitted in the tables on 2,4-D esters. A comparison of the tables for 2,4-D acid with those for 2,4-D ester, illuminates the difference in peak concentrations for 2,4-D esters, which are uniformly lower than those for the corresponding values of the 2,4-D acid. For example, based on GLEAMS modeling of a pond in clay soil at an annual rainfall of 100 inches, the estimated concentration of 2,4-D in water is 111 ppb for the acid/salt (Table 3-7) but only 32.1 ppb for the 2,4-D ester (Table 4-13) – i.e., about a factor of 3.5 lower for the ester, relative to the salt.

The pattern of lesser peak concentrations of 2,4-D esters in water relative to 2,4-D acid is to be expected from the differences in physical and chemical properties between the salts and esters. The greater lipophilicity of the esters – i.e., the higher values for Koc and Kd – lead to greater

binding to soil and hence a lesser amount of both runoff and percolation. Secondly, the much shorter halftime of the esters relative to the salts corresponds to a more rapid degradation of the esters in water.

For the assessment of peak exposures to aquatic organisms from the runoff of 2,4-D esters into ponds or streams, the water contamination rates used in this risk assessment are 0.004 (0.00001 to 0.2) mg/L. These values are included in the G03 worksheets in the workbook for 2,4-D esters (Attachment 2). The upper range is based the modeled concentration in a streams in clay soil at an annual rainfall rate of 200 inches (Table 4-12). As with the values used in the human health risk assessment, the concentration of 201 ppb given in Table 4-12 is converted to units of mg/L, the unit used for all water concentrations in the worksheets. The central value of 0.004 mg/L (i.e., 4 ppb) is the approximate peak concentration in a pond in clay soil at an annual rainfall rate of 25 inches (i.e., 4.16 ppb in Table 4-13). As with the values used in the exposure assessment for 2,4-D acid, the lower bound is somewhat arbitrary. The value of 0.00001 mg/L (0.01 ppb) is in the range of concentrations for ponds and streams in sandy soils at an annual rainfall rate of 50 inches – i.e., 0.02 ppb in Table 4-12 for the stream and about 0.001 ppb in Table 4-13 for the pond.

For comparison, the U.S. EPA/OPP (2004b, Table 12, p. 62) estimated peak concentrations for 2,4-D esters in the range of 0.6-7.2 ppb (0.0006- 0.0072 mg/L) per lb a.e. applied per acre with most values in the range of about 3 ppb or 0.003 mg/L. Thus, the central estimate used in this risk assessment (0.004 mg/L) is consistent with the estimates derived by U.S. EPA/OPP (2004b). The upper range used in this risk assessment, 0.2 mg/L, is much higher than the concentrations estimated by U.S. EPA. As discussed in Section 3.2.3.4.4, the GLEAMS modeling conducted in Forest Service risk assessment will typically estimate higher upper bounds for concentrations in water because of the extremely conservative assumptions built into the GLEAMS modeling (SERA 2004).

Two other concentrations of peak concentrations of 2,4-D esters in water are used in the workbook for the 2,4-D esters: concentrations after an accidental spill (Worksheet 17a) and concentrations after direct applications of 2,4-D ester formulations to water (Worksheet 17c). The scenario for the accidental spill corresponds directly to the scenario used for formulations of 2,4-D salts (Section 3.2.3.4.1). The scenario for the direct application of 2,4-D ester formulations to water for aquatic weed control is detailed in Section 3.2.3.4.6.

4.3. DOSE-RESPONSE ASSESSMENT
4.3.1. Overview
The specific toxicity values used in this risk assessment are summarized in Table 4-14, and the derivation of each of these values is discussed in the various subsections of this dose-response assessment. The available toxicity data support separate dose-response assessments in eight classes of organisms: terrestrial mammals, birds, terrestrial invertebrates, terrestrial plants, fish, aquatic invertebrates, aquatic algae, and aquatic macrophytes. Different units of exposure are used for different groups of organisms depending on how exposures are likely to occur and how the available toxicity data are expressed. In Forest Service risk assessments, it is customary to derive a range of risks based on the most sensitive and most tolerant species within a given group of organisms (e.g. terrestrial mammals or aquatic plants). The risk assessment for 2,4-D considers the salts and esters of 2,4-D as well as the acid; accordingly, the most sensitive or tolerant species within a given group of organisms exposed to 2,4-D is determined from the response of a specific organism to a specific form of 2,4-D. Hence, the toxicity values selected for ecological risk assessment, shown in Table 4-14, are based on the most sensitive or tolerant result obtained within the class of 2,4-D chemicals under consideration (e.g. salts or esters) as well as the species tested.

For terrestrial animals, the various salts and esters of 2,4-D are assumed to be equal in toxicity to the acid. Based on differences in solubility and toxicity, 2,4-D acid and salts are considered separately from 2,4-D esters for terrestrial plants and aquatic organisms. Based on both acute and chronic dietary toxicity values, mammals appear to be more sensitive than birds to 2,4-Ds. Dogs are more sensitive than other mammals, including humans and rodents, due to their limited capacity to eliminate organic acids. On the basis of acute toxicity, mammals are approximately 14 to more than 300 times more sensitive than birds. On the basis of chronic toxicity, mammals are approximately 15-75 times more sensitive than birds.

For non-canine mammals, the dose-response assessment for acute toxicity is based on the same data as the human health risk assessment (i.e., the acute NOAEL of 25 mg a.e./kg for protection of reproductive age females). The acute NOEL of 1.1 mg a.e./kg from the study of Beasley et al. (1991) is used for assessing acute risks to canines and other sensitive carnivorous mammals. For non-canine mammals, the dose-response assessment for chronic toxicity is also based on the same data as the human health risk assessment (i.e., a chronic NOAEL of 5 mg a.e./kg/day). For canines and other sensitive carnivorous mammals, the dose-response assessment for chronic toxicity is based on the canine NOAEL of 1 mg a.e./kg/day.

An acute NOAEL of 415 mg a.e./kg is selected for birds on the basis of a gavage study with bobwhite quail (Hoxter et al. 1990). Based on a reproduction study, the chronic NOAEL for birds is set at 76 mg a.e./kg/day. There is relatively little information is regarding effects on terrestrial insects exposed to 2,4-D. A contact toxicity value of 1075 mg/kg bw (for honey bees) is taken as a NOAEC for terrestrial invertebrates, although a study published in the open literature indicates that parasitic wasps may be more sensitive to 2,4-D at lower doses.

The toxicity of 2,4-D to terrestrial plants is well characterized. 2,4-D acid, salts, and esters are effective at inhibiting seed germination and are toxic after either direct spray or soil application. Based on the available toxicity studies submitted to the U.S. EPA, 2,4-D acid and salts appear to be equally toxic in both pre-emergent and direct spray applications (i.e., seedling emergence versus vegetative vigor studies). In pre-emergent soil applications (i.e., seedling emergence studies), the NOAEC values for the most sensitive and tolerant combination of species and 2,4-D form are 0.0093 lb a.e./acre and >4.2 lb a.e./acre, respectively. The corresponding values for direct spray (post-emergent bioassays) are 0.0075 and 2.1 lb a.e./acre.

Based on the available EPA-required toxicity studies, 2,4-D esters appear to be more toxic following direct spray than by pre-emergent application. In pre-emergent soil applications (i.e., seedling emergence studies), the NOAEC values for the most sensitive and tolerant combination of species and 2,4-D ester are 0.045 lb a.e./acre and >0.96 lb a.e./acre, respectively. The corresponding values for direct spray (post-emergent bioassays) are 0.0075 lb a.e./acre and >0.96 lb a.e./acre.

2,4-D acid, salts, and esters are toxic to aquatic animals, with esters having greater toxicity than 2,4-D acid and salts. 2,4-D esters convert rapidly to 2,4-D acid; consequently acute toxicity is considered only for aquatic organisms exposed to 2,4-D esters. The chronic toxicity of 2,4-D esters is presumed to be covered by the assessments for chronic toxicity to 2,4-D acid. This approach is in agreement with that taken by U.S. EPA/OPP (2005a). With regard to 2,4-D acid and salts, the acute LC_{50} values for sensitive and tolerant fish vary by a factor of about 9, with a range from 96.5 mg a.e./L (carp, 2,4-D acid) to 830 mg a.e./L (rainbow trout, bluegill, DMA salt). For the esters of 2,4-D, the range of toxicity is a factor of 92.7, about an order of magnitude greater than with the acid/salts of 2,4-D, with 96-hour LC_{50} values ranging from 0.1564 (tidewater silverside) to 14.5 mg a.e./L (rainbow trout). For longer-term exposures to 2,4-D acid and salts, NOEC values range from 14.2 to 63.4 mg a.e./L, based on bioassays in fathead minnows. These differences are relatively modest (i.e., a factor of about 4.5). Based on differences in acute toxicity and the assumption of similar differences in chronic toxicity, chronic NOAEC values ranging from 19 mg a.e./L (approximated for carp) to 63.4 mg a.e./L (fathead minnow) are used to represent the most sensitive and tolerant fish species, respectively, exposed to 2,4-D acid and salts.

A similar pattern in the acute toxicity of 2,4-D to aquatic invertebrates is apparent for salts of 2,4-D (less toxic) and esters of 2,4-D (more toxic). For 2,4-D acid, LC_{50} values for aquatic invertebrates range from 25 mg/L for *Daphnia magna* to 1389 mg/L for crayfish. This factor of about 55 is greater than the variability seen in fish – i.e., a factor of about 9. The increased variability in aquatic invertebrates is even more pronounced for 2,4-D esters with acute LC_{50} values ranging from 0.092 mg a.e./L (grass shrimp, *Palaemonetes pugio*) to greater than 66 mg a.e./L (scud, *Gammarus fasciatus*). The chronic data available on 2,4-D acid and salts are not concordant with the acute data on aquatic invertebrates, and the reason or reasons for the lack of concordance are not apparent. For sensitive invertebrate species, the chronic NOEC value of 16.05 mg/L from a daphnid study involving exposure to the diethanolamine salt of 2,4-D is used

to characterize risk. For tolerant species, the NOEC of 75.7 mg/L from a daphnid study using the DMA salt of 2,4-D is used to characterize risk of longer-term exposures in tolerant species of aquatic invertebrates.

In amphibians, toads exposed to 2,4-D acid yielded the most sensitive result, with a 96-hour LC_{50} of 8.05 mg a.e./L; while leopard frogs exposed to 2,4-D acid yielded the most tolerant result, with a 96-hour LC_{50} of 359 mg/L. These values are used to evaluate acute exposures of amphibians to 2,4-D acid and salts. A single 96-hour LC_{50} of 0.505 mg a.e./L is used to evaluate acute exposures to 2,4-D esters. Data regarding chronic exposure of amphibians to 2,4-D are not available.

The sensitivity of aquatic algae to 2,4-D is equal to that of fish and aquatic invertebrates. Limited testing indicates that 2,4-D acid and salts are less toxic than 2,4-D esters to aquatic algae, as is the case with other aquatic species. In survival and growth studies, the NOAEC values used to assess the toxicity of 2,4-D and its various salts range from 1.41 mg a.e./L (freshwater diatom, *Navicula pelliculosa*, DMA salt) to 56.32 mg a.e./L (blue-green algae, *Anabaena flos aquae*, DMA salt), representing the most sensitive and most tolerant species, respectively. Exposure to 2-ethylhexyl ester yielded the most sensitive and most tolerant result with the corresponding NOAEC values of 0.062 mg a.e./L (marine diatom, *Skeletonema costatum*)and 2.48 mg a.e./L (green algae, *Selanastrum capricornutum*). These values are used to assess acute exposures. As stated previously, chronic exposures are evaluated only for 2,4-D acid and salts. In the absence of long-term studies, the acute values are used to assess longer-term exposures to 2,4-D acid and salts.

Aquatic macrophytes are more sensitive than algae, as demonstrated by duckweed (*Lemna gibba*) exposure studies submitted to U.S. EPA as well as a published study on water milfoil. Studies submitted to the U.S. EPA report NOAEC values ranging from 0.0581 mg/L for 2,4-D acid to 0.128 mg a.e./L for the TIPA salt (most tolerant). For 2,4-D esters, the NOAEC values range from 0.062 mg a.e./L for EHE to 0.141mg a.e./L for BEE. A published study by Roshon et al. (1999) suggests that common water milfoil (*Myriophyllum sibiricum*) is substantially more sensitive than duckweed to the effects of 2,4-D acid – i.e., the EC_{50} for the most sensitive endpoint in water milfoil is reported as 0.013 mg/L and the corresponding EC_{25} is 0.005 mg/L.

4.3.2. Toxicity to Terrestrial Organisms
4.3.2.1. Mammals –As summarized in the dose-response assessment for the human health risk assessment (Section 3.3), U.S. EPA/OPP (2005a,b) derived two acute RfDs and a chronic RfD for the protection of human health, which are based on animals studies.

EPA/OPP derived an acute RfD of 0.025 mg/kg/day for the protection of reproductive-age females and a value of 0.067 mg/kg/day for the protection of the general population. The acute dietary RfD for reproductive-age females is based on a maternal NOAEL of 25 mg/kg/day derived from the developmental toxicity study on 2,4-D acid in rats (Nemec et al. 1983a,b; Table 3-3, Appendix 1). The acute RfD for the general population is based on the acute neurotoxicity

screening study in rats, which yielded a NOAEL of 67 mg/kg/day and a LOAEL of 227 mg/kg/day on the basis of gait abnormalities (Mattsson et al. 1994a; summarized in Table 3-3). As discussed in Section 3.3, the acute dietary RfD of 0.025 is adopted in this risk assessment to evaluate acute human exposures. This assessment also adopts the NOAEL of 25 mg/kg/day, upon which this RfD is based, to assess acute toxicity in non-canine mammals exposed to 2.4-D acid, salts, and esters.

As discussed previously, dogs are more sensitive than other mammals to the toxic effects of 2,4-D, due to their limited capacity to excrete organic acids. Consequently, this assessment adopts a separate acute NOAEL of 1.1 mg a.e./kg to assess risk to canid species. The basis for this NOAEL is the study by Beasley et al. (1991) which reports a NOAEL of 1.3 mg/kg/day for dogs exposed to a single oral dose of DMA-4 (formulated dimethylamine salt of 2,4-D). Sub-clinical evidence of myotonia was observed in dogs exposed to doses of 8.8 mg/kg and higher. Steiss et al (1987) demonstrated similar results in a separate study, with a NOEL of 25 mg/kg, and a LOAEL of 50 mg/kg for myotonia (form of 2,4-D used was not identified in the study). Adjusting the NOAEL of 1.3 mg DMA salt/kg/day from the Beasley et al. (1991) study by an acid equivalence factor of 0.831 (from Table 2-3), yields a NOAEL of 1.1 mg a.e./kg. This value is used to assess acute toxicity to dogs and other sensitive carnivorous mammals exposed to 2,4-D acid, salts and esters.

U.S. EPA/OPP (2005a) derives a chronic RfD of 0.005 mg/kg/day based on a NOAEL of 5 mg/kg/day observed in the chronic toxicity/carcinogenicity study for rats (Jeffries et al. 1995). There is an older chronic RfD of 0.01 mg/kg/day on EPA's IRIS database, but this value does not take into account the currently available database. As discussed in detail in Section 3.3.2, this assessment adopts the RfD of 0.005 mg/kg/day to assess risks of chronic human exposure to 2,4-D acid, salts, and esters. Similarly, this assessment adopts the NOAEL of 5 mg/kg/day upon which this RfD is based, to assess toxicity in non-canine mammals exposed to 2,4-D acid, salts, and esters.

As with acute toxicity, a separate value is used to assess chronic toxicity in canines and other sensitive carnivorous mammals. The chronic value used to assess toxicity to sensitive carnivorous mammals exposed to 2,4-D acid, salts, and esters is the NOAEL of 1 mg a.e./kg/day 2,4-D acid from a chronic dog study (U.S. EPA/OPP 2005b, Dalgard 1993d). The study, which is summarized in Table 3-3, reports a LOAEL of 5 mg/kg/day based on decreased body-weight gain (both sexes) and food consumption (females), as well as alterations in clinical chemistry parameters [increased BUN, creatinine, and alanine aminotransferase, decreased glucose] in both sexes, decreased brain weight in females, and histopathological lesions in liver and kidneys.

4.3.2.2. Birds –Standard laboratory studies on birds are usually conducted with bobwhite quail and mallard ducks. On the basis of acute gavage studies with bobwhite quail and mallard ducks exposed to various 2,4-D acid, salts, and esters, the lowest LD_{50}, 415 mg a.e./kg bw, was obtained in a study with bobwhite quail and DMA salt (Hoxter et al. 1990). While Forest Service risk assessments generally prefer to use acute NOEC values rather than LD_{50} values for risk characterization, neither the RED (U.S. EPA/OPP 2005a) nor the supporting Science Chapter (U.S. EPA/OPP 2004b) specify an NOEC from the Hoxter et al. (1990) studys; moreover, this study is not included in the cleared reviews obtained form U.S. EPA/OPP (Section 1). Consequently, the LD_{50} of 415 mg a.e./kg bw is used characterize risk, and the interpretation of the resulting hazard quotients is discussed further in Section 4.4.2.2.

Reproduction studies are generally used to assess the consequences of longer-term exposures for birds. As discussed previously, a single dietary reproduction study (20 weeks) was conducted with bobwhite quail and 2,4-D acid (Mitchell et al. 2000). No effects on reproduction or parental animals were observed in quail exposed to the highest concentration tested, yielding a dietary NOAEC of 962 ppm on the basis of eggs cracked per eggs laid. Assuming that a quail ingests 0.079 kg diet/kg body weight per day (U.S. EPA 1993a: *Wildlife Exposure Factors Handbook*, page 2-127), the NOAEC of 962 ppm is equivalent to a NOAEL of 76 mg a.e./kg body weight per day ,and this value is used to evaluate chronic avian exposure to 2,4-D acid, salts, and esters..

Studies reported in the open literature generally support the notion that birds are less sensitive than mammals with regard to maternal toxicity, developmental, and reproductive effects associated with 2,4-D exposure. No effects were noted on hens' eggs at a dose of 50 ppm injected directly into the egg (Mullison 1981). No reproductive differences were noted between Japanese quail hatched from eggs sprayed with 2,4-D and those hatched from untreated eggs (Hilbig et al. 1976). Laying capacity, fertility, and hatching rate of eggs were measured in this study. Exposure to the butoxyethyl ester of 2,4-D (Esteron 99) at approximately 10 lb a.i./acre caused no adverse effects on White Leghorn chicken eggs (Somers et al. 1978). No effects were noted on laying rates, egg shell thickness, egg or yolk weight, hatching success or viability of offspring when hens were fed 50 or 150 mg/kg/day of 2,4-D (Whitehead and Pettigrew 1972).

4.3.2.3. Terrestrial Invertebrates – There is little information on the toxicity of 2,4-D to terrestrial insects. This is the case with most herbicides, which are generally presumed to be relatively nontoxic to insects and other invertebrates. Palmer and Krueger (1977e) report an acute contact NOAEL of 100 ug/bee for 2,4-D, which corresponds to a dose of approximately 1075 mg/kg bw, which is used to characterize risks for honey bees. There is a study in the open literature which suggests that 2,4-D may be toxic to parasitic wasps fed 300 ppm in honey(Ozkan and Yanikolu 1999); however, it is not possible to quantify an exposure dose for these insects based on the information provided in the study. Earthworm studies conducted with 2,4-D express exposures in terms of lbs a.e./acre, and this information is considered directly in the risk characterization (Section 4.4).

4.3.2.4. Terrestrial Plants (Macrophytes) – The toxicity of 2,4-D to terrestrial plants is well characterized. 2,4-D acid, salts, and esters are effective at inhibiting seed germination and are toxic after either direct spray or soil application.

Based on the available EPA-required toxicity studies (U.S. EPA/OPP 2004b), 2,4-D acid and salts appear to be equally toxic in both pre-emergent and direct spray applications (i.e. seedling emergence versus vegetative vigor studies). In pre-emergent soil applications (i.e., seedling emergence studies), the NOAEC values for the most sensitive and tolerant combination of species and 2,4-D form are 0.0093 lb a.e./acre (mustard, DMA salt, Backus and Crosby 1992) and greater than 4.2 lb a.e./acre (tomato, 2,4-D acid, Backus 1992a), respectively. The corresponding values for direct spray (post-emergent bioassays) are 0.0075 lb a.e./acre (onion, 2,4-D acid, Backus 1992b) and 2.1 lb a.e./acre (corn, 2,4-D acid, Backus 1992b).

Based on the available EPA-required toxicity studies, 2,4-D esters appear to be more toxic following direct spray than by pre-emergent application. In pre-emergent soil applications (i.e., seedling emergence studies), the NOAEC values for the most sensitive and tolerant combination of species and 2,4-D ester are 0.045 lb a.e./acre (radish EC_{25} used as NOAEC because no NOAEC was established, EHE; Backus 1995) and greater than 0.96 lb a.e./acre (tomato, EHE, Backus 1992a), respectively. The corresponding values for direct spray (post-emergent bioassays) are 0.0075 lb a.e./acre (soybean, EHE, Backus 1992a) and greater than 0.96 lb a.e./acre (corn EHE, Backus 1992b).

In this assessment, 2,4-D acid and salts are treated separately from the esters. To assess the potential consequences of exposures to nontarget plants via runoff or direct soil treatment, the NOAEC values reported above from seedling emergence (pre-emergence application) bioassays are used (Attachment 1, Worksheets G04a-c). To assess the impact of drift (accidental direct spray) on nontarget terrestrial vegetation, the NOAEC values reported above from the post-emergent (vegetative vigor) bioassays are used (Worksheets G05a-c, G06a-c, and G07a-c).

4.3.2.5. Terrestrial Microorganisms – Unicellular heterotrophic algae (*Polytoma uvella* and *Polytomella papillata*) respond to increasing concentrations of 2,4-D with decreases in cell numbers, fresh weight, dry weight, and starch content (Pelekis et al. 1987). Changes were observed at 2,4-D concentrations ranging from 10^{-4} to $2 \cdot 10^{-3}$ M – i.e., from 0.0221 to 0.442 mg/L. *Prototheca chlorelloides,* another unicellular heterotrophic alga, was not sensitive to 2,4-D and did not exhibit the types of changes observed for the other species.

Algae living in the soil respond to 2,4-D in almost the same manner as aquatic algae; low concentrations of 2,4-D can be stimulatory; however, high concentrations retard growth and increase mortality. This initial stimulatory effect followed by inhibition is shown by *Chlorella pyrenoidosa*, a green alga found in soil and water. The alga had increased net oxygen uptake and production at 2,4-D concentrations of $1 \cdot 10^{-4}$M (0.0221 mg/L), while at higher concentrations ($1 \cdot 10^{-3}$M or 0.221 mg/L) oxygen uptake and production decreased (Bertagnolli and Nadakavukaren 1974).

Concentrations of 2,4-D greater than 1000 mg/L significantly reduced the radial growth of three species of ectomycorrhizal fungi (*Cenococcum geophilum, Pisolithus tinctorius,* and *Hebeloma longicaudum*) (Estok et al. 1989). 2,4-D at concentrations of 10 ppm had little effect on the growth of *Tricholoma saponaceum, T. pessundatum,* and *Amanita citrina*, three other ectomycorrhizal species; however, the compound was inhibitory at higher concentrations and completely suppressed growth at concentrations more than 1000 ppm (Ibola1978).

4.3.3. Aquatic Organisms

4.3.3.1. Fish – In studies with 2,4-D acid and salts, the most sensitive result for acute toxicity was obtained with carp exposed to 2,4-D acid, with an LC_{50} of 5.1 mg a.e./L (Vardia and Durve 1981). This bioassay, however, was conducted at 39°C (102°F) and heat stress was probably a major factor in the apparent sensitivity of the carp. The study by Rehwoldt et al. (1977) reports an LC_{50} of 96.5 mg/L in carp for the 2,4-D acid tested at 20°C . These results are more closely aligned with LC_{50} data reported for 2,4-D acid in several other species of fish reported by other authors. The most tolerant result was obtained with rainbow trout (MRID 233350, Vilkas, A.G., 1977, as cited in U.S. EPA/OPP 2004b) and bluegills (MRID 234027, Vilkas, A.G., 1978, as cited in U.S. EPA/OPP 2004b) exposed to the DMA salt, with an LC_{50} of greater than 830 mg a.e./L in both studies. For this risk assessment, the LC_{50} value of 96.5 mg/L in carp (Rehwoldt et al. 1977) is used to characterize risks in sensitive fish species, while the LC_{50} value of 830 mg a.e./L is used to characterize risks in tolerant species of fish.

For esters, the 2-ethylhexyl ester yielded both the most sensitive and most tolerant results, with 96-hour LC_{50} values ranging from 0.1564 in tidewater silverside (Ward and Boeri 1991a), to 14.5 mg a.e./L in rainbow trout (Buccafusco 1976). These values are adopted in this risk assessment to evaluate acute toxicity in fish.

The evaluation of longer-term exposures to 2,4-D acid and salts is complicated by the lack of studies in any species other than fathead minnow. These early life-stage studies, reported by U.S. EPA/OPP (2004b) were conducted for 2,4-D acid, DEA salt, DMA salt, EHE, and BEE. As with other types of toxicity studies, the acid and salts were less toxic than the esters. NOEC values ranged from 14.2 mg a.e./L for the DMA salt (Dill et al. 1990) to 63.4 mg a.e./L (Mayes et al. 1990a) for the acid. NOEC values for the esters were 0.05554 mg a.e./L for BEE (Mayes et al. 1989b) and 0.0792 mg a.e./L for EHE (Mayes et al. 1990b).

