



**2,4-Dichlorophenoxyacetic acid Formulations -
Human Health and
Ecological Risk Assessment
FINAL REPORT**

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ACRONYMS, ABBREVIATIONS, AND SYMBOLS

a.e.	acid equivalents
a.i.	active ingredient
AEL	adverse-effect level
ACGIH	American Conference of Governmental Industrial Hygienists
AChE	acetylcholinesterase
AMPA	aminomethylphosphonate
APHIS	Animal and Plant Health Inspection Service
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
bw	body weight
BUN	blood urea-nitrogen
ChE	cholinesterase
cm	centimeter
2,4-D	dichlorophenoxyacetic acid
DEA	Drug Enforcement Administration
EC ₅₀	concentration causing 50% inhibition of a process
EC ₁₀₀	concentration causing complete inhibition of a process
EIS	environmental impact statement
F	female
F ₁	first filial generation
FS	Forest Service
g	gram
GC	gas chromatography
GRAS	generally recognized as safe
HHRA	human health risk assessment
HQ	hazard quotient
IARC	International Agency for Research on Cancer
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
kg	kilogram
K _{o/c}	organic carbon partition coefficient
K _{ow}	octanol-water partition coefficient
K _p	skin permeability coefficient
L	liter
lb	pound
LC ₅₀	lethal concentration, 50% mortality
LD ₅₀	lethal dose, 50% mortality
LD ₉₅	lethal dose, 95% mortality
LOAEL	lowest-observed-adverse-effect level
m	meter
M	male
MCS	multiple chemical sensitivity
mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day

ACRONYMS, ABBREVIATIONS, AND SYMBOLS (continued)

mL	milliliter
MS	mass spectrometry
MW	molecular weight
MOS	margin of safety
NCI	National Cancer Institute
NHL	non-Hodgkin's lymphoma
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NRC	National Research Council
OPP	Office of Pesticide Programs
pK _a	dissociation constant
ppm	parts per million
RBC	red blood cells
R.E.D.	re-registration eligibility document
RfD	reference dose
SARA	Superfund Amendments and Reauthorization Act
SMR	standard mortality ratio
UF	uncertainty factor
U.S.	United States
U.S. EPA	U.S. Environmental Protection Agency
USDA	United States Department of Agriculture
>	greater than
≥	greater than or equal to
<	less than
≤	less than or equal to
=	equal to
≈	approximately equal to

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

To convert ...	Into ...	Multiply by ...
acres	hectares (ha)	0.4047
acres	square meters (m ²)	4,047
atmospheres	millimeters of mercury	760
centigrade	Fahrenheit	1.8C°+32
centimeters	inches	0.3937
cubic meters (m ³)	liters (L)	1,000
Fahrenheit	centigrade	0.556F°-17.8
feet per second (ft/sec)	miles/hour (mi/hr)	0.6818
gallons (gal)	liters (L)	3.785
gallons per acre (gal/acre)	liters per hectare (L/ha)	9.34
grams (g)	ounces, (oz)	0.03527
grams (g)	pounds, (oz)	0.002205
hectares (ha)	acres	2.471
hectares (ha)	square meters	10,000
inches (in)	centimeters (cm)	2.540
kilograms (kg)	ounces, (oz)	35.274
kilograms (kg)	pounds, (lb)	2.2046
kilograms per hectare (kg/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

Introduction

2,4-D is a herbicide used by the Forest Service in vegetation management programs. Several dry and liquid formulations of 2,4-D are available and may be used by the Forest Service primarily for noxious weed control and vegetation management. In 1989, the Forest Service prepared a series of environmental impact statements (EISs) with accompanying risk assessments regarding the use of these products. The updated risk assessments for human health and ecological effects that comprise this document support a reassessment of the environmental consequences of using 2,4-D in future Forest Service programs.

This document has four chapters: the introduction, program description, risk assessment for human health effects, and risk assessment for ecological effects or effects on wildlife species. Each of the two risk assessment chapters has four major sections: an identification of the potential hazards associated with commercial formulations of 2,4-D, an assessment of potential exposure to these products, an assessment of the dose-response relationships, and a characterization of the risks associated with plausible levels of exposure.

Recent human health risk assessments and ecological risk assessments prepared as part of the EIS for the Drug Enforcement Agency (DEA) Cannabis Eradication Program was used extensively in the preparation of this risk assessment, and portions of each of the risk assessments are incorporated into chapters 2, 3, and 4 of this risk assessment.

Program Description

The herbicidal properties, environmental chemistry, and toxicology of 2,4-D have been extensively investigated. There are 20 formulations of 2,4-D available for use. The formulations consist of 2,4-D salts, 2,4-D esters, or combinations of 2,4-D salts and esters, and all but two of these are liquid formulations. Several additional herbicide formulations are available in which 2,4-D is a component. In these formulations, 2,4-D is combined with one or more additional herbicides. The Forest Service uses herbicide mixtures in which 2,4-D is combined with triclopyr, dicamba, picloram, and glyphosate. 2,4-D is registered for both ground and aerial applications. In addition, one formulation of 2,4-D, Aqua-Kleen, can be applied directly to water to control the growth of noxious weeds. 2,4-D is the only phenoxy herbicide that is registered for the treatment of noxious aquatic weeds. Although 2,4-D is registered for aerial applications, the Forest Service does not currently use this method to apply 2,4-D. This application method, nonetheless, is included in this risk assessment to support the potential use of 2,4-D in aerial applications that might be judged appropriate or necessary (i.e., noxious weeds)

In Forest Service programs, herbicide formulations containing 2,4-D are used most commonly in wildlife openings, rights-of-way maintenance, and noxious weed control. For these uses, the most common application methods include backpack (selective foliar), hack-and-squirt, and roadside hydraulic spray. Aerial applications are considered in this risk assessment but are not currently used in or planned for Forest Service programs. The specific application rates used in ground or aerial programs would vary according to local conditions and the nature of the target vegetation.

For ground applications, the U.S. Forest Service uses rates ranging from 0.5 to 2.0 lbs a.e./acre. The typical application rate is 1.0 lb a.e./acre. The same rates are likely to be used for aerial applications, if conducted. Application rates in the higher range are used for activities like 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). Much higher application rates could be used to apply Aqua-Kleen directly to bodies of water.

Human Health Risk Assessment

Hazard Identification

The toxicity of 2,4-D is well studied in experimental mammals. In fact, there are many studies regarding the health status of human populations exposed to 2,4-D. U.S. EPA has scheduled the re-registration of 2,4-D, but the Re-registration Eligibility Document (RED) is not scheduled for completion until the year 2000. Although the mode of action of 2,4-D as a plant toxin is well understood, the mode of action of 2,4-D toxicity in mammals is not clear. After acute lethal exposure, adverse effects in humans include convulsions, vomiting, congestion of various organs, and degenerative changes in nerve cells. In non-lethal but toxic oral exposure to 2,4-D, adverse human health effects include irritation to mouth, throat, and gastrointestinal tract, vomiting, chest and abdominal pain, diarrhea, muscle twitches, tenderness, and stiffness. Similar signs of toxicity were observed in experimental mammals exposed to 2,4-D.

In general, herbicides formulated with 2,4-D esters have higher concentrations of 2,4-D than do herbicides formulated with 2,4-D salts. Using herbicides formulated with 2,4-D esters may involve handling and applying concentrated solutions of 2,4-D. The variation in 2,4-D concentrations among the different formulations has an impact on the exposure assessment for each formulation; however, a more significant factor affecting the exposure assessments is the likelihood that there are formulation-related differences regarding the dermal absorption of 2,4-D. Although structure-activity relationships for dermal permeability suggest that the differences in dermal absorption rates are likely to be substantial, *in vivo* dermal absorption studies in humans and experimental mammals do not report systematic differences in dermal absorption. Consequently there is substantial uncertainty associated with the exposure assessments and risk characterizations for the various formulations of 2,4-D.

There is less uncertainty associated with the dose-response relationships for 2,4-D acid, salts, and esters. The acute toxicity of 2,4-D acid, salts, or esters to mammals is relatively low, with LD₅₀ values ranging from 100 mg/kg (butyl ester) in cattle to 1800 mg/kg (sodium salt) in rats. Moreover, the toxicological equivalence of 2,4-D acid, salts, and esters is supported by teratogenicity screening assays in which the acute lethal potency of 2,4-D acid and its various esters was similar. In addition, dog studies involving subchronic exposure to 2,4-D acid, 2,4-D dimethylamine, or the ethylhexyl ester of 2,4-D, indicate that these various forms of 2,4-D are similar to one another with respect to toxic potency. On the other hand, there is a significant difference in sensitivity to 2,4-D exposure among animal species. This pattern is common in toxicity studies, with smaller animals being less sensitive than larger animals to chemical exposure. Although studies involving acute exposure to 2,4-D indicate that mice are outliers in this pattern,

being somewhat more sensitive than rats, this deviation in the pattern has little impact on the risk assessment.

The subchronic and chronic effects of exposure to 2,4-D are well studied. Most of the information on which this risk assessment is based is taken from the published literature and studies submitted to the U.S. EPA in support of the re-registration of 2,4-D. The current RfD for 2,4-D is based on a dietary study in which rats were exposed to daily doses of 1, 5, 15, or 45 mg/kg bw/day 2,4-D. After exposure, dose-related increases in kidney weight (males and females) and thyroid weights (males only) were noted in addition to decreases in mean hemoglobin, hematocrit, RBC levels, and reticulocyte levels (males only). The increase in thyroid weight was associated with an increase in T₄ (thyroxine) levels in the blood of male rats. U.S. EPA does not consider the thyroid effects observed at 1 mg/kg/day to be treatment related. Based on a review of the individual animal data from this study as well as a review of other studies in which thyroid effects were reported, the weight of evidence suggests that toxicologically significant effects on the thyroid may occur at relatively high dose levels. Hence, U.S. EPA seems justified in classifying the dose of 1 mg/kg/day a NOAEL, based on the dietary study in rats.

Exposure to the n-butyl ester of 2,4-D seems to cause immunological effects that are attributable to the n-butyl moiety rather than to 2,4-D. Since, however, the n-butyl ester of 2,4-D is no longer commercially available, it is not given specific consideration in this risk assessment.

At relatively high doses associated with fetotoxicity or maternal toxicity, 2,4-D might induce fetal malformations. One occupational exposure study reports an association between 2,4-D exposure and sperm damage; however, the data in the study do not support the association. At best, the study demonstrates that the incidence of sperm anomalies in a group of pesticide applicators was higher than in a group of individuals who did not apply pesticides. Whether exposure to 2,4-D was the cause of the observed effects cannot be verified by the data presented in the study. Although some animal studies support an association between 2,4-D exposure and male reproductive impairment, reproductive effects seems to be a much less sensitive endpoint than the effects on which the RfD derived by U.S. EPA is based.

Many epidemiology studies suggest an association between exposure to 2,4-D (and other phenoxy herbicides) and the development of cancer. The studies were reviewed thoroughly by the U.S. EPA and are undergoing additional review as part of the re-registration process. Barring compelling arguments to the contrary, the cancer risk assessment for 2,4-D should be re-examined after the re-registration process for 2,4-D is completed. This recommendation is based on the level of analysis and review that the U.S. EPA decision will receive and the general principle that other government agencies should apply the U.S. EPA assessments consistently in their risk assessments. At this time, it appears that none of the studies regarding the potential carcinogenicity of 2,4-D demonstrate a causal relationship between 2,4-D exposure and the development of cancer.

Although the database on the oral toxicity of 2,4-D is relatively good, there is much less information regarding the inhalation toxicity of 2,4-D or its combustion products. Estimated LC₅₀ values are not available for 2,4-D. As part of the re-registration process for 2,4-D, an acute whole body inhalation study was conducted in rats. When five rats of each sex were exposed to 2.15 mg 2,4-D/L (2150 mg/m³), two of the 10 rats died. The American Conference of Governmental Industrial Hygienists adopted a TLV of 10 mg/m³ for 2,4-D, which is identical to analogous values recommended by other federal agencies and other nations.

Exposure Assessment

The relatively rich database on occupational exposure to 2,4-D indicates that respiratory exposure is negligible, compared with dermal exposure. Because 2,4-D is eliminated unchanged and almost completely in the urine, the absorbed dose of 2,4-D can be estimated from urinary excretion data and related to the amount of 2,4-D handled during a particular application. Thus, occupational exposure rates are expressed in units of mg agent/kg bw · lb agent handled.

For directed ground (backpack) applications, broadcast ground applications, and aerial applications, exposure rates are taken from a relatively detailed review of studies on occupational exposure rates. Central estimates of exposure, expressed as absorbed dose, fall within a relatively narrow range: 0.013-0.022 mg/kg/day. The upper limits of projected exposure are also within a narrow range: 0.08-0.15 mg/kg/day. All of these estimates are based both on application rates of 1 lb a.e./acre and the estimated number of acres that a worker might treat in 1 day.

Aqua-Kleen, is the only 2,4-D formulation registered for direct application to surface waters to control undesirable vegetation. Occupational exposure studies are not available for this formulation. Based on an occupational exposure study involving the application of a liquid formulation of 2,4-D to aquatic media, the central estimate of absorbed dose for workers is 0.017 mg/kg/day, with an upper limit of 0.038 mg/kg/day. Again, these dose estimates are similar to those for other groups of workers. A major difference, however, is that the estimates for applying 2,4-D to aquatic media are based on the treatment of only 1 acre at a typical application rate of 19 lbs/acre, because the number of acres that workers might treat in 1 day has not been estimated.

The potential consequences of accidental worker exposure vary significantly, depending on the nature of the event and the duration of exposure. Typically, after accidental exposure to 2,4-D, the estimated absorbed dose for workers is close to the estimated absorbed dose for workers handling 2,4-D.

Under normal conditions, members of the general public should not be exposed to substantial levels of 2,4-D. Nonetheless, the number of exposure scenarios that can be constructed for the general public is great, based on various assumptions regarding application rates, compound dispersion, canopy interception, and human activity. Several highly conservative scenarios are developed for this risk assessment.

The most plausible exposure scenario for the general public involves walking through a contaminated area shortly after it is sprayed with 2,4-D. Estimates of absorbed dose for this scenario are extremely low, ranging from approximately 0.00042 to 0.0066 mg/kg/day. These estimates are consistent with the lower limits of estimated doses for workers involved in the application of 2,4-D.

A somewhat less likely but plausible exposure scenario involves the consumption of inadvertently contaminated vegetation. Both acute and subchronic exposure scenarios are derived for this scenario. The acute scenario leads to estimates that fall within the range of estimated exposure rates for workers involved in ground applications of 2,4-D. The longer-term scenario leads to substantially lower estimates of exposure.

Given the limited nature of most 2,4-D applications (i.e., relatively small treatment areas), general contamination of groundwater seems unlikely. Nonetheless, under the very conservative assumption that an entire watershed is treated, exposure levels will be relatively low, approximately 0.00002-0.00014 mg/kg/day. Based on accidental spill scenarios, estimates of acute exposure (i.e., 1 day) from the consumption of contaminated water are substantially higher, approximately 0.17-1.3 mg/kg. These acute exposures, however, are dominated by generally arbitrary exposure assumptions about the amount of 2,4-D spilled and the size of the body of water into which the compound is spilled.

Both acute and longer-term exposure scenarios are also developed for the consumption of contaminated fish. Exposure estimates for these scenarios depend on the concentration of 2,4-D in the water, the bioconcentration of 2,4-D by fish, and the amount of fish consumed by an individual. For this exposure scenario, the 2,4-D concentrations in water are identical to those used for the drinking water exposure scenario. The bioconcentration of 2,4-D by fish is based on an experimental determination that is probably conservative. Data are available on the amounts of fish consumed by the general population as well as subsistence populations (i.e., individuals who catch and consume fish as a major source of protein in their diet). Separate exposure assessments are conducted for these two groups. As with the longer-term exposure scenarios for the consumption of contaminated water, estimates of longer-term exposures to 2,4-D in fish are very low for both groups of people (i.e., ranging from 0.0000036 to 0.0011 mg/kg/day). Acute 1-day exposures based on an accidental spill scenario, however, lead to substantially higher estimates (i.e., ranging from approximately 0.2 to 3.1 mg/kg). Again, the spill scenario is dominated by arbitrary variability.

Dose-Response Assessment

In 1988, the U.S. EPA derived an RfD of 0.01 mg/kg/day for 2,4-D. The RfD is based on a NOAEL of 1 mg/kg/day using an uncertainty factor of 100 to account for species to species extrapolation and sensitive individuals in the human population. Since this RfD was developed, a significant amount of new information was made available and is under review by the U.S. EPA as part of the re-registration process for 2,4-D. This information suggests that the current RfD adopted by U.S. EPA is appropriate and protective.

An assessment of the dose/duration/severity data on 2,4-D suggests no apparent or, at least, no strong relationship between exposure duration and the severity of effects at a given dose. In other words, adverse effects, if they were to develop, would develop relatively fast and would not become more severe as the duration of exposure continued. This assessment is confirmed by categorical regression analysis in which the duration of exposure is statistically insignificant.

The dose-severity data on 2,4-D are considered quantitatively using categorical regression analysis, which is a conceptually simple statistical method for relating dose to the probability of observing an effect at a particular level of severity. At the RfD of 0.01 mg/kg/day, the categorical regression analysis indicates that the probability of an adverse effect (AEL) is about 0.009 (9 in 1000). Most likely, this AEL would involve subclinical effects rather than overt signs of toxicity. The probability of a frank effect level would be about 0.00009 (9 in 100,000). Most likely, this effect would involve signs of neurological toxicity.

Risk Characterization

Exposure levels for workers involved in the ground or aerial application of 2,4-D, may exceed the RfD slightly, based on central estimates of exposure, or substantially, based on upper limits of exposure. The central estimates of exposure are not of substantial concern; however, the upper limits of exposure could be associated with covert toxic effects (i.e., adverse effects on organ function or pathology not associated with frank signs of toxicity). Hence, this information suggests that 2,4-D can be applied safely if effective methods are used to protect workers and minimize exposure. If effective measures of hygiene are not employed, occupational exposure to 2,4-D could result in adverse, but probably not frankly toxic, effects.

The general public should not have adverse effects after exposure to 2,4-D, under normal conditions of exposure. After accidental exposure to 2,4-D, estimated exposure levels may be comparable to those resulting from occupational exposure. In addition, the consequences of accidental exposure are similar to the anticipated consequences of occupational exposure, which may entail covert toxic effects but are not likely to entail gross signs of toxicity. The major concern for members of the general public involves the consumption of contaminated vegetation over periods of several months. If such an exposure were to occur, the exposure could result in adverse health effects. The likelihood of such an exposure, however, seems remote.

ECOLOGICAL RISK ASSESSMENT

Hazard Identification

The toxicity of 2,4-D is well-characterized for most groups of potential non-target species. Furthermore, there are detailed studies regarding the toxicity of 2,4-D to experimental mammals. Acute exposure to high levels of 2,4-D is associated with signs of nervous system toxicity. Nonetheless, the acute toxic potency of 2,4-D acid, salts or esters to mammals is relatively low, with LD₅₀ values ranging from 100 mg/kg (butyl ester) in cattle to 1800 mg/kg (sodium salt) in rats.

Although differences in sensitivity to 2,4-D are apparent among mammalian species (i.e., large animals appear to be more sensitive than small animals when dose is expressed as mg/kg body weight), there do not appear to be substantial or systematic differences in toxic potency among the various forms of 2,4-D (i.e., acid, salts, or esters) used in commercial formulations. The subchronic and chronic effects of exposure to 2,4-D are well studied in experimental mammals. The most sensitive effects from chronic exposure to 2,4-D seem to involve the kidney, thyroid, and blood. Of these, kidney effects appear to be most clearly associated with potentially significant toxic effects. Birds appear to be somewhat less sensitive to the acute lethal effects of 2,4-D, with acute oral LD₅₀ values ranging from 300 to 5000 mg/kg.

The toxicity of 2,4-D to terrestrial invertebrates is less well studied than the toxicity of 2,4-D to vertebrates. The estimated LD₅₀ for honey bees is approximately 120-1100 mg/kg, in the range of values reported for experimental mammals. There is some evidence, however, that younger bees may be more sensitive than adult bees to 2,4-D exposure. Nevertheless, adult bees are typically used in toxicity studies. At exposure rates corresponding to high application rates (i.e. about 3-30 lbs a.e./acre), 2,4-D caused death in adult millipedes. The reported responses of earthworms to 2,4-D are varied, with some studies suggesting a potential for decreased growth.

2,4-D is a plant growth regulator and acts as a synthetic auxin or hormone. 2,4-D alters the metabolism and growth characteristics of plants, often causing a proliferation of abnormal growth that interferes with the transport of nutrients throughout the plant. Broad-leaved plants are more susceptible than narrow leaved plants like grasses. Tolerant plants are able to 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. 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 parthenogenic or seedless fruit to develop from unfertilized flowers. Roots are more sensitive than shoots to 2,4-D exposure; however, these signs of plant toxicity are not as obvious as other signs and are not often reported. 2,4-D increases the permeability of root membranes, which can lead to a loss of nutrients and possibly increase risk of invasion by pathogens.

As with effects on terrestrial organisms, aquatic plants are generally more sensitive than fish or other aquatic animals to the effects of 2,4-D. In general, the ester formulations of 2,4-D are more toxic to fish than the amine formulations, and there are up to 1000-fold differences in the acute toxicity of 2,4-D to some fish species, as measured by 24- or 48-hour LC₅₀ tests. When mixed with emulsifiers, however, 2,4-D acid results in LC₅₀ values that are comparable to those reported

for acute exposure to 2,4-D esters. Although some species of aquatic algae are sensitive to levels of approximately 1 mg/L 2,4-D, low levels of the compound may stimulate algal growth in other species.

Exposure Assessment

Terrestrial animals may be exposed to any applied herbicide from direct spray, the ingestion of contaminated media (vegetation, prey species, or water), grooming activities, indirect contact with contaminated vegetation, or inhalation. In a scenario involving exposure to direct spray, the extent of dermal contact depends on the application rate and the surface area of the organism. Because of the relationship of body size to surface area, very small organisms like bees and other terrestrial insects could be exposed to much greater amounts of 2,4-D per unit body weight. Thus, for honey bees, the estimated exposure level from a direct spray scenario is 163 mg/kg, while estimates for a small mammal range from 0.3-24 mg/kg depending, on the specific assumptions regarding the rates and kinetics of dermal absorption.

Exposure scenarios for the consumption of contaminated water or vegetation lead to central estimates of exposure in the range of 1.4 to 5 mg/kg/day over short periods of time. Longer-term exposure scenarios lead to much lower estimates of daily dose: 0.0005-0.56 mg/kg/day.

The principal hazard of 2,4-D exposure to non-target terrestrial plants is from unintended direct deposition or spray drift. Unintended direct spray will result in exposure equivalent to the application rate. The potential for spray drift was investigated several field studies involving low-flight agricultural applications of pesticides by means of various nozzles under differing meteorological conditions. Central estimates of off site drift in these studies were 0.05, 0.02, 0.01, and 0.008 of nominal application rates at distances of 100, 200, 300, and 400 feet downwind. At 400 feet down wind, deposition rates ranged from 0.002 to 0.01 of the nominal application rate.

The herbicide concentration of 2,4-D in soil or litter may be estimated using the Groundwater Loading Effects of Agricultural Management Systems (GLEAMS). For this exposure assessment, the parameters in GLEAMS were selected to minimize the adsorption of the herbicides to organic matter and maximize their potential for loss through runoff or percolation. Only one soil type, a loamy very fine sand, was modeled. The modeling scenario used simulated terrain that sloped downhill from the site of herbicide application. Ground-based broadcast and directed application were simulated by specifying the proportion of the herbicide applied to plants. For both the dimethylamine salt and esters, the greatest concentrations occurred in the uppermost layer of the soil (0-1 cm depth) immediately after application.

For aquatic organism, the estimated levels of 2,4-D in ambient water are based on the data used in the human health risk assessment. After an accidental spill, maximum initial concentrations of 2,4-D in water are estimated at 6 mg/L/lb applied. This concentration will diminish rapidly due to microbial degradation, binding to suspended particulate, or dispersion. Similarly, after the application of Aqua-Kleen, levels in ambient water should not exceed 0.02 mg/L. As with the spill scenario, this concentration will be applied only to estimate the consequences of short-term

peak exposure. For longer-term exposures, the average level in water associated with an application rate of 1 lb a.e./acre is 0.002 mg/L with a range of 0.001 to 0.004 mg/L.

Dose-Response Assessment

The available data on the acute toxicity of 2,4-D to numerous mammalian species weakly supports the conservative assumption that larger mammals are somewhat more sensitive than smaller animals to 2,4-D exposure. The current RfD of 0.01 mg/kg/day can be used to represent the most conservative dose estimate not associated with adverse effects in mammals. Smaller mammals may be less sensitive to 2,4-D, and the NOAEL of 1 mg/kg/day for mammals is not likely to result in adverse effects in most smaller species. Estimates of the likelihood of observing adverse or frank effects in mammalian species may be based on the categorical regression analysis, as in the human health risk assessment. The available oral toxicity studies suggest that birds may be somewhat less sensitive to 2,4-D than mammals. Thus, for exposure scenarios involving the ingestion of 2,4-D from either contaminated vegetation or water, the dose-response relationships for mammals may serve as conservative estimate for avian species. The available toxicity data on terrestrial invertebrates is relatively sparse. Based on oral LD₅₀ values, the acute oral toxicity of 2,4-D to honey bees is comparable to that for experimental mammals and birds.

For terrestrial plants exposed by direct spray or drift, the relevant exposure metameter is the application rate or functional rate of deposition expressed in units of toxicant weight per unit area (e.g., lbs/acre). The maximum broadcast application rate, about 2 lbs a.e./acre, is effective against most species and life stages of terrestrial plants, except grasses. Conversely, application levels of 0.5-1 lb a.e./acre are likely to damage broadleaf vegetation but less likely to affect other species of vegetation. Most terrestrial microorganisms will be exposed to 2,4-D as soil residues. As would be expected from the phytotoxic properties of 2,4-D, soil algae are more sensitive than other types of soil microorganisms.

In aquatic species, the ester formulations of 2,4-D—including the butoxyethyl ester found in Aqua-Kleen—are approximately 200-1000 times more toxic to fish than the amine formulations, when toxicity is measured by acute (24- to 48-hour) LC₅₀ values. Nonetheless, while the esters of 2,4-D are chemically stable, they have a relatively short half-time in natural water due to biological degradation. In natural waters, the rate of conversion of the 2,4-D esters to 2,4-D acid is estimated at 0.02 to 0.07 hour⁻¹; thus, the proportion of 2,4-D ester remaining after 24 hours would range from approximately 0.04 to 0.5. In other words, after 1 day, half to nearly all of the 2,4-D ester would degrade to the 2,4-D acid. For this risk assessment, 1 mg a.e./L is used as a conservative estimate of the exposure level to 2,4-D esters that would likely be associated with mortality in fish. This value is somewhat lower than the lowest reported LC₅₀ for the esters of 2,4-D encountered in the literature. The corresponding value for 2,4-D salts is taken at 100 mg a.e./L. These values are applied to acute exposure scenarios, recognizing that the lower value for the 2,4-D esters may be conservative in natural waters in which the breakdown of the 2,4-D esters to the acid form is very rapid. The toxicity of 2,4-D to amphibians has not been investigated as thoroughly as the toxicity of 2,4-D to fish but the available data suggest the 2,4-D may be more toxic to some amphibians than to fish.

For chronic exposures, it is not reasonable to assume that any substantial amount of 2,4-D esters will remain in natural waters. Even in the lower range of the decay rate for the esters - i.e., a half time of 1 day - only about 0.01 of the original concentration of the ester would remain after 1 week and only about $1 \cdot 10^{-9}$ would remain after 30 days. For longer-term exposures, a NOEL of 10 mg/L will be used based on a NOAEL in sunfish.

For aquatic invertebrates, a level of 1 mg a.e./L is used as an estimate of possible lethal exposure for at least some sensitive species, specifically small free swimming organisms. Larger aquatic invertebrates appear to be no more sensitive to 2,4-D than fish. By analogy to the data on fish, 2,4-D esters could be much more toxic than 2,4-D acid or salts. The limited data on the toxicity of 2,4-D esters and salts to daphnids suggest that the esters are more toxic by a factor of about 10. For chronic reproductive effects, a NOAEL has not been identified.

The toxicity of 2,4-D to aquatic plants does not appear to be remarkably greater than the toxicity of this compound to sensitive invertebrate and amphibian species. Based on the studies summarized in section 4.1.3.4., the no-observed effect concentration for most unicellular algal species appears to be above 1 mg/L. EC_{50} s for growth inhibition are typically in the lower range of LC_{50} s for fish, invertebrates, or amphibians: 10 to about 200 mg/L. Aquatic macrophytes appear to be more sensitive than unicellular algae, with EC_{50} s at about 0.3 mg/L for a reduction in chlorophyll concentrations.

There appear to be substantial differences in the sensitivity of different groups of aquatic non-algal microorganisms to 2,4-D. The most sensitive group appears to be the nitrogen-fixing cyanobacteria which evidence growth inhibition at concentrations as low as 1 mg/L.

Risk Characterization

Except for accidental exposure scenarios, there is relatively little indication that 2,4-D applications are likely to cause any adverse effects in terrestrial animals. For small mammals, a reasonable verbal interpretation of the direct spray scenarios is that signs of frank toxicity are unlikely but subclinical effects could result in some species. The direct spray scenario for the bee is less ambiguous: some populations of bees subject to a direct spray could evidence substantial mortality. An major consideration in all of the direct spray scenarios involves interception of the 2,4-D by vegetation. This would tend to reduce the level of exposure but the magnitude of the reduction would depend on the proportion of the 2,4-D that is intercepted prior to contacting the animal. While this cannot be well quantified in general, it may account for the failure of some field studies to note toxicity in bees after the application of 2,4-D.

Neither of the drinking water scenarios lead to hazard quotients that reach a level of concern. For the longer-term drinking water scenario, the anticipated exposures are far below a level of concern. As in the characterization of risk for potential human health effects, both the acute and longer term exposures of a small mammal to vegetation contaminated with 2,4-D are of some concern. Nonetheless, given the conservative nature of the exposure assumptions as well as the marginal nature of the hazard quotients - i.e., 0.5 to 2 - it seems reasonable to assert that, at least in some and perhaps most instances, actual exposures would be below and sometimes far below a

level of concern. Nonetheless, if contaminated vegetation is the sole diet of the animal, some subclinical toxic effects could occur. No frank signs of toxicity, however, are likely.