It is not possible to use the results from the fathead minnow studies directly to evaluate longer-term exposures to 2,4-D acids and salts among the most sensitive species of fish. Based on acute toxicity, fathead minnows (LC_{50} = 320 mg a.e./L for 2,4-D acid; 318 mg a.e./L for DMA salt; see U.S. EPA/OPP 2004b, Appendix C) are between the most sensitive species, carp (LC_{50} = 96.5 mg a.e./L for 2,4-D acid) (Rehwoldt et al. 1977), and the most tolerant species, rainbow trout and bluegills (LC_{50} greater than 830 mg a.e./L for DMA salt) (U.S. EPA/OPP 2004b). As such it is inappropriate to use the longer- term studies on fathead minnows to assess exposures of sensitive fish species to 2,4-D acids and salts without making some modifications. One way to do this is to use the fathead minnow data to obtain a ratio of acute LC_{50} to chronic NOAEC, then apply this

ratio to the acute LC_{50} for carp to approximate a chronic NOAEC for carp. This is accomplished as follows. Dividing the fathead minnow acute LC_{50} for 2,4-D acid (320 mg a.e./L) by the fathead minnow early life-stage NOAEC for 2,4-D acid (63.4 mg a.e./L), yields a ratio of 5. Dividing the carp LC_{50} of 96.5 mg a.e./L by the acute-chronic ratio of 5 and rounding to two significant figures approximates a longer-sterm NOAEC of 19 mg a.e./L for carp.

Based on the above considerations, NOAEC values ranging from 19 mg a.e./L (approximated for carp) to 63.4 mg a.e./L (fathead minnow) are used to represent the most sensitive and tolerant fish species, respectively, exposed to 2,4-D acid and salts. As stated previously, chronic exposure to 2,4-D esters is not evaluated for aquatic organisms due to the rapid degradation of esters to 2,4-D acid.

4.3.3.2. Amphibians – Both EPA-required studies and studies from the open literature exist from which to assess the toxicity of 2,4-D acids, salts, and esters to amphibians. With regard to acute toxicity, toads (*Bufo melanosticus*) exposed to 2,4-D acid yielded the most sensitive result, with a 96-hour LC_{50} of 8.05 mg a.e./L (Vardia et al. 1984). The most tolerant result among amphibians was observed in leopard frogs (*Rana pipiens*) exposed to 2,4-D acid, with a 96-hour LC_{50} of 359 mg/L (Palmer and Krueger 1997d). These values are used to evaluate acute exposures of amphibians to 2,4-D acid and salts. As stated previously, chronic exposure to 2,4-D esters is not evaluated separately for aquatic organisms due to the rapid degradation of esters to 2,4-D acid.

4.3.3.3. Aquatic Invertebrates – Numerous studies exist from which to assess the toxicity of 2,4-D acids, salts, and esters to aquatic invertebrates. These studies are from both the open literature and from EPA-required testing pursuant to the pesticide registration process. Both freshwater and salt-water species were tested, and the range of sensitivities between saltwater and freshwater species appears to be comparable. Accordingly, the dose-response assessment uses one set of numbers to represent both saltwater and freshwater species.

As discussed in Section 4.1.3.3 and illustrated in Figure 4-1, the most consistent pattern regarding the toxicity of the various forms of 2,4-D to aquatic invertebrates is the distinction between 2,4-D acid and the DMA salt of 2,4-D, relative to the toxicities of the BEE and EHE esters of 2,4-D. For 2,4-D acid, LC_{50} values range from 25 mg/L for *Daphnia magna* (Alexander et al. 1985) to 1389 mg/L for crayfish (Cheah et al. 1980). These values are chosen to represent the range of acute species sensitivities for 2,4-D acid. For the esters, the extreme values for apparent sensitivity are encompassed by the studies submitted to the U.S. EPA (Table 4-4) rather than the studies from the open literature (Table 4-5) with acute LC_{50} values ranging from 0.092 mg a.e./L (grass shrimp, *Palaemonetes pugio*, EHE) (Ward and Boeri 1991f) to greater than 66 mg a.e./L (scud, *Gammarus fasciatus*, BEE) (Mayer and Ellersieck 1986). Thus, consistent with the approach taken in U.S. EPA/OPP (2005a), this range of values is used to assess risks for sensitive and tolerant species of aquatic invertebrates exposed to 2,4-D esters.

As summarized in Table 4-4, the available chronic studies are limited to EPA-required 21-day tests of survival and reproduction conducted with *Daphnia magna* (U.S. EPA/OPP 2005a; U.S. EPA/OPP 2004b). The longer-term NOAEC value for 2,4-D acid is 75.7 mg/L (Ward 1991a). The study is classified by U.S. EPA/OPP (2004b) as *Core*, indicating that it completely satisfies EPA's guidelines for the conduct of a chronic daphnid toxicity study. The study on the DMA salt of 2,4-D (Ward and Boeri 1991c) yielded a longer-term LC_{50} value for survival of 79 mg a.e./L, only somewhat greater than the NOEC value for 2,4-D acid. Note that both of these values are greater than the 25 mg/L acute LC_{50} value for daphnids from the study by Alexander et al. (1985).

As noted above, the 25 mg/L acute LC_{50} value for daphnids is used to characterize acute risks to sensitive species exposed to 2,4-D acid and salts. Therefore, it would not be sensible to use the higher chronic NOEC value of 75.7 mg/L from the study by Ward (1991a) to characterize the effects of longer-term exposures. In addition, the 75.7 mg/L concentration for the NOEC is far too close to the chronic LC_{50} value of 79 mg/L reported by Ward and Boeri (1991c). The reason or reasons for this apparently anomalous data set cannot be clearly defined. One factor may simply be random variability or variability associated with unidentified variables and differences in the available studies. For example, as indicated in Table 4-5, the reported acute LC_{50} values for *Daphnia* and closely related *Ceriodaphnia* range from 25 mg/L (Alexander et al. 1985) to 236 mg/L (Nelson and Roline 1998). As an alternative to the chronic NOEC value of 75.7 mg/L from the study by Ward (1991a), this risk assessment uses the chronic NOEC value of 16.05 mg/L from the chronic daphnid study by Holmes and Peters (1991) and applies the value to sensitive species. Although this study involved the diethanolamine salt of 2,4-D (a form not used in Forest Service programs), the study is classified as *Core* by U.S. EPA (U.S. EPA/OPP 2004b) and results in a chronic NOEC value that is less than the acute NOEC value of 35 mg/L for sensitive species. For tolerant species, the NOEC of 75.7 mg/L from the study by Ward (1991a) is used to characterize the effects of longer-term exposures.

As stated previously, chronic exposure to 2,4-D esters is not evaluated separately for aquatic organisms due to the rapid degradation of esters to 2,4-D acid. It is noted that a much lower NOAEC of 0.2 mg/L for a 2,4-D ester is available (Gerisch et al. 1989). In this study, however, a flow-through system was used to ensure that the daphnids were exposed continuously to the 2,4-D ester over the duration of the study. While there is little doubt that the ester is more toxic under these conditions, the conditions will not occur after the application of the 2,4-D ester in the field. As discussed by U.S. EPA/OPP (2005a, pp. 65-66), the 2,4-D ester will not persist after application. Therefore, consistent with the approach taken by U.S. EPA/OPP (2005a), this risk assessment doess not derive hazard quotients for chronic exposure to2,4-D esters.

4.3.3.4. Aquatic Plants – Limited testing indicates that for aquatic plants, like other aquatic species, 2,4-D acid and salts are less toxic than 2,4-D esters. NOAEC values from EPA-required survival and growth studies are used to assess the toxicity of 2,4-D and its various salts (U.S. EPA 2004b, reported in Appendix C). With regard to 2,4-D acid and salts, the most sensitive result is a NOAEC of 1.41 mg a.e./L (freshwater diatom, *Navicula pelliculosa*, DMA salt)

(Hughes 1990a). The least sensitive result is a NOAEC of 56.32 mg a.e./L (Blue-green algae, *Anabaena flos aquae*, DMA salt) (Hughes 1989). There are no longer-term studies regarding the toxicity of 2,4-D acids and salts to aquatic algae or plants. Consequently, the NOAEC values from the available survival and growth studies are used to evaluate acute and chronic exposures a aquatic algae to 2,4-D acid and salts.

Of the 2,4-D esters tested, 2-ethylhexyl ester yielded the most sensitive and the most tolerant result with the corresponding NOAEC values of 0.062 mg a.e./L (marine diatom, *Skeletonema costatum*) (Hughes 1990b) and 2.48 mg a.e./L (green algae, *Selanastrum capricornutum*) (Hughes 1990c), respectively. These values are used to evaluate acute exposures to 2,4-D esters for the most sensitive and most tolerant aquatic species of algae.

The only studies with which to quantitatively evaluate exposures of aquatic macrophytes to 2,4-D acid, salts, and esters are the EPA-required studies of survival and growth conducted on duckweed, *Lemna gibba* (U.S. EPA 2004a, reported in Appendix C) as well as a published study by Roshon et al. (1999) on water milfoil (*Myriophyllum sibiricum*). These studies suggest that aquatic macrophytes are more sensitive than algae. The range of sensitivities to 2,4-D acid and salts for *Lemna gibba* is represented by NOAEC values of 0.0581 mg/L for 2,4-D acid (most sensitive) (Hughes et al. 1997) and 0.128 mg a.e./L for the TIPA salt (most tolerant) (Hughes et al. 1994). Water milfoil, however, appears to be more sensitive than duckweed.

As discussed in Section 4.1.3.4, the published studies on the toxicity of 2,4-D to aquatic macrophytes cover a broader range than the studies submitted to the U.S. EPA on *Lemna*. In the published study by Roshon et al. (1999), the reported 14-day EC_{50} values are 0.018 mg/L based on shoot growth and 0.013 mg/L based on root length.. This value is about 50 times less than the lowest EC_{50} reported in duckweed – i.e., 0.695 mg/L for 2,4-D acid in the study by Hughes et al. (1997) [0.695 mg/L ÷ 0.013 mg/L = 53.5]. The study by Roshon et al.(1999), however, does not provide an NOAEC, the endpoint preferred by the USDA/Forest Service. As an alternative, the EC_{25} of 0.005 mg/L that is reported by Roshon et al. (1999) is used for sensitive macrophytes. It is recognized that water milfoil is generally classified as an invasive pest species. In the absence of toxicity data on other nontarget aquatic macrophytes, however, the conservative assumption is made that some nontarget plant species may be as sensitive as water milfoil. In the absence of corresponding data on the toxicity 2,4-D esters to water milfoil, this value also is used to characterize the toxicity of 2,4-D esters. For tolerant species, the NOAEC of 2 mg/L in sago pondweed from the study by Sprecher et al. (1998) is used to characterize risk. This approach, however, is confounded by differing exposure conditions. Roshon et al. (1999) exposed milfoil for 14 days, while Sprecher et al. (1998) exposed sago pondweed for 24 hours followed by a 35-day observation period. This difference may account for some the apparent discrepancies in the range of sensitivities. Nonetheless, these studies seem to be the best for considering the potential range of sensitivities of aquatic macrophytes to 2,4-D exposure.

Based on studies submitted to the U.S. EPA on 2,4-D esters, the range of NOAEC values for *Lemna* are bounded by values of 0.062 mg a.e./L for EHE (Hughes 1990d) and 0.141mg a.e./L

for BEE (Hughes 1990e). As with data on 2.4-D acid and salts, this range is much narrower than the range of 0.005 mg/L for common water milfoil (Roshon et al. 1999) to 2 mg/L for sago pondweed (Sprecher et al. 1998). Consequently, the broader range of 0.005-2 mg/L based on studies with 2,4-D salts is used to characterize risks associated with exposure to 2,4-D esters.

4.4. RISK CHARACTERIZATION
4.4.1. Overview
2,4-D acid, salts, and esters have been tested in a variety of organisms. However, by necessity, the available tests represent a limited number of species, and the conditions of the tests may not represent actual conditions of exposure in the wild. These are limitations inherent to any risk characterizations and may result in underestimates or overestimates of actual risk. The methods used in both the exposure and dose-response assessments are intended to consider these uncertainties by using protective assumptions in developing both the exposure and dose-response assessments which form the basis of the risk characterization.

Due to similarities in toxicity, 2,4-D acid, salts, and esters are considered together as a single class for the assessment of terrestrial animals. Based on differences in toxicity and solubility, 2,4-D acid and salts are considered separately from 2,4-D esters for terrestrial plants and aquatic organisms. In general, the esters are more toxic than the acid and salts. However, given that 2,4-D esters degrade rapidly to the acid form, risks for esters are considered only for acute exposure scenarios. Longer-term exposures in this risk assessment are addressed quantitatively only for2,4-D acid/salts, with the assumption that risks due to esters will be no greater than those estimated for 2,4-D acid to which they degrade.

Because 2,4-D is an effective herbicide, unintended effects on nontarget vegetation are plausible. The effective use of 2,4-D is achieved by applying it to target vegetation at a time and in a manner that will minimize effects on nontarget plant species. If applied properly and with care, 2,4-D could have only minor effects on nontarget vegetation. Nonetheless, in the normal course of applying herbicide formulations at rates that are effective in weed control, drift or runoff are likely to cause adverse effects on terrestrial plants.

Damage to aquatic vegetation, particularly aquatic macrophytes, is likely in the event of an accidental spill or in the case of direct application of the ester to control aquatic weeds. Longer-term exposure to concentrations of 2,4-D associated with inadvertent water contamination from runoff could affect sensitive species of macrophytes at the upper range of application rates used in Forest Service programs.

Over the range of 2,4-D acid/salt application rates used in Forest Service programs (0.5 to 4 lb a.e./acre), adverse effects on fish, amphibians, and aquatic invertebrates are likely only in the event of an accidental spill. However, with regard to 2,4-D esters, adverse effects on aquatic animals (fish, invertebrates, amphibians) are plausible in association with runoff (all application rates) and would be expected with direct application for weed control and in cases of relatively large accidental spills.

Over the range of application rates used in Forest Service programs, adverse effects are plausible in mammals that consume contaminated vegetation and insects after 2,4-D is applied at the typical and maximum rates, but at the lower rate. In addition, adverse effects are plausible among carnivorous mammals that consume contaminated small mammals after 2,4-D is applied

at the typical and maximum rates, but not at the lowest anticipated rate. Based on a comparison of NOAEL and LOAEL values, adverse effects would be anticipated in some non-canid mammals, particularly at the highest application rate. There is no indication that substantial numbers of mammals would be subject to lethal exposure to 2,4-D. Consequently, adverse effects such as weight loss and reproductive impairment could occur but might not be readily apparent or easy to detect. Based on reproduction studies, birds appear to more tolerant than mammals to 2,4-D, and no effects appear to be plausible based on the longer-term exposures of birds to 2,4-D.   Adverse effects in birds due to the acute toxicity of 2,4-D is a concern, but the plausibility of adverse in birds is much less compelling than in mammals.

In addition to the direct effects mentioned above, both terrestrial and aquatic animals could be impacted secondarily by the adverse effects of 2,4-D on vegetation. These secondary effects associated with the depletion of vegetation would be likely to vary over time and among different animal species. Certain effects might be detrimental for some species – i.e., a reduction in the supply of preferred food or a degradation of habitat – yet, beneficial to others – i.e., an increase in food or prey availability or an enhancement of habitat.

4.4.2. Terrestrial Organisms
The quantitative risk characterization for mammals and other terrestrial animals is summarized in Worksheets G02a-c of the EXCEL workbook (Attachment 1). These worksheets summarize the hazard quotients for the range of application rates specifically considered in this risk assessment: a typical rate of 1 lb a.e./acre (Worksheet G02a), the lowest anticipated application rate of 0.5 lb a.e./acre (Worksheet G02b), and the highest anticipated application rate of 4 lb a.e./acre (Worksheet G02c). In this and other similar worksheets, risk is characterized as the hazard quotient, the estimated dose (taken from Worksheet G01) divided by the toxicity value. The toxicity values used for each group of animals – mammals, birds, and insects – are summarized in Table 4-14, and the specific toxicity values used for mammals are discussed in Section 4.3.2.1. These toxicity values are repeated in the last column of the worksheets. A hazard quotient of 1 or less indicates that the estimated exposure is less than the toxicity value. The interpretation of the hazard index depends on the nature of the endpoint used. Except for sensitive species of terrestrial plants based on pre-emergence bioassays and acute effects in birds, all toxicity values for terrestrial species are NOAEC values, and a hazard index of less than 1 indicates that there is no basis for asserting that adverse effects are plausible.

4.4.2.1. Mammals – No hazard quotient exceeds the level of concern for terrestrial mammals exposed via scenarios involving the lowest application rate (Worksheet G02b), except for small mammals consuming contaminated insects (hazard quotient = 1.4). For the typical application rate (1 lb a.e./acre), hazard quotients exceed 1 for small mammals consuming contaminated insects (acute exposure; upper bound HQ = 3), large mammals consuming grass (acute exposure; upper bound HQ = 1.9), small mammals consuming grass (acute exposure; upper bound HQ = 1.7), carnivorous mammals consuming small mammals (acute exposure; HQ = 1.9), and large mammals consuming contaminated vegetation onsite (chronic exposure; upper bound HQ = 1.4).

Several hazard quotients for exposure scenarios using the highest anticipated application rate (4 lb a.e./acre) exceed the level of concern (HQ = 1) for a number of scenarios. For acute exposures, these scenarios involve direct spray of small mammals (all HQ values = 4); large mammals consuming contaminated grass (HQs range from 2 to 8); small mammals consuming contaminated grass (HQs range from 2 to 7); small mammals consuming contaminated insects (HQ values range from 4 to 11); and carnivorous mammals consuming small mammals (all HQ values = 8). For longer-term exposure, the upper bound HQ (5) for large mammals consuming contamination onsite exceeds the level of concern.

As withl all of the other groups of organisms covered in this risk assessment, substantial uncertainties are associated with the use of laboratory toxicity studies to characterize potential effects in field populations. The most direct method is based on comparing the NOAEL values on which the HQ values are based to the corresponding LOAEL values.

For non-canid mammals, the acute NOAEL of 25 mg/kg/day is from the developmental study by Nemic et al. (1983a) in which adverse effects (decreased body-weight gains in dams and skeletal abnormalities in offspring) were noted at 75 mg/kg/day. Based on these values, adverse effects could be anticipated at HQ values of 3 or more – i.e., 75 mg/kg/day ÷ 25 mg/kg/day. The potential effects at intermediate HQ values – i.e., >1 to <3 – cannot be characterized. Using this approach, adverse effects could be expected at the typical application rate of 1 lb a.e./acre for small mammals consuming contaminated insects and in several of the scenarios at the highest application rate. The chronic NOAEL of 5 mg/kg/day from the study by Jeffries et al. (1995) is also associated with a LOAEL 75 mg/kg/day at which a number of biochemical endpoints and organ weights were altered (Table 3-3). This ratio of these to values, 15, is very broad, and none of the chronic HQs reach this value. Thus, the potential for observing adverse effects cannot be well characterized.

For canids, the acute NOAEL of 1.3 mg/kg/day is from the toxicity study in dogs by Beasley et al. (1991) in which adverse effects (changes in the behavior of muscles) were noted at 8.8 mg/kg/day. Based on these values, adverse effects could be anticipated at HQ values of about 7 or more – i.e., 8.8 mg/kg/day ÷ 1.3 mg/kg/day. None of the hazard quotients for carnivorous mammals, which would include canids, approach an HQ of 7.

This risk characterization for non-canid mammals is consistent with the risk assessments by the U.S. EPA/OPP (2004b) in which hazard quotients for acute exposure exceed the level of concern for mammals foraging on short and tall grass, broadleaf plants, and small insects, following broadcast spray application of 2,4-D. Chronic exposures involving broadcast spray also exceed levels of concern in small, medium, and large mammals feeding on short grass, broadleaf forage, and insects,(U.S. EPA/OPP 2004b, Appendix F, pp 484 - 521). The U.S. EPA risk assessment does not give risk characterizations for canids, thus no comparison is possible.

As noted in Section 4.1.2.1, the effect of 2,4-D on vegetation may alter habitat, which, in turn, may increase or decrease food availability. These secondary effects are likely to vary over time and among different species of mammals.

4.4.2.2. Birds – Worksheets G02a-c of the EXCEL workbook in Attachment 1 summarize the risk characterization for birds. Based on reasonably comparable toxicity values from reproduction studies in birds and mammals (Table 4-14), birds appear to be substantially less sensitive than mammals to 2,4-D. The reproductive NOAEL for birds is 76 mg/kg/day (Mitchell et al. 1999), a factor of about 15 greater than the corresponding reproductive NOAEL in non-canid mammals – i.e., 5 mg/kg/day from the study by Jeffries et al. (1995). For acute exposures, however, comparable toxicity values are not available and the risk characterization for birds is based on the lowest oral LD_{50} value – i.e., 415 mg/kg bw from the study by Hoxter et al. (1990).

All longer-term HQ valuess for birds are below the level of concern (i.e., 1) even at the highest application rate. Thus, there is no basis for asserting that toxic effects from longer-term exposures to 2,4-D are plausible. For acute exposures, the upper bound of HQ values for birds are 0.1 at the application rate of 0.5 lb a.e./acre, 0.3 at the application rate of 1 lb a.e./acre, and 1.1 at the application rate of 4 lb a.e./acre. All of these hazard quotients are associated with the consumption of contaminated insects. Because these hazard quotients are based on the LD_{50} value rather than an acute NOAEL, the interpretation of the hazard quotient is different. The U.S. EPA (e.g., U.S. EPA/OPP 2005a) adopted the following convention for interpreting HQs based on LD_{50} values in birds and mammals: an HQ of 0.5 or more triggers concern for acute toxic effects and an HQ of 0.1 or more triggers concern for endangered species. Based on this convention, no hazard quotients for birds are of concern at the lowest application rate of 0.5 lb a.e./acre. At the typical application rate of 1 lb a.e./acre, concern for endangered species is triggered. At the highest application rate, the level of concern for acute toxicity is triggered. While the U.S. EPA adopts exposure scenarios different from those used in this risk assessment, this general characterization of risk to birds is consistent with that presented in U.S. EPA/OPP (2005a).

The major reservation with expressing concern for birds involves reliance on the acute gavage LD_{50} value from Hoxter et al. (1990). This is the same value used by U.S. EPA. As noted in U.S. EPA/OPP (2005a, p. 65), *it is likely that the risk estimates associated with the gavage studies overestimate the actual exposure of birds in the field.* In other words, the gavage study involved placing the entire dose of 2,4-D into the stomach of the birds at one time. While in the field, birds will typically be exposed much more gradually as they forage. Thus, while adverse effects due to the acute toxicity of 2,4-D remains a matter of concern, the plausibility of adverse effects in birds is much less compelling than in the risk characterization for mammals.

As with mammals, secondary effects on some species of birds may occur through changes in vegetation that may impact food availability and habitat (Section 4.1.2.2). These effects may be beneficial to some species and detrimental to others, and the magnitude of any effects are likely

to vary over time. In some instances, habitat changes could result in changes at the localized population levels of some bird species.

4.4.2.3. Terrestrial Invertebrates – The studies which address the effects of 2,4-D on terrestrial invertebrates are discussed in Section 4.1.2.3, and involve honey bees, parasitic wasps, millipedes, predacious mites, and earthworms. The large number of terrestrial invertebrate species severely limits the risk characterization.

The study on honey bees is directly useful in estimating hazard quotients for risk characterization. These data, shown in Worksheets G02a through G02c, suggest that honey bees will not be adversely affected by 2,4-D use, even at the highest application rate (i.e., all HQ values are less than 1).

There are several other studies which suggest that 2,4-D can adversely affect survival and growth of terrestrial invertebrate species. GLEAMS modeling suggests that peak soil concentrations of 2,4-D which are likely to result from application at the typical rate of 1 lb a.e./acre are likely to range from 0.002 to 0.174 ppm in the top foot of soil (Table 4-10). Most of the studies that quantify exposure in terms of ppm or lb/acre suggest that adverse effects occur at concentrations or application rates greater than those typically employed by the Forest Service, although adverse effects are plausible in association with the highest anticipated application rate of 4 lb a.e./acre. Adult millipedes, *Scytonotus simplex*, exposed to a dose of 0.34 mg a.i./cm^2 (30 lbs a.e./acre) of the butoxyethanol ester of 2,4-D [Esteron 99] had much higher mortality than the control group that was not exposed to herbicides (Hoy 1985). The greatest mortality (45%) was observed when millipedes were exposed to 2,4-D by contact and through consuming treated food items. Mortality was also observed at a much lower application rate of 0.034 mg a.i./cm^2 (3 lb a.e./acre).

In addition to the above considerations, 2,4-D may have effects on nontarget vegetation that result in secondary effects on terrestrial invertebrates. The extent with which such effects would be regarded as beneficial or detrimental is speculative. No field studies to determine whether changes in the distribution of soil invertebrates occurs following 2,4-D use are available. See Section 4.4.2.5 for a discussion of the impacts of 2,4-D exposure on soil microorganisms.

4.4.2.4. Terrestrial Plants – A quantitative summary of the risk characterization for terrestrial plants is presented in Worksheets G04a-c for runoff, Worksheets G05a-c for drift after low boom ground applications, Worksheets G06a-c for drift after aerial applications, and Worksheets G07a-c for off-site contamination due to wind erosion. As with the worksheets for terrestrial animals, the a-c designations represent groups of three worksheets for the typical application rate (a), the lowest anticipated application rate (b), and the highest anticipated application rate (c). Also analogous to the approach taken for terrestrial animals, risk in these worksheets is characterized as a ratio of the estimated exposure to a benchmark exposure (i.e., exposure associated with a defined response). The benchmark exposure is a NOAEC, as derived in Section 4.3.2.4, for both sensitive and tolerant species. The hazard quotients for 2,4-D acid and salts are presented in Attachment 1. The hazard quotients for 2,4-D esters are presented in

Attachment 2. Estimates associated with runoff are generated only for 2,4-D acid/salts, given that esters rapidly degrade to the acid form in water.

2,4-D is an effective herbicide and adverse effects on some nontarget plant species due to direct application or drift are likely. 2,4-D acid, salts, and esters yield identical patterns of toxicity in terms of hazard quotients associated with application method and distance from site of application. This is true because the NOAEC values for the most sensitive species are identical for 2,4-D acid/salts and 2,4-D esters, and within a factor of two for the tolerant species. Direct spray or application at the lowest anticipated (0.5 lb a.e./acre) and typical application rates (1 lb a.e./acre) is only likely to damage sensitive plant species. Direct application at the highest anticipated application rate is likely to affect both sensitive and tolerant species. Spray drift will affect sensitive species within 100 feet for ground application, and up to 300 feet for aerial application. Again, this observation applies to both acid/salt and ester forms of 2,4-D. Tolerant species are not affected beyond the site of application for either aerial or ground application methods, at any application rate.

Whether or not damage due to drift would actually be observed after the application of 2,4-D depends on a several site-specific conditions, including wind speed and foliar interception by the target vegetation. In other words, in applications conducted at low wind speeds and under conditions in which vegetation at or immediately adjacent to the application site would limit off-site drift, damage due to drift could be inconsequential or limited to the area immediately adjacent to the application site.

Thus, all of these risk characterizations for drift should be viewed as only a crude approximation of the potential for damage during any actual application. AgDrift is a highly parameterized model, and the output of the model is sensitive to a number of site-specific and application specific variables – e.g., wind speed, type of aircraft, and elevation at which the pesticide is released. It is not feasible and would not be particularly useful to elaborate a large number of different drift scenarios based on the many variables subject to modification. The generic drift modeling presented in Worksheets G05a-c and Worksheets G06a-c suggests that efforts should be made to minimize drift.

In contrast to drift that could occur during application, relatively conservative estimates of pesticide transport by wind erosion of soil (Worksheets G07a-c, Attachment 1 for 2,4-D acid/salts; Attachment 2 for esters) suggest that wind erosion is not likely to result in exposures of concern. At the highest application rate for any form of 2,4-D (Worksheet G07c), the upper bound of the hazard quotient for the most sensitive species is only 0.07.

As summarized in Worksheet G04a-c, in Attachment 1, the off-site transport of 2,4-D by runoff and sediment losses could cause substantial damage to sensitive, but not tolerant, species under conditions that favor runoff and sediment loss – i.e., high rainfall rates and clay or loam soil. Based on the generic GLEAMS modeling for off-site pesticide losses (Table 4-7), adverse effects in sensitive species could be expected at the lowest and typical application rates in clay soils in

regions receiving more than 15 inches of rainfall annually. Adverse effects in sensitive species could be expected at the lowest anticipated application rate in regions receiving greater than 25 inches of rainfall annually. Adverse effects could be expected when the highest anticipated application rate is employed in areas with loam soils receiving greater than 100 inches of rainfall annually. In predominantly sandy soils, the major transport mechanism is percolation into the soil with very little risk of off-site loss due to runoff or sediment loss. As with AgDrift, GLEAMS is a highly parameterized model designed for site-specific assessments (Knisel and Davis 2000; SERA 2004b). The use of the generic modeling in the current risk assessment is simply to illustrate factors that could be considered in assessing the potential for significant off-site movement. For 2,4-D, the potential appears to be high, particularly for predominantly clay and loam soils.

This risk characterization is reasonably consistent with the risk characterization given by U.S. EPA/OPP (2004b), though the numerical estimates of risk differ between this assessment and U.S. EPA's, due to differences in toxicological endpoints (i.e., EC_{25} values rather than NOEC values) and modeling scenarios. U.S. EPA/OPP (2004b, pp. 99-100, Table 33 for single spray application; Table 34 for multiple spray applications) hazard quotients range from less than 1 to over 936. The hazard quotients estimated in the worksheets for the current Forest Service risk assessment range from less than 1 to 533.

In summary, this assessment and U.S. EPA (2004a,b; 2005a) conclude that nontarget plant species could be adversely affected by the runoff, sediment loss, or off-site drift of 2,4-D under a variety of different scenarios depending on local site-specific conditions that cannot be generically modeled. If 2,4-D is applied in proximity to sensitive crops or other desirable sensitive plant species, site-specific conditions and anticipated weather patterns need to be considered ,if unintended damage is to be avoided.

4.4.2.5. Soil Microorganisms – As discussed in Section 4.3.2.5, there are several studies regarding the toxicity of 2,4-D to soil bacteria and fungi. The information in provided in the studies is not useful for making definitive conclusions about the toxicity of 2,4-D to soil microorganisms; however, it suggests that when applied at rates at or above those typically used by the Forest Service, 2,4-D could have an at least a transient impact on algae living in the soil. This conclusion is based on reported inhibition of oxygen uptake in *Chlorella pyrenoidosa* at a concentration of 0.221 ppm (Bertagnolli and Nadakavukaren 1974). As noted in Table 4-10, peak concentrations of 2,4-D in the top 12 feet of soil are about 0.17 ppm. Concentrations of 2,4-D at shallower depths are likely to exceed 0.2 ppm at least for a short time.

Effects on other soil microorganisms seem less likely based on the studies by Ibola (1978) – i.e., an LOAEC of 1000 ppm – and Estok et al.(1989) – i.e., an NOEC of 10 ppm. Soil concentrations in this range are not likely to occur; however, if they did occur, they would be maintained in soil for a very brief period of time.

4.4.3. Aquatic Organisms

The risk characterization for aquatic organisms is presented in Worksheets G03a, G03b, and G03c, in Attachment 1 for typical (1 lb a.e./acre), lower (0.5 lb a.e./acre) and maximum anticipated (4 lb a.e./acre) application rates, respectively. Risks to both tolerant and sensitive species are presented where appropriate toxicity data are available (discussed in Section 4.3.3.1). Both acute and chronic risks associated with exposure to 2,4-D acid/salts are presented in Attachment 1. As discussed previously, only acute risks are estimated for 2,4-D esters, due to the rapid degradation of esters to the acid form. Exposure to 2,4-D esters also considers a scenario where a formulation like Aqua-Kleen is applied intentionally and directly to the water to eliminate unwanted aquatic plant species like milfoil. The application rates assumed in the latter scenario range from 19 lb a.e./acre (lower and central estimates) to 38 lb a.e./acre (Attachment 2, Worksheet F17c). The estimated risks for 2,4-D esters are shown in Worksheets G03a through c in Attachment 2. For most aquatic species, the HQ values associated with acute exposures are based on LC_{50} values and risk characterizations for such HQ values vary among the different groups of organisms considered in this risk assessment.

In general, this risk assessment is in agreement with U.S. EPA (2005a, 2004b) although somewhat different approaches are taken to modeling and assessing risk. U.S. EPA (2005a, 2004b) is concerned primarily with the effects of 2,4-D application to bodies of water for weed control. In their assessment, U.S. EPA (2005a, 2004b) start with an application rate, then using the results of field dissipation studies, calculate half-lives, and the concentrations of 2,4-D acid that would result at various time points, for the various salts and esters that could be used. Then using applicable toxicity data, (LC_{50}, EC_{50} and NOAEC values), U.S. EPA estimates the water concentrations needed in order to arrive at a hazard quotient that is below pre-determined levels of concern. Following such an analysis, U.S. EPA (2004b, p 72) states:

"The results of the risk assessment suggest potential concern for aquatic animals and plants primarily for the direct application of 2,4-D and 2,4-D BEE (no other 2,4-D esters are used) to water for aquatic weed control. In addition, there is also the same potential concern for the 2,4-D acid and amine salts for rice use. Potential risk concerns for aquatic animals and plants arise from the fact that available data indicate that the toxicity of the esters is in some cases more than two orders of magnitude more toxic than the amine salts. These toxicity levels combined with screening level exposure values result in LOC [level of concern] exceedances."

4.4.3.1. Fish – The risk characterization for fish differs markedly between the applications of the DMA salt of 2,4-D and the application of 2,4-D esters. This difference is due almost exclusively to the higher acute toxicity of 2,4-D esters (acute toxicity value of 0.15 mg/L) compared with the acute toxicity value used for the DMA salt formulations of 2,4-D (acute toxicity value of 95.6 mg/L). The risk characterization for fish is also influenced by the use of 2,4-D ester formulations for the control of aquatic vegetation. While the concentrations of both the salt and ester forms of 2,4-D are higher in an accidental spill scenario (up to about 18 mg/L at 1 lb/acre) than in a direct application (up to about 2 mg/L at 1 lb/acre), the direct application

scenario is a planned and relatively predictable event – i.e., it will occur as a consequence of program activities – rather than a relatively arbitrary scenario for an event that may not occur.

For the DMA salt of 2,4-D, the risk characterization for fish is simple and unambiguous: there is no basis for suggesting that adverse effects are plausible even at the highest application rate under normal conditions of exposure – i.e., the highest HQ is 0.02. In the accidental spill scenario, the highest HQ value is 0.1 at 0.5 lb a.e./acre, 0.2 at 1 lb a.e./acre, and 0.8 at 4 lb a.e./acre. As with the acute HQ values for birds, the acute HQ values for fish are based on LC_{50} values rather than NOEC values. Similar to the interpretation of HQ values for birds, the U.S. EPA uses standard HQ trigger values: 0.05 for endangered species and 0.5 for acute toxicity. Based on these conventions, concern for acute toxicity is triggered only in the case of an accidental spill at the highest application rate. Concern for endangered species is triggered only for accidental spills across the range of application rates. For non-accidental exposures, none of the levels of concern are triggered.

A very different risk characterization is given for the esters of 2,4-D. Based on peak exposures anticipated in the normal use of 2,4-D esters, hazard quotients based on upper bound estimates of exposure exceed unity for sensitive species of fish at both the typical and at the highest application rate. Concern for acute toxicity (i.e., an HQ greater than 0.5) is triggered across the range of application rates – i.e., an upper bound HQ of 0.6 at an application rate of 0.5 lb a.e./acre.

In the direct application of 2,4-D esters to ponds for aquatic weed control, the HQ values range from 7 to 14 for sensitive fish species. The HQ is based on the LC_{50} value, which indicates that the expected concentrations are in excess of the LC_{50} by factors of 7-14. For such exposures, mortality, and perhaps substantial mortality, would be expected in sensitive fish species. For accidental spills, the HQ values for sensitive fish range from 3 (the lower range at an application rate of 0.5 lb a.e./acre) to 465 (the lower range at an application rate of 5 lb a.e./acre). Again, adverse effects including substantial mortality would be expected. As a comparison, U.S. EPA (2004b) estimates risk quotients (same as hazard quotient) that range from 9.3 to 43.5 for the butoxyethyl ester (BEE) following a direct application scenario.

It should be noted that secondary effects on fish could be associated with damage to aquatic invertebrates and vegetation (Sections 4.4.3.3 and 4.4.3.4). The nature of these effects could be beneficial or detrimental and could be vary over time and probably among different species of fish.

U.S. EPA (U.S. EPA/OPP 2004b) estimates that the target concentration for acute exposure needs to be reduced from an assumed value of 4 mg a.e./L to a value 0.430 mg a.e./L to reduce acute risks to levels below the levels of concern. Similarly, the long-term estimated exposure concentration (2.610 mg a..e./L) would need to be reduced to below 0.060 mg a.e./L to reduce hazard quotients to levels below the levels of concern. This Forest Service risk assessment estimates longer-term water concentrations on the basis of modeled runoff, which are well below

EPA's estimate of 0.06 mg/L (longer-term EEC in association with 4 lb a.e./acre application rate is 0.033 mg a.e./L). In terms of direct application of an ester, the estimated EEC values in this assessment (1.06-2.13 mg a.e./L) are higher than the 0.430 mg/L concentration derived by EPA to reduce risks to levels below the levels of concern. This matter is consistent with the finding in this risk assessment that direct applications of 2,4-D esters to water may cause adverse effects on fish.

4.4.3.2. Amphibians –This assessment evaluates only acute exposures for amphibians since no chronic data are available. As with fish, the ester forms of 2,4-D are much more toxic than the acid or salts. For both forms, the HQ values are based on LC_{50} values rather than NOEC values. For such HQ valules, the U.S. EPA uses triggers for concern that are identical to those for fish: : 0.05 for endangered species and 0.5 for acute toxicity. For 2,4-D acid and salts, the HQ values associated with non-accidental exposures are below the trigger values except for the upper bound of the HQ for sensitive amphibian species at an application rate of 4 lb a.e./acre – i.e., an HQ of 0.2 which triggers concern for endangered species which may be sensitive to 2,4-D acid and salts. For an accidental spill, the LOC for endangered species is triggered across the range of scenarios for sensitive species – i.e., HQ values of 0.06-9 – and it is also triggered at the upper range of exposures from an accidental spill for tolerant species at an application rate of 1 lb a.e./acre (HQ = 0.05) and 4 lb a.e./acre (HQ 0.2). Based on the trigger for acute toxicity in non-endangered species (i.e., a trigger of 0.5), accidental spills are of concern only for sensitive species.

For the 2,4-D esters, the toxicity data used to characterize risks are based only on one species, leopard frogs (Table 4-14). Thus, no distinction is made between sensitive and tolerant species. Based on peak concentrations anticipated in the normal use of 2,4-D esters, the trigger for concern with endangered species (LOC = 0.05) is exceeded across the range of applications at the upper bound of plausible exposures – i.e., upper bound HQ values of 0.2, 0.4, and 1.6 at application rates of 0.5 lb a.e./acre, 1 lb a.e./acre, and 4 lb a.e./acre, respectively. For direct application to water for the control of aquatic weeds, the risk quotients range from 2 to 4 – i.e., factors of 2-4 above the LC_{50} value. These exposures could result in mortality that could be observed in the field. In the case of an accidental spill, the HQ values range from 0.9 (the lower bound at an application rate of 0.5 lb a.e./acre) to 144 (the upper bound at an application rate of 4 lb a.e./acre). Observable mortality in amphibians is plausible at the lower bound of this range and expected at the upper bound.

4.4.3.3. Aquatic Invertebrates – The pattern of risks to aquatic invertebrates is similar to that of fish. As with fish and amphibians, the acute hazard quotients are based on LC_{50} values rather than NOEC values and triggers for concern are 0.05 for endangered species and 0.5 for acute toxicity, following the convention of U.S. EPA.

With regard to 2,4-D acid and salts, no levels of concern are triggered for acute toxicity based on peak or longer-term concentrations – i.e., a hazard quotients are less than 0.5. At the highest application rate of 4 lb a.e./acre, an upper bound HQ of 0.07 is marginally higher than the trigger

of 0.05 for endangered species. Accidental spills trigger concern for endangered species at the lowest application rate (an upper bound HQ of 0.4) and the level of concern for toxicity is triggered by accidental spills at application rates of 1 lb a.e./acre (an upper bound HQ of 0.7) and 4 lb a.e./acre (an upper bound HQ of 3).

With regard to 2,4-D esters, hazard quotients are elevated for sensitive species for every scenario evaluated. For tolerant species, levels of concern are triggered only for an accidental spill. For the scenario involving direct application to water, the upper bound hazard quotient is 23 for sensitive species. These HQ values suggest that adverse effects are plausible for sensitive but not tolerant invertebrate species.

Many ecologically important aquatic invertebrates are primary consumers of aquatic vegetation. It is virtually certain that effects on aquatic vegetation (Section 4.4.3.4) would enhance the detrimental effects on aquatic invertebrates anticipated in association with direct toxicity.

U.S. EPA/OPP (2004b) concludes: *"The potential risks to aquatic invertebrates are similar to the risks to fish for the use of 2,4-BEE for aquatic weed control, but due to the lower toxicity values RQs are much lower. The acute and chronic levels of concern are exceeded for freshwater invertebrates for aquatic weed control."* U.S. EPA/OPP (2004b) concludes further that the target water concentration needs to be 1.250 mg/L or lower for endangered species of invertebrates to be adequately protected from adverse effects associated with 2,4-D. As noted in the previous section on fish (4.4.3.1), the present assessment assumes modeled water concentrations for the accidental spill and direct application scenarios that exceed this value. U.S. EPA/OPP (2004b) also notes that sediment dwelling organisms are likely to be adversely affected by exposure to BEE, and requires that additional testing be conducted to evaluate this potential risk.

4.4.3.4. Aquatic Plants –As with other species, 2,4-D acid and salts are less toxic than 2,4-D esters on the basis of limited testing. Aquatic macrophytes appear to be more sensitive than algae to both acid/salts and esters.

On the basis of hazard quotients shown in Worksheets G03a-c in Attachments 1 (2,4-D acid/salts) and 2 (2,4-D esters), an accidental spill is virtually certain to cause damage to both sensitive algae and sensitive macrophytes. For sensitive algae exposed to 2,4-D acid/salts, upper bound hazard quotients range from 6, at the lowest application rate (1 lb a.e./acre), to 32 at the highest anticipated application rate (4 lb a.e./acre). Tolerant species, such as blue-green algae, *Anabaena flos aquae*, are not likely to be affected from exposure due to an accidental spill of 2,4-D acid/salts, given that all hazard quotients are less than 1. For sensitive algae exposed to 2,4-D esters via an accidental spill, upper bound hazard quotients range from 147 to 1172 in association with the lowest and highest anticipated application rates, respectively. Tolerant species are also likely to be affected following an accidental spill of esters, with hazard quotients ranging from 4 to 29. Notably, the toxicity value for sensitive algae is based on the marine diatom, *Skeletonema costatum*. Spills of 2,4-D into ocean water are not plausible in Forest

Service programs. Nonetheless, as an admittedly conservative assumption, Forest Service risk assessments use data from the most sensitive species – marine or freshwater – unless there is a compelling reason to do otherwise.

For aquatic macrophytes, spill scenarios yield upper bound hazard quotients well in excess of 1000. In non-accidental longer-term exposure scenarios, risks to sensitive aquatic macrophytes could occur at or near the upper range of the application rate – i.e., a hazard quotient of 3 at an application rate of 4 lb a.e./acre.

This risk characterization is qualitatively consistent with that of U.S. EPA/OPP (2004b, p. 76) which concludes:

> *"Using the most toxic definitive aquatic plant study available among the 2,4-D acid and amine salts for each class of aquatic plants it was concluded that aquatic vascular plant endangered species LOCs [levels of concern] are only exceeded from terrestrial use on pasture and apples.*

> *"For the 2,4-D EHE the results from the ester drift analysis scenario, the acute and endangered species LOCs are not exceeded for any of the scenarios. Additionally, the acute and endangered species levels of concern were not exceeded for the IPE which is only registered for use on citrus."*

> *"The direct application to water for weed control for the acid and amine salts indicates potential risk to aquatic vascular plants. These RQs range from 13.33 for acute risk to 83.33 for endangered species risk. These potential risks appear to be due to the high sensitivity and toxicity of the aquatic vascular plants.*"

4.5. CONNECTED ACTIONS AND CUMULATIVE EFFECTS

Under NEPA, the Forest Service is required to consider the potential connected actions and cumulative effects associated with the use of 2,4-D. Connected actions related to potential impacts on risks to ecological receptors would include the presence of inerts, adjuvants, impurities, and metabolites in 2,4-D formulations as well as cumulative effects associated with repeated applications. The potential impacts of metabolites and impurities was discussed previously in this document. The risks presented here take into account the presence of these compounds. The cumulative effects on risks to ecological receptors associated with the use of 2,4-D could include:

1. risks associated with drift from other herbicides used by others (not the Forest Service),

2. physical activities such as mowing, or "acts of nature" such as drought or flooding, which could act in concert with 2,4-D to alter the growth and survival of nontarget plant and animal species,

3.   cumulative risk of repeated 2,4-D application (considered in this assessment as chronic exposure).

The extent to which connected actions or cumulative effects will have an impact the risk characterization is highly dependent on site-specific and application-specific considerations that cannot be well-encompassed in a generic risk assessment. These factors, however, can be further considered in Environmental Assessments conducted for specific applications of 2,4-D.

5. LITERATURE SEARCH

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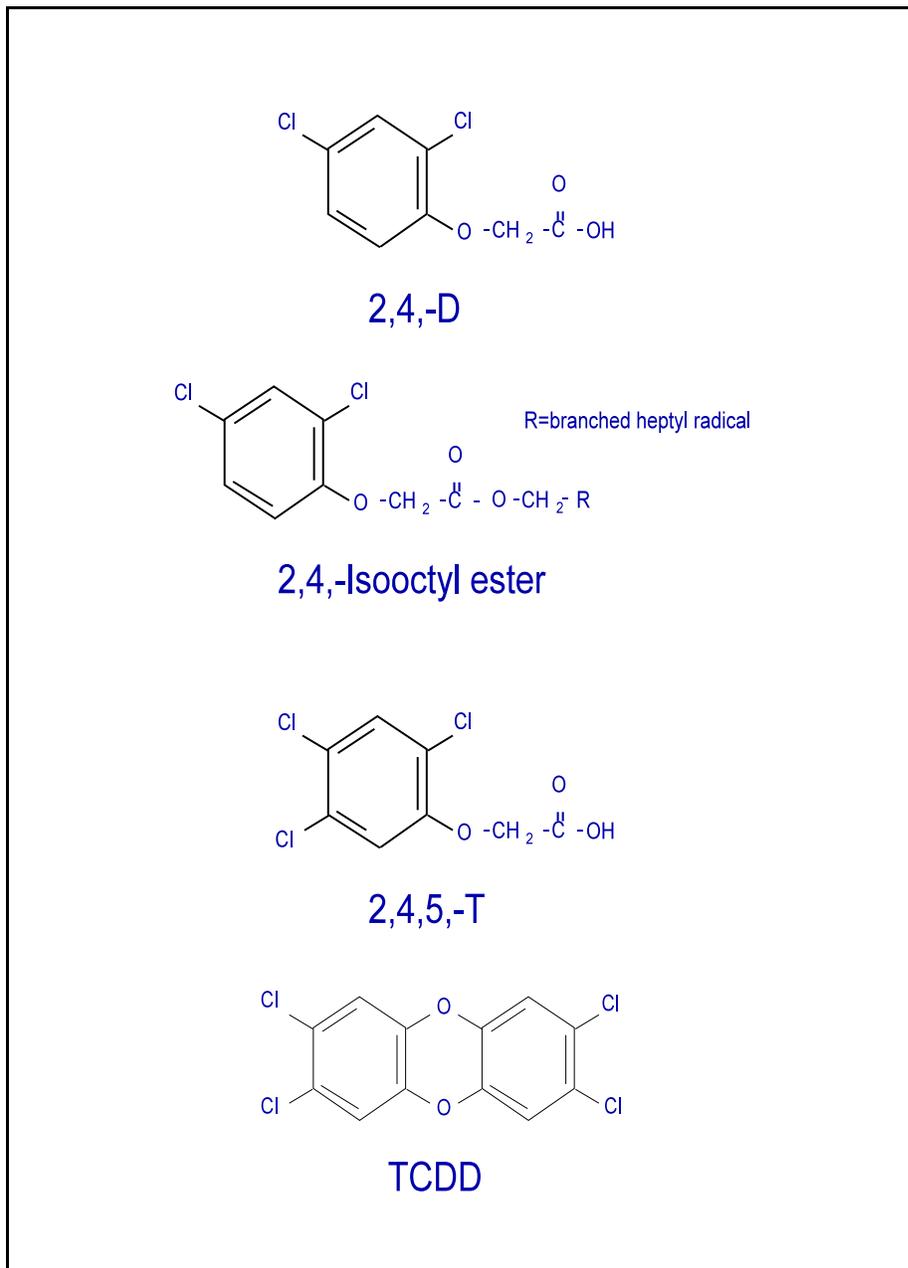
Figure 2-1: Structure of 2,4-D acid and esters as well as structures of 2,4,5-T and TCDD.

Region 9
Eastern <1%

Region 6
Pacific Northwest <1%

Region 1
Northern 33%

Region 4
Inter-mountain 30%

Region 2
Rocky Mountain 36%

Pacific Southwest <1%

Region 5

Region 3
South-western <1%

HAWAII

ALASKA

Region 8
Southern <1%
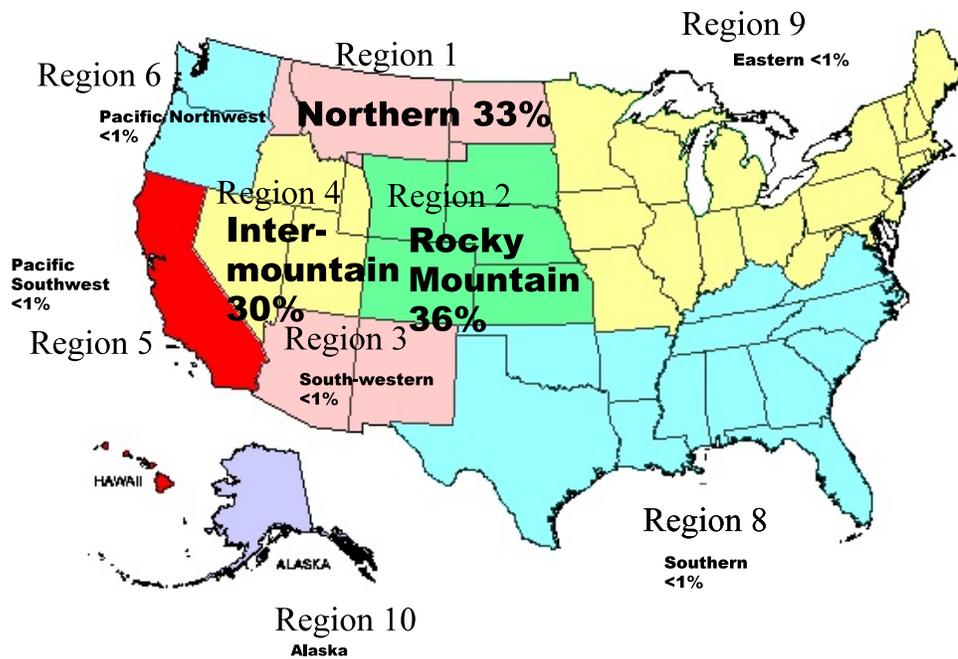
Region 10
Alaska

Figure 2-2: Use of 2,4-D by the Forest Service between 2000 and 2004 by region of the country as a percentage of the total pounds of 2,4-D used in all Forest Service programs (see Table 2-6 for data).