A very conservative multi-route exposure scenario supports a concern for potential although perhaps isolated effects on terrestrial vertebrates. The dose-response assessment on which this hazard characterization is based is most clearly relevant to mammalian species. However, because the dose-response assessment encompasses more sensitive species - i.e., larger mammals - and the exposure assessment is based on a smaller mammal, the assessment is inherently conservative.

Although the data on avian species are not as extensive as those for mammals, acute toxicity studies in birds suggest that avian species are somewhat less sensitive than mammals. In addition, the available studies on the effects of 2,4-D on avian eggs suggest that no effects would be anticipated from a direct spray of avian eggs at application rates of up to 10 lb/acre, a rate that is far in excess of those anticipated by the Forest Service.

Because 2,4-D is an effective herbicide, it is used to control the growth of various broadleaves and other undesirable plants species. When the growth of such plants is inhibited, secondary ecological effects occur due to changes in habitat, food supply, lighting, and other conditions. Such changes are due to differences in vegetation and are not specific to 2,4-D. Similar effects would be induced if the undesirable vegetation were removed by any other herbicide or non-herbicide vegetation management practice. There is a relatively large body of evidence suggesting that vegetation management practices with or without herbicides have a beneficial effect on habitat quality for some species and a detrimental effects on other species. While these field studies cannot be used directly to modify this risk assessment, they probably represent the most predictable and significant terrestrial ecological effect in the use of 2,4-D.

Aquatic macrophytes appear to be the most sensitive to concentrations of 2,4-D in water, with EC_{50} s for the inhibition of chlorophyll levels at about 0.3 mg/L. The direct application of Aqua-Kleen to bodies of water for the control of undesirable vegetation may lead to concentrations on the order of 0.5 to 0.7 mg/L. At this concentration, mortality might be expected in some sensitive fish or invertebrate species, particularly over the first day or two when a substantial proportion of 2,4-D could exist in the ester form. On the other hand, at least some fish species will avoid 2,4-D in water at subtoxic levels. Thus, the presence of toxic levels of 2,4-D in treated bodies of water would not necessarily lead to fish kills or other overt toxic effects. In any event, the treated area of a lake or pond will undoubtedly evidence ecological changes associated with a change in vegetation - i.e., the destruction of the undesirable aquatic macrophytes. As with the secondary effects of vegetation management on terrestrial systems, the extent to which these changes are regarded as adverse may be largely subjective.

The consequences of an accidental spill of 2,4-D into a body of water will depend not on the application rate but on formulation of 2,4-D, the amount spilled into the water and the amount of water that is contaminated. In the case of a spill of a 2,4-D ester, some fish mortality might be anticipated. This would depend on specific local conditions that would influence the rate of dispersion and/or the rate of conversion of the 2,4-D ester to the free acid. Similarly, there is at

least some evidence for 2,4-D that the presence of emulsifiers with 2,4-D amine will substantially enhance the toxicity of the 2,4-D amine to aquatic species.

1. INTRODUCTION

2,4-D is a herbicide used by the Forest Service in vegetation management programs. A number of different dry and liquid formulations of 2,4-D are available and may be used by the Forest Service primarily for noxious weed control and vegetation management. In 1989, the Southern Region of the Forest Service prepared a series of environmental impact statements with accompanying risk assessments that concern the use of these products (USDA 1989a,b,c). The present document provides updated risk assessments for both human health and ecological effects to support a reassessment of the environmental consequences of using these products in future Forest Service 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 the commercial formulations of 2,4-D, an assessment of potential exposure to these products, 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 has been made to ensure that the document can be understood by individuals who do not have specialized training in the chemical and biological sciences. Certain technical concepts and terms common to all parts of the risk assessment are described in as plain a language as possible in a separate document: *The Preparation of Environmental Documentation and Risk Assessments for the Forest Service* (SERA 1995a). In addition, these terms are defined in the glossary to this risk assessment. Moreover, some of the more complicated terms and concepts are defined, as necessary, in the text.

The risk assessments presented in this document are not, and are not intended to be, comprehensive summaries of all of the available information. Much of the early literature is summarized in recent reviews on 2,4-D (Munro et al. 1992, WHO 1988), previous risk assessments and environmental impact statements covering this compound (USDA 1989a,b,c), a Chemical Background Statement prepared by USDA (1989d) as well as recent human health risk assessments (SERA 1997) and ecological risk assessments (USDA 1997) prepared as part of the EIS for the DEA Cannabis Eradication Program. These latter two documents have been used extensively in the preparation of this risk assessment and portions of each of these documents have been incorporated into chapters 2, 3, and 4 of this risk assessment.

As part of the pesticide registration process, manufacturers are required to conduct various studies regarding the toxicity and environmental fate of pesticides. These studies are classified as confidential business information (CBI) and, although these studies are submitted to the U.S. EPA, they are not generally released for public review. As necessary, copies of the original studies have been obtained from the U.S. EPA for the human health risk assessment.

For the most part, the risk assessment methods used in this document are similar to those used in risk assessments previously conducted for the Forest Service as well as risk assessments conducted by other government agencies. Details regarding the specific methods used to prepare the human health risk assessment are provided in SERA (1998a), while detailed explanations of specific methods used in estimating occupational exposure are provided in Rubin et al. (1998).

Risk assessments are usually expressed with numbers; however, the numbers are far from exact. *Variability* and *uncertainty* may be dominant factors in any risk assessment, and these factors should be expressed. Within the context of a risk assessment, the terms *variability* and *uncertainty* signify different conditions.

Variability reflects the knowledge of how things may change. Variability may take several forms. For this risk assessment, three types of variability are distinguished: *statistical*, *situational*, and *arbitrary*. *Statistical variability* reflects, at least, apparently random patterns in data. For example, various types of estimates used in this risk assessment involve relationships of certain physical properties to certain biological properties. In such cases, best or maximum likelihood estimates can be calculated as well as upper and lower confidence intervals that reflect the statistical variability in the relationships. *Situational variability* describes variations depending on known circumstances. For example, the application rate or the applied concentration of a herbicide will vary according to local conditions and goals. As discussed in the following section, the limits on this variability are known and there is some information to indicate what the variations are. In other words, *situational variability* is not random. *Arbitrary variability*, as the name implies, represents an attempt to describe changes that cannot be characterized statistically or by a given set of conditions that cannot be well defined. This type of variability dominates some spill scenarios involving either a spill of a chemical onto the surface of the skin or a spill of a chemical into water. In either case, exposure depends on the amount of chemical spilled and the area of skin or volume of water that is contaminated.

Variability reflects knowledge or at least an explicit assumption about how things may change, while *uncertainty* reflects a lack of knowledge. For example, the focus of the human health dose-response assessment is an estimation of an “acceptable” or “no adverse effect” dose that will not be associated with adverse human health effects. For 2,4-D and for most other chemicals, however, this estimation regarding human health must be based on data from experimental animal studies, which cover only a limited number of effects. Generally, judgment, not analytical methods, is the basis for the methods used to make the assessment. And although the judgments may reflect a consensus (i.e., be used by many groups in a reasonably consistent manner), the resulting estimations of risk cannot be proven analytically. In other words, the estimates regarding risk involve uncertainty. The primary functional distinction between variability and uncertainty is that variability is expressed quantitatively, while uncertainty is expressed qualitatively.

In considering different forms of variability, almost no risk estimate presented in this document is given as a single number. 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. Very simple calculations are included in the body of the document. Some of the calculations, however, are cumbersome. For those calculations, a set of worksheets is included as an attachment to the risk assessment. The worksheets provide the detail for the estimates cited in the body of the 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 non-target organisms.

2. PROGRAM DESCRIPTION

2.1. OVERVIEW

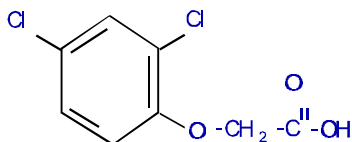
2,4-D, the common name for 2,4-dichlorophenoxyacetic acid, is a selective systemic herbicide used to control broadleaf weeds. The herbicidal properties, environmental chemistry, and toxicology of 2,4-D were investigated extensively. There are 20 herbicide formulations of 2,4-D in which the compound is available as salts, esters, or combinations of salts and esters, and all but two of the formulations are liquid. There are many 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, one formulation of 2,4-D, Aqua-Kleen, can be applied directly to water to control noxious weeds. 2,4-D is the only phenoxy herbicide registered for the treatment of noxious aquatic weeds. Although 2,4-D is registered for aerial applications, the Forest Service does not use the 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, possibly to control the growth 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. Consequently, 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 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 applies between 0.5 and 2.0 lbs a.e./acre with a typical application rate of 1.0 lb a.e./acre. The same rates are likely to be used for aerial applications, if conducted. The higher range of application rates are 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 Aqua-Kleen to bodies of water would involve much higher rates.

2.2. CHEMICAL DESCRIPTION AND COMMERCIAL FORMULATIONS

2,4-D is a selective systemic herbicide used to control broadleaf weeds. Extensive investigations were made regarding the herbicidal properties, environmental chemistry, and toxicology of 2,4-D, 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 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 20 herbicide formulations of 2,4-D in which the compound is present as salts, esters, or combinations of salts and esters (CPR 1997, Kells 1997). All of the

2,4-D herbicides are formulated as liquids, except for Aqua-Kleen and Savage. Aqua-Kleen, which is a granular formulation of 2,4-D butoxyethyl ester in slow dissolving attaclay granules, is intended solely for the treatment of water. Savage is a crystalline formulation of dimethyl amine salt. Most liquid formulations of 2,4-D contain either the dimethyl amine salt (8/18) or the isooctyl ester (9/18). Some isooctyl ester formulations specify that the 2,4-D is conjugated as the 2-ethylhexyl ester (i.e., a specific form of an isooctyl ester). Other ester formulations specify only that 2,4-D is present as the isooctyl ester, which suggests that the ester may be derived from a mixture of isooctyl alcohols.

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. The controversial use of 2,4-D and 2,4,5-T in Agent Orange is a major point of discussion in the literature (Munro et al. 1992, USDA 1989a,b,c, WHO 1984). A major issue in this controversy is the contamination of Agent Orange with TCDD, which is a contaminant in 2,4,5-T. There is no evidence in the literature that 2,4-D contains TCDD as a contaminant. As illustrated in Figure 2-1, 2,4-D is structurally similar to 2,4,5-T, differing by only one chlorine atom at the five position on the aromatic ring. This difference, however, is extremely important: since 2,4-D does not contain adjacent chlorine-substituted carbons on the aromatic ring, the probability of 2,4-D containing TCDD is remote because, as illustrated in Figure 2-1, TCDD requires chlorine substitution on two sets of adjacent carbon atoms. The significance of this feature of 2,4-D is discussed further in the hazard characterization in the human health risk assessment (section 3.2).

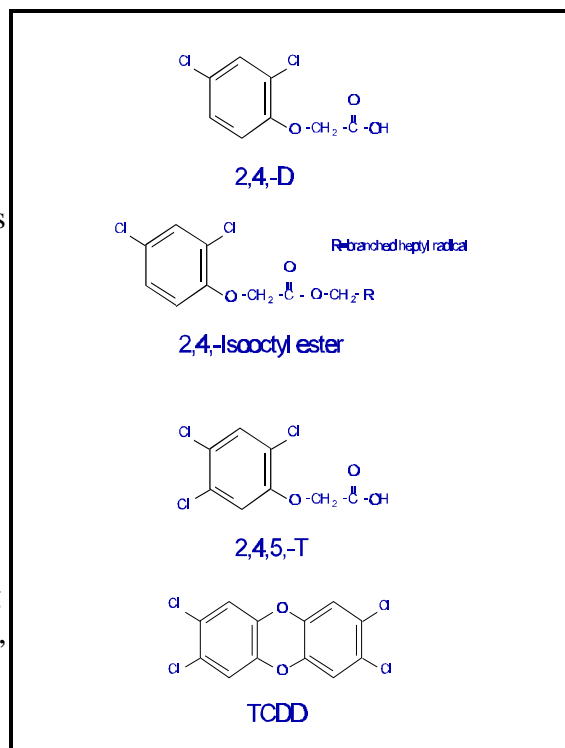


Figure 2-1: Structure of 2,4-D acid and esters as well as structures of 2,4,5-T and TCDD.

Table 2-1. 2,4-D Commercial formulations^a

Commercial Name ^b	Manufacturer	Active Ingredient (%)	Acid Equivalent (lbs/gallon)
Dimethylamine salt			
Amine 4 [P.1938]	United Agri Products	46.5	3.74
Ded-weed® Sulv™ [P.1896]	Uniroyal Chemical	46.6	3.8
Savage® [P.s84]	United Agri Products	95	-- ^c
Weed Rhap A-4d [P.1221]	Helena	46.7	3.8
Weedar® 64 [P.1698]	Rhone-Poulenc	46.8	3.8
Weedar® Ivm 44 [P.1702]	Rhone-Poulenc	46.8	3.8
Triisopropanolamine [T] and Dimethylamine [D] Salts			
Formula 40* [P.1556]	Rhone-Poulenc	34.05[t] 21.97[d]	3.8
Formula 40* Ivm [P.1559]	Rhone-Poulenc	34.05[t] 21.97[d]	3.8
Butoxyethyl Ester [E] and 2,4-d Acid [a]			
Phenoxy 088 [P.1747]	Riverside/Terra Corp	24.5[e] 13.8[a]	2.8
Weedone 638 [P.1704]	Rhone-Poulenc	24.5[e] 13.8[a]	2.8
Butoxyethyl Ester			
Aqua-Kleen® ^d [P.1495]	Rhone-Poulenc	27.6	-- ^d
Isooctyl Ester [N.o.s.]			
Barrage® [P.1180]	Helena	78.1	4.7
Weed Rhap Lv-6d [P.1221]	Helena	89.5	5.6
Weedone® Lo Vol 6 [P.1713]	Rhone-Poulenc	86.6	5.4
Isooctyl (2-ethylhexyl) Ester			
Brush-Rhap® [P.1185]	Helena	65.4	3.76
Esteron* 99 [P.1544]	Rhone-Poulenc	65.9	3.8
Low Vol 4 Ester [P.1969]	United Agri Products	65.5	3.8
Salvo [P.1981]	United Agri Products	81.8	5
Weedone® Lv4 Solventless [P.1708]	Rhone-Poulenc	62.6	3.8

^a Source: CPR 1997

^b Page numbers refer to CPR 1997

^c Acid equivalent specified as 19% by weight

^d Granular aquatic herbicide. Acid equivalent specified as 78.9% by weight.

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

Commercial Name ^b	Manufacturer	Components	Active Ingredient (%)	Acid Equivalent (lbs/gallon)
Scorpion III [p.729]	DowElanco	2,4-D	50.0	N/A ^d
		Clopyralid	25.0	
		Flumetsulam	9.3	
Crossbow [p.691] ^c	DowElanco	2,4-D BEE	34.4	2.0
		Triclopyr BEE	16.5	1.0
Weedmaster [p.1877]	Sandoz	2,4-D DMA	35.7	2.87
		Dicamba DMA	12.4	1.0
2 Plus 2 [p.1231]	ISK	2,4-D DMA	24.5	1.9
	Biosciences	MCPA DMA	24.2	1.8
Tiller [p.113]	AgrEvo	2,4-D IOE	10.35	0.58
		Fenoxaprop-ethyl	4.41	0.375
			32.11	1.75
		MCPA IOE		
Shotgun [p.S87]	UAP	2,4-D IOE	16.58	1.0
		Atrazine	24.24	2.25
Landmaster BW [p.1375]	Monsanto	2,4-D TIPA	20.6	1.5
		Glyphosate TIPA	12.9	0.9
Curtail [p.693]	DowElanco	2,4-D TIPA	34.8	2.0
		Clopyralid	7.5	0.38
		AKA		
Grazon P+D [p.702]	DowElanco	2,4-D TIPA	39.6	2.0
		Picloram TIPA	10.2	0.54
Tordon RTU [p.758]	DowElanco	2,4-D TIPA	20.9	1.06
		Picloram TIPA	5.4	0.29

Table 2-3. Selected physical and chemical properties of 2,4-D acid, and commercially significant salts and esters.				
Chemical	2,4-D (acid)	2,4-D Dimethylamine	2,4-D Butoxyethyl ester	2,4-D Isooctyl esters
CAS Number	94-75-7	2008-39-1	1929-73-3	25168-26-7
Molecular weight	221.0	266.1	321.2	333.3
Acid equivalents factor [221.0/MW]	1	0.831	0.688	0.663
Density (g/cm ³)	1.565 at 30°C (Tomlin 1994)		viscous, colorless liquid when pure (WSSA 1989)	
Vapor pressure (mm Hg)	USDA (1996): 1.42 x 10 ⁻⁷ (25°C) 9.7 x 10 ⁻⁸ (20°C)	8 x 10 ⁻¹⁰ (38°C) (Howard 1991)	4.50 x 10 ⁻⁶ (25°C) (Howard 1991)	
Water solubility (mg/L)	USDA (1996): 311 (25°C, pH1) 20031 (25°C, pH5) 23180 (25°C, pH7) 34196 (25°C, pH9)	3 x 10 ⁺⁶ (20°C) (WSSA 1989)	12 (25°C) (Howard 1991)	0.07 (1-octyl ester of 2,4-D) (Howard 1991)
Henry's law constant (atm-m ³ /mole)	Estimated: ^a 1.33 x 10 ⁻¹⁰ (25°C,pH1) 1.78 x 10 ⁻¹² (25°C,pH7)	Estimated: ^a 2.8 x 10 ⁻¹⁶ (38°C)	Estimated: ^a 1.59 x 10 ⁻⁷ (25°C)	Estimated: ^a 4.6 x 10 ⁻⁵ (25°C)
pKa	2.87 (25°C) (USDA 1996)			
Log K _{ow}	USDA (1996): 2.87 (pH1) -0.75 (pH7)	0.65 (Moody et al. 1987)	4.10 (estimated) ^d	6.73 (estimated) ^d
Dermal permeability coefficient (cm/hour)	Estimated: ^b 0.00932 (log Kow = 2.87) 2.5 x 10 ⁻⁵ (log Kow = -0.75)	Estimated: ^b 0.000131 (log Kow = 0.65)	Estimated: ^b 0.0171 (log Kow = 4.10)	Estimated: ^b 1.34 (log Kow = 6.73)
Soil adsorption K _{oc}	48 (USDA 1996) 60 (Tomlin 1994) 20-109 (Howard 1991)	72 - 136 (avg of 109 in three soils) (Rao and Davidson 1979)	6607-6900 (Reinert and Rodgers 1987) 1100 (Howard 1991)	25000 - 68000 (Howard 1991)
Evaporation rate		very low compared to other esters of 2,4-D and to other pesticides (Que Hee et al. 1975, Que Hee and Sutherland 1974)		
Foliar half-life (days)			the esters of 2,4-D are rapidly converted to the acid by plants (WSSA 1989)	the esters of 2,4-D are rapidly converted to the acid by plants (WSSA 1989)

Table 2-3. Selected physical and chemical properties of 2,4-D acid, and commercially significant salts and esters.				
Chemical	2,4-D (acid)	2,4-D Dimethylamine	2,4-D Butoxyethyl ester	2,4-D Isooctyl esters
Soil half-life (days)	14 (field dissipation; USDA 1996) 5.5 (soil half-life, USDA 1996) 10-30 (Mullins et al. 1993)	4-6 (in agricultural soil) (Howard 1991) 7-23 (in forest soil) (Howard 1991)	hydrolysis in moist soil may occur within a few days (Howard 1991) 0.11-2.3 (biodegradation half-life) (Reinert and Rodgers 1987)	In moist prairie soils (pH5.6-7.3), complete conversion to the acid and alcohol occurred in 2-3 days (Howard 1991)
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)	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
Air half-life (days)	2.42 (estimated) ^c	2.62 (estimated) ^c	0.65 (estimated) ^c	1.40 (estimated) ^c
Plant uptake rate	plant roots absorb polar forms of 2,4-D most readily; leaves absorb non-polar forms (esters) most readily (WSSA 1989)	plant roots absorb polar forms of 2,4-D most readily; leaves absorb non-polar forms (esters) most readily (WSSA 1989)	plant roots absorb polar forms of 2,4-D most readily; leaves absorb non-polar forms (esters) most readily (WSSA 1989)	plant roots absorb polar forms of 2,4-D most readily; leaves absorb non-polar forms (esters) most readily (WSSA 1989)

^a Estimated from the vapor pressure and water solubility

^b Estimated by the method of U.S. EPA (1992)

^c Estimated by the method of Meylan and Howard (1993)

^d Estimated by the method of Meylan and Howard (1995)

2.3. APPLICATION METHODS

The general use of herbicides in silviculture and the various methods of application are discussed in the available literature (e.g., Cantrell and Hyland 1985) and in previously prepared risk assessments (USDA 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, one formulation of 2,4-D, Aqua-Kleen, may be applied directly to water for the control of noxious weeds. 2,4-D is the only phenoxy herbicide that is registered for the treatment of noxious aquatic weeds (Lembi 1997).

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 of 0.25-1.0 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 of 0.25-1.0 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 1989b, p 2-9 to 2-10).

Although 2,4-D is registered for aerial applications, the Forest Service does not currently employ this method with 2,4-D. This application method, nonetheless, is included in this risk assessment to support the potential use of 2,4-D in aerial applications that might be judged appropriate or necessary (i.e., 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.

One formulation of 2,4-D, Aqua-Kleen, is labeled for direct application into bodies of water for the control of noxious weeds. For large areas, a fertilizer spreader or mechanical seeder may be used to disperse the granules over the surface of the water. When repeated applications are necessary, the product is applied in 50-100 foot lanes, separated by buffer lanes of equal width. Re-treatment typically occurs after 6-8 weeks, when the vegetation killed by the initial treatment decays sufficiently to allow a normal oxygen regime to become reestablished.

2.4. MIXING AND APPLICATION RATES

The various formulations of 2,4-D and mixtures of 2,4-D with other herbicides are used primarily for the control of annual and perennial broadleaf weeds and are labeled for uses such as the maintenance of rights-of-way and wildlife openings.

In previously conducted Forest Service vegetation management programs (USDA 1989a,b,c), 2,4-D was applied in relatively small amounts, compared with the application of other herbicides. For example, in Forest Service Region 8 (comprised of Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, North California, Oklahoma, South Carolina, Tennessee, Texas, Virginia, and part of West Virginia), there are approximately 12,000,000 acres of National Forests and Grassland, of which up to 600,000 acres were treated with various herbicides each year. In the late 1980s, 2,4-D amine was applied to about 10,000 acres each year and 2,4-D esters were applied to only about 900 acres per year (USDA 1989b, p.2-4). More recently, the Forest Service use of herbicides in Region 8 decreased to treatment of fewer than 50,000 acres/year.

During 1995, the Forest Service treated approximately 20,000 (20,400.7) acres with 2,4-D nation-wide. About 33% of the acres were treated with 2,4-D alone (6843.7 acres) at an average application rate of 0.8 lbs/acre. The great majority of the acres treated with 2,4-D alone (77% or 5296.7 acres of 6843.7 acres) involved noxious weed control. Of the 13,557.2 acres treated with mixtures of 2,4-D and another herbicide, almost all of the acres treated (96.7% or 13,147.7 acres of 13,557.2 acres) involved noxious weed control. Of the 18,408.9 lbs of 2,4-D containing herbicide mixtures used in 1995, mixtures of 2,4-D with picloram and/or dicamba accounted for about 90% of the herbicide mixture use (16,625.7 lbs of 18,408.9 lbs) (USDA 1996).

The specific application rates used in a ground or aerial program will vary depending on local conditions and the nature of the target vegetation. For ground applications, the U.S. Forest Service will use rates ranging from 0.5 to 2.0 lbs a.e./acre with a typical application rate of 1.0 lb a.e./acre. The same rates would probably be used for aerial applications, if such applications were to be conducted. The higher range of the application rates would be used for activities such as 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).

Much higher application rates could be used with the direct application of Aqua-Kleen to bodies of water. As summarized in Table 2-1, Aqua-Kleen contains the butoxyethyl ester of 2,4-D in attaclay 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 stargress. 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. As noted above, 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.

3. HUMAN HEALTH RISK ASSESSMENT

3.1. HAZARD IDENTIFICATION

3.1.1. Overview. There are extensive investigations regarding the toxicity of 2,4-D to experimental mammals. Moreover, the health status of human populations exposed to 2,4-D was also studied. The U.S. EPA has scheduled the re-registration of 2,4-D, but the Re-registration Eligibility Document (RED) is not scheduled for completion until the year 2000. Although the mode of action of 2,4-D as a plant toxin is well understood, the mode of action of 2,4-D toxicity in mammals is not clear. After acute lethal exposure, the signs of toxicity in humans include convulsions, vomiting, congestion of various organs, and degenerative changes in nerve cells. 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. Similar signs of toxicity were observed in experimental mammals exposed to 2,4-D.

As summarized in Tables 2-1 and 2-2, there are several formulations of 2,4-D available as either amine salts or esters. In general, the ester formulations contain 2,4-D concentrations that are somewhat higher than those found in the salt formulations. In fact, using the ester formulations may involve handling and applying concentrated solutions of 2,4-D. The variation in 2,4-D concentrations among the different formulations has an impact on the exposure assessment for each formulation; however, a more significant factor affecting the exposure assessments is the likelihood that there are formulation-related differences regarding the dermal absorption of 2,4-D. Although structure-activity relationships for dermal permeability suggest that the differences in dermal absorption rates are likely to be substantial, *in vivo* dermal absorption studies in humans and experimental mammals do not report systematic differences in dermal absorption. Consequently there is substantial uncertainty in the exposure assessments and risk characterizations for the different formulations of 2,4-D.

There is less uncertainty concerning the dose-response relationships for 2,4-D acid, salts, and esters. The acute toxicity of 2,4-D acid, salts or esters to mammals is relatively low, with LD₅₀ values ranging from 100 mg/kg (butyl ester) in cattle to 1800 mg/kg (sodium salt) in rats. Moreover, the toxicological equivalence of 2,4-D acid, salts, and esters is supported by teratogenicity screening assays in which the acute lethal potency of 2,4-D acid and its various esters was similar. In addition, dog studies involving subchronic exposure to 2,4-D acid, 2,4-D dimethylamine, or the ethylhexyl ester of 2,4-D, indicate that the toxic potencies of these various forms of the compound are similar to one another. There is a significant difference in sensitivity to 2,4-D exposure among animal species. This pattern is common in toxicity studies, with smaller animals being less sensitive than larger animals to chemical exposure. On the other hand, studies involving acute exposure to 2,4-D indicate that mice are outliers in this pattern, being somewhat more sensitive than rats. Nevertheless, this deviation in the pattern has little impact on the risk assessment.

The subchronic and chronic effects of exposure to 2,4-D are well studied. Most of the information on which this risk assessment is based is taken from the published literature and

studies submitted to the U.S. EPA in support of the re-registration of 2,4-D. The current RfD for 2,4-D is based on a dietary study in which Fisher 344 rats were exposed to daily doses of 1, 5, 15, or 45 mg/kg bw/day 2,4-D. After exposure, dose-related increases in kidney weight (males and females) and thyroid weights (males only) were noted in addition to decreases in mean hemoglobin, hematocrit, RBC levels, and reticulocyte levels (males only). The increase in thyroid weight was associated with an increase in T₄ (thyroxine) levels in the blood of male rats. U.S. EPA does not consider the thyroid effects observed at 1 mg/kg/day to be treatment related. Based on a review of the individual animal data from this study and a review of other studies in which thyroid effects were reported, the weight of evidence suggests that toxicologically significant effects on the thyroid may occur at relatively high dose levels. Hence, U.S. EPA seems justified in classifying the dose of 1 mg/kg/day as a NOAEL, based on the dietary study in rats.

Exposure to the n-butyl ester of 2,4-D seems to cause immunological effects that are attributable to the n-butyl moiety rather than to 2,4-D. Since, however, the n-butyl ester of 2,4-D is no longer commercially available, it is not given detailed consideration in this risk assessment.

At relatively high doses associated with fetotoxicity or maternal toxicity, 2,4-D might induce fetal malformations. One occupational exposure study reports an association between 2,4-D exposure and sperm damage; however, the data in the study do not support the association. At best, the study shows that the incidence of sperm anomalies in a group of pesticide applicators was higher than in a group of individuals who did not apply pesticides. Whether exposure to 2,4-D was the cause of the observed effects cannot be verified by the data presented in the study. Although some animal studies support an association between 2,4-D exposure and male reproductive impairment, reproductive effects seem to be much less sensitive endpoints than the effects on which the RfD derived by U.S. EPA is based.

There are many epidemiology studies regarding an association between exposure to 2,4-D (and other phenoxy herbicides) and the development of cancer. These studies were reviewed thoroughly by the U.S. EPA and are undergoing additional review as part of the re-registration process. Given the level of analysis and review that the U.S. EPA decision will receive and the general principle that other government agencies should apply the U.S. EPA assessments consistently in their risk assessments, barring compelling arguments to the contrary, the cancer risk assessment for 2,4-D should be reexamined after the re-registration process for 2,4-D is completed. At this time, it appears that none of the studies regarding the potential carcinogenicity of 2,4-D demonstrate a causal relationship between 2,4-D exposure and the development of cancer.

Although there is a relatively good database on the acute and chronic oral toxicity of 2,4-D, there is much less information regarding the inhalation toxicity of 2,4-D or its combustion products. Estimates of inhalation LC₅₀ values are not available. As part of the re-registration process for 2,4-D, an acute whole body inhalation study was conducted. In the study, five rats of each sex were exposed to 2.15 mg 2,4-D/L (2150 mg/m³), and two of the 10 rats died. The American Conference of Governmental Industrial Hygienists adopted a TLV of 10 mg/m³ for 2,4-D, which is identical to analogous values recommended by other federal agencies and other nations.

3.1.2. Acute Toxicity and Mechanisms of Action. As summarized in Table 3-1, 2,4-D has a low order of acute toxicity to mammals, with oral LD₅₀ values ranging from approximately 69 mg a.e./kg (100 mg butyl ester/kg) in cattle (Bjorklund and Erne 1966) to 1800 mg/kg (2000 mg sodium salt/kg) in rats (Tucker and Crabtree 1970). Although the mode of action of 2,4-D as a plant toxin is well understood, the mode of action of 2,4-D toxicity in mammals is not clear. 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 (U.S. DOE 1983, Mullison 1981, Lommen 1980). Similar signs of acute toxicity were observed in monkeys (Hill and Carlisle 1947) and pigs (Bjorklund and Erne 1966) exposed to 2,4-D. With the possible exception of neurotoxicity (section 5.1.6), none of these signs or symptoms suggests a highly specific mode of toxic action in mammals.