Figures-2

2,4-D

ESTIMATED ANNUAL AGRICULTURAL USE

Average use of
Active Ingredient
Pounds per square mile
of county per year

☐ No Estimated Use
■ < 3.253
■ 3.253 – 8.881
■ 8.882 – 15.668
■ 15.669 – 23.562
■ >= 23.563

Crops	Total Pounds Applied	Percent National Use
pasture	16,106,460	43.25
wheat and grains	7,926,326	21.29
corn	4,550,447	12.22
other hay	3,718,124	9.98
soybeans	2,044,003	5.49
barley	908,808	2.44
rice	555,462	1.49
sorghum	453,258	1.22
oats	275,812	0.74
sugar cane: sugar & seed	164,703	0.44

Figure 2-3: Agricultural uses of 2,4-D in the United States (USGS 1998).
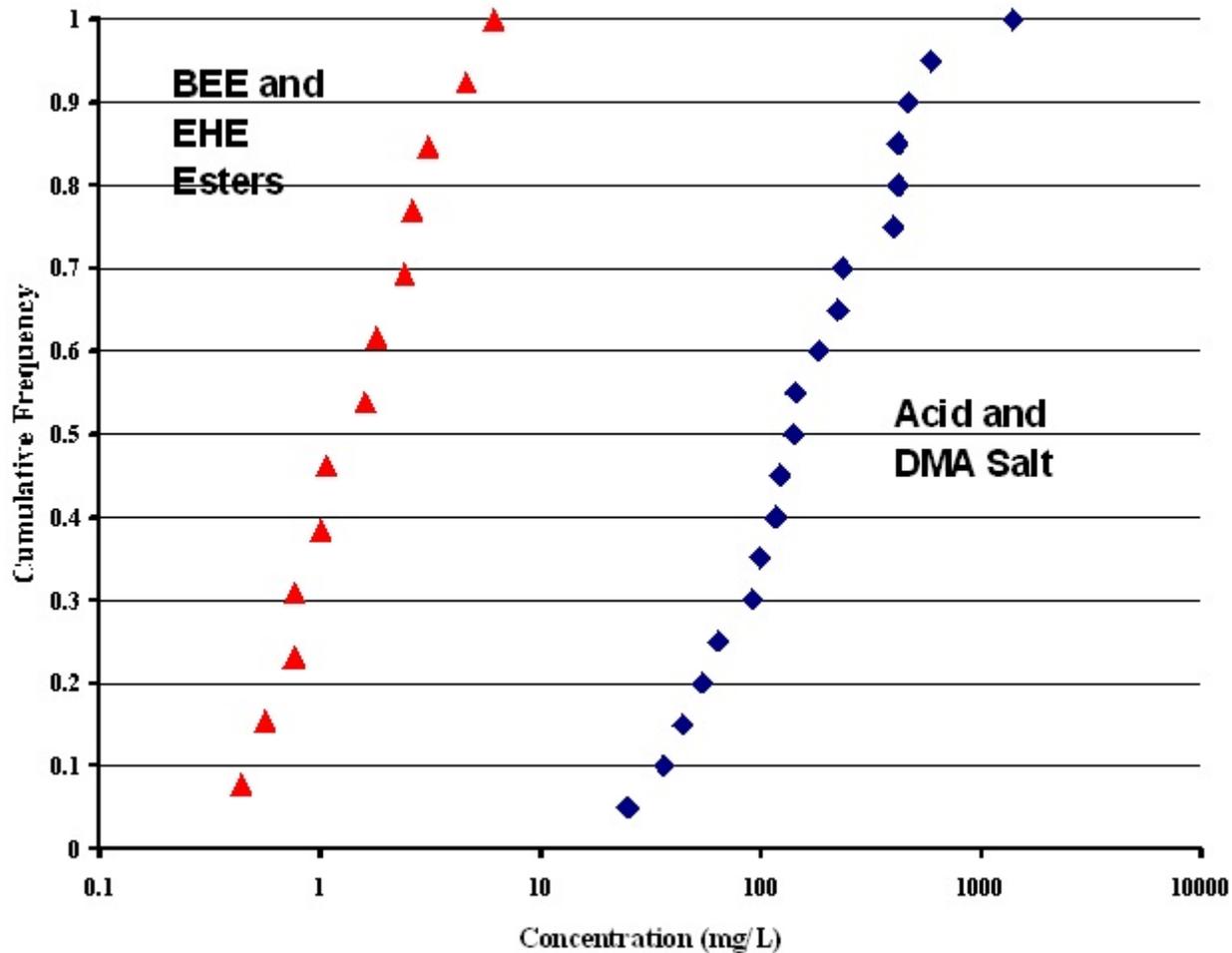
Figures-3

Figure 4-1: Empirical Cumulative Probability Plot of LC$_{50}$ Values for 2,4-D Acid and DMA Salt (triangles) and 2,4-D BEE and EHE esters combined (diamonds). See Table 4-5 for data.

Figures-4

Table 2-1. 2,4-D Commercial formulations covered in this risk assessment [a]

Commercial Name	Manufacturer	Active Ingredient (%)	Acid Equivalent (lbs/gallon)	Label Application Rates (lbs a.e./acre)
Dimethylamine salt				
Amine 4[b]	Wilbur-Ellis formerly United Agri Products]	39.3	3.8	1.9 - 3.8 non-crop 0.24 - 2.85 crop 0.95 - 38 aquatic
2,4-D Amine 4[b]	Agrisolutions	39.3	3.8	0.48 - 3.8 non-crop 0.16 - 1.9 crop
2,4-D Amine 4[d]	Helena	46.8	3.8	0.24 - 7.6 non-crop 0.12 - 2.85 crop 1.2 -38 aquatic
2,4-D 6 Amine	Nufarm	66.8	5.7	0.48 - 4 non-crop 0.1 - 2.85 crop 0.48 - 2.13 aquatic
DMA* 4 IVM[b]	Dow AgroSciences	46.3	3.8	0.48 - 3.8 non-crop 1.9 - 3.8 aquatic
Weed Rhap A-4D[b]	Helena	46.7	3.8	0.24 - 7.6 non-crop 0.24 - 2.85 crop
Weedar® 64[b]	Nufarm Americas [formerly Rhone-Poulenc]	46.8	3.8	0.24 - 2.85
Riverdale® Weedestroy ®AM-40[b]	Nufarm Americas	47.3	3.8	0.48 - 3.2 non-corp 0.24 - 3.8 crop 0.95 - 1.9 aquatic
Triisopropanolamine [*TIPA*] and Dimethylamine [*DMA*] Salts				
Riverdale® Formula 40®[b]	Nufarm Americas [formerly Rhone-Poulenc]	34.0 TIPA 21.9 DMA	3.67	0.11 - 1.8 non-crop 0.23 - 1.8 crop
Butoxyethyl Ester [*e*] and 2,4-d Acid [*a*]				
Phenoxy 088 *[P.1747]*	Riverside/Terra Corp	24.5[*e*] 13.8[*a*]	2.8	
Butoxyethyl Ester (BEE)				
Aqua-Kleen®[c] *[P.1495]*	Rhone-Poulenc	27.6	N/A[c]	Up to 19 aquatic
Isooctyl (2-ethylhexyl) Ester (EHE)				
Brush-Rhap® *[P.1185]*	Helena	65.4	3.76	
Esteron® 99®[b]	Nufarm Americas [formerly Rhone-Poulenc]	65.9	3.8	0.95 - 3.8 non-crop 0.24 - 1.9 crop
Low Vol 4 Ester *[P.1969]*	United Agri Products	65.5	3.8	

Table 2-1. 2,4-D Commercial formulations covered in this risk assessment [a]

Commercial Name	Manufacturer	Active Ingredient (%)	Acid Equivalent (lbs/gallon)	Label Application Rates (lbs a.e./acre)
Riverdale® 2,4-D L.V.4 Ester[b]	Nufarm Americas	67.2	3.84	0.24 - 3.84 non-crop 0.12 - 2.88 crop
Riverdale® 2,4-D L.V.6 Ester[b]	Nufarm Americas	87.3	5.5	0.23 - 4 non-crop 0.23 - 2.75 crop
Weedone® 650[b]	Nufarm Americas	88.8	5.64	0.47 - 3.76 non-crop 0.23 - 1.65 crop
Weedone®LV4 EC[b]	Nufarm Americas	67.2	3.84	0.48 - 3.84 non-crop 0.24 - 2.88 crop
Weedone®LV4 Solventless[b]	Nufarm Americas	62.6	3.8	0.95 - 7.6 non-crop 0.24 - 2.85 crop
Weedone®LV6 EC[b]	Nufarm [formerly Rhone Poulenc]	86.6	5.4	1.35 - 9 non-crop 0.23 - 2.7 crop
Weed Rhap ® LV-6D[b]	Helena	89.5	5.6	0.47 - 5.6 non-crop 0.12 - 2.8 crop

Isooctyl (2-ethylhexyl) Ester {EHE} + EHE of (+)R-2,4-(dichlorophenoxy) propionic acid {EHE-DP}

Commercial Name	Manufacturer	Active Ingredient (%)	Acid Equivalent (lbs/gallon)	Label Application Rates (lbs a.e./acre)
Riverdale Turf Weed & Brush Control[b]	Nufarm Americas	32.1 IEE 16.1 IEE-DP	1.71 IEE 0.87 IEE-DP	not to exceed 4

[a] Source: CPR 1997 unless noted otherwise; Page numbers refer to CPR 1997
[b] Source: www.greenbook.net
[g] Granular aquatic herbicide with 2,4-D BEE at a proportion of 0.276.

Table 2-2. Commercial formulations containing mixtures of 2,4-D with other herbicides [a]

Commercial Name	Manufacturer	Components	Active Ingredient (%)	Acid Equivalent (lbs/gallon)
Scorpion III [p.729]	DowElanco	2,4-D Clopyralid Flumetsulam	50.0 25.0 9.3	N/A[d]
Crossbow [p.691][b]	DowElanco	2,4-D BEE Triclopyr BEE	34.4 16.5	2.0 1.0
Riverdale Veteran 720[e]	Nufarm Americas	2,4-DMA Dicamba DMA	24.58 12.8	1.9 1
Weedmaster [p.1877]	Sandoz	2,4-D DMA Dicamba DMA	35.7 12.4	2.87 1.0
2 Plus 2 [p.1231]	ISK Biosciences	2,4-D DMA MCPP DMA	24.5 24.2	1.9 1.8
Tiller [p.113]	AgrEvo	2,4-D IOE Fenoxaprop-ethyl MCPA EHE	10.35 4.41 32.11	0.58 0.375 1.75
Shotgun [p.S87]	UAP	2,4-D EHE Atrazine	16.58 24.24	1.0 2.25
Landmaster BW [p.1375]	Monsanto	2,4-D TIPA Glyphosate TIPA	20.6 12.9	1.5 0.9
Curtail	Dow AgroSciences [formerly DowElanco]	2,4-D TIPA Clopyralid MEA	39.0 5.1	2.0 0.38
Grazon P+D [p.702]	DowElanco	2,4-D TIPA Picloram TIPA	39.6 10.2	2.0 0.54
Pathway[e]	Dow AgroSciences	2,4-D TIPA Picloram TIPA	20.9 5.4	11.2%[f] 3.0%[f]
Tordon 101 mixture[e]	Dow AgroSciences	2,4-D TIPA Picloram TIPA	39.6 10.2	2 0.54
Tordon RTU[e]	Dow AgroSciences [formerly DowElanco]	2,4-D TIPA Picloram TIPA	20.9 5.4	11.2%[f] 3.0%[f]

BEE = Butoxyethyl ester
DMA = Dimethylamine salt
EHE = 2-ethylhexyl ester
MCPA = methyl-4-chlorophenoxyacetate
MCPP = 2-(2-methyl-4-chlorophenoxy)propionic acid
MEA = monoethylamine salt
TIPA = Triisopropanolamine salt
[a] Source: CPR 1997 unless noted otherwise, page numbers refer to CPR 1997
[b] Contains petroleum distillates
[c] Source: Dow AgroSciences, no date. Label for Curtail.
[d] Water soluble packet
[e] Source: www.greenbook.net
[f] label does not specify further

Tables-3

Table 2-3. Selected physical and chemical properties of 2,4-D acid, and commercially significant salts and esters [a].

Chemical	2,4-D (acid)	2,4-D Dimethylamine	2,4-D Butoxyethyl ester	2,4-D 2-ethylhexyl ester
Synonyms	2,4-D	2,4-D-dimethylam-monium	2,4-D-butoxyl	2,4-D-isoctyl ester
IUPAC Name	(2,4-dichlorophen-oxy)acetic acid	dimethylammonium (2,4-dichlorophen-oxy)acetate	2-butoxyethyl (2,4-dichloropheno xy)acetate	octyl (2,4-dichlorophen-oxy)acetate
CAS Number	94-75-7	2008-39-1	1929-73-3	25168-26-7 1280-20-2 [former]
Molecular weight	221.0	266.1	321.2	333.3
Acid equivalents factor [221.0/MW]	1	0.831	0.688	0.663
Physical state	Colorless powder, with a slight phenolic odor			Yellowish-brown liquid, with a phenolic odor
Density (g/cm^3)	1.508 (20 °C)			1.14-1.17
Foliar half-life (days)	5 (Knisel and Davis 2000) 8.8 (U.S. EPA 2004b)4	9 (Knisel and Davis 2000)	5 (Knisel and Davis 2000 [b])	5 (Knisel and Davis 2000 [b])
Foliar washoff fraction	0.45 (Knisel and Davis 2000) 0.5 (U.S. EPA 2004b)	0.45 (Knisel and Davis 2000)	0.45 (Knisel and Davis 2000 [b])	0.45 (Knisel and Davis 2000 [b])
Henry's law constant (atm-m^3/mole)	9.21×10^{-9} (Meylan and Howard 2000) 4.74×10^{-10} (U.S. EPA 2004b)	1.45×10^{-16} (Meylan and Howard 2000)	1.25×10^{-7} (Meylan and Howard 2000)	5.65×10^{-5} (Meylan and Howard 2000)
Log K_{ow}	2.58-2.83 (pH 1) 0.04-0.33 (pH 5) USDA/ARS 2001: 2.83 (pH1) -0.75 (pH7) Meylan and Howard 2000: 2.81, experimental 2.62, estimate U.S. EPA 2004b: 2.81	0.65 (Moody et al. 1987) Meylan and Howard 2000: 0.65, experimental 0.84, estimate	Meylan and Howard 2000: 4.10, estimate 4.35 (U.S. EPA 2004b)	Meylan and Howard 2000: 6.73, estimate

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Table 2-3. **Selected physical and chemical properties of 2,4-D acid, and commercially significant salts and esters** [a].

Chemical	2,4-D (acid)	2,4-D Dimethylamine	2,4-D Butoxyethyl ester	2,4-D 2-ethylhexyl ester
pKa	2.73 2.87 (25°C) (USDA/ARS 2001)			
Soil adsorption K_{oc}	48 (20-79) (USDA/ARS 2001) 60 (Tomlin 2004) 20 - 109 (Howard 1991) 20 (Knisel and Davis 2000) 29.4 (Meylan and Howard 2000) 61.7 (13.23 - 116.67) (U.S. EPA 2004b)	72 - 136 (avg of 109 in three soils) (Rao and Davidson 1979) 20 (Knisel and Davis 2000) 325.4 (Meylan and Howard 2000)	6607-6900 (Reinert and Rodgers 1987) 1100 (Howard 1991) 100 (Knisel and Davis 2000 [b]) 337 (Meylan and Howard 2000)	25000 - 68000 (Howard 1991) 100 (Knisel and Davis 2000 [b]) 22,800 (Meylan and Howard 2000)
Soil half-life (days)	14 (field dissipation; USDA/ARS 2001) 5.5 (soil half-life, USDA /ARS 2001) 5 days (soil half-life) (Crespin et al. 2001) 10-30 (Mullins et al. 1993) 10 (Knisel and Davis 2000) 6.2 (aerobic soil metabolism) (U.S. EPA 2004b)	4-6 (in agricultural soil) (Howard 1991) 7-23 (in forest soil) (Howard 1991) 10 (Knisel and Davis 2000)	hydrolysis in moist soil may occur within a few days (Howard 1991) 0.11-2.3 (biodegradation half-life) (Reinert and Rodgers 1987) 10 (Knisel and Davis 2000 [b])	In moist prairie soils (pH5.6-7.3), complete conversion to the acid and alcohol occurred in 2-3 days (Howard 1991) 10 (Knisel and Davis 2000 [b])

Table 2-3. **Selected physical and chemical properties of 2,4-D acid, and commercially significant salts and esters** [a].

Chemical	2,4-D (acid)	2,4-D Dimethylamine	2,4-D Butoxyethyl ester	2,4-D 2-ethylhexyl ester
Vapor pressure (mm Hg at 25°C unless otherwise specified)	1.86×10^{-2} mPa USDA/ARS (2001): 1.42×10^{-7} 9.7×10^{-8} 2.79×10^{-5} (Meylan and Howard 2000) 1.47×10^{-7} (U.S. EPA 2004b) 8×10^{-6} (USDA/ARS 2001)	3.98×10^{-8} (Meylan and Howard 2000)	5.29×10^{-6} (Meylan and Howard 2000)	1.02×10^{-5} (Meylan and Howard 2000)
Water solubility (mg/L)	311 (25°C, pH1) [c] 20031 (25°C, pH5)[c] 23180 (25°C, pH7)[c] 34196 (25°C, pH9)[c] Meylan and Howard (2000): 677, experimental 336.2, estimated 569 (U.S. EPA 2004b)	3,000,000 (20 °C) Meylan and Howard (2000): 5353, estimated	Meylan and Howard (2000): 12, experimental 2.89, estimated	10 Meylan and Howard (2000): 0.017 - 0.021, estimated

Table 2-3. Selected physical and chemical properties of 2,4-D acid, and commercially significant salts and esters [a].

Chemical	2,4-D (acid)	2,4-D Dimethylamine	2,4-D Butoxyethyl ester	2,4-D 2-ethylhexyl ester
Water half-life (days)	10 to >50 (Howard 1991) approximately 200 days based upon an average measured dissipation rate of about 17.5% over a 56-day incubation period in various river waters containing no sediment (Wang et al. 1994a) 45 for aerobic aquatic degradation, 231 for anaerobic degradation (U.S. EPA 2004b) concentration dependent: 500 days at low concentration (1 ppb) to 5 days at high concentrations (100 ppb) (Torang et al. 2003)	0.5-6.6 (in various natural waters) (Howard 1991) 10-11 (in plastic-lined pools) (Howard 1991) 3.9-11 (Reinert and Rodgers 1987)	0.1-1.0 (biodegradation to free acid) (Howard 1991) 0.025 (chemical hydrolysis at pH9 and 28°C) (Howard 1991) 26 (chemical hydrolysis at pH6 and 28°C) (Howard 1991) 0.11-2.3 (biodegradation half-life) (Reinert and Rodgers 1987)	specific data not available, but may be similar to other 2,4-D esters

[a] Information from Tomlin (2004) unless otherwise specified.
[b] Knisel and Davis (2000) provide only general values for an ester (NOS) of 2,4-D.
[c] These values reported by Tomlin (2004) are identical to those reported by USDA/ARS 2001.

Table 2-4: Known inerts[a] contained in commercial formulations of 2,4-D that may be used in Forest Service Programs.

Producer/Formulation	Inerts Identified on MSDS[a] [CAS #]	Inert % by Weight
Agriliance		
2,4-D Amine 4[b]	Unspecified proprietary	52.7%
Dow AgroSciences		
Curtail* Herbicide[b]	Triisopropanolamine [000122-20-3] Ethylenediamine tetraacetic acid [00060-00-4]	55.9%, total inerts
DMA* 4 IVM Herbicide	Dimethylamine [000124-40-3]	53.7 %
Pathway* Herbicide	Ethylene Glycol [000107-21-1] Isopropanol [00067-63-0] Triisopropanolamine [000122-20-3] Proprietary surfactant	73.7 %, total inerts
Tordon 101 Mixture*	Isoppropanol [00067-63-0] Triisopropanolamine [000122-20-3	50.2 %, total inerts
Tordon RTU* Herbicide	Isopropanol [00067-63-0] Triisopropanolamine [000122-20-3] Proprietary surfactant	73.7%, total inerts
Helena		
2,4-D Amine 4[b]	Water and unspecified sequestering agents	53.2%
Weed Rhap LV-6D[b]	Unspecified, including petroleum distillates	10.5%
Nufarm/Nufarm Americas		
2,4-D 6 Amine	Water and unspecified sequesterants	33.20 % total inerts
Esteron®99	Kerosene [8008-20-6] Polyglycol 26-3 (ethylene oxide) [69020-39-6][c] Proprietary ingredients	34.1%, total inerts
Riverdale® 2,4-D L.V.4 Ester	Inerts not specified	32.8%
Riverdale® 2,4-D L.V.6 Ester	Inerts not specified	12.7%
Riverdale®Formula®40	Ethylenediamine tetraacetic acid [60-00-4] Triisopropanolamine [000122-20-4]	43.98%, total inerts
Riverdale® Turf Weed & Brush Control	Unspecified, including petroleum distillates [64742-47-8]	51.8%, total inerts
Riverdale® Veteran 720	Water and unspecified sequesterants	62.60%
Riverdale® Weedestroy® AM-40 Amine Salt	Not specified	52.7%
Weedar® 64	Unspecified proprietary ingredients	53.2%
Weedone® 650 Solventless	Unspecified emulsifier and proprietary ingredients	11.2%
Weedone® LV4 EC	Unspecified ingredients	32.8%

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Table 2-4: Known inerts[a] contained in commercial formulations of 2,4-D that may be used in Forest Service Programs.

Producer/Formulation	Inerts Identified on MSDS[a] [CAS #]	Inert % by Weight
Weedone® LV6 EC	Unspecified emulsifier, proprietary ingredients, and petroleum distillates	13.2%
Wilbur- Ellis		
Amine 4[b]	No information	No information

[a] Unless specified otherwise, Specimen labels from C&P Press, http://www.greenbook.net/

[b] Manufacturer's MSDS

[c] The CAS # listed on the label may be in error, and may actually be 69029-39-6. There is no listing for the former CAS # or it's associated name on EPA's list of pesticide inerts. However, the latter # is associated with polyoxyethylene polyoxypropylene nono(di-sec).

Table 2-5: Uses of 2,4-D by the Forest Service between 2000 and 2004 by management objective [*].

Management Objective	Pounds	Acres	Pounds/Acre	Proportion	
				lbs	acres
Agricultural Weed Control	28,809.73	24,131.00	1.19	0.22	0.12
Noxious Weed Control	101,095.80	168,616.93	0.60	0.77	0.86
Right-of-Way Management	744.61	865.76	0.86	0.01	0.00
Facilities Maintenance	243.42	21.00	11.59	0.00	<0.01
Nursery Weed Control	214.95	187.46	1.15	0.00	0.00
Other	202.91	3,197.00	0.06	0.00	0.02
Total use and average application rate	131,311.42	197,019.15	0.67	lb/acre	

Source: http://www.fs.fed.us/foresthealth/pesticide/reports.shtml

Table 2-6: Uses of 2,4-D by the Forest Service between 2000 and 2004 by Forest Service Region between 2000 and 2004.

Region	Pounds	Acres	lb/acre	Proportion	
				Lbs	Acres
1: Northern	43,781.93	58,016.08	0.75	0.33	0.29
2: Rocky Mountain	47,294.08	67,260.46	0.70	0.36	0.34
3: Southwestern	37.50	54.00	0.69	<0.01	<0.01
4: Intermountain	39,800.23	72,669.01	0.55	0.30	0.37
5: Pacific Southwest	28.42	N/A	N/A	<0.01	N/A
6: Pacific Northwest [*]	44.70	1.50	29.80	<0.01	<0.01
8: Southern	175.00	140.00	1.25	<0.01	<0.01
9: Eastern	149.85	338.00	0.44	<0.01	<0.01
Grand Total	131,311.71	198,479.05	0.66		

Source: http://www.fs.fed.us/foresthealth/pesticide/reports.shtml
[*] The very high application rate for Region 6 is a single report of 44.7 lbs of 2,4-D applied to 1.5 acres in Forest 7 during the year 2000. This and other individual reports appear to be reporting errors.

Table 3-1: Total percent cumulative dermal absorption of 2,4-D derivatives over a 14 day post-application observation period (adapted from Moody et al. 1990).

Compound, vehicle	Para-meter[a]	Animal species (anatomical site)[b]						
		RB (B)	TR (B)	RT (T)	MY (FA)	MY (FH)	HN (FA)	HN (FH)
2,4-D acid, acetone	% Rec	36			15	29	6[c]	
	$t_{1/2}$	2.41			1.94	1.47	N/R	
2,4-D amine, water	% Rec	12	20					58
	$t_{1/2}$	1.65	2.55					NR
2,4-D amine, acetone	% Rec			14	6	31		
	$t_{1/2}$			1.35	1.83	2.13		
2,4-D isooctyl, acetone	% Rec	50			40	56		6
	$t_{1/2}$	NR			2.07	2.04		1.33
2,4-D isooctyl, Esteron LV96 blank	% Rec	34						6
	$t_{1/2}$	0.74						1.63

[a] %Rec: percent urinary recovery after 14 days. $t_{1/2}$: halftimes in days for urinary excretion after dermal application.
[b] Abbreviations for species: RB, rabbit; RT, rat; MY, monkey, HN, human.
  Abbreviations for anatomical site: B, back; T, tail, FA, forearm; FH, forehead
[c] Data from Table 2 of Feldmann and Maibach (1974) for a five day post-application period. Reported in Moody et al. (1990) as 6%.

Table 3-2: Summary of acute toxicity of 2,4-D acid, salts, and esters

Route/Active Ingredient	Endpoint[a]	Reference[b]	MRID Cited by Reference
Acute Oral			
2,4-D acid	rat LD_{50} = 639 mg/kg; rat LD_{50} = 699 mg a.e./kg	Johnson et al. 1981a Johnson et al. 1981a	00101605 00101605
DEA salt	rat LD_{50} = 735 mg/kg; rat LD_{50} = 618.8 mg a.e./kg	Shults et al. 1990a Shults et al. 1990a	41920901 41920901
DMA salt	rat LD_{50} = 949 mg/kg; rat LD_{50} = 716 mg a.e./kg	Jeffrey et al. 1986 Johnson et al. 1981b	00157512 00101603
IPA salt	rat LD_{50} = 1646 mg/kg; rat LD_{50} = 1300 mg/kg	U.S.EPA/OPP 2005b U.S.EPA/OPP 2004a	00252291 00252291
IPE ester	rat LD_{50} = 1250 mg/kg	Lilja 1990a	41709901
TIPA salt	rat LD_{50} = 1074 mg/kg rat LD_{50} = 579 mg a.e./kg	Berdasco et al. 1989a Berdasco et al. 1989a	41413501 41413501
BEE ester	rat LD_{50} = 866 mg/kg rat LD_{50} = 598 mg a.e./kg	Jeffrey et al. 1987a Jeffrey et al. 1987a	40629801 40629801
EHE ester	rat LD_{50} = 896 mg/kg rat LD_{50} = 591 mg a.e./kg	Mahlburg 1988a U.S.EPA/OPP 2004a	41209001 "Not available"
Acute Dermal			
2,4-D acid	rabbit LD_{50} > 2000 mg/kg	Mayhew et al. 1981	00101596
DEA salt	rabbit LD_{50} > 2000 mg/kg	Shults et al. 1991	41920911
DMA salt	rabbit LD_{50} > 1829 mg/kg	Carreon et al. 1986	00157513
IPA salt	rabbit LD_{50} > 2000 mg/kg	U.S.EPA/OPP 2005b	00252291
IPE ester	rabbit LD_{50} > 2000 mg/kg	Lilja 1990b	41709902
TIPA salt	rabbit LD_{50} > 2000 mg/kg	Berdasco et al. 1989b	41413502
BEE ester	rabbit LD_{50} > 2000 mg/kg	Jeffrey et al. 1987b	40629802
EHE ester	rabbit LD_{50} > 2000 mg/kg	Mahlburg 1988b	41209002
Acute Inhalation			
2,4-D acid	rat LC_{50} > 1.79 mg/L	Auletta and Daly 1986	00161660
DEA salt	rat LC_{50} > 3.5 mg/L	Jackson and Hardy 1991	41986601
DMA salt	rat LC_{50} > 3.5 mg/L	Streeter et al. 1985	00517514
IPA salt	rat LC_{50} = 3.1 mg/L	Heydens 1986	40085501
IPE ester	rat LC_{50} > 4.97 mg/L	Maedgen 1986d	40352701
TIPA salt	rat LC_{50} = 0.78 mg/Lz	Nitschke and Stebbins 1991	41957601
BEE ester	rat LC_{50} = 4.6 mg/L	Streeter et al. 1987	40629803
EHE ester	rat LC_{50} > 5.4 mg/L	Cieszlak 1992	42605202
Primary Eye Irritation			
2,4-D acid	Severe eye irritant	Kirsch 1983	41125302
DEA salt	Severe eye irritant	Shults et al. 1990d	41920902

Table 3-2: Summary of acute toxicity of 2,4-D acid, salts, and esters

Route/Active Ingredient	Endpoint[a]	Reference[b]	MRID Cited by Reference
DMA salt	Severe eye irritant	Carreon 1986	00157515
IPA salt	Severe eye irritant	U.S.EPA/OPP 2005b	00252291
IPE ester	not an eye irritant	Maedgen 1986a	40352702
TIPA salt	Severe eye irritant	Berdasco and Mizell 1989	41413504
BEE ester	not an eye irritant	Jeffrey 1987a	40629804
EHE ester	not an eye irritant	Cieszlak and Brooks 1998	44725303
Primary Dermal Irritation			
2,4-D acid	study deemed unacceptable by U.S.EPA/OPP 2005b due to failure to moisten test substance prior to application	Berdasco et al. 1990b	42232701
DEA salt	slight irritant	Shults et al. 1990b	41920903
DMA salt	slight irritant	Jeffrey 1986a	00157516
IPA salt	slight irritant	U.S.EPA/OPP 2005b	00252291
IPE ester	very mild irritant	Maedgen 1986b	40352703
TIPA salt	slight irritant	Mizell 1989	41413505
BEE ester	very mild irritant	Jeffrey 1987b	40629805
EHE ester	not an irritant	Mizell 1989	41413505
Dermal Sensitization			
2,4-D acid	not a dermal sensitizer	Gargus 1986	00161659
DEA salt	not a dermal sensitizer	Shults et al. 1990c	41920904
DMA salt	study deemed unacceptable by U.S.EPA/OPP 2005b due to non-use of technical grade substance	Robbins 1989	41642805
IPA salt	not a dermal sensitizer	Carreon and Wall 1984	41233701
IPE ester	not a dermal sensitizer	Maedgen 1986c	40352704
TIPA salt	not a dermal sensitizer	Berdasco 1989	41413506
BEE ester	not a dermal sensitizer	Jeffrey 1986b	40629806
EHE ester	study deemed unacceptable by U.S.EPA/OPP 2005b due to non-use of technical grade substance	Mahlburg 1988c	41209006

[a] units given in U.S.EPA/OPP 2005b are not further qualified (i.e., mg a.e., mg a.i. not specified); U.S. EPA/OPP 2004a specifies units as mg a.e./kg.
[b] information from U.S.EPA/OPP 2005b is taken directly from Table 3; information from USEPA/OPP 2004a is taken from Appendix C: Ecological Hazard Data

Table 3-3: Summary of critical neurotoxicity, subchronic, chronic, developmental and reproductive toxicity data for 2,4-D [a]

Route/Species /Active Ingredient	Study Type, Duration	Toxicity Endpoints[a]	Reference[b]
Oral			
Rat, 2,4-D acid	acute neurotoxicity screening battery	NOAEL = 67 mg/kg/day; LOAEL =227 mg/kg/day based on an increased incidence of in-coordination and slight gait abnormalities [described as forepaw flexing or knuckling] and decreased total motor activity.	Mattsson et al. 1994a (MRID: 43115201)
	subchronic neurotoxicity screening battery	NOAEL = 75 mg/kg/day LOAEL = 150 mg/kg/day based on increased forelimb grip strength.	Mattsson et al. 1994b (MRID 43293901)
	90-day toxicity	NOAEL: 15 mg/kg/day LOAEL: 100 mg/kg/day based on decreases in body weight gain, alterations in hematology and clinical chemistry [decreased T3 and T4], and cataract formation in females.	Schulze 1991c (MRID 41991501)
	chronic toxicity and carcinogenicity	NOAEL = 5 mg/kg/day LOAEL = 75 mg/kg/day based on decreased body-weight gain (females) and food consumption (females), alterations in hematology [decreased RBC, HCT, and HGB (females), platelets (both sexes)] and clinical chemistry [increased creatinine (both sexes), alanine and aspartate aminotransferases (males), alkaline phosphatase (both sexes), decreased T4 (both sexes), glucose (females), cholesterol (both sexes), and triglycerides (females)], increased thyroid weights (both sexes at study termination), and decreased testes and ovarian weights; microscopic lesions in the eyes, liver, adipose tissue, and lungs; no evidence of carcinogenic effect	Jeffries et al. 1995 (MRID 43612001)
	developmental toxicity	Maternal NOAEL = 25 mg/kg/day LOAEL = 75 mg/kg/day based on decreased body-weight gains. No treatment-related effect on survival Developmental NOAEL = 25 mg/kg/day LOAEL = 75 mg/kg/day based on skeletal abnormalities	Nemec et al. 1983a (MRID 00130408); Rodwell et al. 1983 (MRID 00251031)

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Table 3-3: Summary of critical neurotoxicity, subchronic, chronic, developmental and reproductive toxicity data for 2,4-D [a]

Route/Species /Active Ingredient	Study Type, Duration	Toxicity Endpoints[a]	Reference[b]
2,4-D acid/ 97.5%	2-generation reproduction, dietary exposure, doses expressed as mg/kg bw/day	Parental/Systemic NOAEL = 5 mg/kg/day; LOAEL = 20 mg/kg/day. based on decreased female body weight/ body-weight gain [Fl] and renal tubule alteration in males [FO and F1]. Reproductive NOAEL = 20 mg/kg/day; LOAEL = 80 mg/kg/day, based on an increase in gestation length [FO females producing Flb pups]. Offspring NOAEL = 5 mg/kg/day LOAEL = 20 mg/kg/day based on decreased pup body weight [F1b]. Increase in dead pups at 80 mg/kg/day.	Tasker 1985 (MRID 00150557); Brown 1986 (MRID 00163996); WHO 1996
Rat, 2,4-D acid	Immuno-histochemical study of potential CNS damage in pups exposed to 2,4-D via dams milk during lactation ; 25-day-old pup brains analyzed	LOAEL= 70 mg/kg/day, based on significantly decreased staining of tyrosine hydroxylase (TH) neurons in the substantia nigra (SN) of the brain; decreased fiber density of 5-hydroxy tyrosine (5-HT) fiber density in SN and ventral tegmental area (VTA) of brain. At 100 mg/kg/day: significant decreases in body weight as well as brain weight; 25% diminution in SN and 33% diminution in VTA neurons; TH immunostaining and significantly lower 5-HT fiber density.	Garcia et al. 2004
Mouse, 2,4-D acid	chronic toxicity and carcinogenicity	NOAEL = 5 mg/kg/day; LOAEL = 62/150 mg/kg/day based on an increased absolute and/or relative kidney weights and an increased incidence of renal microscopic lesions; no evidence of carcinogenicity.	Stot et al. 1995a,b (MRID 43879801, MRID 43597201)

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Table 3-3: Summary of critical neurotoxicity, subchronic, chronic, developmental and reproductive toxicity data for 2,4-D [a]

Route/Species /Active Ingredient	Study Type, Duration	Toxicity Endpoints[a]	Reference[b]
Mouse, DMA salt	developmental immunotoxicity (7-week-old pups evaluated after gestational exposure [days 6-16] to 2,4-D DMA salt); pregnant dams exposed via drinking water on days 6-16 of gestation	Maternal NOAEL = 370 mg a.e./kg/day Offspring NOAEL = 8.5 mg a.e./kg/day; Offspring LOAEL = 37 mg a.e./kg/day, based on decreased body weight gain and decreased kidney weight in females. Immune LOAEL = 370 mg a.e./kg/day, based on subtle effects on the immune system (both sexes), evaluated 7 weeks after birth. Effects included: suppression of lymphocyte stimulation by concanavalin A; significantly increased relative B cells count (% per spleen); significantly reduced relative % suppressor or cytotoxic T-cells. The humoral immune response, measured as antibody production against sheep red blood cells and peritoneal phagocytic function, were not affected at any 2,4-D dose.	Lee et al. 2001
Dog 2,4-D acid	90-day toxicity	NOAEL = 1 mg/kg/day; LOAEL = 3 mg/kg/day based on decreased body weight/body weight gain and food consumption (males), alterations in clinical chemistry parameters [increased BUN (both sexes), creatinine (males)], and decreased testis weight in males.	Schulze 1990a (MRID 41737301)
	90-day toxicity	NOAEL = 1 mg/kg/day; LOAEL =3.75 mg/kg/day based on decreased body-weight gain (both sexes) and food consumption (males), as well as alterations in clinical chemistry parameters [increased BUN, creatinine, and alanine aminotransferase] in both sexes, and decreased testes weight and slightly higher incidence of hypospermatogenesis /juvenile testis and inactive/juvenile prostate were observed.	Dalgard 1993a (MRID 42780001)

Table 3-3: Summary of critical neurotoxicity, subchronic, chronic, developmental and reproductive toxicity data for 2,4-D [a]

Route/Species /Active Ingredient	Study Type, Duration	Toxicity Endpoints[a]	Reference[b]
	chronic toxicity	NOAEL = 1 mg/kg/day; LOAEL = 5 mg/kg/day based on decreased body-weight gain (both sexes) and food consumption (females), as well as alterations in clinical chemistry parameters [increased BUN, creatinine, and alanine aminotransferase, decreased glucose] in both sexes, and decreased brain weight in females, and histopathological lesions in liver and kidneys.	Dalgard 1993d (MRID 430490001)
Rabbit, 2,4-D acid	developmental toxicity	Maternal NOAEL = 30 mg/kg/day; LOAEL = 90 mg/kg/day based on clinical signs [ataxia, decreased motor activity, loss of righting reflex, cold extremities], abortion (2), decreased body-weight gains. Survival was not affected by treatment. Developmental NOAEL =30 mg/kg/day; LOAEL = 90 mg/kg/day, based on abortions.	Hoberman 1990 (MRID 41747601)
Dermal			
Rabbit, 2,4-D acid	21-day toxicity	NOAEL = 1000 mg/kg/day LOAEL >1000 mg/kg/day (no effects at test limit)	Schulze 1990b (MRID 41735304)

[a] Units expressed in U.S.EPA/OPP 2005b are not specified further.

[b] study summaries referenced to U.S. EPA/OPP 2005b are taken directly from U.S.EPA/OPP 2005b, Table 4

Table 3-4 : Summary of select toxicity information for inert ingredients in 2,4-D formulations.

Inert ingredient [CAS#]	Toxicity Information	Reference	EPA Pesticide Inert List Classification[1]
Dimethylamine [000124-40-3]	Rat oral LD_{50}: 698 mg/kg; Mouse oral LD50: 316 mg/kg; Guinea Pig oral LD50: 240 mg/kg Rat 6 hr inhalation LC_{50}: 4540 ppm Corrosive to eyes/skin/respiratory tract; Chronic exposure can lead to conjunctivitis, dermatitis and lung damage	OSHA, 1996a	3
Ethylenediamine tetraacetic acid [60-00-4]	Mouse oral LD50: 240 mg/kg; Corrosive to eyes/skin/gastrointestinal tract; respiratory tract irritant; Prolonged exposure may cause respiratory tract inflammation, kidney damage, muscle cramps, bone marrow depression and generalized allergic reaction.	Fisher Scientific, 2005	4B
Ethylene Glycol [000107-21-1]	Human Chronic Oral RfD: 2 mg/kg/day; basis is chronic oral rat NOEL: 200 mg/kg/day; LOAEL: 1000 mg/kg/day on basis of kidney damage; Teratogenic in mice; reproductive toxin in rats and mice at doses causing maternal toxicity; RfD is protective of these effects.	U.S. EPA 1989b	3
Isopropanol [00067-63-0]	Acute exposure, humans: eye and mucous membrane irritation; may cause in-coordination and narcosis. Ingestion causes gastrointestinal pain, nausea, vomiting, and may cause coma and death. Chronic exposure causes sensitization and eczema in rare cases. Rat oral LD_{50}: 5,045 mg/kg Rabbit dermal LD_{50}: 12,850 mg/kg	OSHA 1996b	4B
Kerosene [8008-20-6]	Harmful or fatal if swallowed. Harmful if inhaled. Causes Irritation to skin, eyes and respiratory tract. Central nervous system depressant. Rat oral LD_{50}: >500 mg/kg; Guinea pig: Severe skin irritation in Draize test at 500 mg	JT Baker 2003	3

Table 3-4 : Summary of select toxicity information for inert ingredients in 2,4-D formulations.

Inert ingredient [CAS#]	Toxicity Information	Reference	EPA Pesticide Inert List Classification[1]
Petroleum distillates [64742-47-8]	13-week rat gavage study LOAEL: 500 mg/kg, associated with kidney damage in males and liver damage in males and females; also for males: significantly elevated platelet count, significantly decreased serum glucose .	ExxonMobil Chemical Company 2001	2
Polyglycol 26-3 (ethylene oxide) [69020-39-6]	NOTE: This listing, taken from the msds for Esteron®9 (Table 2-4), may be in error, as the given CAS# and name do not appear on EPA's list of pesticide inerts.		
Triisopropanol-amine [000122-20-3	Mouse oral LD_{50}: 2520 mg/kg Moderate skin and eye irritant;	ScienceLab.com 2005	3

The United States Environmental Protection Agency, Office of Pesticide Programs established a policy on inert ingredients in 1987: 52 FR 13305, Inert Ingredients in Pesticide Products Policy Statement (04/22/87)). This policy established four categories of toxicological concern for the inert ingredients in existence at that time. In 1989, List 4 "Inerts of Minimal Concern" was subdivided into List A and List 4B (see 54 FR 48314, Inert Ingredients in Pesticide Products; Policy Statement; Revision and Modification List (11/22/89)). List 1: inert ingredient are known to be toxic, and pesticides must include the label statement "This product contains the toxic inert ingredient (name of inert)."; List 2: Potentially Toxic Other Ingredients/High Priority for Testing inerts ; List 3: inerts of "unknown toxicity" which have no basis for listing in the other categories; List 4A: Minimal risk inert ingredients; List 4B: EPA has sufficient information to reasonably conclude that the current use pattern in pesticide products will not adversely affect public health or the environment. This information is available on the internet at: http://www.epa.gov/opprd001/inerts/lists.html. The USEPA/OPP classifications given here are listed as being current as of August 2004.

Table 3-5: Chemical and site parameters used in GLEAMS modeling for 2,4-D acid.

Chemical Specific Parameters

Parameter	Clay	Loam	Sand	Comment/ Reference
Halftimes (days)				
Aquatic Sediment		231		Hetrick 1995f
Foliar		8.8		Note 1
Soil		6.2		Note 2
Water		45		Note 3
$K_{o/c}$, mL/g		61.7		Note 4
K_d, mL/g	1	1.1	0.08	Note 5
Water Solubility, mg/L		569		U.S. EPA 2004b
Foliar wash-off fraction		0.5		U.S. EPA 2004b
Fraction applied to foliage		0.5		Note 6

Note 1   Value taken from review by Willis and McDowell (1987) and used by U.S. EPA/OPP 2004b. Comparable to range of 5 to 9 days recommended by Knisel and Davis (2000). Substantially longer halftimes have been reported by Newton et al. (1990).

Note 2   Value used by by U.S. EPA/OPP 2004b in PRZM/EXAMS modeling based on soil metabolism studies by Matradone (1988a) and Hetrick (1995g). Somewhat longer halftimes of about 10 to 30 days have been reported. See Table 2-3.

Note 3   Value used by U.S. EPA/OPP 2004b in PRZM/EXAMS modeling. The experimental halftime for aquatic metabolism was 15 days (Hetrick 1995e,h). The U.S. EPA/OPP (2004b) triples this value as a conservative approximation for nature bodies of water. Actual rates of degradation in water appear to be concentration dependant, ranging from 5 days (at 1 ppb) to 500 days (at 100 ppb) (Torang et al. 2003).

Note 4   Value used by U.S. EPA/OPP 2004b in PRZM/EXAMS modeling. Koc values estimated from different studies and soils range from about 10 to 120 (see Table 2-3).

Note 5   Values for clay and loam as reported in USDA/ARS (2001). For sand, the value for sandy loam soil (0.38 % OM or about 0.5% OC) reported by USDA/ARS (2001) is used.

Note 6   A foliar fraction of 0.5 is used by default for liquid formulations.

Site Parameters (see SERA 2004 for details)

Pond	1 hectare pond, 2 meters deep, with a 0.01 sediment fraction. 10 hectare (24.71 acre) square field (1037' by 1037') with a root zone of 60 inches.
Stream	Base flow rate of 710,000 L/day with a flow velocity of 0.08 m/second or 6912 meters/day. Stream width of 2 meters (about 6.6 feet'). 10 hectare square field (1037' by 1037') with a root zone of 60 inches.

Table 3-6: Summary of modeled concentrations in streams (all units are ug/L or ppb per lb/acre applied)

Annual Rainfall (inches)	Clay		Loam		Sand	
	Average	Maximum	Average	Maximum	Average	Maximum
5	0	0	0	0	0	0
10	0	0	0	0	0	0
15	0.423	91.9	0	0	0	0
20	0.753	171	0	0	0	0
25	0.999	236	0	0	1.36E-07	9.97E-06
50	1.42	380	0.000835	0.251	0.00119	0.062
100	1.4	439	0.016	5.14	0.0768	4.12
150	1.17	386	0.0115	3.73	0.164	10.8
200	0.972	320	0.00753	2.43	0.224	17.4
250	0.83	273	0.00527	1.64	0.256	22

Table 3-7: Summary of modeled concentrations in ponds after an application rate of 1 lb a.e./acre (all units are ug/L or ppb per lb/acre applied).

Annual Rainfall (inches)	Clay		Loam		Sand	
	Average	Maximum	Average	Maximum	Average	Maximum
5	0	0	0	0	0	0
10	0	0	0	0	0	0
15	0.771	5.19	0	0	0	0
20	1.12	11.2	0	0	0	0
25	1.44	17.8	0	0	2.55E-07	2.37E-06
50	2.44	50.4	0.00189	0.0422	0.00211	0.0208
100	3.29	111	0.0446	1.49	0.151	1.76
150	3.28	135	0.0361	1.42	0.368	5.26
200	3.01	136	0.0254	1.1	0.554	9.18
250	2.76	133	0.0185	0.836	0.683	12.9

Table 3-8: Estimated environmental concentrations (µg/L or ppb) of 2,4-D in surface and groundwater based on modeling.

Scenario	Peak	Long-Term Average
GLEAMS MODELING FOR THIS RISK ASSESSMENT (1 lb/acre)		
Direct Spray of Pond (Worksheet D10a)	56	N/A
Pond, drift at 100 feet (Worksheet D10a)	1.1	N/A
Direct Spray of Stream (Worksheet D10b)	91	N/A
Stream, drift at 100 feet (Worksheet D10b)	1.8	N/A
GLEAMS Pond, Table 3-8	20 (2 - 140)[a]	0.4 (0.02 - 3.3)[b]
GLEAMS, Stream, Table 3-7	11 (0.25-440)[c]	0.2 (0.01-1.4)[e]
OTHER MODELING (U.S. EPA/OPP 2004b adjusted to 1 application at 1 lb/acre)		
PRZM/EXAMS, Index Reservoir [f]	9.4 (1.7 - 30)	1 (0.65 - 2.2)
Sci-Grow 2.3, groundwater	0.03	N/A

[a]Pond Peak Concentrations: Central estimate based on peak at 25" rainfall in clay. Upper range based on clay at 150" or more. Lower range based on sand at 100" rainfall.

[b]Pond Average Concentrations: Central estimate based on sand at 150" rainfall. Upper range based on clay at 100". Lower range based on loam at 250" rainfall.

[c]Stream Peak Concentrations: Central estimate based on sand at 150" rainfall. Upper range based on clay at 100" rainfall. Lower range based on loam at 50" rainfall.

[d]Stream Average Concentrations: Central estimate based on loam at 100" rainfall. Upper range based on clay at 50" to 100". Lower range based on loam at 150" rainfall.

[e]See U.S. EPA/OPP 2004b, Table 5 (p. 47) and Table 6 (p. 205). Normalized to 1 application at 1 lb/acre by dividing by the product of the application rate and number of applications. Central estimates based on PA application to turf. Upper bounds based on NC application to apples. Lower bounds based on OR application to filberts.

[f]See U.S. EPA/OPP 2004b, p. 212. Note that estimate is substantially lower than monitoring data. See text for discussion.

Table 3-9: Summary of field studies assessing water contamination after the application of 2,4-D and general monitoring not associated with specific applications.