Table 3-1. Comparison of the acute oral toxicity (LD₅₀ values expressed in mg/kg/day) of 2,4-D acid (MW 221) or salts 2,4-D esters

Species	Salt/Acid		Ester		Reference
	Nominal	a.e.	Nominal	a.e.	
Chicken	541	541	1420 ^a	1000	Rowe and Hymas 1954
Chicken	541	541	2000 ^b	1400	
Guinea pig	469	469	550 ^a	400	
Guinea pig	469	469	848 ^b	580	
Mouse	368	368	731 ^b	500	
Rat	805 ^c	730	620 ^b	430	
Rat	2000 ^c	1800	1500 ^c	1000	Tucker and Crabtree 1970

^a Isopropyl ester, MW 307, a.e. = 0.720 · a.i.

^b Butyl ester, MW 321, a.e. = 0.688 · a.i.

^c Sodium salt, MW 243, a.e. = 0.909 · a.i.

At high concentrations *in vitro*, 2,4-D may uncouple oxidative phosphorylation and interfere with other enzymes involved in cellular energetics, calcium regulation (Palmeira et al. 1994a,b, 1995, Pereira et al. 1994), protein and DNA synthesis (Rivarola and Balegno 1991a), and polyamine synthesis (Rivarola and Balegno 1991b, Rivarola et al. 1992).

It is plausible that the target organ specificity of 2,4-D is related to active transport processes in certain organs. Bergesse and Balegno (1995) report that 2,4-D appeared to concentrate in cultured Chinese hamster cells. Although the concentration seemed to involve an active transport process, the process is not identified in the study. Villalobos et al. (1996) report that the transport of 2,4-D in the kidney may involve the Na^+/α -ketoglutarate transport system.

This risk assessment is concerned with both the dimethylamine salt of 2,4-D and 2,4-D esters used in commercial formulations. The available information regarding the acute toxicity of 2,4-D covers various forms of 2,4-D, including the acid, iso-octyl, butyl, and propyl esters, and the sodium and dimethylamine salts. Hence, it is important to consider the extent to which toxicity data on one form of 2,4-D can be used to assess the consequences associated with exposure to other forms of 2,4-D.

A direct comparison of the acute toxicity of different forms of 2,4-D using all of the available data is confounded by several variables, including the different animal species used in the bioassays, the different times at which the tests were conducted, the different experimental conditions of the studies, and the different numbers of animals used by the various investigators.

Table 3-1 summarizes the acute oral LD_{50} data involving various forms of 2,4-D in which the LD_{50} values were determined in the same species, by the same investigators. The table provides data regarding the animal species tested, the reported LD_{50} values (both as a.i. and a.e.), and the reference.

These data are illustrated in Figure 3-1. The acid equivalents of the acid or salt form are plotted on the x-axis; the acid equivalents of the ester form are plotted on the y-axis. The thick, dashed diagonal line is the line to be expected if the LD_{50} in acid equivalents of the salt/acid were identical to that of the ester.

As Figure 3-1 illustrates, there are no substantial or systematic differences in toxicity, when expressed as acid equivalents. The greatest difference in toxicity involves the chicken studies (Rowe and Hymas 1954), in which the LD_{50} values for 2,4-D esters are factors of 2- to 3-fold higher than the LD_{50} values for 2,4-D acid. Both mixed butyl and isopropyl esters were used in this study. In rats, the relationship is reversed; the LD_{50} values of the esters are lower by a factor of less than two. In mice and guinea pigs, none of the differences in the LD_{50} values between the acid and ester forms are greater than a factor of 1.4. As indicated in section 5.1.3,

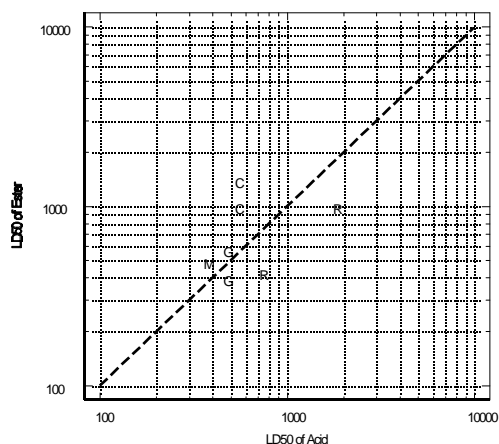


Figure 3-1: Comparison of acute oral LD_{50} values for various species of 2,4-D in chickens (C), guinea pigs (G), mice (M), and rats (R).

this assessment is consistent with the results of a dog study involving subchronic exposure to 2,4-D acid, 2,4-D dimethylamine, and the ethylhexyl ester of 2,4-D. The results of the study suggest that the various forms of 2,4-D have a similar toxic potency (Charles et al. 1996a). Further support for this conclusion can be found in teratogenicity screening assays in which the toxicity of 2,4-D acid was similar to that of the various esters (Courtney 1977, Kavlock et al. 1987).

On the other hand, some esters, particularly the butyl ester, may be more toxic than 2,4-D acid or salts for reproductive endpoints (Schwetz et al. 1971) (section 5.1.4) and neurological endpoints (de Moro et al. 1993) (section 5.1.6). The difference may be attributable to the toxicity of the salt or ester moiety (e.g., the formation of n-butanol).

Although substantial differences in the acute lethal potency of the various forms of 2,4-D are not apparent, there is a significant pattern in sensitivity among species, indicating that, in general, smaller animals are less sensitive (i.e., have higher LD₅₀ values), compared with large animals. This pattern is common in toxicology and is often used to extrapolate across species (e.g., Davidson et al. 1986) based on the general allometric relationship:

$$LD_{50} = aW^b$$

or

$$\log(LD_{50}) = b \cdot \log(W) + \log(a)$$

where **W** is the body weight and **b** is the slope parameter. Larger animals, such as humans, are considered more sensitive than small animals such as rats and mice when **b** is statistically significant and negative.

Data regarding the acute oral LD₅₀ values of various 2,4-D formulations are summarized in Table 3-2. As in Table 3-1, the oral LD₅₀ values are expressed as the salt or ester (a.i.) and the acid equivalents (a.e.). The allometric analysis illustrated in Figure 3-2 is conducted only on the LD₅₀ values expressed as acid equivalents.

Table 3-2 summarizes 23 data points in eight species. All but one of these points are standard LD₅₀ estimates. The data point for humans (Mullison 1981) is a reported lethal dose in one individual. Other acute oral toxicity data regarding human exposure to 2,4-D cannot be used to characterize the acute lethal potency of 2,4-D for several reasons, including inadequate information about dose levels (Minnesota Department of Health 1978, Keller et al. 1994), co-exposure to 2,4-D and other toxic agents (Minnesota Department of Health 1978, Mullison 1981), and the lack of evidence of lethal exposure (U.S. DOE 1983, Mullison 1981, Lommen 1980).

Table 3-2. Studies used for developing allometric relationships for the LD₅₀ values (mg/kg) for 2,4-D

Animal Species	2,4-D Species	Oral LD ₅₀		Animal Body Weight (kg) ^a	Reference
		a.i.	a.e.		
Cat	butyl ester	820	564	1.7	Konstantinova 1970
Cattle	butyl ester	100	69	425	Bjorklund and Erne 1966
Dog	acid	100	100	5.15	Drill and Hiratzka 1953
Guinea pig	sodium salt	551	501	0.445	Rowe and Hymas 1954
Guinea pig	propyl ester	550	396	0.445	Rowe and Hymas 1954
Guinea pig	butyl ester	848	583	0.445	Rowe and Hymas 1954
Guinea pig	acid	469	469	0.445	Rowe and Hymas 1954
Human ^b	dimethylamine	80	66	70	Mullison 1981
	e				
Mouse	sodium salt	360	327	0.022	Loktionov et al. 1973
Mouse	butyl ester	731	503	0.022	Rowe and Hymas 1954
Mouse	butyl ester	380	261	0.022	Konstantinova 1970
Mouse	acid	368	368	0.022	Rowe and Hymas 1954
Mouse	propyl ester	541	390	0.022	Rowe and Hymas 1954
Rabbit	sodium salt	800	727	2.88	Rowe and Hymas 1954
Rabbit	butyl ester	424	292	2.88	Rowe and Hymas 1954
Rat	butyl ester	620	427	0.171	Rowe and Hymas 1954
Rat	propyl ester	700	504	0.171	Rowe and Hymas 1954
Rat	sodium salt	2000	1818	0.171	Schillinger 1960
Rat	sodium salt	805	732	0.171	Rowe and Hymas 1954
Rat	butyl ester	1500	1032	0.171	Schillinger 1960
Rat	acid	375	375	0.171	Rowe and Hymas 1954
Rat	sodium salt	730	664	0.171	Loktionov et al. 1973
Rat	butyl ester	920	633	0.171	Konstantinova 1970

^a From U.S. EPA (1989) and Durkin (1989)

^b Lethal to one individual. Plotted in Figure 3-2 but not used in statistical analysis.

a.i. = Active ingredient; a.e. = acid equivalent

Data regarding dose levels associated with human suicides are not entirely consistent with the estimates provided in Table 3-2. For example, Durakovic et al. (1992) investigated four poisoning cases in which individuals who ingested doses of 1429-2286 mg/kg were treated

successfully by hemodialysis. Although these reports suggest that the approximate acute lethal dose of 2,4-D is higher than the value used in the allometric analysis cited above, it is likely that the survival of the individuals can be attributed to prompt and effective medical intervention. Thus, it is not appropriate to use these data to estimate the relative sensitivities to 2,4-D across species. Lethal overdoses of 2,4-D, 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). Total blood levels of about 7 mg/L are reported in other suicide cases (Smith and Lewis 1987).

The statistical analysis of these data is illustrated in Figure 3-2. The labeled points correspond to the data in Table 3-2. The thick solid line is the maximum likelihood estimate for the allometric relationship using data on all species. The thick dashed line is the maximum likelihood estimate for the allometric relationship using data on all species except the mouse.

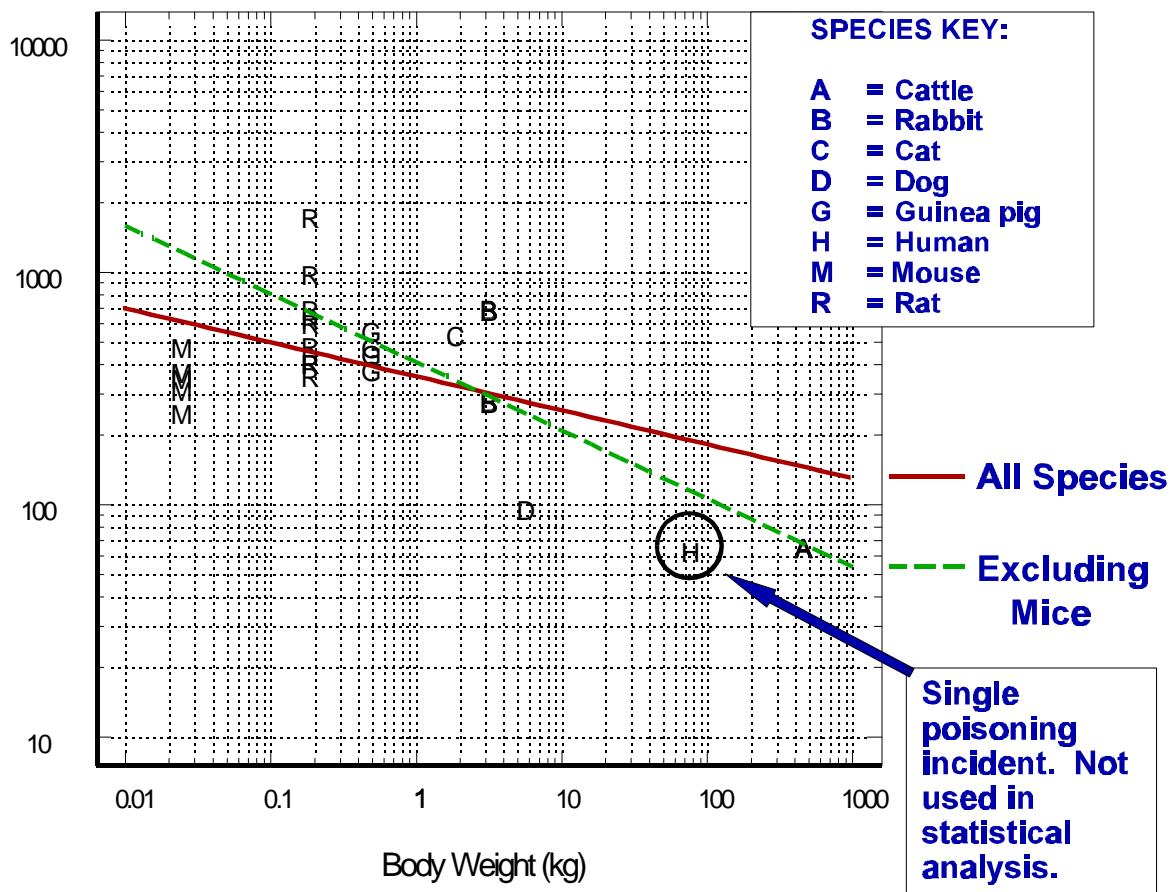


Figure 3-2: Relationship of acute oral LD₅₀ values for various formulations of 2,4-D to body weight (see Table 3-2).

Although the squared correlation coefficient is low (0.24) when all species are considered, the slope parameter, *b*, is statistically significant at $p=0.021$ (slope = -0.14, intercept = 5.9).

Considering that this analysis is based on various studies conducted at different times, by different investigators, the general scatter does not seem remarkable.

Mice deviate from the general relationship, appearing to be more sensitive than rats to 2,4-D after acute exposure; yet, it does not seem reasonable to attribute the apparent deviation from the general allometric relationship to random scatter in the data. The five data points on mice are from three different studies involving four different forms of 2,4-D, and the mouse data are relatively consistent within the studies. On the other hand, subchronic exposure studies suggest that mice are somewhat less sensitive than rats to 2,4-D (section 3.1.3). When the data on mice are excluded, the squared correlation coefficient is substantially higher (0.60) and the slope parameter is highly significant ($p=0.00025$, slope=-0.29, intercept=6.01).

As discussed above, a suicide incident involving the ingestion of 2,4-D by one individual suggests that the acute lethal potency of 2,4-D is 10 times greater for humans than for rats. This estimate, however, is based on only one poisoning incident reported in a secondary source (Mullison 1981). Thus, the value is not an LD_{50} and was not used in the statistical analysis from which the allometric equations were developed (see Figure 3-2).

3.1.3. Subchronic or Chronic Systemic Toxic Effects. Information regarding the subchronic and chronic toxicity of 2,4-D in mammals is summarized in Table 3-3. Most of this information is taken from studies recently submitted to U.S. EPA as part of the re-registration of 2,4-D. Some early published studies (e.g., Drill and Hiratzka 1953, Hansen et al. 1971) are not included in the analysis because the more recent studies provide a much greater level of detail. Qualitatively, these early studies are consistent with the more recent studies submitted to U.S. EPA.

The current RfD for 2,4-D is based on the study by Serota et al. (1983b) using Fisher 344 rats (U.S. EPA 1997). The RfD was last revised on May 5, 1988. A June 30, 1988 entry in IRIS indicates that the RfD was under review and that changes to the RfD were possible (U.S. EPA 1997). In the dietary study by Serota et al. (1983b), rats were exposed to daily doses of 1, 5, 15, or 45 mg/kg bw/day 2,4-D. The investigators observed dose-related increases in kidney weight (males and females) and thyroid weight (males only) as well as decreases in mean hemoglobin, hematocrit, RBC levels, and reticulocyte levels (males only). The increase in thyroid weight was associated with an increase in T_4 (thyroxine) levels in the blood of male rats. In addition, levels of various enzymes associated with liver function (LDH, SGOT, SGPT, and alkaline phosphatase) were decreased rather than increased. The U.S. EPA considered the dose of 1 mg/kg/day the NOAEL, identifying the critical effects as "hematologic, hepatic, and renal toxicity." Although the RfD record indicates that the effects on the thyroid and T_4 levels were taken into consideration, the record also indicates that these effects "were not considered to be treatment-related." As discussed more fully in the dose-response assessment (section 3.3), the NOAEL of 1 mg/kg/day is supported by more recent subchronic and chronic studies in dogs, which indicate a LOAEL of 5 mg/kg/day and a NOAEL of 1 mg/kg/day for kidney effects (Dalgard 1993a,b).

Table 3-3. Dose/Duration/Severity Relationships for 2,4-D in experimental mammals.

Species	Sex	Dose (mg/kg/day)	Duration (Days)	Effect	Severity Level	Reference
Cattle	M	100	1	none	NOAEL	Paulino and Palermo-Neto 1995
	M	300	1	clin. chem. kidney and musc damage	AEL	Paulino and Palermo-Neto 1995
	M	600	1	weakness and lethargy	FEL	Paulino and Palermo-Neto 1995
Dogs	N	1.3	1	none	NOEL	Arnold et al. 1991
Dogs	N	8.8	1	abnormal EMG (+)	AEL	Arnold et al. 1991
Dogs	N	43.7	1	abnormal EMG (++)	AEL	Arnold et al. 1991
Dogs	N	175	1	depression, ataxia, EEG, EMG (++++)	FEL	Arnold et al. 1991
Dogs	N	230	1	depression, ataxia, EEG, EMG (++++)	FEL	Arnold et al. 1991
Dogs	M	1.3	1	NOEL for myotonia.	NOEL	Beasley et al. 1991
Dogs	M	8.8	1	subclinical myotonic discharges	AEL	Beasley et al. 1991
Dogs	M	43.7	1	subclinical myotonic discharges	AEL	Beasley et al. 1991
Dogs	M	86.7	1	subclinical myotonic discharges	AEL	Beasley et al. 1991
Dogs	M	175	1	clinical myotonia and vomiting	FEL	Beasley et al. 1991
Dogs	M	220	1	clinical myotonia and vomiting	FEL	Beasley et al. 1991
Dogs	F	0.5	91	none	NOEL	Charles et al. 1996b
Dogs	F	1	91	slight decrease in weight gain	NOAEL	Charles et al. 1996b
Dogs	F	3.75	91	decreased body weight gain	AEL	Charles et al. 1996b
Dogs	F	7.5	91	decreased body weight gain, increased BUN	AEL	Charles et al. 1996b
Dogs	M	0.5	91	none	NOEL	Charles et al. 1996b
Dogs	M	1	91	slight decrease in weight gain	NOAEL	Charles et al. 1996b
Dogs	M	3.75	91	decreased body weight gain, decreased testes wgt	AEL	Charles et al. 1996b

Table 3-3. Dose/Duration/Severity Relationships for 2,4-D in experimental mammals.
(continued)

Species	Sex	Dose (mg/kg/day)	Duration (Days)	Effect	Severity Level	Reference
Dogs	F	1	365	none	NOEL	Dalgard 1993b
Dogs	F	5	365	reduced body weight gain, increased BUN, liver/kidney path	AEL	Dalgard 1993b
Dogs	F	7.9	365	reduced body weight gain, increased BUN, liver/kidney path	AEL	Dalgard 1993b
Dogs	M	1	365	none	NOEL	Dalgard 1993b
Dogs	M	5.2	365	reduced body weight gain, increased BUN, liver/kidney path	AEL	Dalgard 1993b
Dogs	M	8.2	365	reduced body weight gain, increased BUN, liver/kidney path	AEL	Dalgard 1993b
Dogs	M	25	1	no clinical myotonic or abnormal EMG	NOEL	Steiss et al. 1987
Dogs	M	50	1	myotonia and/or abnormal EMG	AEL	Steiss et al. 1987
Dogs	M	75	1	myotonia and/or abnormal EMG	AEL	Steiss et al. 1987
Dogs	M	100	1	myotonia and/or abnormal EMG	AEL	Steiss et al. 1987
Dogs	M	125	1	myotonia and/or abnormal EMG	AEL	Steiss et al. 1987
Mice	F	5	91	increase in pituitary and adrenal weight	AEL	Serota et al. 1983a
Mice	F	15	91	none	NOEL	Serota et al. 1983a
Mice	F	45	91	none	NOEL	Serota et al. 1983a
Mice	F	90	91	increased pituitary and adrenal weight	AEL	Serota et al. 1983a
Mice	M	5	91	none	NOEL	Serota et al. 1983a
Mice	M	15	91	increase in adrenal and pituitary weight	AEL	Serota et al. 1983a
Mice	M	45	91	increased kidney weight	NOAE L	Serota et al. 1983a

Table 3-3. Dose/Duration/Severity Relationships for 2,4-D in experimental mammals. (*continued*)

Species	Sex	Dose (mg/kg/day)	Duration (Days)	Effect	Severity Level	Reference
Rats	M	300	1	changes in locomotion and rearing frequency	AEL	de Duffard et al. 1993 (<i>continued</i>)
Rats	M	15	91	no effect on kidneys	NOAEL	Eisenbrandt et al. 1986a,b
Rats	M	60	91	degenerative kidney lesions	AEL	Eisenbrandt et al. 1986a,b
Rats	M	100	91	Degenerative kidney lesions	AEL	Eisenbrandt et al. 1986a,b
Rats	M	150	91	Degenerative kidney lesions	AEL	Eisenbrandt et al. 1986a,b
Rats	N	125	1	no effect on blood/brain barrier	NOAEL	Elo et al. 1988
Rats	N	300	1	albumin permeation into blood/brain barrier	NOAEL	Elo et al. 1988
Rats	F	5	365	none	NOEL	Jeffries et al. 1995
Rats	F	75	365	decreased body weight, pathologic changes in the heart, eye, lungs, and liver	AEL	Jeffries et al. 1995
Rats	F	150	365	decreased body weight, pathologic changes in the heart, eye, lungs, and liver	AEL	Jeffries et al. 1995
Rats	M	5	365	none	NOEL	Jeffries et al. 1995
Rats	M	75	366	decreased body weight, pathologic changes in the heart, eye, lungs, and liver	AEL	Jeffries et al. 1995
Rats	M	150	365	decreased body weight, pathologic changes in the heart, eye, lungs, and liver	AEL	Jeffries et al. 1995
Rats	F	15	1	no neurologic effects.	NOEL	Mattsson et al. 1994
Rats	F	75	1	no neurologic effects.	NOEL	Mattsson et al. 1994
Rats	F	250	1	transient changes in gait/coordination.	AEL	Mattsson et al. 1994
Rats	M	15	1	no neurologic effects.	NOEL	Mattsson et al. 1994
Rats	M	75	1	no neurologic effects.	NOEL	Mattsson et al. 1994
Rats	M	250	1	transient changes in gait/coordination.	AEL	Mattsson et al. 1994
Rats	F	1	91	increased ovary weight	NOAEL	Serota et al. 1983b
Rats	F	5	91	increased ovary weight	AEL	Serota et al. 1983b

Unlike the pattern observed in acute toxicity studies (see Figure 3-2), mice seem less sensitive than rats to subchronic or chronic exposure to 2,4-D. The subchronic or chronic NOAEL for exposure to 2,4-D is approximately 5 mg/kg/day in mice (Serota et al. 1983a, Stott et al. 1995). Although 5 mg/kg/day is reported as the NOAEL in a chronic rat study (Jeffries et al. 1995), this study result does not lower concern for the LOAELs reported at 5 mg/kg/day in other studies on rats (Serota et al. 1983b) or dogs (Dalgard 1993a,b).

The NOAEL of 1 mg/kg/day is well supported; however, the potential effects on the thyroid deserve further consideration. U.S. EPA (1997) indicates that these effects "were not considered to be treatment-related," which contradicts the conclusion of the study authors, Serota et al. (1983b):

Compound related increase in both absolute and relative thyroid weights were observed. ... The thyroid effects appeared to be correlated to some degree with increased T₄ values in several of the treated male groups (Serota et al. 1983b, p. 2).

These authors also note that there were no dose-related pathology changes in the thyroid; however, at the lowest tested dose of 1 mg/kg/day, the absolute thyroid weights were increased significantly in male rats. Increases in circulating thyroxine levels were statistically significant at 5 and 15 mg/kg/day but not at 1 and 45 mg/kg/day.

Thyroid weight increases associated with increases in circulating thyroxine levels are important because increases in circulating thyroxine levels are associated with increased carbohydrate use, decreased body fat, and body weight loss (Capen 1996, Capen et al. 1991). Body weight loss is often observed in 2,4-D treated animals and cannot always be explained by decreased food consumption (e.g., Charles et al. 1996a,b). Furthermore, 2,4-D exposure is associated with decreases in blood glucose (Dalgard 1993b) and decreased body fat (Jeffries et al. 1995). Thus, an argument can be made for classifying 1 mg/kg/day as a LOAEL, based on an increase in circulating thyroxine levels because it may be critical to the effects observed at higher doses. Also, changes in circulating thyroxine levels may be associated with several neurological effects, many of which are consistent with 2,4-D intoxication (section 3.1.6).

While increased thyroxine levels and increased thyroid weights are reported in a more recent 2-year rat feeding study (Jeffries et al. 1995), these effects occurred only at relatively high dose levels (75 and 150 mg/kg/day) and not at a dose level of 5 mg/kg/day. Moreover, pathological lesions to the thyroid were observed only in female rats exposed to doses of 150 mg/kg/day. In addition, although statistically significant increases in relative thyroid weights were observed in male rats at all dose levels in the Serota et al. (1983b) study, the dose-response relationship is uneven, with the maximal response observed at 5 mg/kg/day (30% above controls) and lesser responses observed at 15 mg/kg/day (22% above controls) and 45 mg/kg/day (27% above controls). In a 90-day dog feeding study (Dalgard 1993a), only females exposed to 7.5 mg/kg/day (highest dose tested) had a significant increase in thyroid weight relative to body

weight. The increase in thyroid weight was not significant in terms of total organ weight or organ weight relative to brain weight. There were no statistically significant effects on thyroid weight in females given lower doses of 0.5-3.75 mg/kg/day or in males at any dose level (i.e., 0.05-7.5 mg/kg/day). In a 1-year dog study, 2,4-D doses ranging from 1 to 8.2 mg/kg/day did not effect thyroid weight in males or females (Dalgard 1993b).

In terms of toxicologically significant effects on the thyroid, the weight of evidence suggests that effects may occur at relatively high dose levels. Thus, the decision by the U.S. EPA (1997) to classify the dose of 1 mg/kg/day from Serota et al. (1983a) as a NOAEL seems justified.

There is some evidence that certain forms of 2,4-D may cause immunological effects; however, most of the studies involve exposure to the n-butyl ester of 2,4-D, and it is not clear whether the observed responses are attributable to the 2,4-D moiety or the formation of n-butanol. At acutely toxic levels, the n-butyl ester was shown to inhibit immune function in mice, assayed as antibody production against sheep red blood cells. Subtoxic doses, however, had no effect. Thus, the suppression of antibody production may be secondary to other toxic effects (Blakley and Schiefer 1986).

As discussed in section 3.3.3., there is no strong time-response relationship for 2,4-D. The similarities in doses associated with similar subchronic and chronic effects can be explained, in part, by the pharmacokinetics of 2,4-D. Like other phenoxy-herbicides, such as 2,4,5-T and Silvex, 2,4-D is absorbed rapidly, distributed within the body, bound to endogenous proteins, and rapidly eliminated (Arnold and Beasley 1989). Thus, steady-state levels are reached relatively fast and there is little difference with regard to body burdens in subchronic and chronic studies.

3.1.4. Reproductive and Teratogenic Effects. As detailed in Appendix 1 and several literature reviews (e.g., Munro et al. 1992), teratology studies on 2,4-D indicate that malformations are likely to occur only at doses that are fetotoxic or maternally toxic. Data on non-mammalian species also indicate that 2,4-D may be fetotoxic but is not teratogenic (e.g., chick embryos) (Arias 1994). Further evidence that 2,4-D is not teratogenic is provided in a recent aquatic toxicity screening test (FETAX) conducted by Morgan et al. (1996). One of the lesions observed in teratology studies is abdominal hemorrhage (e.g., Aleksashin et al. 1973). This effect may be attributable to the inhibition of platelet aggregation by 2,4-D rather than an overt effect on development (Elo et al. 1991).

There is evidence that 2,4-D may adversely affect male reproductive capacity. 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. It is questionable whether 2,4-D had anything to do with the observed effects.

Studies on experimental animals, nonetheless, support a concern for the effects of 2,4-D on the testes. In a 2-year feeding study, Jeffries et al. (1995) observed that two of 10 rats exposed to 150 mg/kg/day (the highest dose tested) had testicular atrophy at the 12-month interim sacrifice. The effect was not observed in rats exposed to 5 or 75 mg/kg/day. At the 2-year sacrifice, testicular weights were decreased in a dose-related manner at 75 and 150 mg/kg/day. In some dogs, 2,4-D exposure levels as low as 3.75 mg/kg/day resulted in decreased testes weights (Charles et al. 1996a).

Without specifically addressing the rat and dog studies discussed 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.*"

It is true that severely and chronically poisoned male animals may undergo a number of pathological changes, including effects on sperm morphology, which are secondary to other effects and not relevant to exposure at lower doses. In the dog study by Charles et al. (1996b), however, animals exposed to 3.75 mg/kg/day showed no overt signs of toxicity; yet, testicular weight was decreased. Similarly, dogs exposed to 7.5 mg/kg/day had a statistically significant decrease in testicular weight but no significant decrease in body weight. Therefore, the statement by Munro et al. (1992) that effects on sperm could be "easily accounted for" as secondary to changes in thyroid hormone status is somewhat puzzling. The relationship of thyroid function to testicular function is well documented (e.g., Thomas 1981). As indicated in section 3.1.3, the thyroid may be one of the more sensitive sites for 2,4-D toxicity. It is not clear, therefore, why the development of abnormal sperm or decreased testicular weight would be of less cause for concern since the effect is associated with this sensitive endpoint.

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). 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.

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 is questionable.

3.1.5. Carcinogenicity and Mutagenicity. An association has been made but no causal relationship has been shown between the use of herbicides and an increase in non-Hodgkin's Lymphomas (NHL) in the United States since 1953 (Ballester et al. 1993). Herbicide use has also been considered as a factor in the relatively high incidence of NHL in farmers (Bond and Rossbacher 1993). Associations between NHL and herbicide exposure are reported in other countries, as well (Vineis et al. 1991).