Concentrations Related to Application Rates

Application	Concentration (ppb)	Water Contamination Rate	Reference
765 lbs in 6920 acre watershed [0.11 lbs/acre]	0.19 - 0.24, groundwater[a] 0.08 - 0.19, pond water[a] 0.17 - 0.42, spring runoff[a]	1.7 - 2.1 0.72 - 1.7 1.5 - 3.8	Waite et al. 1992
904 lbs in 6920 acre watershed [0.13 lbs/acre]	0.08 - 0.12, pond water[a]	0.61 - 0.91	Waite et al. 1992
461 lbs in 6920 acre watershed [0.067 lbs/acre]	0.15 - 0.30, groundwater[a] 0.30 - 0.51, pond water[a] 0.15 - 0.29, spring runoff[a]	2.3 - 4.5 4-5 - 7.6 2.3 - 4.3	Waite et al. 1992
Stream water, 3 kg/9 km^2 (6.6 lbs/2223 acres)	0.02	6.7	Kreuger et al. 1999

General Background Concentrations

Water Body	Concentrations (ppb)	Notes	Reference
Surface water	58	Maximum concentrations from NAWQA[b]	U.S. EPA/OP 2005a
Groundwater	14.8		
Ground water	8	Maximum concentration from NCOC [c] data	U.S. EPA/OPP 2005a
Flowing water	1.45	Highest annual mean concentration from NAWQA[b]	U.S. EPA/OPP 2005a
Lakes in Saskatchewan, Canada	0.04-0.1168		Donald and Syrgiannis 1995
Creeks in Canadian Great Lake's watersheds	0.7 to 4.63	Peaks during periods of agricultural applications	Hall et al. 1993
Ponds in Canadian agricultural areas	0.29		Waite et al. 2002

[a]Values give as mean to maximum.
[b]USGS National Water Quality Assessment
[c]National Drinking Water Contaminant Occurrence Database

Table 3-10: Concentrations of 2,4-D in surface water used in this risk assessment (see Section 3.2.3.4.7 for discussion).

At application rate:	**1**	**lb/acre**	
		Peak Concentration (ppb or µg/L) [a]	Longer-term Concentration (ppb or µg/L) [b]
	Central	20	0.4
	Lower	2	0.02
	Upper	440	3.3
Water contamination rate [c]	**mg/L per lb/acre applied**		
		Peak Concentration (mg/L per lb/acre)	Longer-term Concentration (mg/L per lb/acre)
	Central	2.00e-02	4.00e-04
	Lower	2.00e-03	2.00e-05
	Upper	4.40e-01	3.30e-03

[a]Central estimate and lower bound based on GLEAMS modeling of pond. Upper bound based on GLEAMS modeling of stream. Upper bound encompasses estimates used by U.S. EPA/OPP (2005a, Table 8, p. 27): 70 to 118 ppb. Most monitoring data near lower bound (Table 3-X2).
[b]Based on long-term average from GLEAMS modeling of small pond. Encompasses annual mean concentrations estimated by U.S. EPA/OPP (2004b) as summarized in Table 3-8.
[c]Water contamination rates – concentrations in units of mg/L expected at an application rate of 1 lb/acre. Units of mg/L are used in workbook.

Table 3-11: EPA/OPP (2005a) Risk Numbers for Human Health

EPA Exposure Scenario	Dose Used, Uncertainty Factor	FQPA SF	Basis Study and Effect Levels
Acute Dietary, Females aged 13-49	NOAEL = 25 mg/kg/day UF = 1000 Acute RfD = 0.025 mg/kg/day	FQPA SF = 1X aPAD = 0.025 mg/kg/day	Rat developmental toxicity; NOAEL = 25 mg/kg/day; LOAEL = 75 mg/kg/day; Nemec et al. 1983a (MRID 00130408)
Acute Dietary, General Population	NOAEL = 67 mg/kg/day UF = 1000 Acute RfD = 0.067 mg/kg/day	FQPA SF = 1X aPAD = 0.067 mg/kg/day	Acute neurotoxicity study in rats, NOAEL = 67 mg/kg/day, LOAEL = 227 mg/kg/day Mattsson et al. 1994a (MRID 43115201)
Chronic Dietary, All populations	NOAEL = 5 mg/kg/day UF = 1000 Chronic RfD = 0.005 mg/kg/day	FQPA SF = 1X cPAD = 0.005 mg/kg/day	Rat Chronic Toxicity study; NOAEL = 5 mg/kg/day LOAEL = 75 mg/kg/day Rowland 1996a (MRID 43612001)

aPAD = acute population adjusted dose; NOAEL ÷ (UF x FQPA SF)
cPAD = chronic population adjusted dose; NOAEL ÷ (UF x FQPA SF)
FQPA = food quality protection act
SF = safety factor
UF = uncertainty factor

Table 3-12: Studies and Toxicity Values Which Cause Uncertainty* in EPA's Derivation of Acute RfDs for Reproductive-Age Females

Reference	Type of 2,4-D	Species	Exposure/ Duration	Effect
Rodwell 1991b (MRID 42055501)	DEA salt (50% a.e.)	Rabbit	Guideline Developmental Toxicity	NOAEL = 10.2 mg a.e./kg/day LOAEL = 20.3 mg a.e./kg/day, based on decreased bw gain and food consumption and one death in dams, and number of litters containing fetuses with 7th cervical ribs
Rowland 1992 (MRID 42158704)	IPA salt (39.6% a.e.)	Rabbit	Guideline Developmental Toxicity	Maternal LOAEL = 10 mg a.e./kg/day based on decreased body wt gain; Developmental NOAEL = 75 mg a.e./kg/day
Schroeder 1990a (MRID 4157102)	TIPA salt (38.7% a.e.)	Rat	Guideline Developmental Toxicity	LOAEL = 17 mg a.e./kg/day, based on decreased body weight and death in dams, and significant incidence of skeletal malformations in fetuses.
Rowland 1992 (MRID 42158705)	TIPA salt (39.2% a.e.)	Rabbit	Guideline Developmental Toxicity	Maternal NOAEL = 10 mg a.e./kg/day, Maternal LOAEL = 30 mg a.e./kg/day based on mortality, morbidity and clinical signs Developmental NOAEL = 75 mg a.e./kg/day
Schroeder 1990b (MRID 41527101)	BEE ester (65.8% a.e.)	Rabbit	Guideline Developmental Toxicity	Maternal NOAEL = 10 mg a.e./kg/day Maternal LOAEL = 30 mg a.e./kg/day, based on mortality, morbidity, clinical signs (decreased activity, prostration etc.) and decreased bw gain; Developmental NOAEL = 110 mg a.e./kg/day
Martin 1992d (MRID 42304601)	2-ethylhexyl ester (2-EHE) (63.25% a.e.)	Rat	Guideline Developmental Toxicity	NOAEL = 10 mg a.e./kg/day LOAEL = 30 mg a.e./kg/day, based on decreased bw and food consumption and increased clinical signs (ataxia, decreased motor activity, bradypnea) in dams, and increased incidence of delayed sternebrae ossification in fetuses. One abortion occurred.

* EPA's acute RfD for Reproductive-age females is based on a maternal NOAEL of 25 mg/kg/day for 2,4-D acid. Studies listed in this table have LOAEL values which are either lower than the NOAEL of 25 mg/kg/day, or are very close to it.

Table 4-1. Summary of 2,4-D critical ecological toxicity data for terrestrial animals

Organism/Study Type/Active Ingredient	Endpoint	Toxicity Value (all units in a.e.)	Reference
Birds			
Acute oral (gavage), most sensitive Bobwhite quail (*Colinus virginianus*) (DMA salt)	LD_{50}	415 mg/kg bw	Hoxter et al. 1990) (MRID 41546201)
Acute oral (gavage), least sensitive Mallard duck (*Anas platyrhynchos*) (DMA salt)	LD_{50}	>3851.2 mg/kg bw	Fink 1978 (MRID 233351, as cited in U.S. EPA/ OPP 2004b; full citation not provided)
Sub-acute dietary, most sensitive Bobwhite quail (*Colinus virginianus*) Mallard duck (*Anas platyrhynchos*) (TIPA salt)	5-day LC_{50}	>3035 mg/kg diet	Driscoll et al. 1990a (MRID 416444)
Sub-acute dietary, least sensitive Mallard duck (*Anas platyrhynchos*), Bobwhite quail (*Colinus virginianus* (2-ethylhexyl ester)	5-day LC_{50}	>6600 mg/kg diet	Fink 1977 (MRID 45070,as cited in U.S. EPA/ OPP 2004b; full citation not provided) Fink 1976 (MRID 226397,as cited in U.S. EPA/ OPP 2004b; full citation not provided)
Chronic oral Bobwhite quail (*Colinus virginianus*) (2,4-D acid)	NOEC LOEC (eggs cracked per eggs laid)	962mg/kg diet >962 mg/kg diet	Mitchell et al. 1999 (MRID 45336401)
Mammals			
acute toxicity, most sensitive species, Dog (DMA salt)	NOAEL	1.3 mg DMA salt/kg bw	Beasley et al. 1991
	LOAEL(sub-clinical myotonia)	8.8 mg DMA salt/kg bw	
acute toxicity, most sensitive non-canine Laboratory rat (*Rattus norvegicus*) (TIPA salt)	LD_{50}	579 mg/kg bw	Berdasco et al. 1989 (MRID 41413501)
acute toxicity, least sensitive non-canine Laboratory rat (*Rattus norvegicus*) (DMA salt)	LD_{50}	716 mg/kg bw	Johnson et al. 1981b (MRID 00101603)
acute neurotoxicity Rat (2,4-D acid)[a]	NOAEL LOAEL	67 mg/kg bw 227 mg/kg bw	Mattsson et al. 1994a (MRID: 43115201)

Table 4-1. Summary of 2,4-D critical ecological toxicity data for terrestrial animals

Organism/Study Type/Active Ingredient	Endpoint	Toxicity Value (all units in a.e.)	Reference
chronic toxicity, most sensitive Dog (2,4-D acid)[b]	NOAEL LOAEL	1 mg/kg/day 5 mg/kg/day	Dalgard 1983a (MRID 43049001)
chronic toxicity, least sensitive Rat (2,4-D acid)[c]	NOAEL LOAEL	5 mg/kg/day 75 mg/kg/day	Jeffries et al. 1995 (MRID 43612001)
Developmental toxicity, most sensitive Rat (TIPA salt)	LOAEL (bw gain; mortality)	17 mg/kg/day	Schroeder 1990a (MRID 41527102)
Developmental toxicity, least sensitive Rat (2,4-D acid)[d]	NOAEL LOAEL (bw gain/food consumption)	25mg/kg/day 75 mg/kg/day	Nemec et al. 1983a (MRID 00130408); Nemec et al. 1983b (MRID 000251031)
2-generation reproduction Rat (2,4-D acid)	Parental and reproductive NOAEL Parental and Reproductive	5 mg/kg/day	U.S. EPA/OPP 2004b (MRID Nos 259442, 259446, and 265489; no reference citations provided)
	LOAEL (body weight gain and male renal tubule alterations; increased gestation length)	20 mg/kg/day	
Insects			
Acute contact toxicity Honey bee (DMA salt, EHE)	LD$_{50}$	> 100 ug/bee	Palmer and Krueger 1997e (MRID 44517304) Palmer and Krueger 1997a (MRID 44517301)

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Table 4-1. Summary of 2,4-D critical ecological toxicity data for terrestrial animals

Organism/Study Type/Active Ingredient	Endpoint	Toxicity Value (all units in a.e.)	Reference
Studies on hatching success and larval development; exposure via direct treatment of host pupae and via parental feeding in honey for 10 days; Parasitic hymenopteran (*Pimpla turionellae*); 2,4-D (NOS)	NOAEC (host pupa treatment)	40 ppm	Ozkan and Yanikaolu 1999
	LOAEC (host pupa treatment)	50 ppm, significantly reduced eggs hatched in sprayed pupae of host; significantly reduced glycogen level in eggs; no eggs hatched at concentrations of 100 ppm and higher.	
	NOAEC (maternal exposure via food)	not determined	
	LOAEC (maternal exposure in food)	300 ppm; significantly reduced glycogen levels in eggs associated with failure of eggs to hatch	

[a]LOAEL based on increased incidence of in-coordination and slight gait abnormalities [described as forepaw flexing or knuckling] and decreased total motor activity

[b]LOAEL based on decreased body-weight gain (both sexes) and food consumption (females); increased BUN, creatinine, and alanine aminotransferase; decreased glucose, decreased brain weight in females, histopathological lesions in liver and kidneys.

[c]LOAEL based on decreased body-weight gain (females) and food consumption (females), alterations in hematology [decreased RBC, HCT, and HGB (females), platelets (both sexes)] and clinical chemistry [increased creatinine (both sexes), alanine and aspartate aminotransferases (males), alkaline phosphatase (both sexes), decreased T4 (both sexes), glucose (females), cholesterol (both sexes), and triglycerides (females)], increased thyroid weights (both sexes at study termination), and decreased testes and ovarian weights; microscopic lesions in the eyes, liver, adipose tissue, and lungs

[d]This study is the basis for EPA's acute RfD for reproductive-age females.

Table 4-2. Summary of 2,4-D critical toxicity data for terrestrial plants

Study Type/Organism/Active Ingredient	Endpoint	Toxicity Value (lb a.e./Acre)	Reference
2,4-D Acid and Amine Salts			
Seedling emergence: most sensitive monocot Sorghum (DMA salt)	NOEC EC_{25}	0.015 0.026	Backus and Crosby 1992 (MRID 42389501)
Seedling emergence: least sensitive monocot Oats, Corn (2,4-D acid)	NOEC EC_{25}	2.1 >4.2	Backus 1992a (MRID 42416802)
Seedling emergence: most sensitive dicot Mustard (DMA salt)	NOEC EC_{25}	0.00953 <0.015	Backus and Crosby 1992 (MRID 42389501)
Seedling emergence: least sensitive dicot Tomato (2,4-D acid)	NOEC EC_{25}	>4.2 >4.2	Backus 1992a (MRID 42416802)
Vegetative vigor: most sensitive monocot Onion (2,4-D acid)	NOEC EC_{25}	<0.0075 <0.0075	Backus 1992b (MRID 42416801)
Vegetative vigor: least sensitive monocot Corn (2,4-D acid)	NOEC EC_{25}	2.1 >4.2	Backus 1992b (MRID 42416801)
Vegetative vigor: most sensitive dicot Tomato (2,4-D acid)	NOEC EC_{25}	<0.0075 0.0075	Backus 1992b (MRID 42416801)
Vegetative vigor: least sensitive dicot Buckwheat (2,4-D acid)	NOEC EC_{25}	0.023 0.0075	Backus 1992b (MRID 42416801)
2,4-D Esters			
Seedling emergence: most sensitive monocot Onion (BEE)	NOEC EC_{25}	0.22 0.36	Narnish 1994 (MRID 43197001, as cited in U.S. EPA/OPP 2004b; no full citation provided)
Seedling emergence: least sensitive monocot Corn (EHE)	NOEC EC_{25}	>0.96 >0.96	Backus et al. 1992 (MRID 42449201, as cited in U.S. EPA/OPP 2004b; no full citation provided)
Seedling emergence: most sensitive dicot Radish (EHE)	NOEC EC_{25}	NR 0.045	Backus 1995 (MRID 43526901, as cited in U.S. EPA/OPP 2004b; no full citation provided)
Seedling emergence: least sensitive dicot Tomato (EHE)	NOEC EC_{25}	>0.96 >0.96	Backus 1992a (MRID 42416802)
Vegetative vigor: most sensitive monocot Onion (BEE)	NOEC EC_{25}	0.03 0.19	Narnish 1993 (MRID 4306701, as cited in U.S. EPA/OPP 2004b; no full citation provided)

Table 4-2. Summary of 2,4-D critical toxicity data for terrestrial plants

Study Type/Organism/Active Ingredient	Endpoint	Toxicity Value (lb a.e./Acre)	Reference
Vegetative vigor: least sensitive monocot Oats, Corn (EHE)	NOEC EC_{25}	>0.96 >0.96	Backus et al. 1992 (MRID 42343902, as cited in U.S. EPA/OPP 2004b; no full citation provided)
Vegetative vigor: most sensitive dicot Soybean (EHE)	NOEC EC_{25}	0.0075 0.02	Backus et al. 1992 (MRID 42343902, as cited in U.S. EPA/OPP 2004b; no full citation provided)
Vegetative vigor: least sensitive dicot Buckwheat (EHE)	NOEC EC_{25}	0.015 0.21	Backus et al. 1992 (MRID 42343902, as cited in U.S. EPA/OPP 2004b; no full citation provided)

Table 4-3. Critical data from the open literature which address the acute toxicity of 2,4-D acid, salts, and esters to fish

Species	Time	Formulation	LC$_{50}$ Dose (mg/L)	Reference
American eel	96 h	acid	300	Rehwoldt et al. 1977.
Banded Killifish	96 h	acid	26.7	Rehwoldt et al. 1977.
Carp	96 h	acid	5.1	Vardia and Durve 1981.
			15.3	Vardia and Durve 1981.
			20	Vardia and Durve 1981.
			24.15	Vardia and Durve 1981.
			31.25	Vardia and Durve 1981.
			35	Vardia and Durve 1981.
			96.5	Rehwoldt et al. 1977.
Coho salmon	96 h	amine	662	Wan et al. 1991.
Chum salmon	96 h	ethylhexyl ester	1 NOEL for fry	Meehan et al. 1974.
Coho salmon	96 h	ethylhexyl ester	1 NOEL for fry	Meehan et al. 1974.
	96 h	ethylhexyl ester	5 NOEL for smelt	Meehan et al. 1974.
Cutthroat trout	96 h	acid	64	Johnson and Finley 1980.
Cyprinid fish	96 h	acid	5.6	Vardia and Durve 1981.
Dolly Varden	96 h	ethylhexyl ester	10 NOEL for fingerlings	Meehan et al. 1974.
Fathead minnow	96 h	acid	263	Alexander et al. 1985.
Goldfish	96 h	acid	>187	Birge et al. 1979.
Lake trout	96 h	acid	45	Johnson and Finley 1980.
Largemouth bass	3.5 d	acid	160.7	Birge et al. 1979.
Pumpkinseed	24 h	acid	94.6	Rehwoldt et al. 1977.
Rainbow trout	96 h	acid	358	Alexander et al. 1985.
		ethylhexyl ester	10 NOEL for fingerlings	Meehan et al. 1974.
Pink Salmon	96 h	amine	438	Wan et al. 1991.
		ester	21	Wan et al. 1991.
	96 h	ethylhexyl ester	10 NOEL for fry	Meehan et al. 1974.
Striped bass	96 h	acid	70.1	Rehwoldt et al. 1977.
White perch	96 h	acid	40	Rehwoldt et al. 1977.
Zebrafish	96 h	acid	160	Benijts-Claus and Persoone 1975.

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Table 4-4. Summary of 2,4-D critical toxicity data for aquatic animals based on EPA studies

Study Duration/Organism/Active Ingredient	Endpoint	Toxicity Value (mg a.e./L)	Reference
	FISH		
2,4-D Acid and Amine Salts			
Acute, most sensitive	NOAEC	NR	Mayes et al. 1989c
Rainbow trout, *Oncorhynchus mykiss*,	96 hr LC$_{50}$	162	(MRID 41353803)
(TIPA salt)			
Acute, least sensitive	NOAEC	NR	Vilkas 1997
Rainbow trout, *Oncorhynchus mykiss*, Bluegill	96 hr LC$_{50}$	830	(MRID 232630),
sunfish, *Lepomis macrochirus*			Vilkas 1978
(DMA salt)			(MRID 234027, as cited
			in U.S. EPA/OPP
			2004b)
Chronic, most sensitive	NOAEC	14.2	Dill et al. 1990
Fathead minnow, *Pimphales promelas*	LOAEC	23.6	(MRID 417667701)
(DMA salt)	(length)		
Chronic, least sensitive	NOAEC	63.4	Mayes et al. 1990a
Fathead minnow, *Pimphales promelas*	LOAEC(larval	>102	(MRID 41737304)
(2,4-D acid)	survival)		
2,4-D Esters			
Acute, most sensitive	NOAEC	NR	Ward and Boeri 1991a
Tidewater silverside, *Menidia beryllina*	96 hr LC$_{50}$	>0.1564	(MRID 41835205)
(2-ethylhexyl ester)			
Acute, least sensitive	NOAEC	NR	Buccafusco 1976b
Rainbow trout, *Oncorhynchus mykiss*	96 hr LC$_{50}$	14.5	(MRID 45068)
(2-ethylhexyl ester)			
Chronic , most sensitive	NOAEC	0.0555	Mayes et al. 1989b
Fathead minnow, *Pimphales promelas*)	LOAEC	0.0791	(MRID 41345701)
(BEE)	(Survival)		
Chronic, least sensitive	NOAEC	0.0792	Mayes et al. 1990b
Fathead minnow, *Pimphales promelas*)	LOAEC	>0.1452	MRID 41737305)
(Ethylhexyl ester)	(Larval		
	survival)		
	AMPHIBIANS		
2,4-D Acid and Amine Salts			
Acute, most sensitive	NOAEC	NR	Palmer and Krueger
Leopard frog tadpoles, *Rana pipiens*	96-hr LC$_{50}$	278	1997c
(DMA salt)			(MRID 44517306)
Acute, least sensitive	NOAEC	NR	Palmer and Krueger
Leopard frog tadpoles, *Rana pipiens*	96-hr LC$_{50}$	359	1997d
(2,4-D acid)			(MRID 44517307)
2,4-D Esters			
Acute, most/least sensitive	NOAEC	NR	Palmer and Krueger
Leopard frog tadpoles, *Rana pipiens*	96-hr LC$_{50}$	0.505	1997b
(2-ethylhexyl ester)			(MRID 44517305)

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Table 4-4. Summary of 2,4-D critical toxicity data for aquatic animals based on EPA studies

Study Duration/Organism/Active Ingredient	Endpoint	Toxicity Value (mg a.e./L)	Reference
	INVERTEBRATES		
2,4-D Acid and Amine Salts			
Acute invertebrate, most sensitive Water flea, *Daphnia magna*, (2,4-D acid)	NOAEC 48-hr LC$_{50}$	NR 25	Alexander et al. 1983d (MRID 41158301)
Acute invertebrate, least sensitive Fiddler crab, *Uca pugilator* (DMA salt)	NOAEC 96-hr LC$_{50}$	NR 830	Vilkas 1997 (MRID 232630, as cited in U.S. EPA/OPP 2004b)
Chronic invertebrate Water flea, *Daphnia magna* (DMA salt)	NOAEC 21-day LC$_{50}$ (Survival)	not established 75.7	Ward 1991a (MRID 41835210)
Chronic invertebrate Water flea, *Daphnia magna*) (2,4-D acid)	NOAEC LOAEC (# of young)	79 151	Ward and Boeri 1991c (MRID 41853211)
Chronic invertebrate Water flea, *Daphnia magna*) (2,4-D diethanolamine salt)	NOAEC LOAEC	16.05 25.64	Holmes and Peters 1991 (MRID 42018303)
2,4-D Esters			
Acute , most sensitive Grass shrimp, *Palaemonetes pugio* (2-ethylhexyl ester)	NOAEC 96-hr LC$_{50}$	NR 0.092	Ward and Boeri 1991f (MRID 41835206)
Acute, least sensitive Eastern oyster, *Crassostrea virginica* (2-ethylhexyl ester)	NOAEC 96-hr LC$_{50}$	NR >66	Ward and Boeri 1991d (MRID 41835204)
Chronic, most/least sensitive Water flea, *Daphnia magna* (BEE)	NOAEC LOAEC (Survival and reproduction)	0.20 0.483	Gerisch et al. 1989 (MRID 41353802)

Table 4-5. Studies from the open literature on the acute toxicity of 2,4-D to aquatic invertebrates [1]

Common Name	Scientific Name	Form [2]	Dura-tion (Days)	LC$_{50}$ (mg/L)	Reference [3]
ACID and DMA Salt					
Water flea	*Daphnia magna*	Acid	2	25	Alexander et al. 1985
Water flea	*Daphnia magna*	Acid	2	36.4	Alexander et al. 1985
Stonefly	*Pteronarcys californicus*	Acid	2	44	Sanders and Cope, 1968
Copepod	*Acanthocyclops vernalis*	DMA	4	54.8	Robertson and Bunting 1976
Oyster	*Crassostrea virginica*	DMA	14	64.2	Davis and Hidu 1969
Mosquito	*Culex tritaeniorhynchus*	Acid	1	91.8	Shim and Self 1973
Scud	*Gammarus fasciatus*	DMA	4	100	Mayer and Ellersieck1986
Rotifer	*Brachionus calyciflorus*	Acid	1	117	Snell 1991
Oligochaete, worm	*Lumbriculus variegatus*	Acid	2	122	Bailey and Liu 1980
Copepod	*Acanthocyclops vernalis*	DMA	4	142	Robertson and Bunting 1976
Copepod	*Eudiaptomus gracilis*	Acid	4	144	Presing and Ponyi 1986
Water flea	*Daphnia magna*	DMA	2	184	Alexander et al. 1985
Copepod	*Acanthocyclops vernalis*	DMA	2	226	Robertson and Bunting 1976
Water flea	*Ceriodaphnia dubia*	Acid	2	236	Oris et al. 1991
Crab	*Uca uruguayensis*	Acid	2	400	Rodriguez and Lombardo1991
Rotifer	*Brachionus calyciflorus*	Acid	1	422	Nelson and Roline1998
Water flea	*Ceriodaphnia dubia*	Acid	2	422	Nelson and Roline1998
Shrimp	*Penaeus duorarum*	Acid	4	467	U.S. EPA/ORD 2006
Rotifer	*Brachionus plicatilis*	Acid	1	598	Snell et al. 1991
Crayfish	*Procambarus clarkii*	DMA	4	1389	Cheah et al. 1980
Esters of 2,4-D					
Scud	*Gammarus fasciatus*	BEE	4	0.44	Mayer and Ellersieck 1986
Shrimp	*Palaemonetes kadiakensis*	BEE	4	0.56	Mayer and Ellersieck 1986
Scud	*Gammarus lacustris*	BEE	2	0.76	Sanders 1969
Shrimp	*Palaemonetes kadiakensis*	BEE	1	1	Mayer and Ellersieck 1986
Crab	*Chasmagnathus granulata*	BEE	2	1.06	Rodriguez and Amin 1991
Stonefly	*Pteronarcys californicus*	BEE	4	1.6	Sanders and Cope 1968
Stonefly	*Pteronarcys californicus*	BEE	2	1.8	Sanders and Cope 1968
Scud	*Gammarus fasciatus*	EHE	4	2.4	Mayer and Ellersieck 1986

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Table 4-5. Studies from the open literature on the acute toxicity of 2,4-D to aquatic invertebrates [1]

Common Name	Scientific Name	Form [2]	Dura-tion (Days)	LC$_{50}$ (mg/L)	Reference [3]
Esters of 2,4-D *(continued)*					
Sowbug	*Asellus brevicaudus*	BEE	4	2.6	Mayer and Ellersieck 1986
Copepod	*Nitocra spinipes*	BEE	4	3.1	Linden et al. 1979
Scud	*Gammarus lacustris*	EHE	2	4.6	Sanders 1969
Scud	*Gammarus fasciatus*	BEE	4	6.1	Mayer and Ellersieck 1986

[1] See Section 4.1.3.3 for discussion. Sorted by LC$_{50}$ values, lowest to highest.
[2] DMA=Dimethylamine salt; BEE = butoxyethyl ester; EHE=2-ethylhexyl ester

Table 4-6. Summary of 2,4-D critical toxicity data for aquatic plants based on EPA studies

Organism/Active Ingredient	Endpoint	Toxicity Value (mg a.e./L)	Reference
2,4-D Acid and Amine Salts			
Water Milfoil(*Myriophyllum sibiricum*), most sensitive vascular (2,4-D acid)	EC_{25}	0.005	Roshon 1999
	EC_{50}	0.013	
Duckweed (*Lemna gibba*), least sensitive vascular (TIPA salt)	NOAEC	0.128	Hughes, et. al. 1994
	EC_{50}	1.28	(MRID 43488602)
Freshwater diatom (*Navicula pelliculosa*), most sensitive non-vascular (DMA salt)	NOAEC	1.41	Hughes 1990a
	EC_{50}	3.88	(MRID 41505903)
Blue-green algae (*Anabaena flos-aquae*), least sensitive non-vascular (DMA salt)	NOAEC	56.32	Hughes 1989
	EC_{50}	156	(MRID 41505902)
2,4-D Esters			
Water Milfoil(*Myriophyllum sibiricum*), most sensitive vascular (2,4-D acid data used in the absence of data on esters)	EC_{25}	0.005	Roshon 1999
	EC_{50}	0.013	
Duckweed (*Lemna gibba*), least sensitive vascular (BEE)	NOAEC	0.141[a]	Hughes 1990e
	EC_{50}	0.3974	(MRID 420688402)
Marine diatom (*Skelotonema costatum*), most sensitive non-vascular (2-ethylhexyl ester)	NOAEC	0.062	Hughes 1990b
	EC_{50}	0.066	(MRID 41735204)
Green algae (*Selanastrum capricornutum*), least sensitive non-vascular (2-ethylhexyl ester)	NOAEC	2.48	Hughes 1990c
	EC_{50}	>19.8	(MRID 41735206)

[a] In U.S. EPA/OPP 2004b, this value is incorrectly reported as 0.281 in "Table 7. Aquatic Plant Toxicity Summary". The correct value is found in the summary table for 2,4-D 2-butoxyethyl ester (BEE) on p. 246.