There are many epidemiology studies that examine the association between exposure to 2,4-D and other phenoxy herbicides and the development of various forms of cancer. These studies are the subject of several reviews sponsored or prepared by industries associated with the manufacture and/or distribution of 2,4-D (Bond and Rossbacher 1993, Carlo et al. 1992, Hammond 1995, Ibrahim et al. 1991, Munro et al. 1992) as well as a recent review sponsored by the USDA (Johnson and Wattenberg 1996). The most relevant case control studies are summarized in Table 3-4, and several relevant cohort studies are summarized in Table 3-5.

The U.S. EPA is in the process of reviewing data on the carcinogenicity of 2,4-D. In 1994, the Science Advisory Board of the U.S. EPA reviewed the agency's analysis of 2,4-D carcinogenicity and concluded the following:

while there is some evidence that NHL may occur in excess in populations which are likely to be exposed to 2,4-D, the data are not sufficient to conclude that there is a cause and effect relationship between the exposure to 2,4-D and NHL. The data are, however, sufficient to require

continued examination of the issue through further studies
(U.S. EPA 1994).

The same conclusion is reached by industry (Bond and Rossbacher 1993, Carlo et al. 1992, Hammond 1995, Ibrahim et al. 1991, Munro et al. 1992) and the USDA (Johnson and Wattenberg 1996). There is no causal relationship between 2,4-D exposure and cancer.

The follow-up of a prospective study of 1,909 Finnish workers who applied mixtures of 2,4-D and 2,4,5-T found no increase in cancer risk (Asp et al. 1994). On the other hand, a follow-up (Bloemen et al. 1993) of a U.S. retrospective study of 878 workers (Bond et al. 1988) who manufactured 2,4-D salts and esters reports an almost 2-fold increased death rate from cancer (SMR 196) in exposed workers; however, the increase is not statistically significant (95% confidence interval on SMR of 24-708).

There is a marginally significant association between the development of canine malignant lymphomas and 2,4-D exposure, as a consequence of dog owners applying 2,4-D to turf. The relative risk and 95% confidence interval is 1.3 (1.04-1.67) (Hayes et al. 1991). More recent studies conducted by the same investigator yielded odds ratios of 1.2-1.4, although the lower limits on these ratios were less than unity (i.e., not statistically significant) (Hayes et al. 1995). As discussed by Sternberg (1992), the magnitude of the exposure of the dogs to 2,4-D and the route of exposure in the Hayes et al. (1991) study are uncertain.

Fleming et al. (1997) examined 1,266 cases of cancer in 33,669 pesticide workers in Florida. There were significant increases in cervical cancer in female workers and prostate cancer in male workers. This study, however, did not differentiate between exposure to phenoxy herbicides, including 2,4-D, and exposure to other pesticides.

Blakely et al. (1992) report that the induction of murine lymphocytic leukemia in mice was unaffected by exposure to drinking water concentrations of up to 0.163% 2,4-D. These results suggest that 2,4-D is not a T-lymphocyte suppressor and is not likely to contribute to the development of NHL in humans via the suppression of T-lymphocytes. Similarly, 2,4-D was not a factor in the activation of c-N-ras alleles in canine malignant lymphoma (Edwards et al. 1993).

Much of the controversy concerning the potential carcinogenicity of 2,4-D involves exposure to Agent Orange, a mixture of 2,4-D, 2,4,5-T, and TCDD used as a herbicide in Vietnam during the Vietnam war (Pierce 1995, Schechter et al. 1995). An increased incidence of NHL was observed in some studies of Vietnam veterans (O'Brien et al. 1991, DeStefano 1995). Nevertheless, the O'Brien et al. (1991) study is particularly puzzling because the job activities of the Vietnam veterans do not suggest that exposure to chlorinated phenoxy herbicides was likely. Other studies on Vietnam veterans reported no association between pesticide exposure and NHL (Dalager et al. 1991, 1995). The National Academy of Sciences' Institute of Medicine recently reviewed the available epidemiology data on the effects of exposure to Agent Orange and concluded that 'there is limited/suggestive evidence for *no* association between exposure to phenoxy-herbicides and brain tumors' (Goetz et al. 1994).

Table 3-4. Epidemiology studies involving populations exposed to phenoxy herbicides^a

Reference	Cancer Type	Cases (Population)/ Controls (Population)	Years (Survey Type)	Odds Ratio (95% Confidence Interval)	
				Phenoxy Herbicide s	2,4-D
Cantor et al. 1992	non-Hodgkin's lymphoma	622 population based /1245 general population	1980-1983 (in-person)	1.2 (0.9-1.6)	1.2 (0.9-1.6)
Dalager et al. 1991, 1995	non-Hodgkin's lymphoma	283 veterans with NHL and 404 veterans with other diagnoses	1969-1985	1.28 (0.9-1.8)	N/A
Eriksson et al. 1981	soft tissue sarcoma	110 population based /220 general population	1974-1978 (mail/phone)	6.8 (2.6-17.3)	NA
Hardell and Sandstrom 1979	soft tissue sarcoma	52 hospital based/208 general population	1970-1977 (mail/phone)	5.3 (2.4-11.5)	NA
Hardell and Eriksson 1988	soft tissue sarcoma	54 population based/311 general population and 179 patients with other cancers	1978-1983 (mail/phone)	3.3 ^h (1.4-8.1) and 2.2 ^b (0.9-5.3)	NA
Hardell et al. 1981	Hodgkin's disease and non-Hodgkin's lymphoma	169 hospital based/338 general population	1974-1978 (mail/phone)	4.8 (2.9-8.1)	NA
Hoar et al. 1986	soft tissue sarcoma	133 population based /948 general population	1976-1982 (telephone)	1.4 ^d (NS)	1.3 ^d (NS)
Hoar et al. 1986	non-Hodgkin's lymphoma	170 population based /948 general population	1979-1981 (phone)	2.2 (1.2-4.1)	2.3 (1.3-4.3)
Hoar et al. 1986	Hodgkin's disease	121 population based /948 general population	1976-1982 (phone)	1.0 ^d (NS)	1.0 ^d (NS)

Table 3-4. Epidemiology studies involving populations exposed to phenoxy herbicides^a (continued)

Reference	Cancer Type	Cases (Population)/ Controls (Population)	Years (Survey Type)	Odds Ratio (95% Confidence Interval)	
				Phenoxy Herbicide s	2,4-D
Persson et al. 1989	Hodgkin's disease	54 hospital based/275 general population	1964-1986 (mail)	3.8 (0.7-1.5) ^c	NA
Smith and Pearce 1986	soft tissue sarcoma	51 population based/315 patients with other cancers	1981-1982 (phone)	0.7 ^b (0.3-1.5) ^c	NA
Smith and Christophs 1992	soft tissue sarcoma	30 population based/82 general population and 82 patients with other cancers	1976-1987 (in-person)	0.8 ^h (0.2-3.7) and 2.5 ^b (0.5-12.9)	NA
Smith et al. 1983, 1984	soft tissue sarcoma	82 population based/92 patients with other cancers	1976-1980 (phone)	1.3 ^b (0.7-2.5) ^c	NA
Smith and Christophs 1992	Hodgkin's disease and non-Hodgkin's lymphoma	52 hospital based/82 general population and 82 patients with other cancers	1976-1987 (in-person)	0.8 ^h (0.3-2.2) and 1.8 ^b (0.5-6.0)	NA
Vineis et al. 1986	soft tissue sarcoma	68 population based /158 general population	1981-1983 (in-person/mail)	2.4 ^e (0.6-10.3) ^c and 1.1 ^f (0.2-5) ^c	NA
Woods 1989, Wood et al. 1987	non-Hodgkin's lymphoma	576 population based /694 general population	1981-1984 (in-person)	0.9 (0.5-1.5)	0.7 (0.4-1.3)
Woods 1989, Wood et al. 1987	soft tissue sarcoma	128 population based /694 general population	1981-1984 (in-person)	0.9 ^g (0.4-1.9)	NA

Table 3-5. Key cohort studies involving soft tissue sarcomas, Hodgkin's disease, and non-Hodgkin's lymphoma in populations exposed to phenoxy herbicides^a

Reference	Cohort/Comparison Population	Follow-up (years)	Relative Risk (95% Confidence Interval)		
			Soft Tissue Sarcoma	Hodgkin's Disease	non-Hodgkin's Lymphoma
Bloemen et al. 1993	878 2,4-D manufacturers/U.S. general population and internal referents	1945-1986	none	NR ^d	2.0 ^c (0.2-7.1) and 3.0 ^e (0.8-11.9)
Bond et al. 1988	878 2,4-D manufacturers/U.S. general population and internal referents	1945-1982	none	2.7 ^c (0.0-14.7)	3.9 ^c (0.4-14.1)
Coggon et al. 1986	5754 phenoxy herbicide manufacturers and applicators/England and Wales general population	1947-1983	1.1 (0.0-5.9)	0.3 0.0-1.6	0.4 (0.0-1.3)
Lynge 1985, 1987, 1993	4461 phenoxy herbicide manufacturers (2119 potentially exposed)/Denmark general population	1947-1987	2.3 ^f (0.6-5.8)	NS	1.3 ^f (0.4-3.3)
Ott et al 1987	2187 phenoxy herbicide manufacturers and applicators/U.S. general population	1940-1982	2.5 ^b (0.1-13.9)	0.9 ^b (0.0-5.1)	1.9 (0.6-4.5)
Wiklund et al. 1987, 1987, 1989	20,245 applicators/Sweden general population	1965-1984	0.9 (0.4-1.9)	1.5 (0.8-2.4)	1.1 (0.7-1.6)

^aAdapted from NAPIAP 1996

^bResults not reported in original study. As cited by Blair and Zahm 1990, Blair et al. 1990

^cRelative risk calculated using general population as comparison group

^dOne case of Hodgkin's disease was observed in initial study but not reported in update

^eRelative risk calculated using internal referents as comparison group

^fAmong cohort members with potential exposure to phenoxy herbicides

NS = not specified; NR = not reported

Nonetheless, a few studies raise interest about the carcinogenic potential of 2,4-D. Kale et al. (1995) report a positive mutagenic response to 2,4-D in the *Drosophila* sex-linked recessive lethal mutation assay. This finding is consistent with an earlier report on the genotoxicity of 2,4-D indicating effects in both somatic and germ-line cells in *Drosophila* (Tripathy et al. 1993).

Furthermore, a recently published abstract indicates that both TCDD and 2,4-D increase the levels of transforming growth factor- α in human breast cancer cells (Lorick et al. 1995).

As part of the re-registration process, the U.S. EPA will develop a final position on the potential carcinogenicity of 2,4-D and decide whether a quantitative risk assessment for this endpoint is justified. Some more recent mutagenicity studies submitted in support of the re-registration of 2,4-D are summarized in Appendix 2.

The EPA's decision regarding the carcinogenicity of 2,4-D will be subject to extensive review and analysis. Thus, the cancer risk assessment included in the current document should be re-examined after the re-registration process for 2,4-D is completed.

3.1.6. Neurotoxicity. As noted above, the National Academy of Sciences' Institute of Medicine reviewed the epidemiology data regarding the effects of exposure to Agent Orange and found no evidence that exposure to these herbicides causes neurological effects (Goetz et al. 1994). This review, however, does not include neurotoxicity studies on laboratory animals.

In experimental animals, 2,4-D exposure is associated with myotonia: the development of tonic muscle spasms in which the muscle remains contracted for a prolonged period. This endpoint may reflect a neurotoxic rather than muscular effect because denervation of the muscle results in a blockage of the myotonic response (Al-Sulaiman et al. 1986). As detailed in Appendix 3 and summarized in Table 3-3, myotonia was elicited in dogs after acute and chronic exposure to 2,4-D. These studies are discussed further in the dose-response assessment (section 3.3).

2,4-D distributes rapidly to brain tissue, particularly when administered at high dose levels (e.g., Elo and Ylitalo 1977, 1979). One hour after the oral administration of 200 mg/kg of 2,4-D acid to rats, concentrations in brain tissue were 22.5 $\mu\text{g/g}$, compared with 478.8 $\mu\text{g/g}$ in serum. At 3 hours after dosing with 10-200 mg/kg, detectable levels of 2,4-D were found in both serum and brain tissue. At the lowest dose, 2,4-D in brain tissues was about 1.6% of that in serum; at the highest dose, 2,4-D in brain tissue was 5.6% of that in serum (Oliveira and Palermo-Neto 1995). Both 2,4-D Na salt and 2,4-D butyl ester were shown to affect brain levels of biogenic amines in rats (de Duffard et al. 1990a, Elo and MacDonald 1989).

Rats, however, may be atypically sensitive to the neurotoxic effects of 2,4-D. Elo et al. (1988) exposed rats, mice, guinea pigs, Syrian hamsters, rabbits, and chickens to comparable single oral doses of 2,4-D acid (300-600 mg/kg). Although signs of toxicity were observed in all species, only rats showed evidence of histopathological changes in the brain. Subchronic exposure to 2,4-D acid was associated with changes in neurological function (Squibb et al. 1983).

Specific nerve damage (i.e., demyelination) was associated with exposure to the n-butyl ester of 2,4-D (de Moro et al. 1993) but not the amine salt. Exposure to the n-butyl ester of 2,4-D caused ataxia, which was attributable to the formation of n-butanol rather than the 2,4-D moiety (Schulze 1988, Schulze and Dougherty 1988a,b), and changes the levels of brain serotonin and 5-

hydroxyinolacetic acid (de Duffard et al. 1990b). Furthermore, the n-butyl ester of 2,4-D appears to form side chain metabolites that would not be formed by a 2,4-D salt (Schulze et al. 1985).

Kim et al. (1988) found that after intraperitoneal injection of 2,4-D, compound residues were detectable in brain tissue; moreover, the concentrations of 2,4-D residue in brain tissue were enhanced by 2,4-D pre-treatment (Kim et al. 1988). More recently, a pharmacokinetic model for the distribution of 2,4-D into the brain tissue of rats was developed by Kim et al. (1994, 1995); however, the model has not been extended to other species.

Table 3-6: Time-course of 2,4-D urinary excretion in humans following intravenous injection and dermal application (data from Feldmann and Maibach 1974).

3.1.7. Effects on the Skin and Eyes. 2,4-D is an organic acid. Like all organic acids, 2,4-D in aqueous solution can be highly irritating to the eyes and can also cause skin irritation. Exposure to powdered 2,4-D acid is much less irritating (Mullison 1981), probably because the irritant effect of 2,4-D solutions is attributable to pH.

More unusual, however, is the potential for an effect on the eyes through systemic absorption. In a 2-year feeding study in rats, degenerative changes in eyes were noted at a dose of 150 mg/kg/day (Jeffries et al. 1995). This effect was not observed at dose levels of 5 or 75 mg/kg/day and is not reported in other subchronic or chronic studies.

3.1.8. Systemic Toxic Effects from Dermal Exposure. There are two key studies regarding the dermal absorption of 2,4-D, Feldmann and Maibach (1974) and Moody et al. (1990). The Feldmann and Maibach (1974) study is useful for estimating key kinetic parameters for 2,4-D acid, while the Moody et al. (1990) study is useful for evaluating the dermal absorption of 2,4-D esters, relative to 2,4-D acid or salts.

As summarized in Table 3-6, Feldmann and Maibach (1974) assayed the urinary excretion of ¹⁴C-labeled 2,4-D acid. 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

Period After Dosing (hours)		Elimination in the Urine as Proportion of Applied or Injected Dose/hour	
Start	End	I.V.	Dermal
0	4	0.03001	0.00009
4	8	0.04003	0.00012
8	12	0.05312	0.00020
12	24	0.01728	0.00029
24	48	0.00737	0.00068
48	72	0.00275	0.00082
72	96	0.00153	0.00042
96	120	0.00097	0.00027
Total:		1.000	0.058
SD:		0.025	0.024
Half-life (h):		13	NR

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 <15 seconds. The total applied dose of radioactivity ranged from 1 to 5 μCi . The 2,4-D was applied at a rate of 4 $\mu\text{g}/\text{cm}^2$ 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.

The urinary excretion rates for each of the collection periods are illustrated in Figure 3-3 (intravenous exposure) and Figure 3-4 (dermal exposure). In Figures 3-3 and 3-4, the excretion rates are illustrated both as bar graphs, covering the period of collection, as well as points, giving the absorption rate at the mid-point of the collection period. Given the experimental design, these rates were probably estimated by measuring the concentrations of 2,4-D in urine of each individual over each collection interval. This concentration multiplied by the volume of urine was probably used to estimate the amount excreted during the collection period. This amount divided by the duration of the collection period in hours would give a rate in units of amount/hour. This rate divided by the total administered dose would give the rates in units of proportion of applied dose/hour that are illustrated in Figure 3-3 and Figure 3-4.

As summarized in Table 3-6, results for each collection period are reported as mean elimination rates in units of proportion of

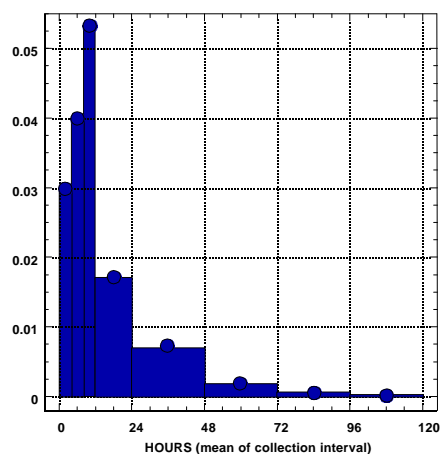


Figure 3-3: Urinary excretion of 2,4-D after intravenous injection (data from Feldmann and Maibach 1974).

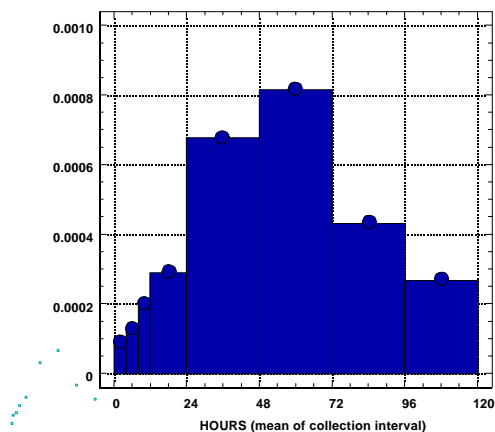


Figure 3-4: Urinary excretion of 2,4-D after dermal application (data from Feldmann and Maibach 1974).

total applied dose/hour. In addition, the cumulative excretion of the 5-day collection period is given as the mean and standard deviation in units of the proportion of the applied dose. The cumulative excretion is equivalent to the rate (proportion/hour) during each collection period multiplied by the duration of each period.

For the intravenous study, Feldmann and Maibach (1974) also report a calculated half-time of 13 hours from a semi-log plot of mean values (Figure 3-3).

Although Feldmann and Maibach (1974) do not report absorption data for each volunteer, they do provide standard deviations for the total amount excreted in both the intravenous and dermal studies (see Table 3-6). As indicated in Table 3-6, 100% of the administered intravenous dose of 2,4-D was recovered in the urine over the 5-day collection period, and the coefficient of variation was only 0.025. For the dermal applications, only 0.058 of the administered dose was recovered over the 5-day period, and the coefficient of variation was about 0.4,

$$0.024/0.058=0.0414$$

which is substantially higher than that noted for the intravenous study. Feldmann and Maibach (1974) discuss the variability of 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).

Based on the differences in the coefficients of variation between the intravenous and dermal studies, the variability in the dermal exposure study is substantially greater than the variability associated with the intravenous exposure 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 3-7. For each of the 2,4-D compounds tested, this table gives the percent recovery of the compound in the urine and the $t_{1/2}$ reported by the investigators.

Moody et al. (1990) 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

Table 3-7. 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	Parameter ^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%.

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 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 discussed in section 2, 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.

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

(see Table 3-7), 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 (Moody et al. 1992). 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.

The dermal absorption of 2,4-D is likely to decrease dramatically when the compound is present in a soil matrix. Dermal absorption of 2,4-D from contaminated soil was estimated at 0.0018-0.0164 day⁻¹ based on *in vitro* measurements using abdominal skin from human cadavers (Duff and Kissel 1996). In addition, Wester et al. (1996) recorded an 8-hour time lag between exposure to soil contaminated with 2,4-D and dermal absorption, using *in vitro* human skin preparations.

The Feldmann and Maibach (1974) study does not provide a kinetic analysis of the 2,4-D dermal absorption rate; however, it does provide sufficient information to allow for approximations of k_a : specifically, average urinary excretion rates expressed as a proportion of applied dose over various time periods after exposure (see Table 3-6).

Two methods can be used to estimate a first-order absorption coefficient for 2,4-D from the Feldmann and Maibach (1974) study. The simplest method is based on the 'flip flop' principle (O'Flaherty 1981). Using this method, the natural log of the proportion of unexcreted dose is plotted against time (Figure 3-5) and the slope of the line is determined.

Using this method and constraining the proportion of the unexcreted dose to be unity [$\ln(1)=0$] at time zero, the estimated k_a is 0.000515 hour⁻¹ (Figure 3-5). While a sigmoidal deviation from the best estimate of the line is apparent, this simple model fits the data relatively well ($r^2=0.98$, $p<0.0001$). The estimate of k_a does not change markedly, 0.000565 hour⁻¹, if the model is unconstrained ($r^2=0.99$, $p<0.00001$).

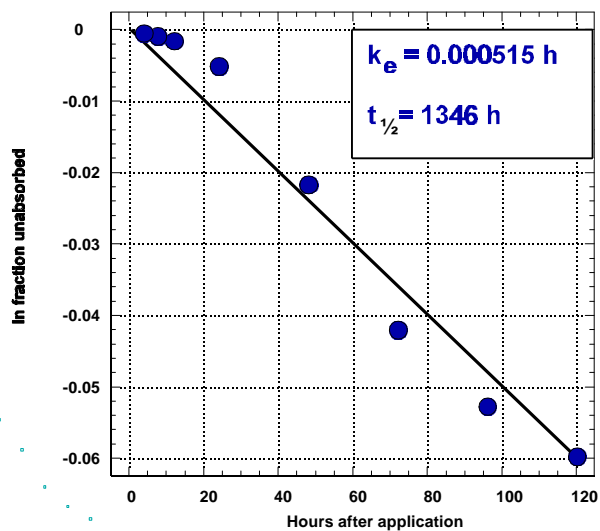


Figure 3-5: The kinetics of the cumulative excretion of 2,4-D acid after dermal application to humans (data from Feldmann and Maibach 1974).

Although the above analysis is based on urinary excretion data, the negative of the slope of cumulative elimination can be taken as the absorption rate coefficient because the intravenous study conducted by Feldmann and Maibach (1974) demonstrates that the excretion rate coefficient ($k_e \approx 0.05 \text{ hour}^{-1}$) is about a factor of one-hundredth higher than the apparent excretion rate after dermal exposure (i.e., the 'flip flop' principle) (Gibaldi and Perrier 1982). In other words, the rate of absorption is the rate limiting step in the rate of excretion.

A more complex method involves estimating the absorption rate directly from the excretion rate data (see Table 3-6, column four). Because the data in Table 3-6 are expressed as average rates of excretion as proportion of administered dose/hour ($k_e X$) rather than the amount excreted (X), the form of the equation used in this analysis is:

$$\text{Excretion Rate} = k_e X = k_e \left(\frac{k_a A_0}{k_e - k_a} \right) (e^{-k_a t} - e^{-k_e t}).$$

For this non-linear equation, the SOLVER function in EXCEL was used to estimate k_a . For each collection interval, t was taken as the average of the start and end times of the collection interval, and the sum of the square (SSQ) of the difference between the model estimates and observed rates was minimized. Both the quasi-Newton and gradient search methods were used, and other estimation factors (e.g., starting estimates of k_e , error tolerance, precision, the use of tangent versus quadratic extrapolation, and the use of forward versus central derivatives) were varied to avoid false minima. The only constraint applied to the model is that the first-order coefficient, k_a , must be greater than or equal to zero. As illustrated in Figure 3-6, the estimated k_a , $0.00058 \text{ hour}^{-1}$, is almost the same as the rate estimated from the cumulative elimination data (see Figure 3-5).

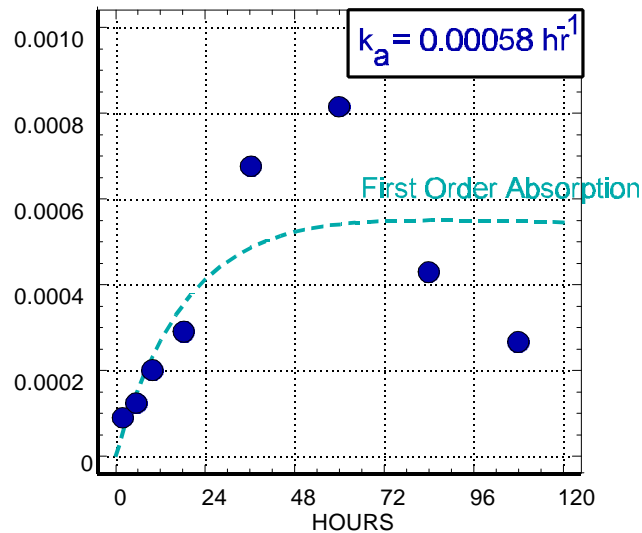


Figure 3-6: Estimates of a first-order rate constant for 2,4-D acid after dermal application in humans based on excretion rates in the urine (data from Feldmann and Maibach, 1974).

For this risk assessment, the central estimate of the dermal absorption rate coefficient will be taken as 0.0005 hour^{-1} with a range of $0.00017 \text{ hour}^{-1}$ to 0.0026 hour^{-1} . This corresponds to rates of 0.012 day^{-1} , with a range of 0.004 day^{-1} to 0.062 day^{-1} . The central estimate is based on the above absorption rates using the simple assumption of first-order absorption with first-order elimination from the Feldmann and Maibach (1974) study.

The upper limit of the range is based on the range of absorption rates for 2,4-D in humans and experimental mammals (see Table 3-7). The upper limit is about 5 times higher than the central estimate, which is more conservative than the recommendation by Feldmann and Maibach (1974) that a factor of 3 is adequate as an approximate 95% upper limit. The more conservative approach is taken for the upper limit because the study by Feldmann and Maibach (1974) does not provide a discussion about how the factor of 3 was calculated. The factor of 5 used in this risk assessment is based on the general variability in absorption estimates (Durkin et al. 1995, U.S. EPA 1992).

The lower limit on the range of dermal absorption rates is based on the estimate of inter-individual variability regarding pesticide absorption in humans, which is provided by Feldmann and Maibach (1974). Using a small factor for the lower limit is, perhaps, too conservative, however, it does not have a substantial impact on the risk characterization.

Figure 3-7 illustrates how these estimates encompass the observations from the study by Feldmann and Maibach (1974). In Figure 3-7, the data points from the human study are plotted along with the central estimate (solid line) and upper and lower limits (dashed lines) based on the absorption rate coefficients of 0.0005 (0.00017 - 0.0026) hour^{-1} and an elimination rate coefficient of 0.0535 hour^{-1} taken from the intravenous study by Feldmann and Maibach (1974). As shown in Figure 3-7, the central estimate of the absorption rate coefficient provides a reasonable fit to the observed values during the first 24 hours, and the upper limit encompasses rates in the day 2 and day 3 collection periods by a substantial margin (i.e., about a factor of 2). Thus, the central estimate should provide a plausible basis for estimating typical risks, and the upper range will provide a conservative (but not too conservative) 'worse' case estimate.

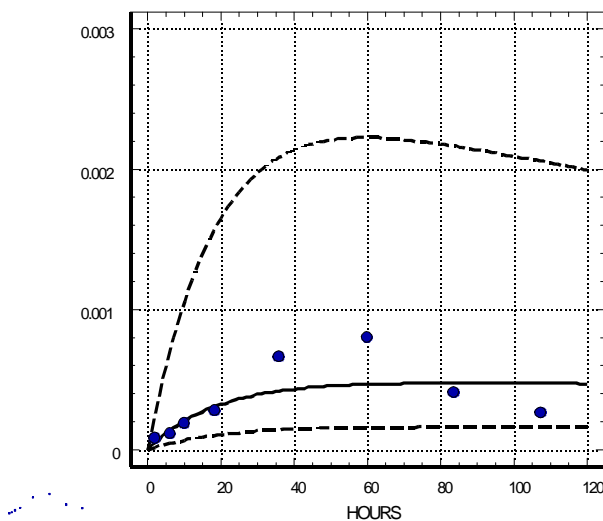


Figure 3-7: Comparison of absorption rate coefficients used in risk assessment to observations from Feldmann and Maibach (1974).

As discussed in Durkin et al. (1995), scenarios that use Fick's first law require an estimate of the permeability coefficient, K_p , expressed in cm/hour. Experimentally determined permeability coefficients for 2,4-D acid, salts, and esters are not available in the literature. Based on the methods recommended by U.S. EPA (1992), estimates of the dermal permeability of 2,4-D with 95% confidence intervals are 0.0000242 (0.0000102-0.0000575) cm/hour (worksheet 10).

The U.S. EPA (1992) method is based on measured K_p values for 95 organic compounds with log K_{ow} values ranging from approximately -2.5 to 5.5 and molecular weights ranging from about 30 to 770. As summarized in Table 2-3, 2,4-D acid has a molecular weight of 221 and a log K_{ow} of about -0.75 at a pH of 7 (neutral pH) and a K_{ow} of 2.87 at a pH of 1. Given its molecular weight of 221, 2,4-D is within the range in which the empirical relationship of molecular size and lipophilicity to dermal permeability is approximately linear. Thus, it is reasonable to use the method recommended by U.S. EPA (1992) to estimate the K_p for 2,4-D.

When applying this method to 2,4-D acid, selecting the appropriate K_{ow} is not necessarily intuitive. At a neutral pH, 2,4-D acid, which has a pK_a of 2.87 (see Table 2-3), will exist predominately in the ionized form, which is relatively polar and insoluble in organic solvents, such as octanol. A far lesser amount will exist in the unionized form, which is much more polar than the ionized form. Thus, the K_{ow} at pH 1 is about 4000 times greater than the K_{ow} at pH 7

$$10^{2.87} \div 10^{-0.75} \approx 4169.$$

For exposure scenarios involving immersion with the direct use of K_p values and Fick's first law, the vehicles will usually have a pH that is closer to neutral (pH 7), rather than highly acidic (pH 1). Thus, the estimate of K_p detailed in worksheet 10 is based on the K_{ow} at pH 7

In deposition scenarios, K_p values are not used directly. Nonetheless, estimates of K_p might be useful in comparing apparent absorption rates for related compounds, such as 2,4-D amine and 2,4-D isooctyl ester. For deposition scenarios, however, the selection of a K_p for 2,4-D acid or salts is somewhat less clear. If a solution of 2,4-D amine or salt is spilled onto the skin, the liquid will evaporate leaving behind crystals or amorphous particles of 2,4-D combined with whatever cations are available either from the solution/formulation or from cations or other positively charged biomolecules on the skin surface. The absorption of any crystal or particle should be negligible. Nonetheless, 2,4-D crystal/particles will be absorbed, and the absorption process will probably involve an initial re-solubilization into body water followed by partitioning of the 2,4-D from an aqueous phase into body lipids. Body fluids tend to be at approximately neutral pH. Hence, for deposition scenarios involving exposure to 2,4-D acid or its salts, it seems appropriate to use the K_p of 0.0000242 cm/hour based on the K_{ow} at pH 7, as derived in worksheet 10.

The isooctyl ester of 2,4-D has a molecular weight of 333.3 and a log K_{ow} of about 6.73. This K_{ow} is about a factor of 17 greater than the highest K_{ow} used to develop the U.S. EPA (1992) method. Because of the relatively high molecular weight and lipophilicity of 2,4-D isooctyl ester, K_p values estimated using the methods detailed in worksheet 10 may not be reliable.

For example, using the equation above, the estimated K_p for 2,4-D isooctyl ester is about 1.0 cm/hour:

$$\begin{aligned}\log K_p &= -2.72 + (0.71 \cdot 6.73) - (0.0061 \cdot 333.3) \\ &= 0.02517 \\ K_p &= 10^{0.02517} = 1.059\end{aligned}$$

or more than 40,000 times that of 2,4-D acid at neutral pH. Using the upper limit of -1.5 for the $\log K_p$ of compounds with a molecular weight of more than 150 and a K_{ow} of more than 3.5 (U.S. EPA 1992), a more reasonable estimate of the K_p for the isooctyl ester would be about 0.032 cm/hour ($10^{-1.5}$), which is still about 1300 times greater than the K_p of 2,4-D acid at neutral pH:
 $0.032 \text{ cm/hour} \div 0.000025 \text{ cm/hour} = 1280.$

Commercial formulations of 2,4-D esters may contain 2,4-D at concentrations of up to 5.6 lbs a.e./gallon or 670 g a.e./L (see Table 2-3). Commercial formulations of 2,4-D esters contain carriers, the identity of which is considered proprietary information. Clearly, however, these formulations must contain solvents and/or emulsifiers. For example, Esteron LV96 contains kerosene and proprietary emulsifiers (Moody et al. 1990), and it is likely that other commercial formulations of 2,4-D esters contain similar materials.

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.

Similarly, the available data do not suggest a consistent difference between the dermal absorption of 2,4-D acid and its esters. Because the reported differences in absorption rates among species, sites, and forms of 2,4-D are not consistent (Moody et al. 1990, 1992) (see Table 3-7), no attempt is made to account for such differences explicitly in this risk assessment. Nevertheless, these uncertainties are implicit in the nearly 16-fold difference between the upper and lower range of the absorption rate coefficients used for 2,4-D.

3.1.9. Inhalation Exposure. Compared with oral exposure data, data regarding the inhalation toxicity of 2,4-D are extremely limited. Estimates of inhalation LC_{50} values are not available. As part of the re-registration effort for 2,4-D, an acute whole body inhalation study was conducted in which rats (five of each sex) were exposed to 2.15 mg 2,4-D/L (2150 mg/m^3) (Douds 1995, MRID 43611401). Two of the 10 rats died. [Two Russian studies regarding the inhalation toxicity of the octyl ester of 2,4-D are discussed in the previously prepared EIS, p. B-13.]

ACGIH (1993) adopted a TLV of 10 mg/m³. 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:

By interpretation from the animal feeding studies and use experience, the TLV Committee has recommended a TLV-TWA of 10 mg/m³ since 1954 (ACGIH 1993, p 337).

This TLV is, nonetheless, identical to analogous values recommended by other federal agencies and other nations (ACGIH 1993).

3.1.10. Impurities, Adjuvants, and Metabolites.

3.1.10.1. 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-dichlorophenoxyacetic 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 concern regarding the use of 2,4-D is the possibility of contamination with chlorinated dioxins. Agent Orange, which is a mixture of 2,4-D and 2,4,5-T, was used during the Vietnam war, and it did contain 2,3,7,8-TCDD. Moreover, commercial formulations of 2,4,5-T have been known to contain 2,3,7,8-TCDD. 2,4-D formulations, however, were shown not to contain 2,3,7,8-TCDD (Cochrane et al. 1981, Hansen et al. 1971). Thus, it appears that 2,3,7,8-TCDD contamination in commercial preparations of herbicides containing 2,4-D is attributable to the contamination of 2,4,5-T with 2,3,7,8-TCDD rather than the presence of 2,3,7,8-TCDD in 2,4-D.

Some commercial samples of 2,4-D amine have been shown to contain polychlorinated dibenzo-p-dioxins, chiefly di-, tri-, and non-2,3,7,8-tetrachlorinated isomers. Most samples contained <1 µg/L, and the highest concentration of any dioxin in the samples of 2,4-D amine was 0.32 mg/L. All but one of the 2,4-D esters, however, were found to contain dioxin residues, and the levels were much higher, ranging from 0.1 to 23 mg/L (Cochrane et al. 1981). Additional studies regarding dioxin contamination in 2,4-D commercial samples are not available in the more recent literature.

3.1.10.2. Nitrosamines -- Some early samples of commercial formulations of 2,4-D were shown to contain N-nitrosodimethylamine (DMA) at levels <1-5 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.

DMA has also been found in mixtures of 2,4-D amine and fertilizers at levels <0.05-0.25 ppm and attributed to the contamination of the 2,4-D amine salt with DMA (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).

3.1.10.3. Metabolites -- 2,4-D does not appear to be metabolized extensively in mammals; however, the compound 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 has been 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 about 3 times lower than the U.S. EPA RfD for 2,4-D. 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.

3.2. EXPOSURE ASSESSMENT

3.2.1. Overview. The relatively rich database on occupational exposure to 2,4-D indicates that respiratory exposure is negligible, compared with dermal exposure. Because 2,4-D is eliminated unchanged and almost completely in the urine, the absorbed dose of 2,4-D can be estimated from urinary excretion data and related to the amount of 2,4-D handled during a particular application. Thus, occupational exposure rates are expressed in units of mg agent/kg bw · lb agent handled.

For directed ground (backpack) applications, broadcast ground applications, and aerial applications, exposure rates are taken from a relatively detailed review of studies on occupational exposure rates (Rubin et al. 1998). Based on this analysis, central estimates of exposure, expressed as absorbed dose, fall within a relatively narrow range: 0.013-0.022 mg/kg/day. The upper limits of projected exposure are also within a narrow range: 0.08-0.15 mg/kg/day. All of these estimates are based on application rates of 1 lb a.e./acre as well as estimates of how many acres a worker might treat in 1 day.

One formulation of 2,4-D, Aqua-Kleen, is registered for direct application of surface waters to control undesirable vegetation. No occupational exposure studies are available on this formulation. Based on an occupational exposure study involving the aquatic application of a liquid formulation of 2,4-D, the central estimate of absorbed dose for workers is 0.017 mg/kg/day, with an upper limit of 0.038 mg/kg/day. Again, these are similar to the estimates for other worker groups. A major difference, however, is that the estimates for aquatic applications are based on the treatment of only 1 acre at a typical application rate of 19 lbs/acre. This approach is taken because no estimates are available on the number of acres that workers might treat in a single day.

The consequences of accidental worker exposure may vary significantly, depending on the nature of the event and the duration of exposure. Typically, estimates of absorbed dose in workers after accidental exposure to 2,4-D are close to the estimates of absorbed dose in workers handling 2,4-D.

Under normal conditions, members of the general public should not be exposed to substantial levels of 2,4-D. 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 highly conservative scenarios are developed for this risk assessment.

The most plausible exposure scenario for the general public involves walking through a contaminated area shortly after it is sprayed with 2,4-D. Estimates of absorbed dose for this scenario are extremely low, ranging from approximately 0.00042 to 0.0066 mg/kg/day. These estimates are consistent with the lower limits of estimated doses for workers involved in the application of 2,4-D.

A somewhat less likely but plausible exposure scenario involves the consumption of inadvertently contaminated vegetation. Both acute and subchronic exposure scenarios are derived for this scenario. The acute scenario leads to estimates that fall within the range of estimated exposure rates for workers involved in ground applications of 2,4-D. The longer term scenario leads to substantially lower estimates of exposure.

Given the limited nature of most 2,4-D applications (i.e., relatively small treatment areas), general contamination of groundwater seems unlikely. Nonetheless, under the very conservative assumption that an entire watershed is treated, exposure levels will be relatively low, about 0.00002-0.00014 mg/kg/day. Based on accidental spill scenarios, estimates of acute exposure (i.e., 1 day) from the consumption of contaminated water are substantially higher, approximately 0.17-1.3 mg/kg. These acute exposures, however, are dominated by rather arbitrary exposure assumptions concerning the amount of 2,4-D spilled and the size of the body of water into which the compound is spilled.

Both acute and longer term exposure scenarios are also developed for the consumption of contaminated fish. These estimates are dependent on the concentration of 2,4-D in the water, the bioconcentration of 2,4-D by fish, and the amount of fish consumed. The concentrations of 2,4-D in water used for this assessment are identical to those used for the drinking water exposure scenario. The estimate of the bioconcentration of 2,4-D by fish is based on an experimental determination that is probably conservative. There is information pertaining to the amount of fish consumed by the general population as well as subsistence populations (i.e., individuals who may catch and consume fish as a major source of protein in their diet). Separate exposure assessments are conducted for these two groups. As with the longer term exposure scenarios for the consumption of contaminated water, estimates of longer term exposures to 2,4-D in fish are very low for both groups of people (i.e., 0.0000036-0.0011 mg/kg/day). Acute (1-day) exposure

based on an accidental spill scenario leads to substantially higher estimates (i.e., in the range of 0.2-3.1 mg/kg). Again, the spill scenario is dominated by arbitrary variability.

3.2.2. Workers.

A summary of the occupational exposure estimates for workers are given in Table 3-8. Two types of exposure assessments are provided: those based on total exposure attributed to handling differing amounts of 2,4-D by different application methods as well as various accidental exposure scenarios. Each type of exposure assessment is detailed in the following subsections.

Plausible levels of exposure for workers involved in backpack, hydraulic rig, and aerial applications are estimated as the product of the typical application rate of 2,4-D used by the Forest Service (1 lb a.e./acre), the area treated per hour (acres treated/hour by a worker), and the exposure rate (mg/kg bw/lb a.e.). For these workers, the amount of herbicide handled per day is the product of the application rates, and the number of acres treated per hour, assuming an 8-hour exposure duration in the workday. As noted in section 2, the Forest Service may vary the application rate by a factor of 2 (i.e., 0.5-2.0 lbs a.e./acre). Given the high degree of variability in the estimated rates for occupational exposure, the relatively modest variations in application rates do have a profound effect on this risk assessment. The consequences of variations in the application rates are considered further in the risk characterization (section 3.4). Regarding aquatic applications, 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 an application rate of 19 lbs. a.e./acre. Variations from these assumptions are also considered in the risk characterization (section 3.4.).

3.2.2.1. Job Categories -- As outlined in the program description (see section 2), this risk assessment is concerned primarily with backpack and boom spray ground applications. Although aerial applications are possible, they are not currently being conducted by the Forest Service. Similarly, direct aquatic applications may occasionally be conducted but they are uncommon.

As discussed in Rubin et al. (1998), occupational exposure to pesticides usually occurs when a chemical agent is inhaled by a worker or comes into contact with the skin surface of the worker (i.e., dermal exposure). Generally, occupational exposure to the pesticide is more likely to involve dermal exposure. Specifically with respect to 2,4-D, inhalation exposure during ground applications is negligible, compared with dermal exposure (Abbott et al. 1987). For this exposure assessment, the absorbed dose of 2,4-D is estimated as a function of the amount of material handled and exposure factors specific to 2,4-D. The absorbed dose is expressed in units of mg agent/kg bw · lb agent handled.

The worksheets included with this risk assessment detail the exposure assessments for four types of applications: ground directed foliar/backpack (worksheet 12a), ground broadcast/boom spray (worksheet 12b), aerial broadcast (worksheet 12c), and direct aquatic (worksheet 12d and 12e). The exposure assessments of backpack, boom spray, and aerial are all based on the typical application rate of 1 lb a.e./acre as well as estimates of how many acres a worker could be involved in treating in a single day. Variability is encompassed by a range of occupational

exposure rates (absorbed dose in units of mg/kg bw per lb a.e. of 2,4-D that is handled) and estimates of the variability in the numbers of acres that might be treated in a single day. Differences in the estimates of absorbed dose will be related directly to differences in application rates. This variability is considered in the risk characterization (section 3.4).

As discussed in section 2, Aqua-Kleen, which is a granular formulation of 2,4-D can be applied at relatively high rates (i.e., 19-38 lbs a.e./acre) 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. As discussed by the investigators in the study, 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 lead to lesser 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 the study by Nigg and Stamper (1983), absorbed doses were assayed as total urinary elimination of 2,4-D over a 24-hour period in four workers applying a liquid formulation of 2,4-D amine for the control of water hyacinths using airboat handguns. As detailed in worksheet 12d, occupational exposure rates can be estimated at 9×10^{-4} (4×10^{-4} to 2×10^{-3}) mg/kg bw per lb a.e. handled. These rates are between those from Rubin et al. (1998) for backpack workers (worksheet 03a) and workers involved in hydraulic ground broadcast applications (worksheet 03b). 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) as detailed in worksheet 12d.

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

Durkin et al. (1995), scenarios that use Fick's first law require an estimate of the permeability coefficient, K_p , expressed in cm/hour.

While the immersion of hands may not be a plausible scenario in some respects, it may approximate worst case exposure scenarios involving contaminated gloves. As discussed by Moody and Nadeau (1994), protective gloves (nitrile butyl rubber) may act as a reservoir for 2,4-D if the 2,4-D is applied in an organic solvent. This effect, however, is much less evident for commercial formulations of 2,4-D amine.

The literature does not provide experimentally determined K_p values for 2,4-D acid, salts, or esters. Nonetheless, a K_p of $2.42 \cdot 10^{-5}$ cm/hour for an aqueous solution of 2,4-D acid can be estimated (worksheet 10), based on structure-activity relationships (U.S. EPA 1992). As discussed in section 3.1.8, this K_p is applied to aqueous formulations of both the amine salt and ester formulations.

Commercial formulations of 2,4-D amine usually contain 2,4-D at levels of 3.8 lbs a.e./gallon or 455 g a.e./L (see Table 2-1 and worksheet 08), which is somewhat greater than the reported water solubility of 2,4-D amine, which is approximately 300 g/L (see Table 2-3 and worksheet 08). It is unclear whether the commercial formulations of 2,4-D are supersaturated solutions, contain undissolved 2,4-D crystals, or whether adjuvants are used to increase the solubility of 2,4-D in the commercial solution. For this risk assessment, the nominal concentration of 455 g a.e./L or 455 mg/cm³ is used as a conservative approximation for concentrated solutions of 2,4-D amine.

During the handling process, a worker may, either through mischance or imprudent handling, immerse part of the body into the pesticide formulation for a short time. The worst case scenario involves placing both hands in the concentrated formulation of 2,4-D amine (455 g a.e./L) or a 2,4-D ester (670 g a.e./L). Details of these exposure assessments are presented in worksheets 13a and 13b.

Very little confidence should be placed in either of these estimates. As noted above and discussed more fully in section 3.1.8, structure activity relationships suggest that the K_p for 2,4-D esters should be much higher than the K_p for 2,4-D amine. This presumed relationship, however, is contrary to the available data regarding the *in vivo* dermal absorption of 2,4-D in humans and experimental mammals. Thus, the absorbed dose for the 2,4-D ester is estimated using the K_p for 2,4-D amine.

An additional source of uncertainty in estimating absorption rates for the 2,4-D ester concerns the potential influence of carriers on dermal absorption. In general, the partitioning of a lipophilic chemical from an aqueous solution into the skin is favored by the lipid content of the skin (i.e., lipophilic chemicals, by definition, will partition into a lipid rich media). Thus, carriers that functionally increase the solubility of a compound in solution will tend to reduce the partitioning process. Conversely, carriers may directly influence skin permeability to favor an increase in the

absorption rate. Although these competing processes can be characterized qualitatively, their influence on the dermal absorption of 2,4-D cannot be quantified.

Estimated doses for immersion of other areas of the body and for other exposure durations can be calculated in a manner similar to the examples given in worksheets 13a or 13b.

3.2.2.3. Accidental Spills -- First-order absorption rate coefficients are estimated for exposure scenarios involving 2,4-D spilled on the lower legs or hand, as detailed in worksheet 14a for amine formulations of 2,4-D and worksheet 14b for ester formulations of 2,4-D.

These scenarios involving spills onto the hands with a 1-hour exposure period can be compared with the immersion scenario discussed in the previous section. As summarized in Table 3-8, the central estimates for these two scenarios indicate greater absorption for the contaminated gloves scenario than the scenario for a spill onto the hands. Intuitively, this supposition seems reasonable because a scenario that is functionally equivalent to immersion (i.e., contaminated gloves) seems to offer greater potential exposure than a simple spill onto the hands. Hence, the higher estimate from the contaminated gloves scenario seems reasonable.

3.2.3. General Public.

3.2.3.2. General Considerations– Under normal conditions, members of the general public should not be exposed to substantial levels of 2,4-D because members of the general public are not normally in an area when 2,4-D is being applied. Nonetheless, any number of exposure scenarios can be constructed for the general public, depending on various assumptions regarding application rates, dispersion, and human activity. Several highly conservative scenarios are developed for this risk assessment and are summarized in Table 3-9. As discussed below, most of these scenarios should be regarded as extreme, some to the point of limited plausibility. The general methods and assumptions used to develop the scenarios are identical to those used for the other herbicides addressed in this risk assessment (see section 3.2.3.1).

3.2.3.2. Direct Spray– It is assumed that during a broadcast ground or aerial application, a naked child is sprayed directly with 2,4-D. These scenarios also assume that the child is completely covered (i.e., 100% of the surface area of the body is exposed) with either an amine (worksheet 15a) or ester (worksheet 15b) formulation. These extremely conservative exposure scenarios are likely to represent upper limits of plausible exposures. An additional set of scenarios are included involving a young woman who is accidentally sprayed over the feet and legs with either an amine (worksheet 16a) or ester (worksheet 16b) formulation. As discussed in section 3.1.8, first- and zero-order dermal absorption rates are based on 2,4-D acid. Thus, differences in the exposure assessments for the acid and ester formulations are attributable solely to differences in the

anticipated concentrations in the field solutions (i.e., the commercial formulation diluted/mixed prior to application).

Table 3-8. Summary of worker exposure scenarios

Scenario	Dose (mg/kg/day or event)			Worksheet
	Typical	Lower	Upper	
General Exposures (dose in mg/kg/day) [acid or ester formulations]¹				
Directed ground spray (Backpack)	1.3e-02	4.5e-04	8.0e-02	12a
Broadcast ground spray (Boom spray)	2.2e-02	6.6e-04	1.5e-01	12b
Aerial Applications	1.5e-02	2.4e-04	8.0e-02	12c
Aquatic Applications ^a	1.7e-02	7.6e-03	3.8e-02	12e
Accidental/Incidental Exposures (dose in mg/kg/event)				
Acid				
Immersion of Hands, 1 minute	2.2e-03	9.2e-04	5.3e-03	13a
Contaminated Gloves, 1 hour	1.3e-01	5.5e-02	3.2e-01	13a
Spill on lower legs, 1 hour	5.4e-02	1.8e-02	2.8e-01	14a
Spill on hands, 1 hour	2.2e-02	7.4e-03	1.1e-01	14a
Ester				
Immersion of Hands, 1 minute	3.2e-03	1.4e-03	7.8e-03	13a
Contaminated Gloves, 1 hour	1.9e-01	8.2e-02	4.7e-01	13a
Spill on lower legs, 1 hour	7.9e-02	2.7e-02	4.1e-01	14a
Spill on hands, 1 hour	3.2e-02	1.1e-02	1.7e-01	14a

¹ Based on an application rate of 1 lb a.e./acre unless otherwise specified.

^a Based on treating 1 acre at 19 lbs a.e./acre.

Table 3-9: Summary of Exposure Scenarios for the General Public.

Scenario	Target	Dose (mg/kg/day)			Worksheet
		Typical	Lower	Upper	
Acute/Accidental Exposures					
Direct spray, acid formulation , entire body	Child	0.01086	0.00074	0.226	15a
Direct spray, ester formulation , entire body	Child	0.01358	0.002954	0.141	15b
Direct spray, acid formulation , lower legs	Woman	0.0011	0.000074	0.023	16a
Direct spray, ester formulation , lower legs	Woman	0.0014	0.0003	0.014	16b
Dermal, contaminated vegetation	Woman	0.0013	0.00043	0.0066	17
Contaminated fruit, acute exposure	Woman	0.035	0.0071	0.21	18
Contaminated water, acute exposure	Child	0.426	0.1663	1.28	20
Consumption of fish, general public	Man	0.32	0.2	0.64	22
Consumption of fish, subsistence populations	Man	1.6	1	3.1	22
Chronic/Longer Term Exposures					
Contaminated fruit	Woman	0.0023	0.00046	0.032	19
Consumption of water	Man	0.000057	0.00002	0.00014	21
Consumption of fish, general public	Man	0.000007	0.0000036	0.00023	23
Consumption of fish, subsistence populations	Man	0.000058	0.000029	0.0011	23

3.2.3.3. Dermal Exposure from Contaminated Vegetation -- In this exposure scenario, it is assumed that the herbicide is sprayed at a given application rate and that an individual comes in contact with sprayed vegetation or other contaminated surfaces at some period after the spray

operation. As discussed in Durkin et al. (1995), some estimate of dislodgeable residue of the herbicide must be available.

Immediately after the spray application, levels of exposure may approximate those involving contact with direct spray, as estimated above. Generally, after the liquid carrier dries, exposure levels are expected to decrease. For example, in a study by Harris and Solomon (1992), 2,4-D was applied to turf at a nominal rate of $11 \mu\text{g}/\text{cm}^2$. Immediately after the liquid carrier dried, the dislodgeable residue of 2,4-D was $0.92 \mu\text{g}/\text{cm}^2$, about 10 times less than the nominal rate. The relatively low dislodgeable residue may be largely but not solely attributable to rapid uptake of 2,4-D. As noted by Cessna (1993) for example, about 20% of deposited 2,4-D could be washed off wheat plants shortly after spraying.

At the typical application rate of 1 lb a.e./acre [$112.1 \text{ mg}/\text{m}^2$ or $11.21 \mu\text{g}/\text{cm}^2$] and using the factor of 0.1 to estimate dislodgeable residue (Harris and Solomon 1992), the dislodgeable residue immediately after the liquid carrier dries will be approximately $1.12 \mu\text{g}/\text{cm}^2$. According to the methods proposed by Durkin et al. (1995, equation 4, p. 68), the transfer rate would be about $1.27 \mu\text{g}/(\text{cm}^2 \cdot \text{hour})$, as detailed in worksheet 17.

This estimate of dislodgeable residue may then be used to estimate absorbed dose for the scenario based on a 1 hour exposure period in the contaminated vegetation and removal of the 2,4-D from the surface of the skin after 24 hours.

Since the estimated doses are based directly on measured uptake values for 2,4-D from contaminated turf as well as measured values for dermal absorption rates of 2,4-D in humans, they may be regarded with a relatively high level of confidence. The considerable range in the estimate of absorbed dose (i.e., about a factor of 15) reflects the statistical uncertainties in the estimated first-order absorption rate coefficient for 2,4-D, as detailed in section 3.1.8.

The potential differences between the amine salt and ester formulations of 2,4-D with respect to environmental degradation and transport kinetics may have an impact on the uncertainties in the exposure assessments for scenarios involving dermal exposure from contaminated vegetation as well as scenarios involving the consumption of contaminated water and contaminated vegetation. In general, amine salts are not expected to be absorbed by vegetation as rapidly as herbicide esters are expected to be absorbed. Furthermore, because the hydrolysis of 2,4-D esters is less rapid than the dissociation of 2,4-D salts (Johnson et al. 1995, Smith and Aubin 1991a,b, Wojtalik et al. 1971), there may be differences in dissipation from turf, soil, and water. Nonetheless, within a 24-hour period, the ester linkage should be cleaved either through hydrolysis, photosensitization, or microbial activity (Hoeppel and Westerdahl 1983).

There is, however, no documentation regarding systematic differences between the environmental transport of 2,4-D amine and 2,4-D esters. To the contrary, a recently published series of field studies regarding the fate of 2,4-D in soil finds no consistent or substantial differences between the soil dissipation kinetics of 2,4-D amine and the 2-ethylhexyl ester of 2,4-D (Wilson et al. 1997). It is reasonable to conclude, therefore, that differences in the environmental transport of

2,4-D salts and 2,4-D esters are potentially significant for very short-term exposure scenarios. And, as is the case for the dermal absorption of 2,4-D esters and amines, the differences cannot be quantified. For longer-term exposure scenarios, the differences are likely to be nonexistent—the salt will dissociate and the esters will hydrolyze, and both will form the free 2,4-D acid or ion.

3.2.3.4. Contaminated Water– Water can be contaminated from runoff, as a result of leaching from contaminated soil, from a direct spill, or from unintentional contamination from aerial applications. In addition, direct aquatic applications of 2,4-D will obviously result in ambient surface water contamination. Although 2,4-D is chemically stable in pure aqueous solutions, it is degraded in natural waters by microbial activity, and water levels are further reduced by dispersal.

The concentration of 2,4-D in ambient water after a chemical spill or other accident will vary with the amount of the chemical released, the proximity of the spill to water, and the physical characteristics of the body of water into which the spill occurs.

As an extreme scenario, it is assumed that 200 gallons of a 2,4-D solution at a typical concentration of 7490 mg/L and a range of concentrations of 4790-14,980 mg/L is released into a shallow pond that has an average depth of 1 m and a surface area of about one-quarter of an acre (1000 m²). The concentrations of 2,4-D in the spill solution represent the likely concentrations in a field solution (i.e., the recommended dilutions of the ester formulation as detailed in worksheet 08).

The volume of water in the pond is about 1000 m³ or 1,000,000 L. Assuming complete and instantaneous mixing, the *typical* concentration of 2,4-D in the water will be about 6 mg/L,

$$7490 \text{ mg/L} \times 3.785 \text{ L/gal} \times 200 \text{ gal} \div 1,000,000 \text{ L} = 5.6699 \text{ mg/L}$$

with a range, similarly calculated, of 3.6-11 mg/L.

This range is somewhat above the range of maximum observed concentrations of 2,4-D in water, 0.68-3.6 mg/L, noted by Hoeppe and Westerdahl (1983) after the application of 2,4-D dimethylamine and a granular formulation of 2,4-D butoxyethyl ester, Aqua-Kleen, at very high rates (i.e., 20-40 lbs a.e./acre) in a lake with a depth of 1-2 m.

The data from the Hoeppe and Westerdahl (1983) study are illustrated in Figure 3-8 [adapted from Table 1, p. 200, in Hoeppe and Westerdahl (1983)]. In Figure 5-8, the concentrations are normalized for application rates of 22.5 and 45 kg a.e./ha, equivalent to about 20 and 40 lbs a.e./acre, and these concentrations are plotted against time in days after application.

Although kinetic data are limited, they suggest that the initial levels of the 2,4-D ester formulation were less, compared with 2,4-D amine, and dissipated more slowly. This apparent relationship may have little to do with differences between the acid and ester form of the 2,4-D and more to do with the granular nature of the 2,4-D ester formulation. The granular formulation probably resulted in a slower release of the 2,4-D with a consequent increase in apparent persistence. Wilson et al. (1977) reported a similar pattern in differences between the soil residues of 2,4-D after the application of amine and granular ester formulations.

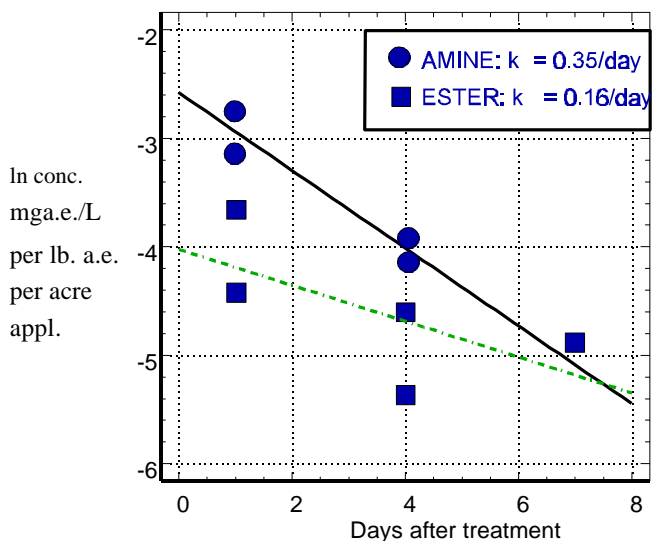


Figure 3-8: The dissipation of 2,4-D from water after the application of 2,4-D amine or 2,4-D butoxyethanol ester (data from Hoeppe and Westerdahl 1983).

In the spill scenario described above, the functional 'treatment rate' is 2 lbs. a.e./0.25 acre pond or about 8 lbs. a.e./acre. Thus, in terms of 2,4-D treatment rates, Hoeppe and Westerdahl (1983) monitored less 2,4-D in the water than might be expected from the instantaneous mixing scenario described above. Although the difference in concentration might be the result of 2,4-D binding to sediments, it also might be the result of dissipation, since Hoeppe and Westerdahl (1983) did not take the first measurement until 1 day after treatment. Similarly, in a 2.13 m deep lagoon treated with 2,4-D amine at 4.48 kg a.i./ha (4 lbs a.i./acre or about 2.9 lbs a.e./acre), initial water levels were about 0.083 mg/L (Averitt and Gangstad 1976). An application rate of 2.9 lbs a.e./acre corresponds to about 325 mg/m². Thus, at a mixing depth of 2.13 m, an application of 325 mg/m² would lead to a concentration of 152 mg/m³ or 0.152 mg/L

$$325 \text{ mg/m}^2 \div 2.13 \text{ m} = 152 \text{ mg/m}^3,$$

assuming instantaneous mixing, which is about 2 times the measured value of 0.083 mg/L. Again, the measurements were not taken until 1 day after treatment, so it is possible that earlier concentrations were closer to nominal estimates.