Table 4-7: Summary of the cumulative loss from soil runoff and sediment as a proportion of the application rate

Annual Rainfall (inches)	Clay	Loam	Sand
5	0	0	0
10	0	0	0
15	0.0147	0	0
20	0.032	0	0
25	0.0511	0	0
50	0.147	0.000115	0
100	0.312	0.00444	0
150	0.407	0.00475	0
200	0.457	0.00408	0
250	0.492	0.00342	0

Table 4-8: Summary of modeled maximum depth of 2,4-D in the soil column**.**

Annual Rainfall (inches)	Clay Depth	Loam Depth	Sand Depth
5	6.5	6.5	6.5
10	6.5	6.5	6.5
15	12	18	30
20	12	24	36
25	18	24	42
50	18	36	60
100	18	54	60
150	18	60	60
200	18	60	60
250	18	60	60

Table 4-9: Summary of modeled concentrations in the entire 60 inch soil column (all units are mg/kg soil or ppm per lb/acre applied)

Annual Rainfall (inches)	Clay Average	Clay Maximum	Loam Average	Loam Maximum	Sand Average	Sand Maximum
5	0.00133	0.0349	0.00113	0.0321	0.00113	0.0321
10	0.00146	0.0349	0.00129	0.0321	0.0012	0.0321
15	0.00142	0.0345	0.00125	0.0321	0.00119	0.0321
20	0.00138	0.0339	0.00124	0.0321	0.00119	0.0321
25	0.00134	0.0333	0.00122	0.0321	0.00119	0.0321
50	0.00117	0.0295	0.00121	0.0321	0.00126	0.0321
100	0.000876	0.0262	0.00123	0.032	0.00141	0.0321
150	0.000701	0.0262	0.00126	0.032	0.00145	0.032
200	0.000604	0.0262	0.00128	0.032	0.00141	0.0316
250	0.000533	0.0262	0.00131	0.032	0.00135	0.0311

Table 4-10: Summary of modeled concentrations in the top 12 inches of the soil column (all units are mg/kg soil or ppm per lb/acre applied)

Annual Rainfall (inches)	Clay		Loam		Sand	
	Average	Maximum	Average	Maximum	Average	Maximum
5	0.00663	0.174	0.00564	0.161	0.00565	0.161
10	0.0073	0.174	0.00643	0.161	0.00598	0.161
15	0.0071	0.172	0.00627	0.161	0.00595	0.161
20	0.0069	0.17	0.00618	0.161	0.00582	0.16
25	0.00672	0.166	0.00611	0.161	0.0057	0.159
50	0.00586	0.148	0.00591	0.16	0.00502	0.148
100	0.00438	0.131	0.00561	0.156	0.00388	0.124
150	0.00351	0.131	0.00541	0.153	0.00315	0.116
200	0.00302	0.131	0.00526	0.15	0.00266	0.116
250	0.00266	0.131	0.00514	0.148	0.00232	0.116

Table 4-11: Chemical and site parameters used in GLEAMS modeling for 2,4-D Esters.

Chemical Specific Parameters				
Parameter	Clay	Loam	Sand	Comment/ Reference
Halftimes (days)				
Aquatic Sediment		9999		Note 1
Foliar		5		Knisel and Davis 2000
Soil		10		Knisel and Davis 2000
Water		1		Howard 1991
$K_{o/c}$, mL/g		100		Knisel and Davis 2000
K_d, mL/g	3	1.5	0.3	Note 2
Water Solubility, mg/L		12		2,4-D BEE, Table 2-3
Foliar wash-off fraction		0.5		Default value
Fraction applied to foliage		0.5		Default value

Note 1 By analogy to the approach used by U.S. EPA (2004b, Appendix A, p. 61), 2,4-D esters are considered stable in aquatic sediments and the halftime is set sufficiently high to have a negligible impact on modeling.

Note 2 Estimated from the Koc: $KD = Koc \times P$, where P is the proportion of organic carbon in the soil. Default values for P taken from SERA (2004): 0.003 for sand, 0.015 for loam, and 0.030 for clay.

Table 4-12: Summary of modeled peak concentrations of 2,4-D esters in streams (all units are ug/L or ppb per lb/acre applied)

Annual Rainfall (inches)	Clay	Loam	Sand
5	0	0	0
10	0	0	0
15	32.6	0	0
20	62.3	0	0
25	87.6	0	0
50	151	0.261	0.0202
100	184	8.82	2.37
150	195	9.13	6.11
200	201	7.74	9.68
250	186	6.41	12.4

Table 4-13: Summary of modeled peak concentrations of 2,4-D esters in ponds (all units are ug/L or ppb per lb/acre applied)

Annual Rainfall (inches)	Clay	Loam	Sand
5	0	0	0
10	0	0	0
15	1.06	0	0
20	2.47	0	0
25	4.16	0	0
50	13.8	0.0305	0.00119
100	32.1	1.78	0.197
150	47	2.42	0.698
200	59.1	2.44	1.4
250	62.5	2.27	2.18

Table 4-14: Summary of 2,4-D toxicity values used in ecological risk assessment (all amounts expressed as a.e.)

Organism / 2,4-D Class / Exposure Duration [a]	Endpoint	Toxicity Value	Reference
Terrestrial Animals, 2,4-D acid, salts, and esters			
Dogs, Acute (DMA salt)	Acute NOAEL, 1.3 mg DMA salt/kg bw, single oral (capsule) dose, subclinical neurotoxicity	1.1 mg a.e./kg[b]	Beasley et al. 1991
Non-canine Mammals, Acute (rats, 2,4-D acid)	Acute NOAEL, oral, maternal toxicity (body weight gain)	25 mg a.e./kg	Nemec et al. 1983 (MRID 00130408)
Dogs, Chronic (2,4-D acid)	Chronic NOAEL	1 mg a.e./kg/day	Dalgard 1993d (MRID 430490001)
Non-canine Mammals, Chronic (rats, 2,4-D acid)	Chronic NOAEL, chronic oral toxicity and 2-generation reproduction studies	5 mg a.e./kg/day	Jeffries et al. 1995 (MRID 43612001); U.S. EPA 2004b (MRID 259442, 259446, 265489)
Birds, acute (bobwhite quail, DMA salt)	Acute gavage study LD_{50}	415 mg a.e./kg/day	Hoxter et al. 1990 (MRID 41546201)
Birds, chronic (bobwhite quail, 2,4-D acid)	Chronic NOAEC, cracked eggs per eggs laid, 962 mg a.e./kg diet	76 mg a.e./kg/day[c]	Mitchell et al. 1999 (MRID 45336401)
Invertebrates, acute (honey bee)	Acute contact NOAEL, 100 ug/bee[d]	1075 mg a.e./kg bw	Palmer and Krueger 1997e (MRID 44517304); Palmer and Krueger 1997a (MRID 445173-01)
Terrestrial Plants, 2,4-D acid and salts			
Terrestrial Plants - Pre-emergence assay (seedling emergence study: soil treatment)			
Sensitive (mustard, DMA salt)	NOAEC, all effects	0.0093 lbs a.e./acre	Backus and Crosby 1992 (MRID 42389501)
Tolerant (tomato, 2,4-D acid)	NOAEC, all effects	>4.2 lbs a.e./acre	Backus 1992a (MRID 42416802)
Terrestrial Plants - Post-emergence assay (vegetative vigor study: direct spray)			
Sensitive (onion, 2,4-D acid)	LOAEC, all effects	0.0075 lbs a.e./acre	Backus 1992b (MRID 42416801)
Tolerant (corn, 2,4-D acid)	NOAEC, all effects	2.1 lbs a.e./acre	Backus 1992b (MRID 42416801)

Table 4-14: Summary of 2,4-D toxicity values used in ecological risk assessment (all amounts expressed as a.e.)

Organism / 2,4-D Class / Exposure Duration [a]	Endpoint	Toxicity Value	Reference
Terrestrial Plants, 2,4-D esters			
Terrestrial Plants - Pre-emergence assay (seedling emergence study: soil treatment)			
Sensitive (radish, EHE)	EC_{25}, all effects (No NOAEC was established)	0.045 lbs a.e./acre	Backus 1995 (MRID 43526901, as cited in U.S. EPA/OPP 2004b; no full citation provided)
Tolerant (tomato, EHE)	NOAEC, all effects	>0.96 lbs a.e./acre	Backus 1992a (MRID 424168-02)
Terrestrial Plants - Post-emergence assay (vegetative vigor study: direct spray)			
Sensitive (soybean, EHE)	NOAEC, all effects	0.0075 lbs a.e./acre	Backus et al. 1992 (MRID 42343902, as cited in U.S. EPA/OPP 2004b; no full citation provided)
Tolerant (corn, EHE)	NOAEC, all effects	>0.96 lbs a.e./acre	Backus 1992b (MRID 42416801)
Aquatic Animals, 2,4-D acid and salts			
Amphibians, Acute			
Sensitive (Toad, *Bufo melanosticus*, 2,4-D acid)	96-hr LC_{50}	8.05 mg a.e./L	Vardia et al. 1984
Tolerant (leopard frog tadpoles, 2,4-D acid)	96-hr LC_{50}	359 mg a.e./L	Palmer and Krueger 1997d (MRID 44517307)
Fish, Acute			
Sensitive (carp, 2,4-D acid)	96 hr LC_{50}	96.5 mg a.e./L	Rehwoldt et al. 1977
Tolerant (rainbow trout, bluegill, DMA salt)	96 hr LC_{50}	830 mg a.e./L	Vilkas 1997 (MRID 232630), Vilkas 1978 (MRID 234027, as cited in U.S. EPA/OPP 2004b)
Fish, Chronic			
Sensitive (Carp, 2,4-D acid)	NOAEC based on acute LC_{50} = 95.6 mg/L ÷ 5 on basis of fathead minnow ratio of acute to chronic toxicity of 2,4-D acid [e]	19 mg a.e./L	Mayes et al. 1990a (MRID41737304); Rehwoldt et al. 1977
Tolerant (fathead minnow, 2,4-D acid)	NOAEC, larval survival	63.4 mg a.e./L	Mayes et al. 1990a (MRID 41737304)

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Table 4-14: Summary of 2,4-D toxicity values used in ecological risk assessment (all amounts expressed as a.e.)

Organism / 2,4-D Class / Exposure Duration [a]	Endpoint	Toxicity Value	Reference
Aquatic Invertebrates, Acute			
Sensitive (Water flea, *Daphnia magna*, 2,4-D acid)	48-hr LC_{50}	25 mg a.e./L	Alexander et al. 1983d and Alexander et al. 1985
Tolerant (Crayfish, *Procambarus clarkii,* DMA salt)	96-hr LC_{50}	1389 mg a.e./L	Cheah et al. 1980
Aquatic Invertebrates, Chronic			
Sensitive (Water flea, *Daphnia magna*, 2,4-D diethanolamine)	NOAEC	16.05 mg a.e./L	Holmes and Peters 1991
Tolerant (Water flea, *Daphnia magna*, 2,4-D acid)	NOAEC	75.7 mg a.e./L	Ward 1991a
Aquatic Animals, 2,4-D esters			
Amphibians, Acute			
Sensitive/tolerant (leopard frog tadpoles, 2-ethylhexyl ester)	96-hr LC_{50}	0.505 mg a.e./L	Palmer and Krueger 1997b (MRID 44517305)
Fish Acute			
Sensitive (tidewater silverside, *Menidia beryllina*, EHE)	96 hr LC_{50}	0.1564 mg a.e./L	Ward and Boeri 1991a (MRID 41835205)
Tolerant (rainbow trout, EHE)	96 hr LC_{50}	14.5 mg a.e./L	Buccafusco 1976b (MRID 45068)
Aquatic Invertebrates, Acute			
Sensitive (grass shrimp, *Palaemonetes pugio*, EHE)	96-hr LC_{50}	0.092 mg a.e./L	Ward and Boeri 1991f (MRID 41835206)
Tolerant (Eastern oyster, *Crassostrea virginica*, EHE)	96-hr EC_{50} for shell deposition	>66 mg a.e./L	Ward and Boeri 1991d (MRID 41835204)
Aquatic Plants, 2,4-D Acid and Salts			
Aquatic Algae			
Sensitive (freshwater diatom, *Navicula pelliculosa*, DMA salt)	NOAEC, survival and growth	1.41 mg a.e./L	Hughes 1990a (MRID 41505903)
Tolerant (Blue-green algae, *Anabaena flos-aquae*, DMA salt)	NOAEC, survival and growth	56.32 mg a.e./L	Hughes 1989 (MRID 41505902)
Aquatic Macrophytes			
Sensitive (*Myriophyllum sibiricum*, 2,4-D acid)	EC_{25}, survival and growth	0.005 mg a.e./L	Roshon 1999
Tolerant Sago pondweed, (*Potamogeton pectinatus*, DMA salt)	NOAEC, survival and growth	2 mg a.e./L	Sprecher et al. 1998

Table 4-14: Summary of 2,4-D toxicity values used in ecological risk assessment (all amounts expressed as a.e.)

Organism / 2,4-D Class / Exposure Duration [a]	Endpoint	Toxicity Value	Reference
Aquatic Plants, 2,4-D Esters			
Aquatic Algae			
Sensitive (marine diatom, *Skelotonema costatum*, 2,4-D EHE)	NOAEC, survival and growth	0.062 mg a.e./L	Hughes 1990b (MRID 41735204)
Tolerant (green algae (*Selanastrum capricornutum*, EHE)	NOAEC, survival and growth	2.48 mg a.e./L	Hughes 1990c (MRID 41735206)
Aquatic Macrophytes			
Sensitive (*Myriophyllum sibiricum*, 2,4-D acid)	EC_{25}, survival and growth	0.005 mg a.e./L	Roshon 1999
Tolerant Sago pondweed, *Potamogeton pectinatus*, DMA salt)	NOAEC, survival and growth	2 mg a.e./L	Sprecher et al. 1998

[a] The use of the terms "sensitive" and "tolerant" refer to the combination of species and form of 2,4-D giving the most sensitive or tolerant result.

[b] mg DMA salt/kg x acid equivalence factor of 0.831 (from Table 2-3) = 1.08 mg a.e./kg

[c] NOAEL = 962 mg/kg diet x 0.079 kg diet/kg bw/day (U.S. EPA 1993a: *Wildlife Exposure Factors Handbook*, page 2-127).

[d] 100 ug/bee ÷ 9.3E-5 kg/bee x 1E-3 mg/ug = 1075 mg/kg bw.

[e] The acute LC_{50} for 2,4-D acid in carp (selected as the sensitive species) is 96.5 mg/L. No chronic testing was conducted with carp. Acute and chronic testing with 2,4-D acid in fathead minnows yielded an acute LC_{50} of 320 mg/L and a chronic NOAEC of 63.4 mg/L. The acute to chronic ratio based on these numbers is 5.0 [320 ÷ 63.4]. Dividing the Carp LC_{50} of 96.5 mg/L by the acute to chronic ratio for fathead minnow and rounding to two significant places, yields an estimated chronic NOAEC of 19 mg/L for carp [96.5 mg/L ÷ 5 = 19.3].

Appendix 1. Reproductive effects and teratogenicity of 2,4-D to mammals and chickens

Reference	Type of 2,4-D	Species	Exposure/ Duration	Effects
Binns and Johnson 1970	2,4-D (NOS)	sheep (NOS)	2 g (40 mg/kg) by gavage daily for 30, 60, or 90 days after breeding	no birth defects in lambs; no clinical signs of toxicity in ewes during treatment; no histopathological lesions in internal organs of ewes or lambs
Bjorklund and Erne 1966	2,4-D amine (commercial formulation), 2,4-D K-Na salt, 2,4-D butyl ester (commercial formulation)	sow (Swedish Lantras; 200 kg; 6-years-old) F_1 generation: 5 piglets	sow: 500 ppm in diet throughout gestation and for 6 weeks after parturition F_1 generation: 500 ppm in diet for 7-8 months	maternal toxicity included anorexia (but no signs of indigestion or other illness); locomotor disturbances after parturition which progressed to lameness of one hind limb by week 6; decreases in hematocrit and hemoglobin values; slight increases in GOT, albumin, and albumin-globulin ratios; albuminuria; no gross or histopathological changes

effects on offspring included decreased pup weights and underdevelopment; 10/15 piglets (2 males and 8 females) died during the first day; autopsy indicated generalized anemia, and embryonic haemotopoietic foci in the livers were detected histopathologically; 2,4-D concentrations (15-80 µg/g) were detected in livers, kidneys and lungs of piglets that died

F_1 generation effects: no indigestion; growth depression; persistent anemia; locomotor disturbances less severe than those observed in maternal experiment observed at 3 months; fissures and ulcerations on abaxial surface of the hoof wall, but length of hoofs remained normal; decreases in hematocrit and hemoglobin values; slight increases in GOT, albumin, and albumin-globulin ratios; albuminuria; moderate degenerative changes in liver and kidneys |
| Bjorklund and Erne 1966 | 2,4-D (NOS) | rats (Sprague-Dawley; 10 pregnant; 350 g) | 0 or 1000 ppm in drinking water throughout gestation and for additional 10 months | no adverse clinical signs or adverse morphology; normal pregnancy, birth, and litter sizes [offspring continued on treatment for 2 years - see entry below] |

Appendix 1-1

Appendix 1. Reproductive effects and teratogenicity of 2,4-D to mammals and chickens

Reference	Type of 2,4-D	Species	Exposure/ Duration	Effects
Bjorklund and Erne 1966	2,4-D (NOS)	rats (Sprague-Dawley; 10 male and 12 female offspring)	1000 ppm in drinking water for 2 years	no adverse clinical signs or adverse morphology; lower food and water consumption, and lower growth rate, and higher mortality rate, compared with controls [control group consisted of 9 untreated males and 8 untreated females]
Chernoff et al. 1990	2,4-D acid	rats (Sprague-Dawley), 5 females, days 6-15 of gestation	115 mg/kg body weight/day by gavage	maternal toxicity (reduced body weight and 15% mortality); significant increase in incidence of supernumerary ribs in fetus
Collins and Williams 1971	three commercial samples of 2,4-D (no sample contained detectable amounts of dioxin)	hamsters (female; Syrian golden)	0, 40, 60, or 100 mg/kg/day by gavage (daily) on days 6-10 of gestation (one sample also tested at 20 mg/kg)	occasional teratogenicity; decrease in fetal viability in one of the three samples; neither effect clearly related to dose; [vehicle = corn oil: carboxymethyl cellulose (1:5.8:10); resorption sites, corpora lutea, and fetal anomalies evaluated by gross examination and microscopically]
Courtney 1977	2,4-D acid and several esters (90-99.9% purity)	mice (CD-1)	0.56-1 mM/kg (123-221 mg a.e./kg body weight/day) by gavage during gestations days 7-15 or a fraction of that period	sporadic decreases in maternal body weight in increases in liver weight observed at all dose levels and did not follow a clear dose-response pattern; fetal body weights were decreased significantly at all dose levels except the low dose n-butyl ester group and the high dose 2,4-D acid in DMSO vehicle group; at 124 mg/kg body, weight/day, 2,4-D acid and PGBE induced cleft palates; at approximately 221 mg/kg body weight/day all the esters tested induced cleft palates; no cleft palates were observed in the control mice.

Appendix 1-2

Appendix 1. Reproductive effects and teratogenicity of 2,4-D to mammals and chickens

Reference	Type of 2,4-D	Species	Exposure/ Duration	Effects
de Moro et al. 1993	2,4-D butyl ester	chicken (hen eggs; fertile)	single topical application of 3.1 mg/egg immediately prior to incubation	no difference in wet weight of brains of treated chicks, compared with controls, except on embryonic day 14; beginning with embryonic day 14 there was a significant decrease in the rate of galactolipids deposition (30-45%), due mostly to alterations in cerebrosides levels (42-55%); the treated group had significant decreases (50%)in brain cholesterol content beginning on embryonic day 16 that diminished to 35% at 1 day post hatching; total brain protein content and CNP activity in treated group were decreased, compared with controls; DNA content in the treated group decreased at embryonic day 12, but increased significantly from embryonic day 14 to day 1 post hatching.
Fofana et al. 2000	2,4-D sodium salt (99.9% pure)	rat (Wistar, 12-week old, 200-360g)	0, 50, 70, 110 or 150 mg/kg/day by gavage in water to each of three test groups: days 6-15 gestation; days 6-10 gestation; or days 11-15 of gestation	Conclusion: 2,4-D is embryo-lethal and induces kidney and genital malformations in the early developmental stage of the neurological system. Maternal LOAEL = 50 mg/kg/day, based on significant weight loss; Offspring NOAEL = 50 mg/kg/day Offspring LOAEL = 70 mg/kg/day, based on significant increases in ureteric dilatation, hydronephrosis, and renal urogenital aplasia; embryo-lethality was dose-related and correlated with maternal weight loss.
Fofana et al. 2002	2,4-D (99.9% pure)	rat (Wistar, 12-week old, 200-300 g)	administration via gavage in water: gestation days 6-15: 0, 70 or 110 mg/kg/day; gestation days 6-10: 0, 70 or 110 mg/kg/day; gestation days 11-15: 0 or 150 mg/kg/day	Significantly increased incidence of urogenital malformations at each 2,4-D dose and gestational period of exposure. Significant increase in postnatal death (birth to 4 weeks) at all 2,4-D doses and gestational exposure periods. 2,4-D had no effect on the post-natal growth (measured as body weight)of survivors in comparison with controls.