While this accidental spill scenario is likely to be conservative in typical ground or aerial applications of 2,4-D, the direct aquatic applications of Aqua-Kleen to water might be expected to lead to higher ambient water concentrations. As noted in section 2, application rates might range from 19 to 38 lbs a.e./acre when Aqua-Kleen is applied. However, as discussed above and illustrated in Figure 3-8, only modestly higher concentrations of 2,4-D in water, 0.68-3.6 mg/L, were noted by Hoeppe and Westerdahl (1983) after the application of 2,4-D amine and Aqua-

Kleen at rates of 19-38 lbs a.e./acre and these higher levels were associated with the amine form of 2,4-D rather than Aqua-Kleen. As illustrated in Figure 3-8, the natural log of the estimated t_0 concentration of 2,4-D after the application of Aqua-Kleen is about -4. This corresponds to a water concentration of about $[e^{-4}=0.02 \text{ mg a.e./L}]$. The monitored concentrations of 2,4-D in water inside of the treated area 24 hours after the application of Aqua-Kleen were 0.54 (0.28-0.68) mg a.e./L at an application rate of 45 kg a.e./ha or 40 lbs. a.e./acre. At an application rate of 22.5 kg a.e./ha or 20 lbs a.e./acre, the monitored concentrations at 24 hours after application were essentially the same, 0.58 (0.51-0.65) mg/L. Normalized for application rate, the central estimates correspond to 0.012-0.026 mg a.e./L per lb a.e. applied. This low concentration may be due to the slow release of 2,4-D from the clay matrix of the granules. Thus, the central estimate of about 6 mg a.e./L used for the accidental spill scenario will likely grossly overestimate the concentration of 2,4-D in the water after the intentional application of Aqua-Kleen to surface water, by a factor of about 300 $[6 \text{ mg/L} \div 0.02 \text{ mg/L}]$.

The estimated doses associated with the concentrations of 2,4-D in water after an accidental spill are detailed in worksheet 20. The scenario assumes that a young child (2-3 years old) consumes 1 liter of contaminated water shortly after the spill. As summarized in Table 3-8, the estimated dose levels are substantial: 0.426 (0.1663-1.28) mg/kg. The relatively narrow range reflects variability only in the estimated field dilution rates.

As noted above, this exposure scenario will overestimate exposure to 2,4-D from treatments involving Aqua-Kleen by a factor of about 600. In other words, the doses of 2,4-D associated with the use of Aqua-Kleen are likely to be only in the range of 0.0007 (0.0002-0.002) mg/kg at an application rate of 1 lb a.e./acre [i.e., $0.426 (0.1663-1.28) \div 600$]. This issue is given further consideration in the risk characterization.

The consumption of contaminated ambient water in runoff from plots treated with 2,4-D will result in exposure concentrations that are much lower than those estimated in the accidental exposure scenario described above. 2,4-D concentrations in drainage outflow from no till fields treated at 1.68 kg/ha (1.5 lbs/acre) agent and irrigated at a rate of 0.9 cm hour⁻¹ ranged from about 20 to 100 µg/L (Shalit and Steenhuis 1996). Comparable concentrations were monitored in runoff from turf, ranging from 12 to about 200 µg/L at application rates of about 1 lb/acre (Harrison et al. 1993).

Any number of higher or lower exposure scenarios could be constructed by varying the dimensions of the body of water or the amount of 2,4-D spilled into the water. The relevance of any of these assessments to a practical estimate of human exposure is questionable. Information regarding the organoleptic properties of 2,4-D in water is not available. It is reasonable to suppose, however, that human consumption of large quantities of highly contaminated water is unlikely due to the objectionable taste of water contaminated with 2,4-D. In addition, there are no case reports concerning accidental consumption of water highly contaminated with 2,4-D.

For this risk assessment, the exposure scenario described above in which 2,4-D is spilled into a shallow pond is used to characterize the risks associated with the consumption of 2,4-D in water

immediately after a spill. As discussed in the risk characterization (section 3.4), this exposure assessment is probably very conservative.

A more plausible and relevant exposure scenario involves background contamination of water associated with the application of 2,4-D. The most relevant study for assessing such exposure involves a 2800 hectare (~6900 acres) watershed in Saskatchewan, Canada in which ambient water levels can be associated with known application rates (Waite et al. 1992). For the years 1984, 1985, and 1986, the amounts of 2,4-D applied in this watershed were 347, 410, and 209 kg, respectively. Water samples were collected weekly in 1985 and 1986 and four times in 1987. The levels of 2,4-D in water are summarized in Table 3-10.

A tremendous amount of monitoring data is available on 2,4-D. For example, levels of 2,4-D in ambient water were detected at 0.04-0.1168 µg/L (Donald and Syrgiannis 1995).

These measurements were taken from prairie lakes in Saskatchewan, Canada. Although the investigators indicate that 2,4-D is the most used herbicide, they do not specify the amount used per unit time in the study area. Somewhat higher concentrations (between 0.7 and 4.63 µg/L) of 2,4-D were detected in urban creeks (Hall et al. 1993). Again, the investigators do not provide enough data to permit an association between the amount of 2,4-D used and the concentrations detected in water.

Table 3-10. Levels of 2,4-D in various types of waters from a watershed in which 2,4-D was applied (Waite et al. 1992)^a

Sample Type	Year (Amount applied to 6900 acre watershed)		
	1985 (347 kg)	1986 (410 kg)	1987 (209 kg)
Groundwater	0.19 (0.22-0.24)	nd (nd-2.07)	0.15 (0.11-0.30)
Pond water	0.08 (0.08-0.19)	0.08 (0.07-0.12)	0.30 (nd-0.51)
Spring runoff	0.17 (0.14-0.42)		0.15 (0.13-0.29)

^a Values shown as mean (median-maximum). All concentrations expressed in µg/L.

For assessing chronic exposure to water contaminated with 2,4-D, a contamination rate of 0.002 mg/L per lb a.e. applied is used. This rate is based on the average level in groundwater of 0.19 µg/L during 1985 in the study by Waite et al. (1992) (see Table 3-9). Over the 3-year period, the average amount of 2,4-D applied to the 6900 acre watershed was 322 kg or 708 lbs. Thus, the average application rate over the entire area was about 0.1 lb/acre (708 lbs/6900 acres). Thus, the monitored level of 0.19 µg/L is rounded to 0.0002 mg/L and divided by 0.1 lb/acre to yield a rate of 0.002 mg/L per 1 lb applied/acre. Based on the variability noted over all three (Table 3-

10) lower and upper limits of 0.001 mg/L per 1 lb applied/acre to 0.004 mg/L per 1 lb applied/acre will be used. These values along with estimates of the amount of water consumed per are used in worksheet 20 to calculate the estimated doses for this scenario that are summarized in Table 3-8.

As indicated in section 2, the Forest Service will typically apply 2,4-D at a rate of about 1 lb a.e./acre. As a very conservative approximation, the nominal application rate of 1 lb a.e./acre is used assuming that the herbicide is broadcast over a very large area (i.e., the entire watershed). Thus, the ambient water level is calculated as 0.002 mg/L. It is likely that the actual level of water contamination would be less because 2,4-D will not be applied over an entire watershed. As discussed in section 3.4, however, this extremely conservative approach has a minimal effect on the characterization of risk because the estimated levels of 2,4-D in ambient water do not approach a level of concern.

3.2.3.5. Oral Exposure from Contaminated Fish -- Consistent with the information on biokinetics in mammals (see section 3.1), 2,4-D appears to reach steady-state rapidly and seems also to have a limited capacity to bioconcentrate in fish. At ¹⁴C-labelled 2,4-D concentrations in water ranging from 0.05 to 0.5 ppm, BCFs of about 10-40 were detected in carp and tallapia over periods of exposure ranging from 0.5 to 14 days (Wang et al. 1994b). In this study, bioconcentration is defined 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 possible that some of the apparent bioconcentration was associated with metabolites of 2,4-D.

Due to the lack of a strong time-concentration relationship in the bioconcentration of 2,4-D, a BCF of 25, the midpoint of the range (10-40) is used for short-term and longer-term exposure scenarios. The variability in the BCF is relatively modest compared to other sources of variability and uncertainty but is considered further in the risk characterization.

A more substantial area of uncertainty involves the qualitative judgment to use the data from Wang et al. (1994b). This approach probably results in an extremely conservative estimate. Field studies indicate that the application of 2,4-D amine or ester to a lake, at very high application rates, did not result in the bioconcentration of 2,4-D in game fish (Hoeppe and Westerdahl 1983). Similarly, the biokinetics of 2,4-D in catfish suggest that the compound has a low potential for bioaccumulation (Plakas et al. 1992). Again, these uncertainties are discussed further in the risk characterization.

As with the drinking water scenarios, the estimates of 2,4-D exposure from the consumption of contaminated fish are made for both acute (worksheet 22) and chronic (worksheet 23) scenarios. As detailed in these worksheets, estimates of the amount of fish consumed are available for both the general public as well as subsistence populations (U.S. EPA 1996 as detailed in worksheet 04). The term *subsistence population* refers to individuals who chose or may need to acquire a substantial portion of their protein from catching fish. The U.S. EPA (1996) bases the estimates

for the consumption of fish in subsistence populations on data from Native American populations. These estimates, however, might be more widely applicable to any economically disadvantaged population.

Because the water levels used to estimate 2,4-D residues in fish are identical to the water levels used for the contaminated drinking water scenarios, the results of the exposure assessments for the consumption of contaminated fish follow a pattern similar to that for drinking water (Table 3-8). Short term (i.e., 1 day) exposures associated with an accidental spill lead to relatively high dose estimates: 0.32 (0.2-0.64) mg/kg bw for the general population and 1.6 (1.0-3.1) mg/kg bw for subsistence populations. Longer term exposures lead to much lower dose estimates: 0.0.00001 (0.0000036-0.00023) mg/kg bw for the general population and 0.000058 (0.000029-0.0011) mg/kg bw for subsistence populations.

3.2.3.6. Oral Exposure from Contaminated Vegetation -- It is possible to construct an exposure scenario involving the consumption of fruit, such as berries, consumed shortly after a pesticide spray. The amount of herbicide on the surface of the fruit will depend on the application rate. An application rate of 1 lb a.e./acre corresponds to 0.0112 mg/cm². Because of the relationship of surface area to volume, smaller fruits will tend to be more contaminated than larger size fruits in terms of residues expressed as mg of contaminant per kg of fruit (worksheet 05b).

Empirical relationships can be drawn from data regarding initial pesticide residues on fruits and vegetables shortly after application. These relationships, which are based on the use of several pesticides and various methods of application, are detailed in worksheet 05a. The relationships suggest residue rates of 125 mg/kg·lb a.i./acre on leaves and leafy crops and extreme residue rates of 240 mg/kg·lb a.i./acre on range grass. For fruits, grains, and seed pods, the corresponding estimates of pesticide residue range from 1.5 to 12 mg/kg (Hoerger and Kenaga 1972). Average residues of 30 mg/kg were monitored on triticale—a cross between wheat and rye—after an application of 2,4-D amine at a rate of 0.56 kg a.e./ha, equivalent to 0.5 lb a.e./acre (Cessna 1990), which corresponds to a residue rate of about 60 mg a.e./kg per lb a.e. applied per acre, reasonably consistent with the estimates from Hoerger and Kenaga (1972).

For this exposure assessment, the estimated amount of residue on berries immediately after 2,4-D is applied ranges from about 5 (typical) to 30 mg/kg (upper range). This range is based on calculations for different sized fruits, adjusted slightly to accommodate empirical ranges for different kinds of edible vegetation presented by Hoerger and Kenaga (1972). Because the investigators do not estimate the lower range of contamination, a lower range of 1 mg/kg is used in this risk assessment. This value is less than the typical level by a factor of 5, about the same factor as the upper range with respect to the typical level, 6.

In this risk assessment, assumptions concerning the amount of vegetation that is consumed per day are specified in worksheets 18 and 19 and are based on a recent and detailed review by the U.S. EPA (1996).

To estimate the doses associated with longer term exposures, an estimate of the dissipation/degradation rate from plants is necessary. Morton et al. (1967) 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. This rate is consistent with the foliar half-time of 9 days for 2,4-D amine that is reported by Davis et al. (1990). After 21 days, residues on 2,4-D treated triticale ranged from 0.001 to 0.15 of initial values, which correspond 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, which correspond to decay rates of 0.051-0.095 days⁻¹ or half-times of approximately 7.3-13.5 days (Cessna 1990).

For this risk assessment, a foliar half-time of 14 days ($k_e=0.05$ days⁻¹) is used. This half-time is at the upper limit of the reported values and results in conservative yet plausible exposure estimates. Details regarding these calculations are presented in worksheet 19.

For longer term exposure, a duration of 90 days is used. This somewhat arbitrarily selected exposure duration is intended to represent the consumption of contaminated vegetation that might be available during a single season. Although longer durations could be used for certain kinds of vegetation, using them would lower the estimated dose (i.e., would result in a less conservative exposure assessment).

As detailed in worksheet 19, the fraction, f , of the initial residue remaining at time, t , is calculated assuming first-order degradation/dissipation,

$$f = e^{-k_e t}$$

where k_e is the decay rate.

For this exposure scenario, the central estimate of dose between days 0 and 90 will be taken as the geometric mean of the concentrations or doses between two time intervals:

$$Conc_{TWA} = (C_{t_1} \cdot C_{t_2})^{0.5}$$

This approach is taken because the geometric mean is the median daily dose (i.e., the doses or concentrations are above this level on half of the days and below this level on the other half). Using a time-weighted arithmetic average dose would tend to overemphasize the early exposure

period, which is covered by the exposure assessment for time zero (i.e., immediately after spraying).

As summarized in Table 3-8, the acute exposure scenario for the consumption of contaminated vegetation leads to much higher dose estimates, 0.035 (0.0071-0.21) mg/kg, than the longer term scenario, 0.0023 (0.00046-0.032) mg/kg/day. The plausibility of these exposure assessments for contaminated vegetation is questionable. 2,4-D is a herbicide; consequently treating (i.e., contaminating) plants with 2,4-D is likely to cause obvious damage, which might not be apparent on day 0. Nevertheless, other signs of treatment might discourage the consumption of contaminated vegetation. The longer term scenario may be particularly conservative because plant damage would be apparent unless all of the vegetation to be consumed were harvested shortly after treatment. The plausibility of these exposure scenarios has a substantial impact on the risk characterization for 2,4-D (section 3.4).

3.3. DOSE-RESPONSE ASSESSMENT

3.3.1. Overview. In 1988, the U.S. EPA derived an RfD of 0.01 mg/kg/day for 2,4-D (U.S. EPA 1997). The RfD is based on a NOAEL of 1 mg/kg/day using an uncertainty factor of 100 to account for species to species extrapolation and sensitive individuals in the human population. Since this RfD was developed, a significant amount of new information was made available and is under review by the U.S. EPA as part of the re-registration process for 2,4-D. This information suggests that the current RfD adopted by U.S. EPA is appropriate and protective. As discussed in the hazard identification, the potential effects of 2,4-D on the thyroid merit detailed consideration. The NOAEL that forms the basis for the RfD was associated with a significant increase in thyroid weight as well as an increase in circulating thyroxine levels. Nonetheless, consistent with the interpretation of this study by the U.S. EPA, the dose-response relationship for this effect suggests that the thyroid changes observed at 1 mg/kg/day are not toxicologically significant.

An assessment of the dose/duration/severity data on 2,4-D suggests no apparent or, at least, no strong relationship between exposure duration and the severity of effects at a given dose. In other words, adverse effects, if they are to develop, will develop relatively fast and will not become more severe as the duration of exposure continues. This assessment is confirmed by categorical regression analysis in which the duration of exposure is statistically insignificant. The weak duration-response relationship for 2,4-D may be explained, in part, by the pharmacokinetics of 2,4-D. For compounds like 2,4-D that are eliminated rapidly, the time to approximate steady-state is relatively brief. Although somewhat speculative, this explanation suggests that 2,4-D does not exert cumulative toxic damage (i.e., at low levels of exposure, the rate of injury is less than the rate of repair).

The dose-severity data on 2,4-D was considered quantitatively using categorical regression analysis, which is a conceptually simple statistical method for relating dose to the probability of observing an effect at a particular level of severity. At the RfD of 0.01 mg/kg/day, the categorical regression analysis indicates that the probability of an adverse effect (AEL) is about 0.009 (9 in 1000). Most likely, this AEL would involve subclinical effects rather than overt signs of toxicity.

The probability of a frank effect level would be about 0.00009 (9 in 100,000). Most likely, this effect would involve signs of neurological toxicity, such as myotonia.

3.3.2. Existing Guidelines. As discussed, U.S. EPA derived an RfD of 0.01 mg/kg/day for 2,4-D in 1988 (U.S. EPA 1997). The RfD is based on a purported NOAEL of 1 mg/kg/day from the 90-day rat feeding study by Serota et al. (1983b). U.S. EPA applied an uncertainty factor of 100. The dose of 5 mg/kg/day was considered a LOAEL based on hematological, hepatic, and renal toxicity.

The uncertainty factor of 100 is a composite factor: 10 for extrapolating from experimental animals to humans and 10 for inter-individual variability in human populations. Although the U.S. EPA sometimes uses an additional factor of 10 to extrapolate from a 90-day study to lifetime exposures, this factor was not used with 2,4-D. The IRIS record does not discuss this decision, but it is a reasonable approach because the subchronic study is supported by several additional chronic studies.

The IRIS entry for the RfD classifies confidence in the RfD as medium using the following rationale:

Confidence in the principal study is medium because a fair number of animals of each sex was used, four doses were given, and a good number of parameters were measured. Confidence in the data base is medium because several studies support both the observation of critical toxic effects and the levels at which they occur. Medium confidence in the RfD follows.

Although U.S. EPA noted that 1 mg/kg/day was associated with a statistically significant increase in thyroid weight in male rats, this effect was "not considered to be treatment-related" by the agency. As discussed in section 3.1.3, the effect was acknowledged as treatment related by the authors of the study but does not appear to be toxicologically significant in the low dose region.

Nonetheless, an argument could be made for lowering the RfD based on the increased thyroid weight observed in rats exposed to 1 mg/kg/day dose (Serota et al. 1983b). Admittedly, the database on the toxicity of 2,4-D is complex and subject to various interpretations. It may be argued that the dose of 1.0 mg/kg/day from the Serota et al. (1983b) study is a legitimate NOAEL because the magnitudes of the observed effects were not great: a 16% increase in thyroid weight and an 11% increase in circulating thyroxine levels. Conversely, the 9% decrease in testicular weight observed in the dogs at a dose of 0.5 mg/kg/day (Dalgard 1993a) could be classified as an adverse effect because it suggests a clear effect on the testes and is consistent with a more pronounced response at higher dose levels.

This kind of uncertainty is common in risk assessment and is exacerbated by the expression of the RfD as a single value. There would probably be broad agreement that a non-hazardous exposure

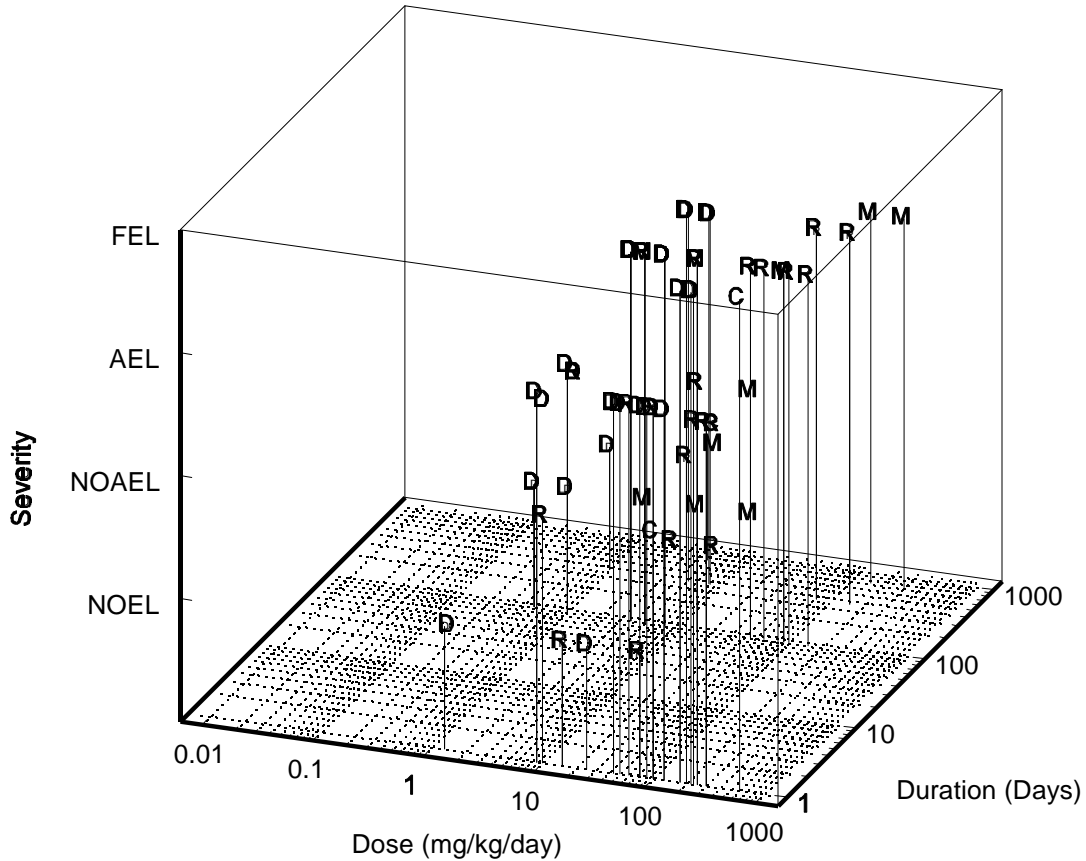


Figure 3-9: Dose-Duration-Severity Relationships for 2,4-D (see Table 3-3 for data).

to 2,4-D falls in the range of 0.0005-0.01 mg/kg/day. The upper end of the range based on the current RfD derived by U.S. EPA. The lower end based on the application of an additional uncertainty factor for the decreased testicular weight loss seen at the dose of 0.5 mg/kg/day (i.e., 0.5 mg/kg/day ÷ 1000). This kind of uncertainty is considered in the following section on dose-severity relationships.

3.3.3. Dose-Response and Dose-Severity Relationships. As summarized in the exposure assessment (see section 3.2) there is substantial uncertainty in the estimates of exposure doses and absorbed doses for workers and the general public. Nonetheless, it is apparent that several of the exposure levels will lead to absorbed doses that are greater than the current RfD derived by U.S. EPA or any plausible alternative RfD. Thus, some effort must be made to characterize the health consequences of such exposures. Two complimentary approaches are taken in this section. One method is largely qualitative. It describes the apparent relationship between increasing dose and exposure duration and the severity of the response. The other method, categorical regression, is more quantitative and based on an explicit mathematical model of the relationship between dose and duration and the severity of the effect.

The dose-duration-severity relationship for experimental mammals is illustrated in Figure 3-9. The animal data on which this figure is based are summarized in Table 3-3. Although most of these studies involve dietary exposure, the doses are plotted in terms of mg a.e./kg bw/day using food consumption and body weight data provided in the studies.

The animal responses are categorized using four standard severity levels: NOEL (no-observed-effect level), NOAEL (no-observed-adverse-effect level), AEL (adverse-effect level), and FEL (frank-effect level). The plotted points represent general clinical damage, including effects on the thyroid and adrenals as well as neurological effects from shorter-term studies. No LD₅₀ values are used. Although LD₅₀ values may be considered frank effects levels (FELs), they represent a different category of response from the other FELs plotted in Figure 3-9 (i.e., ataxia, myotonia, and vomiting).

Judgment must be used when determining categories of response. This point is exemplified in the discussion of thyroid and testicular weights in section 3.3.2. For this analysis, a dose group displaying an adverse effect is classified as an AEL only if the effect is statistically significant with respect to the controls and is biologically substantial. In this analysis, the change in thyroid weight at 1 mg/kg/day is treated as a NOAEL because the magnitude of the effect was relatively modest. As discussed below, this approach has a relatively minor effect on the characterization of risk because, unlike the RfD approach, the dose-response assessment based on severity is not tied to a single number in the expression of risk.

As illustrated in Figure 3-10, there is no apparent or at least no apparently strong relationship between the exposure duration and the severity of the effects at a given dose. In other words, adverse effects, if they are to develop, will develop relatively fast and will not become more severe as the duration of exposure continues. This pattern becomes apparent when several of the subchronic studies are compared with corresponding chronic studies conducted by the same investigator or laboratory. For example, in the 90-day and 1-year dog feeding studies conducted by Dalgard (1993a,b), the dose-response relationships for testicular weights were comparable for both periods of exposure, being somewhat more pronounced in the study of shorter duration.

The weak duration-response relationship for 2,4-D may be explained, in part, by the pharmacokinetics of 2,4-D. For compounds like 2,4-D that are eliminated rapidly, the time to approximate steady-state is relatively brief. For any compound, the number of doses (n) required to reach a certain fraction (f) of the eventual plateau when administered at a certain interval (t), can be calculated as:

$$n = \frac{\ln(1-f)}{-k_e t}$$

where k_e is the first order or terminal elimination coefficient, which is inversely related to the half-time of the compound [$k_e = \ln(2) \div t_{1/2}$] [e.g., Goldstein et al.(1974)]. The first order or terminal half-times reported for 2,4-D is 15.5-101.5 hours for rats and 7.29-48 hours for humans (Munro

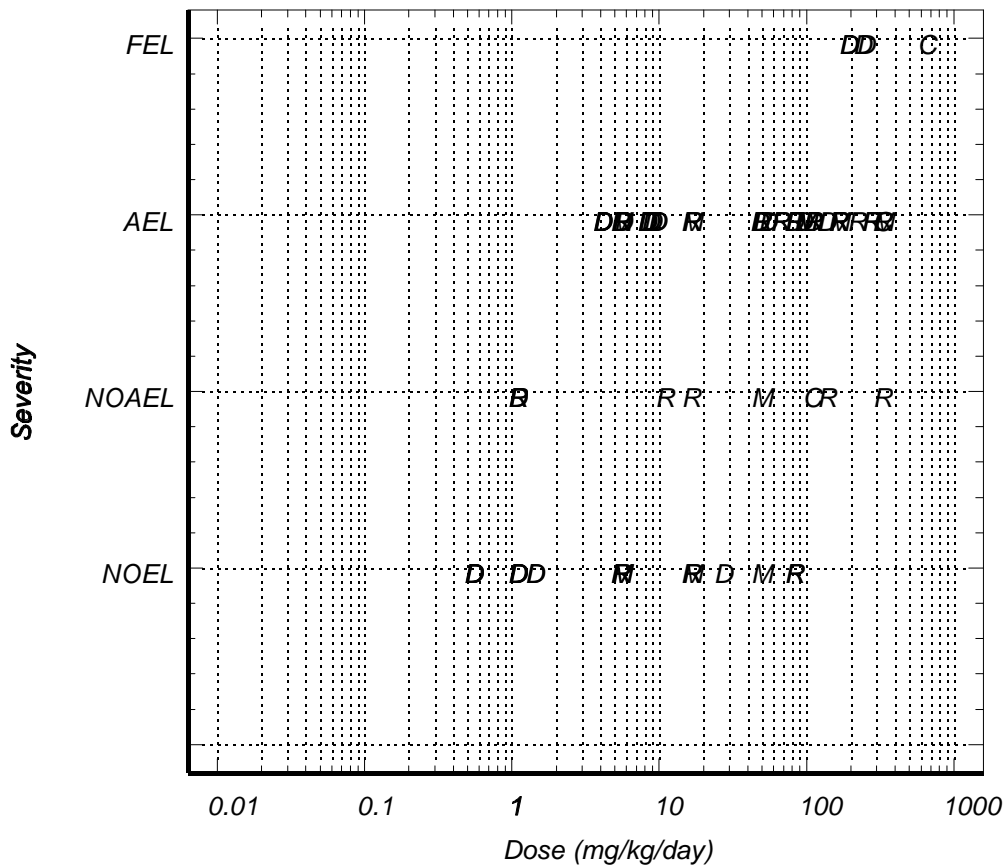


Figure 3-10: Dose-Severity Relationships for 2,4-D over durations from 1 day to 2 years (see Table 3-3 for data).

et al. 1992). After rounding, a range of 10 hours ($k_e=0.07 \text{ hours}^{-1}$ or 1.66 days^{-1}) to 100 hours ($k_e=0.007 \text{ hours}^{-1}$ or 0.166 days^{-1}) encompasses the variability observed in both species. Thus, the time required to reach a 90% steady-state concentration is about 32-320 hours or 1-14 days. Consequently, unless the endpoint involves cumulative damage or late developing effects, there is not likely to be a major difference between subchronic and chronic exposure. This assessment is consistent with U.S. EPA's decision not to use an additional uncertainty factor for the subchronic study used to derive the RfD.

A qualitative summary of the dose-severity relationship based on these data is presented in Table 3-11. The the animals doses are presented ranges, and the ranges correspond to the doses given in Table 3-3. The column labeled 'estimated human dose' is the animal dose divided by 10 (i.e., the uncertainty factor used for species to species extrapolation in deriving RfDs). Using the allometric relationships developed in section 3.1.2 to adjust for interspecies extrapolation would make more use of the available data; however it is not clear whether the approach would provide a better estimate of effective doses, particularly for subchronic and chronic exposure. Notably,

the column giving estimated human doses in Table 3-11 does not incorporate an additional uncertainty factor for sensitive subgroups, which is incorporated into the RfD. The purpose of Table 3-11 is to provide dose estimates associated with effects of varying severity. Effects like these in sensitive subgroups exposed to 2,4-D cannot be quantified from the available data.

Table 3-11. Qualitative Summary of dose-severity relationships for 2,4-D.