Appendix 1-3

Appendix 1. Reproductive effects and teratogenicity of 2,4-D to mammals and chickens

Reference	Type of 2,4-D	Species	Exposure/ Duration	Effects
Garcia et al. 2004 (Developmental neurotoxicity study)	2,4-D from Sigma Chemical Co., St. Louis, MO. (Analytical grade; purity not specified)	rats (Wistar; 100 dams total; litters culled randomly to 8 pups each; 5 25-day-old pups per litter were analyzed)	dams injected intraperitoneally with 70 or 100 mg/kg/day of 2,4-D in DMSO for 16 days during lactation (days 9 to 25 post-partum); controls received DMSO only	Immuno-histochemical study of potential CNS damage in pups exposed to 2,4-D via dams milk during lactation; 25-day-old pup brains analyzed. LOAEL= 70 mg/kg/day, based on significantly decreased staining of tyrosine hydroxylase (TH) neurons in the substantia nigra (SN) of the brain; decreased fiber density of 5-hydroxy tyrosine (5-HT) fiber density in SN and ventral tegmental area (VTA) of brain. At 100 mg/kg/day: significant decreases in body weight as well as brain weight; 25% diminution in SN and 33% diminution in VTA neurons as well as brain weight; 25% diminution in SN and 33% diminution in VTA neurons; also TH immunostaining and significantly lower 5-HT fiber density.
Hansen et al. 1971	acid (96.7%) pure	rats (Osborne-Mendel; 10 males and 20 females)	100, 500, or 1500 ppm (5, 25, or 75 mg/kg/day) in diet for 3 generations	no adverse effects on fertility, mean litter size or viability of pups during first 21 days of age at 100 or 500 ppm; t 1500 ppm, sharp reduction in survival rate of offspring to day 21 and sharp decreased weanling weight; no adverse effects on litter size or fertility and no birth defects at 1500 ppm; liver enzyme activity did not differ from controls
Nemec et al. 1983b (MRID 000251031)	2,4-D, acid (97.5%)	rats Fischer	0, 8, 25, or 75 mg/kg body weight/day to dams on days 6 through 15 of gestation	no embryotoxic or teratogenic effects at any dose level; slight maternal toxicity (manifested as reduced body weight) at 75 mg/kg body weight/day.
ITF 1992	2,4-D acid	rats (NOS)	5, 20 or 80 mg/kg body weight	5 mg/kg body weight = NOEL; at 20 mg/kg body weight, no adverse effects except for a slight decrease in F_{1b} pup body weights during lactation; at 80 mg/kg body weight, decreases in maternal body weight and food consumption, decreases in gestational length and in F_{1a} and F_{1b} body weights during lactation, and litter sizes; excessive pup mortality occurred in the F_{1b} generation

Appendix 1-4

Appendix 1. Reproductive effects and teratogenicity of 2,4-D to mammals and chickens

Reference	Type of 2,4-D	Species	Exposure/ Duration	Effects
ITF 1992	2,4-D DMA	rats (CRI:DC BR VAP/Plus)	12.5, or >50 mg/kg body weight/day	maternal NOAEL = 12.5 mg/kg body weight/day; developmental NOAEL = 50 mg/kg body weight/day; 50 mg/kg body weight/day caused decreases in maternal body weight and food consumption; highest dose (NOS) caused decreases in fetal body weight and delayed bone ossification
ITF 1992	2,4-D acid	rabbits (New Zealand White; pregnant)	0, 30, or 90 mg/kg body weight/day, by gavage during gestations day 6 to 18	no developmental effects at any dose level; maternal NOAEL = <90 mg/kg body weight/day; developmental NOAEL = >90 mg/kg body weight/day; 90 mg/kg body weight/day resulted in abortion of 2/20 does; ataxia in 2/20 does (1 doe common to both effects), and decreases in maternal body weights; effects not observed at lower doses.
Kavlock et al. 1987	2,4-D acid, propylene glycol butyl ester, or isooctyl ester	mice (CD-1; 60-days-old; pregnant)	87.5 mg/kg body weight/day during gestation days 8-12	statistically significant decrease in weight gain of fetuses on postnatal day 1, but not on postnatal day 3
Khera and McKinley 1972	acid, dimethyl-amine salt, butyl ester, isooctyl ester, butoxy ethanol ester	rats (Wistar; 200-250 g; pregnant)	single daily gavage doses of 25-150 mg/kg on days 6-15 of gestation	no apparent effect on maternal body weight; no significant teratogenic effects at 25-50 mg/kg; skeletal anomalies (including wavy ribs, extra ribs, delayed ossification, and abnormalities in sternum morphology) and fetotoxicity such as decreased litter size, fetal weight, and survival of newborn at doses of 100 or 150 mg/kg/day
Kim et al. 1988	[^{14}C]2,4-D	mice (CD-1; pregnant; pretreated with 0, 40, or 80 mg/kg on gestation days 15 and 16)	0.2 mg/kg injected intraperitone-ally on day 17 of gestation	at 1 hour after exposure, concentrations of radiolabeled 2,4-D in the maternal and fetal brain were ~4% and ~8%, respectively, of plasma concentrations; steady state was achieved over the next 5 hours (maternal and fetal concentrations did not change, relative to plasma concentrations) pre-exposure to 40 or 80 mg/kg unlabeled 2,4-D caused a marked increase in the accumulation of radiolabeled 2,4-D in the brain

Appendix 1-5

Appendix 1. Reproductive effects and teratogenicity of 2,4-D to mammals and chickens

Reference	Type of 2,4-D	Species	Exposure/ Duration	Effects
Kim et al. 1988	[^{14}C]2,4-D	mice (CD-1; pregnant; pretreated with 80 mg/kg unlabeled 2,4-D in DMSO on gestation days 15 and 16)	0.4 mg/kg injected intraperitone-ally on day 17 of gestation	autoradiography indicated that pretreatment of dams resulted in marked increases of radiolabeled 2,4-D concentrations in the brains of mothers and fetuses, compared with controls. Brain concentrations of 2,4-D were, however, still below 2,4-D concentra-tions in most other tissues; liver and kidney showed the greatest accumulation of 2,4-D, but levels in the kidney were decreased by pretreatment.
Lee et al. 2001 (Develop-mental immuno-toxicity study)	commercial formulation of 2,4-D DMA salt (C.I.L. Dandelion Killer; NU-GRO Corp., Canada)	mouse (CD-1; 5-week-old; pregnant)	0, 0.02, 0.1 or 1.0 % 2,4-D DMA formulation in drinking water (0, 8.5, 37 or 370 mg a.e./kg bw/day) on days 6-16 of gestation)	Maternal NOAEL = 370 mg a.e./kg/day Offspring NOAEL = 8.5 mg a.e./kg/day; Offspring LOAEL = 37 mg a.e./kg/day, based on decreased body weight gain and decreased kidney weight in females. Immune LOAEL = 370 mg a.e./kg/day, based on subtle effects on the immune system (both sexes), evaluated 7 weeks after birth. Effects included: suppression of lymphocyte stimulation by concanavalin A; significantly increased relative B cells count (% per spleen); significantly reduced relative % suppressor or cytotoxic T-cells. The humoral immune response, measured as antibody production against sheep red blood cells and peritoneal phagocytic function, were not affected at any 2,4-D dose.

Appendix 1. Reproductive effects and teratogenicity of 2,4-D to mammals and chickens

Reference	Type of 2,4-D	Species	Exposure/ Duration	Effects
Lee et al. 2000 (Develop-mental immunotox-icity and carcino-genicity)	commercial formulation of 2,4-D DMA salt (C.I.L. Dandelion Killer; NU-GRO Corp., Canada)	(CD-1; 5-week-old; pregnant)	0, 0.02, 0.1 or 1.0 % 2,4-D DMA formulation in drinking water (0, 8.5, 37 or 370 mg a.e./kg bw/day) on days 6-16 of gestation)	2,4-D had slight anti-neoplastic activity in surviving offspring exposed in-utero to 370 mg a.e./kg/day. 2,4-D exposure did not reduce the number of urethane-induced lung adenomas, but there was a significant decrease in tumor diameter among pups exposed to the highest dose in-utero. The authors attribute this effect to 2,4-D inhibition of enzymatic or metabolic pathways necessary for cellular growth and tissue development. 2,4-D had no effect on urethane-induced sleeping times. Maternal NOAEL = 370 mg a.e./kg/day Offspring NOAEL = 8,5 mg a.e./kg/day; Offspring LOAEL = 37 mg a.e./kg/day, based on reduced body weight; there were no clinical signs of toxicity.
Schwetz et al. 1971	propylene glycol butyl ether ester	rats (pregnant adult Sprague-Dawley; 225 g)	12.5, 25.0, 50.0, 75.0, or 87.5 mg/kg/day on days 6-15 of gestation	no teratogenicity at any dose level; no effects on fertility, gestation, viability, or survival of newborns; high doses caused embryotoxic and fetotoxic effects including subcutaneous edema, delayed ossification, decreased fetal weight, lumbar ribs, and wavy ribs; no adverse effects on fertility, but highest dose decreased litter size and survival rate of newborn to the end of weaning; NOEL = equivalent of 25 mg/kg/day 2,4-D acid
Schwetz et al. 1971	EHE	rats (pregnant adult Sprague-Dawley; 225 g)	12.5, 25.0, 50.0, 75.0, or 87.5 mg/kg/day on days 6-15 of gestation	no teratogenicity at any dose level; no effects on fertility, gestation, viability, or survival of newborns; high doses caused embryotoxic and fetotoxic effects including subcutaneous edema, delayed ossification, decreased fetal weight, lumbar ribs, and wavy ribs; no adverse effects on fertility, but highest dose decreased litter size and survival rate of newborn to the end of weaning; NOEL = equivalent of 25 mg/kg/day 2,4-D acid

Appendix 1-7

Appendix 1. Reproductive effects and teratogenicity of 2,4-D to mammals and chickens

Reference	Type of 2,4-D	Species	Exposure/ Duration	Effects
Schwetz et al. 1971	acid	rats (pregnant adult Sprague-Dawley; 225 g)	12.5, 25.0, 50.0, 75.0, or 87.5 mg/kg/day on days 6-15 of gestation	no teratogenicity at any dose level; no effects on fertility, gestation, viability, or survival of newborns; high doses caused embryotoxic and fetotoxic effects including subcutaneous edema, delayed ossification, decreased fetal weight, lumbar ribs, and wavy ribs; no adverse effects on fertility, but highest dose of 87.5 mg/kg/day decreased litter size; NOEL = 25 mg/kg/day
Nemic et al. 1983a (MRID 00130408); Nemec et al. 1983b (MRID 000251031)	2,4-D acid (97.7% a.i.)	rat (Fischer 344, no other details given by USEPA)	0, 8, 25 and 75 mg/kg/day, "acceptable guideline study" for developmental toxicity per USEPA pesticide registration requirements	Maternal NOAEL = 25 mg/kg/day LOAEL = 75 mg/kg/day based on decreased body-weight gains. No treatment-related effect on survival Developmental NOAEL = 25 mg/kg/day LOAEL = 75 mg/kg/day based on skeletal abnormalities
Hoberman 1990 (MRID 41747601)	2,4-D acid (96.1% a.i.)	rabbit (no other details given by USEPA)	0, 10, 30 and 90 mg/kg/day, "acceptable guideline study" for developmental toxicity per USEPA pesticide registration requirements	Maternal NOAEL = 30 mg/kg/day; LOAEL = 90 mg/kg/day based on clinical signs [ataxia, decreased motor activity, loss of righting reflex, cold extremities], abortion (2), decreased body-weight gains. Survival was not affected by treatment. Developmental NOAEL =30 mg/kg/day; LOAEL = 90 mg/kg/day, based on abortions.

Appendix 1-8

Appendix 1. Reproductive effects and teratogenicity of 2,4-D to mammals and chickens

Reference	Type of 2,4-D	Species	Exposure/ Duration	Effects
Tasker 1985 (MRID 00150557); Brown 1986 (MRID 00163996}; U.S. EPA/ OPP 2004b (MRID 259442, 259446, 265489); WHO 1996	2,4-D acid (97.5% a.i)	rat (Fischer 344, 30 male, 30 female F0; treated 105 days prior to mating and through gestation and lactation of two litters, and for 30 days post-weaning for last litter	dietary exposure equivalent to doses of 0, 5, 20 and 80 mg/kg bw/day, "acceptable guideline study for 2-generation reproduction per USEPA pesticide registration requirements	Parental/Systemic NOAEL = 5 mg/kg/day; LOAEL = 20 mg/kg/day. based on decreased female body weight/ body-weight gain [Fl] and renal tubule alteration in males [FO and F1]. Reproductive NOAEL = 20 mg/kg/day; LOAEL = 80 mg/kg/day, based on an increase in gestation length [FO females producing Flb pups]. Offspring NOAEL = 5 mg/kg/day LOAEL = 20 mg/kg/day based on decreased pup body weight [F1b]. Increase in dead pups at 80 mg/kg/day.

NOTE: EFED (U.S. EPA/OPP 2004b) cites a number of developmental toxicity studies on rats conducted with salts and esters of 2,4-D. HED (U.S.EPA/OPP 2005a) states that they reviewed all of these studies, and concluded that the toxicity of the class of 2,4-D acid, salts, and esters is appropriately represented by 2,4-D acid. HED relies upon the studies conducted with 2,4-D acid as the basis for it's conclusions about the maternal and developmental toxicity of 2,4-D. Consequently, U.S. EPA/OPP (2005a) uses the NOAEL of 25 mg/kg/day derived from the rat developmental toxicity study with 2,4-D acid as the basis for the acute RfD of 0.025 mg/kg/day for dietary exposure to 2,4-D among reproductive age females (See Table 7 of U.S. EPA/OPP (2005a), (Reregistration Eligibility Decision for 2,4-D). Neither U.S.EPA/OPP (2005a) nor U.S.EPA/OPP (2005b) explains why the studies conducted with 2,4-D salts and esters which provide LOAEL values (in units of mg a.e./kg/day) below the 2,4-D acid rat NOAEL of 25 mg/kg/day are not used as the basis for this RfD. For the sake of completeness, all studies conducted with the salts and esters of 2,4-D are summarized below, as cited by EFED (U.S. EPA/OPP 2004b).

Reference	Type of 2,4-D	Species	Exposure/ Duration	Effects
Siglin 1990 (MRID 41920906)	DEA salt (45.6% a.e.)	Rat	NR	NOAEL = 10.2 mg a.e./kg/day; LOAEL = 50.6 mg a.e./kg/day, based on decreased body wt gain in dams and skeletal variations in fetuses
Rodwell 1991b (MRID 42055501)	DEA salt (50% a.e.)	Rabbit	NR	NOAEL = 10.2 mg a.e./kg/day LOAEL = 20.3 mg a.e./kg/day, based on decreased bw gain and food consumption and one death in dams, and number of litters containing fetuses with 7th cervical ribs

Appendix 1-9

Appendix 1. Reproductive effects and teratogenicity of 2,4-D to mammals and chickens

Reference	Type of 2,4-D	Species	Exposure/ Duration	Effects
Lochry 1990 (MRID 41735201)	DMA salt (55.5% a.e.)	Rat	NR	NOAEL = 12.5 mg a.e./kg/day LOAEL = 50 mg a.e./kg/day, based on decreased body wt gain and reduced food consumption in dams and decreased fetal body weight
Martin 1991a (MRID 42224001)	DMA salt (55.5% a.e.)	Rabbit	NR	Maternal NOAEL = 30 mg a.e./kg/day; Maternal LOAEL = 90 mg a.e./kg/day, based on clinical signs (ataxia etc.) mortality and morbidity in dams; Developmental NOAEL = 90 mg a.e./kg/day
Schroeder 1990f (MRID 41527103)	IPA salt (50.2% a.e.)	Rat	NR	NOAEL = 5 mg a.e./kg/day; LOAEL = 150 mg a.e./kg/day based on decreased body wt gain and reduced food consumption in dams and slight increase in incidence of skeletal and external malformations in fetuses.
Breslin et al. 1991b (MRID 42158704)	IPA salt (39.6% a.e.)	Rabbit	NR	Maternal LOAEL = 10 mg a.e./kg/day based on decreased body wt gain; Developmental NOAEL = 75 mg a.e./kg/day
Schroeder 1990a (MRID 41527102)	TIPA salt (38.7% a.e.)	Rat	NR	LOAEL = 17 mg a.e./kg/day, based on decreased body weight and death in dams, and significant incidence of skeletal malformations in fetuses.
Rowland 1992 (MRID 42158705)	TIPA salt (39.2% a.e.)	Rabbit	NR	Maternal NOAEL = 10 mg a.e./kg/day, Maternal LOAEL = 30 mg a.e./kg/day based on mortality, morbidity and clinical signs Developmental NOAEL = 75 mg a.e./kg/day
Zablotny 1991 (MRID 42158706)	BEE ester (65.1% a.e.)	Rat	NR	NOAEL = 51 mg a.e./kg/day LOAEL - 125 mg a.e./kg/day, based on decreased body wt, food consumption and RBCs; and increased reticulocytes in dams; significant increase in incidence of skeletal malformations in fetuses.

Appendix 1-10

Appendix 1. Reproductive effects and teratogenicity of 2,4-D to mammals and chickens

Reference	Type of 2,4-D	Species	Exposure/ Duration	Effects
Schroeder 1990b (MRID 41527101)	BEE ester (65.8% a.e.)	Rabbit	NR	Maternal NOAEL = 10 mg a.e./kg/day Maternal LOAEL = 30 mg a.e./kg/day, based on mortality, morbidity, clinical signs (decreased activity, prostration etc.) and decreased bw gain; Developmental NOAEL = 110 mg a.e./kg/day
Martin 1992d (MRID 42304601)	2-ethylhexyl ester (2-EHE) (63.25% a.e.)	Rat	NR	NOAEL = 10 mg a.e./kg/day LOAEL = 30 mg a.e./kg/day, based on decreased bw and food consumption and increased clinical signs (ataxia, decreased motor activity, bradypnea) in dams, and increased incidence of delayed sternebrae ossification in fetuses. One abortion occurred.
Martin 1992c (MRID 42304603)	2-ethylhexyl ester (2-EHE) (63.5% a.e.)	Rabbit	NR	Maternal NOAEL = 30 mg a.e./kg/day Maternal LOAEL = 75 mg a.e./kg/day, based on mortality, morbidity and clinical signs of toxicity and decreased bw gain; Developmental NOAEL = 75 mg a.e./kg/day

Appendix 2. Published neurotoxicity studies of 2,4-D in mammals

Reference	2,4-D Species	Animal Species/ Strain/Sex	Exposure/ Duration	Effects
Beasley et al. 1991	DMA-4	dog (English pointer; approximately 1-year-old)	6 mL (approximately 2.5 g 2,4-D) on three 4x4 gauze pads taped to the right cranial tibial muscle	no electromyography alterations or other abnormalities
Beasley et al. 1991	DMA-4	dogs (6 castrated male and female English Pointers; approximately 1-year-old)	single oral dose (capsule) of 1.3, 8.8, 43.7, 86.7, 220, or 175 mg/kg/body weight	1.3 mg/kg body weight = NOEL for development of subclinical myotonia; 8.8, 43.7, or 86.7 mg/kg body weight produced no clinical signs of toxicosis, but induced subclinical myotonic discharges that peaked between 7 and 24 hours after exposure; 175 or 220 mg/kg body weight resulted in toxicosis (vomiting episodes that occurred several times during the 26-hour observation period) and clinical myotonia; by 23-24 hours after dosing the dogs appeared normal.
Steiss et al. 1987	2,4-D (NOS)	dogs (4/ dose group; NOS)	0, 25, 50, 75, 100, 125 mg/kg body weight	25 mg/kg body weight = NOEL for development of myotonia, based on absence of clinical myotonia and aberrations in electromyograph
Kim et al. 1988	[^{14}C]2,4-D	rabbits (young adult New Zealand White; pretreated for 2 hours with 0, 40, 80, 160 mg/kg 2,4-D in DMSO	0.2 mg/kg injected intraperitoneally (2 hours after pretreatment)	radiolabeled 2,4-D concentrations in the brains of control rabbits were very low (~3-5% of plasma concentrations); pretreatment with 2,4-D increased brain concentrations to 7-8% (40 mg/kg), 13-16% (80 mg/kg), and 23-27% (160 mg/kg) of plasma concentrations
de Duffard et al. 1990a	2,4-D n-butyl ester	rats (Wistar male and female)	69 mg/kg body weight in the diet for 15 or 45 days	changes in brain concentrations of, 5-hydroxytryptamine and 5-hydroxyindolacetic acid
de Duffard et al. 1990b	2,4-D n-butyl ester	rats (Wistar)	69 mg/kg body weight in the diet for 15 or 17 days	poorer scores in behavioral tests including active avoidance learning and rotarod and open field tests

Appendix 2-1

Appendix 2. Published neurotoxicity studies of 2,4-D in mammals

Reference	2,4-D Species	Animal Species/ Strain/Sex	Exposure/ Duration	Effects
Elo and Ylitalo 1979	radiolabled 2,4-D sodium salt	rats (pretreated with 250 mg/kg unlabeled 2,4-D sodium salt)	20-50 mg/kg body weight by intra-peritoneal injection	concentrations of 2,4-D in the brain and cerebrospinal fluid were 7-fold and 22-fold greater, compared with concentrations reported for rats that were not pretreated with unlabled 2,4-D.
Elo et al. 1988	2,4-D acid	rats (NOS)	single gavage dose of 150 or ≥300 mg/kg body weight	no evidence of damage to blood/brain barrier at 150 mg/kg body weight; at concentrations ≥300 mg/kg body weight there was evidence of albumin permeation of the CNS (indicative of damage to blood/brain barrier)
Elo and MacDonald 1989	2,4-D sodium salt	rats (Wistar)	200 mg/kg by single sub-cutaneous injection	significant increase in brain concentrations of 5-hydroxyindolacetic acid
Oliveira and Palermo-Neto 1993	2,4-D dimethyla mine (U-46 D-Fluid®, Basf)	rats (25 male Wistar weighing 230-250 g)	10, 60, 100, or 200 mg/kg single oral dose (controls received distilled water)	2,4-D concentrations in brains and serum of all treated rats were dose dependent
Oliveira and Palermo-Neto 1993	2,4-D dimethyla mine (U-46 D-Fluid®, Basf)	rats (30 male Wistars weighing 230-250 g)	200 mg/kg single oral dose (controls given distilled water)	exposure to 200 mg/kg did not alter homovanilic acid or dopamine striatal levels up to 4 hours after administration, but decreased the striatal levels of serotonin 3 and 4 hours after treatment and increased 5-hydroxyindoleacetic acid striatal levels 4 hours after treatment. in the brain stem experiment, exposure to 200 mg/kg significantly increased 5-hydroxy-indoleacetic acid levels in the brain stem of treated rats but did not alter serotonin levels, compared with controls
Oliveira and Palermo-Neto 1993	2,4-D dimethyla mine (U-46 D-Fluid®, Basf)	rats (30 male Wistars weighing 230-250 g)	10, 60, 100, or 200 mg/kg single oral dose (controls given distilled water)	no effects observed at 10 mg/kg; 60, 100, or 200 mg/kg increased levels of 5-hydroxy-indoleacetic acid in the brain stem but had no effect on serotonin levels

Appendix 2-2

Appendix 2. Published neurotoxicity studies of 2,4-D in mammals

Reference	2,4-D Species	Animal Species/ Strain/Sex	Exposure/ Duration	Effects
Oliveira and Palermo-Neto 1993	2,4-D dimethylamine (U-46 D-Fluid®, Basf)	rats (80 male Wistars weighing 230-250 g)	60, 100, 200, or 300 mg/kg single oral dose (controls given distilled water)	locomotion and rearing frequency were decreased at all dose levels, but the decreases were statistically significant after exposure to 100, 200 or 300 mg/kg; there was a statistically significant increase in immobility duration at all dose levels
Oliveira and Palermo-Neto 1993	2,4-D dimethylamine (U-46 D-Fluid®, Basf)	rats (140 male Wistars weighing 230-250 g)	200 mg/kg single oral dose (controls given distilled water)	locomotion and rearing frequency were decreased at all dose levels, but the decreases were statistically significant after exposure to 100, 200 or 300 mg/kg; there was a statistically significant increase in immobility duration at all dose levels; effects lasted 24 hours, with peak effects occurring at 3 hours, at which time, compared with controls, treated rats registered the highest changes recorded in the three parameters, differing ($p<0.05$) from those detected in the subsequent hours of observation
Oliveira and Palermo-Neto 1993	2,4-D dimethylamine (U-46 D-Fluid®, Basf)	rats (35 male Wistars weighing 230-250 g)	200 mg/kg single oral dose (controls given distilled water)	concentrations of 2,4-D in the brain increased from 1 to 4 hours after administration
Rosso et al. 2000a	2,4-D acid	rat cell cerebellar granule cell cultures	1 mM to 2 mM (221 mg/L to 442 mg/L)	dose-dependent inhibition of nerve cell development
Rosso et al. 2000a	2,4-D acid	neonatal rats	25, 70, and 100 mg/kg/day for 13 to 14 days at varying times after birth	signs of neurological impairment as well as neuropathology.

Appendix 2. Published neurotoxicity studies of 2,4-D in mammals

Reference	2,4-D Species	Animal Species/ Strain/Sex	Exposure/ Duration	Effects
Schulze 1988	2,4-D acid, 2,4-D n-butyl ester, or 50:50 mixture n-butyl ester and isobutyl ester	rats (5 male Wistars; approximately 200-days-old)	120 mg/kg (2,4-D), or 150 mg/kg (n-butyl ester), or 150 mg/kg (mixed butyl ester) by single sub-cutaneous in-jection for 3 consecutive days	administration of 2,4-D n-butyl ester caused statistically significant increases in landing foot splay; administration of 2,4-D acid or 2,4-D mixed butyl esters did not produce the effect
Squibb et al. 1983	2,4-D acid (NOS)	rats (NOS)	20-80 mg/kg body weight by gavage 2 times/week for 5 weeks	increases in hind limb and forelimb grip strength, which suggests myotonia

Appendix 2-4