Animal Dose	Estimated Human Dose	Effect
<0.01-0.1	<0.001-0.01	No effects are likely.
0.1-1	0.01-0.1	At the upper end of the range, a slight increase in thyroid weight and/or decrease in testicular weight may be noted. Possible decrease in whole body weight gain. (Charles et al. 1996a).
1-10	0.1-1	In addition to above, subclinical signs of neurologic toxicity are possible. Subclinical pathology to the kidney and liver.
10-100	1-10	Subclinical signs of neurologic toxicity are likely and mild signs of toxicity are plausible (60 mg/kg/day) (de Duffard et al. 1993). Degenerative or other pathological changes to several organs are likely. Upper limit of the range may be lethal.
100-1,000	10-100	Frank neurological and/or reproductive effects, including terata are likely. Upper limit of the range will be lethal without prompt and effective medical intervention.

All doses in units of mg a.e./kg bw/day.

As an alternative or supplement to this judgmental approach, categorical regression analyses were conducted on the animal data summarized in Table 3-3. The concept of categorical regression is easy to understand. With increasing dose, observed effects tend to become increasingly more severe. Categorical regression assumes that each effect level can be associated with a distribution (e.g., normal or logistic) and that the shape of the distributions of the various severity levels are identical. At any given dose, the probability of observing an effect at a particular level of severity can be expressed. Although, categorical regression is not a novel method in statistics (McCullagh 1980), it was not applied to dose-severity relationships in risk assessment until recently (Dourson et al. 1997, Durkin et al. 1993).

When categorical regression was conducted on both dose and duration of exposure, the effect of duration was not statistically significant ($p=0.7229$) and its inclusion in the model resulted in a rejection of the overall model fit (Chi-square of 13.5 with 4 degrees of freedom, $p=0.009$). This results seems reasonable, given the data regarding the influence of exposure duration on toxicity

and the kinetics of 2,4-D. A plot of the dose-severity relationship for 2,4-D (i.e., collapsing/omitting the duration axis in Figure 3-10) is given in Figure 3-11.

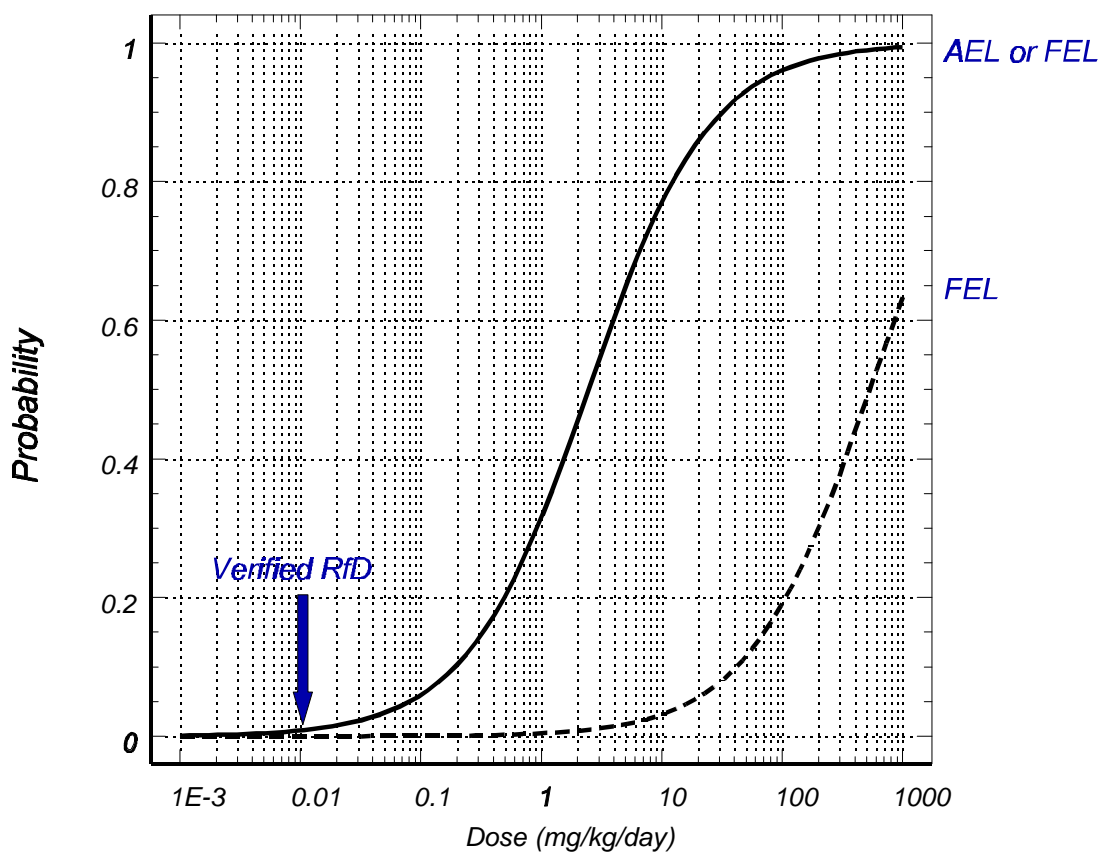


Figure 3-11: Categorical regression of dose/severity relationships for 2,4-D.

When the analysis was run a second time using only dose as the independent variable, the four-category model adequately fit the available data (Chi-square of 4.3 with 2 degrees of freedom, $p=0.1144$). The results of this analysis are illustrated in Figure 3-11.

At the RfD of 0.01 mg/kg/day, the probability of an adverse effect (AEL) is about 0.009 (9 in 1000). Given the classifications used in Table 3-3, the AEL would involve subclinical changes rather than overt signs of toxicity. The probability of a frank effect level (FEL) would be about 0.00009 (9 in 100,000). This effect would most likely involve signs of neurological toxicity, such as myotonia.

3.3.4. Carcinogenicity. For this risk assessment, no quantitative risk assessment for the potential carcinogenicity of 2,4-D is recommended. Some *in vitro* or invertebrate studies indicate that 2,4-D may have genotoxic potential; however, neither the epidemiology data nor the data on

experimental mammals appears adequate to support the qualitative assessment that 2,4-D is a likely human carcinogen. Moreover, the data do not appear to be adequate for a quantitative risk assessment (see section 3.1.5.).

In a recent risk assessment of the use of 2,4-D in seed orchards (USDA 1997), the Forest Service (1997) used a cancer potency factor of $0.019 \text{ (mg/kg/day)}^{-1}$ derived by U.S. EPA (1988). The cancer potency factor is based on the incidence of astrocytomas, a type of brain tumor, in rats. This potency factor is not included on U.S. EPA's IRIS database and does not seem to represent the current position of the agency. As noted above, the National Academy of Sciences' Institute of Medicine recently reviewed the available epidemiology and experimental data on the effects of phenoxy herbicides and concluded that 'there is limited/suggestive evidence for *no* association between exposure to herbicides and brain tumors' (Goetz et al. 1994). Using this old cancer potency factor appears to be an administrative decision.

Previously, Forest Service risk assessments on 2,4-D (USDA 1989a,b,c) used a cancer potency factor of $0.00503 \text{ (mg/kg/day)}^{-1}$ based on the study by Hansen et al. (1971), in which the total number of tumors, both malignant and benign, was taken into consideration. In this study, groups of 25 rats of either sex were given 2,4-D acid in the diet at levels of 0, 5, 125, 625, or 1250 ppm for 2 years. Although there is a marginally significant dose-response relationship in the incidence of total malignant tumors in male rats, the only statistically significant increase (i.e., Fisher exact test) is in the incidence of total malignant tumors in the high dose males (6/25 vs 3/25, $p=0.05$). Nonetheless, there are no statistically significant increases in malignant tumors at any specific site.

3.4. RISK CHARACTERIZATION

3.4.1. Overview. Exposure levels for workers involved in the ground or aerial application of 2,4-D, may exceed the RfD slightly, based on central estimates of exposure, or substantially, based on upper limits of exposure. The central estimates of exposure are not of substantial concern; however, the upper limits of exposure could be associated with covert toxic effects (i.e., adverse effects on organ function or pathology not associated with frank signs of toxicity). Hence, this information suggests that 2,4-D can be applied safely if effective methods are used to protect workers and minimize exposure. If effective measures of hygiene are not employed, occupational exposure to 2,4-D could result in adverse, but probably not frankly toxic, effects.

The general public should not have adverse effects after exposure to 2,4-D under normal conditions of exposure. After accidental exposure to 2,4-D, estimated exposure levels may be comparable to those resulting from occupational exposure. In addition, the consequences of accidental exposure are similar to the anticipated consequences of occupational exposure, which may entail covert toxic effects but are not likely to entail gross signs of toxicity. The major concern for members of the general public involves the consumption of contaminated vegetation over periods of several months. If such an exposure were to occur, the exposure could result in adverse health effects. The likelihood of such an exposure, however, seems remote.

3.4.2. Workers. Table 3-12 provides a quantitative summary of the risk characterization for workers. The hazard quotients are based on the RfD of 0.01 mg/kg/day derived by U.S. EPA (see section 3.3.2).

The uncertainties inherent in this RfD have relatively little impact on the substance of the risk characterization, given the wide variability in the exposure assessments, several of which estimate exposures that far exceed the RfD. When an RfD is exceeded, particularly by the magnitudes summarized in Table 3-12, it is important to try to assess the health consequences of such exposures.

As discussed in the dose-response and dose-severity relationships (see section 3.3.3), there is no evidence that overt signs of toxicity are plausible at exposures to dose levels less than 1 mg/kg/day of 2,4-D, which is 2 times greater than the NOAEL on which the lower RfD is based (see Table 3-11). This assessment is supported by the categorical regression analysis of the animal toxicity data on 2,4-D. Thus, overt signs of toxicity are not expected to occur in workers involved in ground or aerial applications of 2,4-D, for which central estimates of the absorbed dose range from 0.013 to 0.022 mg/kg/day (Table 3-8). This assessment is consistent with data regarding human experience with the use of 2,4-D. Even at the upper limits of exposure (i.e., 0.08-0.15 mg/kg/day) there are not likely to be overt signs of toxicity. For workers involved in ground or aerial applications of 2,4-D, all of the exposure assessments are based on an application rate of 1 lb a.e./acre. Nonetheless, even at the highest anticipated application rate of 2 lbs a.e./acre, no overt signs of toxicity would be expected.

The exposure assessment for workers who apply 2,4-D to bodies of water is somewhat different because the area that might be treated in 1 day, and hence the amount of 2,4-D that a worker might handle, cannot be well characterized. Thus, the exposure assessment is based on treating 1 acre at an application rate of 19 lbs a.e./acre. As summarized in Table 3-8, the estimated absorbed dose associated with treating 1 acre is 0.017 (0.0076-0.038) mg/kg or 0.017 (0.0076-0.038) mg/(kg•acre). Thus, a worker would reach an absorbed dose of 1 mg/kg/day by treating 58 (26-131) acres,

$$1 \text{ mg/kg} \div 0.017 (0.0076-0.038) \text{ mg/(kg}\bullet\text{acre)}.$$

That overt toxic effects are not likely to be seen even at the upper limits of exposure in no way implies that such exposures are prudent or acceptable. At doses ranging from 0.1 to 1 mg/kg/day, effects could occur in the kidney, liver, thyroid, and/or the testes, and subclinical signs of neurotoxicity are possible. While it cannot be demonstrated from the available data that such subclinical effects would lead to behavioral effects, or neurological or reproductive impairment, such effects are plausible. Similarly, the central estimates of absorbed doses for all worker groups (i.e., 0.013-0.022 mg/kg/day) could adversely affect the thyroid (probably thyroid enlargement) and cause testicular weight to decrease. Although these effects would not be profound and might not, in themselves, lead to functional impairment, they are undesirable and represent a source of toxic stress that might compromise an individual's ability to tolerate exposure to other agents or stressors.

Table 3-12: Summary of Quantitative Risk Characterization for Workers

Scenario	Hazard Quotient			Exposure Assessment Worksheet
	Typical	Lower	Upper	
General Exposures [acid or ester formulations]: Hazard Quotients^a				
Directed ground spray (Backpack)	1	0.05	8	12a
Broadcast ground spray (Boom spray)	2	0.1	15	12b
Aerial Applications	1	0.02	8	12c
Aquatic Applications ^b	2	0.8	4	12e
Accidental/Incidental Exposures: Hazard Quotients^a				
Acid				
Immersion of Hands, 1 minute	0.2	0.1	0.5	13a
Contaminated Gloves, 1 hour	13	6	32	13a
Spill on lower legs, 1 hour	5	2	28	14a
Spill on hands, 1 hour	2	1	11	14a
Ester				
Immersion of Hands, 1 minute	0.3	0.1	0.8	13a
Contaminated Gloves, 1 hour	19	8	47	13a
Spill on lower legs, 1 hour	8	3	41	14a
Spill on hands, 1 hour	3	1	17	14a

^a Exposures are based on an application rate of 1 lb a.e./acre and estimates of acres covered per day unless otherwise specified. The exposures are divided by the U.S. EPA RfD of 0.01 mg/kg/day to calculate the hazard quotient.

^b Based on treating 1 acre at 19 lbs a.e./acre.

At the lower end of the range of exposure for all groups of workers, the RfD is not exceeded (i.e., the hazard quotient in Table 3-12 is less than 1) and there is no apparent cause for concern. For workers involved in the aquatic application of 2,4-D, however, this interpretation must consider the limited nature of the application scenario used in the exposure assessment (i.e., treating 1 acre at an application rate of 19 lbs a.e./acre).

The best interpretation of this somewhat complex risk characterization for workers is that 2,4-D can be applied safely if thorough and effective methods are used to protect workers and minimize exposure. If effective measures of hygiene are not employed, occupational exposure to 2,4-D could result in adverse but probably not overtly toxic effects. For workers involved in the aquatic application of 2,4-D additional protective measures may be necessary including limitations on the amount of 2,4-D that is handled.

The accidental scenarios for workers, as summarized in Table 3-12, result in central estimates for hazard quotients that are comparable to the upper limits of the hazard quotients for different job categories. This result is consistent with the assumption that the upper limits of exposures based on job categories are probably associated with mishandling of the chemical during use (Rubin et al. 1998). Like the exposure analyses involving job categories, accidental and incidental exposure analyses suggest that mishandling 2,4-D is not likely to result in overt systemic toxicity. In other words, the upper range of the exposure assessments are less than 1 mg/kg (see Table 3-8).

The major concern regarding human health effects associated with the upper limits of occupational exposure based on different job categories and accidental exposure is that these exposure levels may be associated with covert toxic effects (i.e., adverse effects on organ function or pathology not associated with frank signs of toxicity). Mostly likely, the covert effects would entail relatively subtle changes in neurological parameters and, perhaps, effects on circulating thyroxine levels as well as decreases in testicular weight. Human experience involving occupational exposure to 2,4-D under normal conditions of use supports the argument that 2,4-D does not cause overt adverse effects in workers. Moreover, the dose-response assessment and exposure assessment both suggest that overt adverse effects are not likely to result from occupational exposure to 2,4-D. Nevertheless, the human experience cannot rule out the possibility and perhaps the plausibility that less obvious (i.e., covert) adverse effects could be caused by occupational exposure to 2,4-D in which poor handling or application practices were employed.

2,4-D and its various salts and esters are skin and eye irritants (see section 3.1). Usually, quantitative risk assessments for irritation are not derived; however, from a practical perspective, eye or skin irritation is likely to be the only overt effect as a consequence of mishandling 2,4-D.

3.4.3. General Public. The quantitative hazard characterization for the general public is summarized in Table 3-13. Like the worker exposure scenarios, some accidental public exposures are at doses that substantially exceed the RfD (i.e., direct spray, consumption of contaminated water, fish, or fruit shortly after application). These exposures, however, will be relatively short term. In addition, as discussed in the exposure assessment, many of the exposure scenarios

Table 3-13: Summary of quantitative risk characterization for the general public

Scenario	Target	Hazard Quotient ^a			Exposure Assessment Worksheet
		Typical	Lower	Upper	
Acute/Accidental Exposures					
Direct spray, acid formulation , entire body	Child	1	0.07	23	15a
Direct spray, ester formulation , entire body	Child	1	0.3	14	15b
Direct spray, acid formulation , lower legs	Woman	0.1	0.01	2	16a
Direct spray, ester formulation , lower legs	Woman	0.1	0.03	1	16b
Dermal, contaminated vegetation	Woman	0.1	0.04	0.7	17
Contaminated fruit, acute exposure	Woman	4	1	21	18
Contaminated water, acute exposure	Child	43	17	128	20
Consumption of fish, general public	Man	32	20	64	22
Consumption of fish, subsistence populations	Man	160	100	310	22
Chronic/Longer Term Exposures					
Contaminated fruit	Woman	0.2	0.05	3	19
Consumption of water	Man	0.006	0.002	0.01	21
Consumption of fish, general public	Man	0.0007	0.0004	0.02	23
Consumption of fish, subsistence populations	Man	0.006	0.003	0.1	23

^a Exposures are based on an application rate of 1 lb a.e./acre. The exposures are divided by the U.S. EPA RfD of 0.01 mg/kg/day to calculate the hazard quotient.

associated with these higher levels of exposure are dominated by *arbitrary uncertainty*. In other words, the amount of exposure is dependent on the magnitude of a spill or some other accidental

event. These arbitrary assessments are included in this risk assessment to illustrate the potential consequences of such accidents but the likelihood of such event occurring is probably very low.

The exposures associated with the longer-term consumption of contaminated water are much more plausible and based on modest modeling extrapolations from monitoring studies. Although 2,4-D is not a highly persistent chemical in water, compared with compounds like PCBs, it is persistent enough that it might contaminate groundwater and surface waters. As illustrated in Table 3-13, however, the plausible levels of longer term exposures—based on conservative assumptions—are substantially below a level a concern.

Two separate sets of exposure scenarios are presented and used for the consumption of contaminated vegetation (see section 3.2.3.6). The higher estimates are based on an assumed consumption rate of 1 lb or 0.454 kg of contaminated fruit/day. While this is a somewhat arbitrary value, it is based on the approximate mid range of the typical and upper limits for the consumption of vegetables in the U.S. population (U.S. EPA 1996), as detailed in worksheet 04 based on the 64 kg body weight.

The chronic estimates of 2,4-D exposure from contaminated vegetation are based on the full range of consumption estimates from the U.S. EPA (1996) and lead to estimates of exposure that range from 0.00046 to 0.032 mg/kg/day with a central value of 0.0023 mg/kg/day. The variability in these estimates encompasses differences in anticipated residues of 2,4-D on vegetation and differences in the amount of vegetation consumed. As detailed in worksheet 19, the central and upper estimates of exposure as based on typical consumption levels because the U.S. EPA (1996) does not provide lower ranges for vegetation consumption. Thus, the lower estimate of exposure is conservative. Since both the lower and central estimates of exposure are below a level of concern (i.e., hazard quotients of 0.05-0.2) this conservative approach has no impact on the risk characterization.

At the upper limit, however, the estimated dose of 2,4-D from exposure to contaminated vegetation is 0.032 mg/kg/day, about a factor of 3 above the RfD. Based on the qualitative dose-severity relationships (see Table 3-10), the upper range of the estimated dose, 0.3 mg/kg/day, would not be associated with any adverse effects in experimental mammals or humans. Based on the categorical regression analyses of the animal toxicity data, illustrated in Figure 3-11, the probability of subclinical adverse effects is about 15% but the probability of a frank effect is very small.

The verbal interpretation of the quantitative risk characterization for this scenario is unambiguous: if fruits or vegetables were to be contaminated with 2,4-D at anticipated application rates, the consumption of the fruits or vegetable would be highly undesirable and could lead to covert health effects.

A more difficult yet very important question, however, is the likelihood of such an event occurring. The plausibility of the scenario for the longer-term consumption of contaminated vegetation is questionable. First, 2,4-D is a herbicide. If 2,4-D is applied at a rate that will

effectively kill target vegetation, consumable vegetation is also likely to be damaged to the point that it will not be available for consumption over prolonged periods. Although short-term consumption may occur after an unintentional direct spray, longer-term consumption is unlikely. In addition, the exposure assessment is based on residues in vegetation (berries) of 5-30 mg 2,4-D a.e./kg fruit (acute exposure scenario) and on terminal residues in vegetation (berries) of 0.055-0.33 mg 2,4-D a.e./kg fruit (90-day exposure scenario). As discussed by Ingellog et al. (1977) residues in the range of 0.5 mg of 2,4-D/kg berries impart an objectionable taste to berries. Thus, particularly for the acute scenario but also for most of the 90-day scenario, the 2,4-D would impart an objectionable taste to the berries that would decrease the likelihood of human consumption.

The most reasonable verbal interpretation for these conflicting risk characterizations is that, except for accidental exposures or extremely atypical and perhaps implausible ambient exposures to 2,4-D in vegetation, the risk assessment suggests that the normal use of 2,4-D will not pose any identifiable risk to the general public.

3.4.4. Sensitive Subgroups. While this risk assessment encompasses neurotoxicity as an endpoint (see section 3.1.6), the assessment is based on standard animal bioassay data that focuses on myotonia. As reviewed by Mullison (1981), there is anecdotal information (case histories) suggesting that some individuals may be sensitive to 2,4-D. These individuals may develop neuropathy/impaired nerve function after exposure to 2,4-D at levels that are not expected to cause adverse health effects in the general population. The effects reported in the case studies are debilitating, and recovery may be prolonged and incomplete. On the other hand, the case studies do not rule out the possibility that the neuropathy was caused by other unidentified agents.

Probenecid enhances the acute toxicity of 2,4-D and 2,4,5-T to rats (Ylitalo et al. 1990). This effect is attributable, in part, to the displacement of the phenoxy acids from proteins. Thus, individuals taking probenecid, as an adjuvant to penicillin therapy, may be more sensitive than others to the acute toxicity of 2,4-D.

Laboratory studies demonstrate that there is substantial variation among individual animals within a species in their response to 2,4-D (Arnold and Beasley 1989). Also, there is evidence of human variability regarding dermal absorption rates. Individuals who absorb 2,4-D more rapidly or eliminate the compound more slowly are likely to be more sensitive than others to 2,4-D exposure.

3.4.5. Connected Actions. By analogy to other phenoxy herbicides, the elimination of 2,4-D may depend somewhat on urinary pH. The consumption of compounds that increase urinary pH (e.g., acetates) results in an increased rate of elimination (Arnold and Beasley 1989).

The use of surfactants could influence the toxicity of 2,4-D. For example, Cascorbi and Foret (1991) demonstrated that co-exposure to 2,4-D and sodium dodecyl benbenesulfonate

substantially enhances the *in vitro* activity of 2,4-D on the inhibition of D-glucose transport as well as Na⁺/K⁺ ATPase.

2,4-D is commonly mixed with picloram. There is some indication that co-exposure to 2,4-D and picloram may induce effects not associated with exposure to 2,4-D alone. For example, mixtures of 2,4-D and picloram are associated with an increased incidence of lung adenomas in female mice exposed to concentrations of up to 0.3% mixture in drinking water for 15 weeks. Because this effect was not associated with a change in urethane metabolism, the investigators speculate that the enhanced tumor formation might result from an immunological mechanism (Adams et al. 1991). Also, after male mice were exposed to toxic levels of a mixture of 2,4-D and picloram, teratogenic effects and fetal death were observed in the offspring (Blakley et al. 1989, 1992).

Jacobi and coworkers (Jacobi and Witte 1991, Jacobi et al. 1996, Witte et al. 1995) reported a synergistic interaction between 2,4-D and several compounds, using the induction of DNA synthesis in human fibroblasts as an endpoint. The design of these studies involved exposing the *in vitro* preparations to a fixed level of 2,4-D and to a fixed level of each of the other compounds, then comparing that response with the response from exposure to 2,4-D alone. Consequently, additivity cannot be ruled out as the mode of joint action.

3.4.6. Cumulative Effects. As noted above, this risk assessment specifically considers the effect of repeated exposure in that the chronic RfD is used as an index of acceptable exposure. As discussed in the dose-response and dose-severity relationships (see section 3.3.3), the daily dose rather than the duration of exposure determines the toxicological response. Consequently, repeated exposure to levels below the toxic threshold should not be associated with cumulative effects. There is some information to suggest that repeated exposure to 2,4-D and other phenoxy herbicides may result in an increased rate of elimination (Arnold and Beasley 1989). This assessment is consistent with some of the animal studies that demonstrate an apparent decrease in the magnitude of effects as the duration of exposure increases.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview. The toxicity of 2,4-D has been well-characterized for most groups of potential non-target species. As indicated in section 3.1 (Hazard Identification for Human Health Effects), the toxicity of 2,4-D to experimental mammals has been examined in detail. Acute exposure to high levels of 2,4-D is associated with signs of nervous system toxicity from degenerative changes of nerve cells to convulsions and myotonia. Nonetheless, the acute toxic potency of 2,4-D acid, salts or esters to mammals is relatively low, with LD₅₀ values ranging from 100 mg/kg (butyl ester) in cattle to 1800 mg/kg (sodium salt) in rats.

Although there are apparent differences in sensitivity to 2,4-D among mammalian species, with larger animals being more sensitive than small animals when dose is expressed as mg/kg body weight, there are no substantial or systematic differences in the toxic potency of the different types of 2,4-D (i.e., acid, salts, or esters) used in commercial formulations. The subchronic and chronic effects of exposure to 2,4-D are well studied in experimental mammals. The most sensitive effects in chronic exposures appear to involve the kidney, thyroid, and blood. Of these, kidney effects are most clearly associated with potentially significant toxic effects. Birds are somewhat less sensitive to the acute lethal effects of 2,4-D, with acute oral LD₅₀ values ranging from 300 to 5000 mg/kg.

The toxicity of 2,4-D to terrestrial invertebrates is less well studied than the toxicity of 2,4-D to vertebrates. The estimated LD₅₀ for honey bees is about 120-1100 mg/kg, in the range of values reported for experimental mammals. There is some evidence, however, that younger bees may be more sensitive to 2,4-D than adult bees, the latter of which are typically used in toxicity studies. At exposure rates that correspond to high application rates (i.e. about 3-30 lbs a.e./acre) 2,4-D caused mortality in adult millipedes. The reported responses of earthworms to 2,4-D are variable, with some studies suggesting a potential for decreased growth.

2,4-D is a plant growth regulator and acts as a synthetic auxin or hormone. 2,4-D alters the plant's metabolism and growth characteristics, often causing a proliferation of abnormal growth that interferes with the transport of nutrients throughout the plant. Dicotyledonous plants (broad-leaved) are more susceptible than monocotyledonous plants (grasses). 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. 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 parthenogenic or seedless fruit to develop from unfertilized flowers. Roots are more sensitive to 2,4-D than shoots; however, few symptoms are reported because they are

less obvious. 2,4-D increases the permeability of root membranes, which can lead to a loss of nutrients and possibly increase risk of invasion by pathogens.

As with effects on terrestrial organisms, aquatic plants are usually more sensitive to 2,4-D than fish or other aquatic animals. In general, the ester formulations of 2,4-D are more toxic to fish than the amine formulations and differences in the acute LC₅₀ values, either 24- or 48- hour, to some fish species can reach a factor of nearly 1000. When mixed with emulsifiers, however, the acute LC₅₀ values of 2,4-D acid are comparable to those of the esters of 2,4-D. Some species of aquatic algae are sensitive to 2,4-D at levels of about 1 mg/L although, in some cases, low levels may stimulate algal growth.

4.1.2. Toxicity to Terrestrial Organisms.

4.1.2.1. Mammals-- As summarized in the human health risk assessment (see section 3), the toxicity of 2,4-D has been examined extensively in experimental mammals. Although the mode of action of 2,4-D as a plant toxin is well understood, the mode of action of 2,4-D toxicity in mammals is not clear. After acute lethal exposure, the signs of toxicity include convulsions, vomiting, congestion of various organs, and degenerative changes in nerve cells. In non-lethal but toxic oral exposure to 2,4-D, the signs and symptoms of toxicity include irritation to mucus membranes, vomiting, diarrhea, muscle twitches, and myotonia.

The acute toxicity of 2,4-D acid, salts or esters to mammals is relatively low, with LD₅₀ values ranging from 100 mg/kg (butyl ester) in cattle to 1800 mg/kg (sodium salt) in rats. Moreover, the toxicological equivalence of 2,4-D acid, salts, and esters is supported by teratogenicity screening assays in which the acute lethal potency of 2,4-D acid and its various esters was similar. In addition, dog studies involving subchronic exposure to 2,4-D acid, 2,4-D dimethylamine, or the ethylhexyl ester of 2,4-D, indicate that the toxic potencies of these various forms of the compound are similar to one another. There is a significant difference in sensitivity to 2,4-D exposure among animal species. This pattern is common in toxicity studies, with smaller animals being less sensitive than larger animals to chemical exposure. On the other hand, studies involving acute exposure to 2,4-D suggest that mice are outliers in this pattern, being somewhat more sensitive than rats.

The subchronic and chronic effects of exposure to 2,4-D are well studied in experimental mammals. The most sensitive effects in chronic exposures appear to involve the kidney, thyroid, and hematopoietic system. Of these, kidney effects are most clearly associated with potentially significant toxic effects. As detailed further in section 4.3, the NOAEL for these endpoints in chronic exposures is 1 mg/kg/day. At relatively high doses associated with fetotoxicity or maternal toxicity, 2,4-D might induce fetal malformations.

4.1.2.2. Birds – Birds may be somewhat less sensitive to the acute lethal effects of 2,4-D with acute oral LD₅₀ values ranging between 300 and 5000 mg/kg (Gangstad 1987). 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 1977) at approximately 10 lbs 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).

Differences in hatching rates were observed in chicken eggs treated with 3.1 mg of 2,4-D butyl ester (de Cantarini et al 1992). Some chicks hatching from the treated eggs had motor dysfunction, curled claws, and other alterations. All of the chicks from treated eggs had residues of 2,4-D in all of the tissues studied with the highest concentrations in the brain and kidney.

Toxic effects on chick embryos were noted at all dose levels of 2,4-D (Arias 1994). Changes in the liver were observed in all of the chicks exposed to 2,4-D. In this experiment, 2,4-D was injected into developing eggs. The 15-day LD₅₀ was 8.6 mg/egg.

4.1.2.3. Reptiles--Exposure to 2,4-D acid did not affect several parameters measured to assess alterations to the endocrine system of alligators (Crain et al. 1997). Specifically, 2,4-D did not affect plasma hormone concentrations, gonadal-adrenal mesonephros (GAM) aromatase activity or gonadal histopathology of the alligators. No other toxicological data were found for 2,4-D and reptiles.

4.1.2.4. Terrestrial Invertebrates--Few studies have examined the effects of 2,4-D on terrestrial invertebrates. As summarized in the Forest Service Chemical Background Statement on 2,4-D (Sassaman et al. 1984), acute oral LD₅₀ for bees has been reported to range from 11.525 to 105 µg/bee. Using a body weight of 0.093 g for the honey bee (USDA 1993), these values correspond to doses of about 124 mg/kg

0.011525 mg/0.000093 kg
to 1129 mg/kg
0.105 mg/0.000093 kg,

These estimates are near LD₅₀ values reported in experimental mammals (i.e.,100-1800 mg/kg) (see section 3.1.2).

The bee mortality after the isooctyl ester of 2,4-D was applied at a rate of 2.5 lbs a.i./acre was not significantly different from bee mortality in untreated areas, although mortality rates were lower in the treated area (Moffett and Morton 1971). 2,4-D is reported to be toxic to bees in the study by Leppik (1951). The herbicide acts slowly, and symptoms can take several days to appear. Death occurs 5-7 days after exposure to toxic levels. Volckaert and Van Laere (1984) reported toxic effects associated with the spray of 2,4-D mixed with the herbicide MCPA. They report an LD₅₀ of 850 ppm for this mixture for bee larvae.

2,4-D was found to induce cytochrome P450 in the southern armyworm (*Spodoptera eridania*) and cause 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.

Adult millipedes, *Scytonotus simplex*, exposed to a dose of 0.34 mg a.i./cm² (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%) came 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./cm² (3 lbs a.e./acre). Slugs took up 2,4-D not only through ingestion of contaminated food, but also through contact with contaminated soil (Haqu 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). However, other studies report that 2,4-D in soil can decrease earthworm growth (Martin 1982) and growth can also be decreased when earthworms are immersed in a solution of 2,4-D (Ghabbour and Iman 1967).

Mortality of coccinellid 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. Nonetheless, not all soil invertebrates appear to be affected by 2,4-D. Collembolan and mite populations were not affected by application of the sodium salt of 2,4-D at rates of 1.34 and 2.68 lbs/acre (Prasse 1979).

4.1.2.5. Terrestrial Plants (Macrophytes)--The herbicide 2,4-D is a plant growth regulator and acts as a synthetic auxin or hormone (Stevens and Sumner 1991). 2,4-D alters the plant's 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 (WSSA 1989). The ester forms of 2,4-D are readily absorbed through the leaves (WSSA 1989). Both the ester and the amine forms of 2,4-D are translocated, usually via the phloem, to the meristematic regions of the plant (WSSA 1989). Both the amine and ester forms of 2,4-D are rapidly metabolized by plants to 2,4-D acid.

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).

Dicotyledonous plants (broad-leaved) are more susceptible than monocotyledonous plants (grasses). 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 parthenogenic or seedless fruit to develop from unfertilized flowers. Roots are more sensitive to 2,4-D than shoots; however, few symptoms 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.

4.1.2.6. 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, while at higher concentrations ($1 \cdot 10^{-3}$ M) oxygen uptake and production decreased (Bertagnolli and Nadakavukaren 1974).

Concentrations of 2,4-D as low as 1 mg/L may have an inhibitory effect on soil algae (Peterson et al. 1994)

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 (Ibola 1978).

4.1.3. Aquatic Organisms.

4.1.3.1. Fish – Studies on the acute toxicity of 2,4-D to various species of fish are summarized in Table 4-1. Overall, the ester formulations of 2,4-D are more toxic to fish than the amine formulations (Sigmon 1979, Gangstad 1987, Vardia and Durve 1984, Zaffaroni et al.1986). As summarized by Sassaman et al. (1984, Table 2-3, p. D-33), the difference in the acute LC₅₀ values, either 24 or 48 hours, to bluegill sunfish can reach a factor of nearly 1000 (i.e., about 1 mg/L for various butyl, propyl, or octyl ester versus nearly about 200-1000 mg/L for various alkanolamine or dimethylamine salts). When mixed with emulsifiers, however, the acute LC₅₀ values of 2,4-D acid are comparable to those of the esters of 2,4-D (Sassaman et al.1984).

Neither the acid nor commercial formulations using the amine salt were toxic to green sunfish at a concentration of $5 \cdot 10^{-4}$ M (110 mg a.e./L), while at the same concentration the butoxyethanol ester produced toxicity (Sargent et al.1970). The LC₅₀ for the propylene glycol butylether ester was 4.5 ppm, while the LC₅₀ for the butoxyethanol ester was 4.5 mg/L. No effects were observed for the acid at 50 mg/L, the dimethylamine salt at 15 mg/L, or the 2-ethylhexyl ester at 10 mg/L.

Isopropyl and butyl esters are more toxic than octyl esters (Meehan et al.1974). Butyl ester and propylene glycol butyl ester were more toxic to lake and cutthroat trout than isooctyl esters (Woodward and Mayer 1978). The LC₅₀ values for butyl ester and propylene glycol butyl ester were 490-1220 µg/L, while isooctyl ester was not toxic at concentrations below 60,000 µg/L.

Rainbow trout exposed to a butoxyethanol ester of 2,4-D experienced 20% mortality at a 9 mg/L concentration, 50% mortality at 10 mg/L, and 90% mortality at 10.5 mg/L (Dodson and Mayfield 1979). The smallest fish were the least affected while larger fish were the first to die. Brief exposure (30–60 minutes) to 3 ppm of the butoxyethanol ester of 2,4-D did not affect the oxygen consumption of bluegills (*Lepomis macrochirus*) (Sigmon 1979). The mean uptake by bluegills of the butoxyethanol ester of 2,4-D was low, resulting in herbicide concentrations of less than 0.05 ppm after 8 days of exposure (Sigmon 1979).

Fish (fathead minnows, bluegills and rainbow trout) were more sensitive to 2,4-D acid than to the dimethylamine formulation over 24- to 96-hour exposure periods (Alexander et al. 1985). The LC₅₀ values for the acid ranged between 260 and 358 mg/L. For the dimethylamine LC₅₀ values ranged from 250 to more than 600 mg/L. Using the acid as a surrogate for the amine may be a conservative assumption.

2,4-D has been 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). 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).

Table 4-1. LC₅₀ values of 2,4-D acid, salts and esters to various species of fish

Species	Time	Formulation	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.
Chinook salmon	96 h	butyl ester	0.303 fry	Finlayson and Verrue 1985.
			0.327 fry	Finlayson and Verrue 1985.
			0.332 smolt	Finlayson and Verrue 1985.
			0.418 smolt	Finlayson and Verrue 1985.
Chum salmon	96 h	Isooctyl ester	1 NOEL for fry	Meehan et al. 1974. [cited by Sassman 1984]
Coho salmon	96 h	Isooctyl ester	1 NOEL for fry	Meehan et al. 1974.
	96 h	Isooctyl ester	5 NOEL for smolts	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	Isooctyl ester	10 NOEL for fingerlings	Meehan et al. 1974.
Fathead minnow	96 h	acid	263	Alexander et al. 1985.
		butyl ester	3.3	Johnson and Finley 1980.
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.
		Isooctyl ester	10 NOEL for fingerlings	Meehan et al. 1974.
		butyl ester	0.452 smolt	Finlayson and Verrue 1985.
			0.484 smolt	
			0.512 fry	
			0.525 fry	
			1.206 smolt	
			3.689 smolt	
Pink Salmon	96 h	amine	438	Wan et al. 1991.
		ester	21	Wan et al. 1991.
	96 h	Isooctyl 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.

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 dimethylamine of 2,4-D (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 (PAH) acid by cell cultures of winter flounder proximal tubules (Dawson and Renfro 1993). This 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 has also been 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).

4.1.3.2. Amphibians--2,4-D has been shown to produce teratogenic effects to *Xenopus* frog embryos only at high concentrations (>200 mg/L) that are unlikely to occur in natural waters (Pyles 1995). 2,4-D appears to be more embryotoxic than teratogenic to *Xenopus* frog embryos (Pyles 1995).

Adult crested newts (*Triturus cristatus carnifex*) had increased mortality following exposure to the isooctyl ester of 2,4-D in the 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 LC₅₀ for 2,4-D acid was 8.05 mg/L. Amphibian eggs are more resistant to pesticides and herbicides than larvae. The median survival time was 10.5 hours for the highest 2,4-D concentration tested (11 mg/L). The incipient lethal level was determined to be 6.1 mg/L.

Xenopus frog embryos were killed by high concentrations of 2,4-D in a buffered solution (LD₅₀ 254) (Pyle 1995). In natural waters, both the EC₅₀ and the LC₅₀ were greater than 270 mg/L. The difference between the buffered solution and the natural water sample may be due to the presence of dissolved organic matter in the natural water sample. Dissolved organic matter has been shown to reduce the bioavailability and toxicity of organic compounds.

4.1.3.3. Aquatic Invertebrates--The relative toxicity of the various formulations of 2,4-D can vary considerably for different species of aquatic crustaceans (Table 4-2). 2,4-D uncouples respiratory chain-oxidative phosphorylation (Rodriguez and Monserrat 1991). In other

Table 4-2. Acute toxicity of 2,4-D acid, salts, and esters to aquatic invertebrates

Species	Time	Formulation	LC ₅₀ (mg/L)	Comments	Reference
Calanoid copepod <i>Eudiaptomus gracilis</i>	96 h	acid	144.1		Presing and Ponyi 1986.
Red swamp crayfish	96 h	acid	1389	juvenile	Cheah et al. 1980.
Scud <i>Gammarus fasciatus</i>	96 h	butyl ester	5.9	Early instar	Sanders 1970a.
			6.5	Early instar	Sanders 1970a.
			6.1	Mature	Johnson and Finley 1980.
			6.4	Mature	Sanders 1970a.
Scud <i>Gammarus lacustris</i>	96 h	butyl ester	0.440	2 months old	Sanders 1970a.
			1.4	2 months old	Sanders 1969.
Harpacticoid copepod	96 h	butyl ester	3.1		Linden et al. 1979.
Aquatic sowbug (<i>Asellus brebicaudus</i>)	48 h	butyl ester	3.2	Early instar	Sanders 1970a.
	96 h		2.6	Mature	Johnson and Finley 1980.
Grass shrimp (<i>Palaemonetes kadiakensis</i>)	48 h	butyl ester	1.4	Early instar	Sanders 1970a.
<i>Daphnia magna</i>	48 h	acid	25	Neonate	Alexander et al. 1985.
			36.4	Neonate	Alexander et al. 1985.
			135		Benijts-Claus and Persoone 1975.
<i>Daphnia magna</i>	48 h	butyl ester	5.6	Early instar EC ₅₀	Sanders 1970a.
			6.4	1st instar EC ₅₀	Johnson and Finley 1980.
<i>Daphnia magna</i>	96 h	acid	3390		Elcey and Tiwari 1991.
<i>Culex tritaeniorhynchus</i>	24 h	acid	91.8	larvae	Shim and Self 1973.
	96 h	acid	1.6	nymph	Cope 1965.
<i>Pteronarcys californica</i>			15	adult	Sanders and Cope 1968.
		butyl ester	1.6	adult	Sanders and Cope 1968.
<i>Chironomus</i> sp.	96 h	acid	1785		Elcey and Tiwari 1991.
Dragon fly nymph	96 h	acid	1540		Elcey and Tiwari 1991.
<i>Branchiura sowerbii</i>	96 h	acid	2000		Elcey and Tiwari 1991.
<i>Planorbis exustus</i>	96 h	acid	2375		Elcey and Tiwari 1991.
<i>Pila globosa</i>	96 h	acid	2450		Elcey and Tiwari 1991.
<i>Lymnaea leuteola</i>	96 h	acid	2600		Elcey and Tiwari 1991.
<i>Cyclops viridis</i>	96 h	acid	3330		Elcey and Tiwari 1991.
<i>Viviparus bengalensis</i>	96 h	acid	2440		Elcey and Tiwari 1991.
<i>Diaptomus</i> sp.	96 h	acid	3445		Elcey and Tiwari 1991.
Common bay mussel	96 h	acid	259	Adult	Liu and Lee 1975.
Virginia oyster	12 d	butyl ester	0.740	larvae, 2 day old	Davis and Hidu 1979.
Ciliate (<i>Stylonychia mytilus</i>)	72 h	acid	104		Benijts-Claus and Persoone 1975.
			485		
Rotifer (<i>Brachionus calyciflorus</i>)	24 h	acid	5		George et al. 1982.

uncouplers, this results in an initial increase in oxygen consumption followed by a decrease as the enzymes in the Krebs cycle are inhibited (Rodriguez and Monserrat 1991).

The dimethylamine formulation of 2,4-D was less toxic than the acid to a planktonic cladoceran, *Daphnia magna* (Alexander et al. 1985). The LC50 for the acid was greater than 100 mg/L for a 24-hour exposure and ranged between 25 and 36.5 mg/L for a 48-hour exposure. The 24-hour exposure LC50 for the dimethylamine was 406 mg/L. An increase in exposure time caused the LC50 to decline to 184 for the 48-hour exposure period.

The triethanolamine salt of 2,4-D caused acute toxic effects at high concentrations (48-hour LC50=98 mg/L); however, after 3 weeks of exposure, reproductive effects were observed at much lower concentrations (ED50=1.25 mg/L) (Claus 1976). Juveniles were more susceptible than adults to 2,4-D.

Acute exposure (15 days) of the crab *Chaemagnathus granulata* to concentrations of up to 3.0 mg/L of the isobutoxyethanol ester of 2,4-D did not result in any change in oxygen consumption rate, which is an overall indicator of metabolic rate (Rodriguez and Monserrat 1991). Chronic exposure (30 days) to low concentrations (5 ppm) of isobutoxyethanol ester of 2,4-D resulted in an increase in oxygen consumption while at higher concentrations (50 ppm) there was a decrease in oxygen consumption.

Acute toxicity tests exposing the cladoceran, *Simocephalus vetulus*, to the sodium salt of 2,4-D show complete mortality following 96 hours of exposure to concentrations ranging from 0.5 to 5.0 mM (Kaniewska-Prus 1975). Exposure for 48 hours to a 6.98 mM concentration resulted in 50% mortality 2 days after transfer of the organisms to untreated water. The sodium salt of 2,4-D decreased the respiratory rate of the cladoceran (Klekowski and Zvirgzds 1971; Kaniewska-Prus 1975). Respiration rate is depressed even at the lowest concentrations tested. At higher concentrations, the respiration rate increases after the first 12 hours as the organism attempts to compensate for the effects of the herbicide. A permanent decrease in oxygen consumption was observed following exposure to the highest concentrations tested (4.65 and 6.98 mM). Active life stages of the copepods *Cyclops strenuus* and *Eudiaptomus graciloides* had high mortality rates at concentrations of 2.5 to 20 mM/L of the sodium salt of 2,4-D; however, the resting stages (copepodids) of the two organisms were not affected (Wierzbicka 1974). Similar effects of the sodium salt of 2,4-D have also been reported for *Asellus aquaticus*, another aquatic isopod (Zimakowska-Gnoinska 1977).

Toxicity of 2,4-D varies between groups of organisms. Ranked in order of greatest to least sensitivity to 2,4-D are insect larvae, oligochaetes, snails, and zooplankton (Sarkar 1991). No difference in oxygen consumption was observed between untreated *Daphnia pulex* and those exposed to 3 mg/L of the butoxyethanol esters of 2,4-D (Sigmon 1979). However, at the same concentration of butoxyethanol esters of 2,4-D, the midges (*Chironomus spp.*) experienced greater mortality and lower pupation and emergence rates following exposure (Sigmon 1979).

The zoeae stage of the Dungeness crab, *Cancer magister*, is the most sensitive stage (Caldwell et al. 1979). The maximum acceptable toxicant concentration (MATC) for *Cancer magister* for 2,4-D acid is <1000 µg/L (Caldwell et al. 1979). The zoeae stage of the crab *Chaemagnathus granulata* is more sensitive to the isobutoxyethanol ester of 2,4-D than juvenile or adult crabs

(Rodriguez and Amin 1991). This type of response has been reported for some pesticides and may be expected for other 2,4-D formulations as well. The LC50 values for 96 hours of exposure were 3370 mg/L for adults, 2890 mg/L for juveniles, and 2.89 mg/L for zoeae (Rodriguez and Amin 1991; Rodriguez and Lombardo 1991). Chronic exposure for 4 weeks decreased the LC50 values to >50 mg/L for adults and 30.36 mg/L for juveniles (Rodriguez et al. 1992). *Uca uruguayensis* showed greater sensitivity to the isobutylethanol ester of 2,4-D (Rodriguez and Lombardo 1991, Rodriguez et al. 1992). Crabs chronically exposed to 2,4-D had a decrease in the size of mature oocytes and an increase in the proportion of atretic oocytes (Rodriguez et al. 1994). The size of the mature oocyte determines the viability of the larvae hatched at the end of the incubation period. A decrease in size of the oocytes may affect larval viability. In addition, atretic oocytes in fish have been linked to decreases in gonadotrophin and sexual steroids delaying gonadal maturation (Rodriguez et al. 1994). If the similar changes occur in crabs, 2,4-D could cause changes to the reproductive cycle.

The toxic response to 2,4-D may increase under certain environmental conditions that effect metabolism (e.g., temperature). The LC50 observed at high temperature was uniformly lower for a variety of invertebrates tested including zooplankton, snails, oligochaetes, and insect larvae (Sarkar 1991). Lower LC50 and EC50 were also observed in unaerated treatment tanks than were observed in aerated treatment tanks (Claus 1976).

Susceptibility to a herbicide containing the sodium salt of 2,4-D varied seasonally in the planktonic copepod, *Eudiaptomus gracilis* (Présing and Ponyi 1986). Populations of the copepod were more susceptible to the herbicide in the spring than any other season.

4.1.3.4. Aquatic Plants--The freshwater fern, *Salvinia natans*, is affected by 2,4-D at low concentrations in water (Göncz and Senčič 1994). The EC₅₀ values for changes in growth of leaves, wet weight, length of stems, and chlorophyll *a* and chlorophyll *b* are low (6, 6.5, 6.5, 0.3, 0.3 mg/L, respectively). 2,4-D primarily affected the concentration and ratio of the chlorophylls. At higher concentrations (10 mg/L), curled roots and narrow, hairless leaves also developed. 2,4-D also inhibits the growth of other *Salvinia* species at greater than 0.5 mg/L (*Salvinia auriculata*, Rao and Narayana 1968) and greater than 1.0 mg/L (*Salvinia rotundifolia*, Gaudet and Koh 1968).

2,4-D can cause stimulatory effects (increasing growth rates) on some algae at low concentrations (<1-5 ppm) (El-Ayouty et al. 1980, Fargasova 1994, Okay and Gaines 1996, Wong and Chang 1988). Not all studies show a stimulatory effect at low concentrations, however. Concentrations of 1 ppm or less of 2,4-D did not change the growth patterns of 36 isolates of planktonic algae; however, at 4 ppm, growth was inhibited in some replicates (Butler et al. 1975).

Chlorococcal green algae are more sensitive than filamentous green algae or cyanobacteria (Bednarz 1981). When grown together, more tolerant algal species can ameliorate the toxic effects of 2,4-D for sensitive species (Bednarz 1981).

At concentrations of 18 ppm of an unspecified formulation of 2,4-D, changes in cell size and numbers of cells per coenobia were observed in the freshwater algae *Microcystis*, *Oscillatoria*, *Scenedesmus* and *Coelastrum* (El-Ayouty et al. 1980). *Scenedesmus quadricauda* coenobia production was reduced by 30% at concentrations of 70 mg/L, and more than 50% of the coenobia were disintegrated at concentrations of 100 mg/L (Fargasova 1994). The concentration at which half of the algae were affected (EC₅₀) was 98 mg/L (Fargasova 1994). Growth, photosynthesis, and chlorophyll *a* synthesis were inhibited in the freshwater alga, *Chlamydomonas reinhardtii* (mt++) at concentrations of 10, 20, and 40 ppm (Wong and Chang 1988). Growth of the unicellular cyanobacterium *Gloecapsa* was inhibited at concentrations of 175 [50% inhib.] and 200 [75% inhib.] ppm, while no inhibition was observed at concentrations of 100, 125, or 150 ppm (Tozum-Calgan and Sivaci-Guner 1992).

Exposure for 1 week to a 220 ppm (10⁻³ M) concentration of 2,4-D did not affect the mortality of 16 species of marine and freshwater algae (Elder et al. 1970). In two other species, *Pediastrum* and *Chlorella*, growth [really bizarre growth curves] was inhibited at 10⁻³ M but was normal at 10⁻⁴ M (22 ppm). Exposure for approximately 1 day to 2.917 mg/L of 2,4-D did not affect four species of freshwater algae and six species of cyanobacteria (Peterson et al. 1994).

The NOEL of 2,4-D acid ranges between 10 and 1000 mg/L for day-old or week-old individuals of *Phyllospora comosa*, a marine alga (Burrige et al. 1995). Similar values have also been identified for freshwater algae (2.0 mg/L for *Microcystis aeruginosa* and 3.6 mg/L for *Scenedesmus quadricauda*), protozoans (0.5 mg/L for *Entosiphon sulcatum* and 1.6 mg/L *Uronema parduzci*), and bacteria (6.0 mg/L for *Psuedomonas putida*) (Burrige et al. 1995).

Greater than 75 mg/L of butoxyethanol ester of 2,4-D was necessary to reduce the growth of four marine algae by 50% (Walsh 1972). Significant effects on growth of the marine diatom, *Phaeodactylum tricornutum* [LD₅₀ 362 ± 9] and green alga, *Dunaliella tertiolecta* [LD₅₀ 184 ± 11] were observed at concentrations of 100 mg/L of either the acid or the amine formulation (Okay and Gaines 1996). Cultures of the diatom can adapt slowly over a few days to increasing concentrations of 2,4-D such that stable cultures in concentrations as high as 500 mg/L were produced. It has been suggested that the amine formulation of 2,4-D is consumed by marine phytoplankton in continuous culture in preference to nitrate (Okay and Gaines 1996).

4.1.3.5. Other Aquatic Microorganisms-- Fungal propagule levels in the water column or the sediment are probably not directly affected by application of 2,4-D (Sherry 1994). Application of the amine and the butoxyethyl ester to a set of man-made experimental ponds did not have clear effects on the numbers of yeasts, molds, or total fungi. Mean mold, yeast, and total fungal levels were depressed in the treated ponds for up to 114 days following treatment; however, more experimental replicates were required to determine whether this trend was real or an artifact.

The chemoattraction ability of the protozoan ciliate *Tetrahymena pyriformis* was affected during 1- and 5-hour exposures to 2,4-D (Roberts and Berk 1993). The EC₅₀ for the 1-hour exposure was 158.3 mg/L and was 182.0 mg/L for the 5-hour exposure. The 24-hour LC₅₀ was greater than 500 mg/L. Effects begin to show up at concentrations much lower than the LC₅₀.

The heterotrophic bacteria community in fish ponds was stimulated [increased growth rate and biomass] by a twice monthly dose of 2,4-D (unspecified formulation) at 500 mg/L (Jana and De 1982).

Growth of the nitrogen-fixing cyanobacterium, *Nostoc linckia*, was stimulated at low concentrations (<100 µg/L) of 2,4-D, while at higher concentrations (1000 µg/L) growth was reduced (Pandey and Tiwari 1986). At 1500 µg/L the herbicide was lethal. Formation of heterocysts, the specialized cells for nitrogen fixation, was also stimulated at 100 µg/L and reduced at concentrations of 1000 µg/L or greater. Growth and heterocyst formation in *Cylindrospermum*, a cyanobacterium, was inhibited at 300 µg/L or greater concentrations of 2,4-D (Singh 1974). The cyanobacterium, *Microcystis aeruginosa*, could tolerate 2,4-D concentrations of more than 500 µg/L for 12 days (Swain et al. 1994). Sublethal effects were observed at 2,4-D concentrations of 1000 µg/L and lethal effects were observed at higher concentrations. Ammonia (NH₄⁺) production was stimulated in *Nostoc linckia* at low concentrations of 2,4-D (100 and 500 µg/mL) while inhibited at higher concentrations (Mishra and Pandey 1989).

Concentrations of 2,4-D between 10 and 40 ppm may stimulate the nitrogen-fixing cyanobacteria in paddy soil (Mishra and Tiwari 1986). At low concentrations, 2,4-D stimulated the growth and nitrogen fixation of *Nostoc linckia*, *N. calciocola*, *Nostoc* spp., and *Anabaena doliolum* (Mishra and Pandey 1989). At 100 µg/mL, 2,4-D stimulated uptake of nitrate, but not ammonia. Lower pH enhanced the toxicity of 2,4-D to cyanobacteria (Mishra and Pandey 1989). Concentrations of 2,4-D more than 5.0 micromoles (µM) inhibited the growth of the cyanobacteria, *Anabaena doliolum* and *Anacystis nidulans* found in rice paddy soil (Singh and Singh 1989).

4.2. EXPOSURE ASSESSMENT

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

In this exposure assessment, estimates of oral exposure are expressed in the same units as the available toxicity data (i.e., oral LD₅₀ and similar values). As in the human health risk assessment, these units are usually expressed as mg of agent per kg of body weight and abbreviated as mg/kg body weight. For dermal exposure, the units of measure are usually expressed in mg of agent per cm of surface area of the organism and abbreviated as mg/cm². 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/cm² and the amount of surface area exposed), which can be expressed either as mg/organism or mg/kg body weight. The *absorbed dose* is the proportion of the exposure dose that is actually absorbed by the animal. Inhalation exposure is calculated, in a similar way, as the proportion of the compound retained in the animal after exposure. Sometimes, it is appropriate to combine oral, dermal, or inhalation exposure to estimate the total impact on the organism, as discussed further in the risk characterization (section 4.4).

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

$$y = aW^x$$

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

For most allometric relationships used in this exposure assessment, such as the relationship of body weight to surface area as well as the consumption of food and water, **x** ranges from approximately 0.65 to 0.75. These relationships dictate that, for a fixed level of exposure (e.g., levels of a chemical in food or water), small animals will receive a higher dose, in terms of mg/kg body weight, than large animals will receive.

For many compounds, allometric relationships for interspecies sensitivity to toxicants indicate that for exposure levels expressed as mg toxicant per kg body weight (mg/kg body weight), large animals, compared with small animals, are more sensitive. As discussed in section 4.3, this general relationship appears to hold for 2,4-D. For this exposure assessment, however, generic estimates of exposure are given for a small mammal. A body weight of 20 g is used for a small animal, which approximates the body weight of small mammals such as mice, voles, shrews, and bats. All body weight values are taken from U.S. EPA (1989) unless otherwise specified. In some scenarios, the available toxicity data support specific assessments for other species, such as birds or invertebrates. In the risk characterization, these exposure estimates are compared with the dose/response estimates based on the most sensitive species, regardless of body weight. This approach is admittedly conservative but, as detailed in section 4.3, the differences in sensitivity among species, while significant, are not substantial. Thus, this conservative approach has only a minor impact of the characterization of risk, as detailed in section 4.4.

A summary of the exposure assessments for terrestrial animals is given in Table 4-3. The scenarios covered in this table include all of those for the 20 g mammal as well as the direct spray scenario for the honeybee. Details of the assumptions and calculations involved in these scenarios may be found in the worksheets that accompany this risk assessment, as specified in the last column of Table 4-3.

Table 4-3. Summary of exposure scenarios for terrestrial animals

Scenario	Dose (mg/kg/day)			Worksheet
	Typical	Lower	Upper	
Acute/Accidental Exposures				
Direct spray, small mammal, first-order absorption	0.3	0.1	1.5	24
Direct spray, small animal, 100% absorption	24	12	49	25
Direct spray, bee, 100% absorption	163	81	325	26
Consumption of contaminated vegetation, acute exposure	5	1.5	19	27
Consumption of contaminated water, acute exposure	1.4	0.91	2.8	29
Chronic Exposure Scenarios				
Consumption of contaminated vegetation, chronic exposure	0.566	0.162	2.02	28

4.2.1.1. *Direct Spray* -- In the broadcast application of any herbicide, wildlife species may be sprayed directly. This is similar to the accidental exposure scenarios for the general public discussed in section 3.2.3.2.

In a scenario involving exposure to direct spray, the extent of dermal contact depends on the application rate and the surface area of the organism. As discussed in section 2, the Forest Service usually uses 2,4-D at an application rate of 1 lb a.e./acre or approximately 0.0112 mg a.e./cm². Most application rates will not exceed 2 lbs a.e./acre or 0.0224 mg/cm². As with the human health risk assessment, all exposure assessments are based on an application rate of 1 lb a.e./acre and the consequences of varying this exposure rate are considered further in the risk characterization.

As detailed in worksheets 24 and 25, two sets of exposure assessments are developed for the 20 g mammal, one based on first-order dermal absorption kinetics (worksheet 24) and the other based on 100% dermal absorption (i.e., complete absorption during the first 24 hours after exposure).

Estimates of absorbed doses using first order dermal absorption brackets plausible levels of exposure for small mammals based on uncertainties in the dermal absorption rate of 2,4-D. There are, however, other and perhaps substantial additional uncertainties that could suggest higher estimates for absorbed dose. For example, some animals, particularly birds and some mammals, groom frequently, and grooming may contribute to the total absorbed dose by the direct ingestion of the compound during grooming of fur or feathers. Furthermore, other vertebrates, particularly amphibians, may have skin that is far more permeable than the skin of most mammals (Moore 1964). Quantitative methods for considering the effects of grooming or increased dermal permeability have not been found in the available literature. As a conservative upper limit, this risk assessment will assume complete absorption as an upper limit of exposure to account for the effects of grooming or unusually high dermal permeability (worksheet 25). The impact of this extremely conservative assumption on the characterization of risk is discussed further in section 4.4.

Because of the relationship of body size to surface area, very small organisms such as bees and other terrestrial insects could be exposed to much greater amounts of 2,4-D per unit body weight. Using a body weight of 0.093 g for the honey bee (USDA 1993) and assuming 100% absorption, an exposure assessment for the direct spray of a bee is developed in worksheet 26.

4.2.1.2. *Indirect Contact* -- As in the human health risk assessment (see section 3.2.3.3), the only approach for estimating the potential significance of indirect dermal contact is to assume a relationship between the application rate and dislodgeable foliar residue. The study by Harris and Solomon (1992), discussed in section 3.2.3.3, is used to estimate that the dislodgeable residue will be approximately 100 times less than the nominal application rate. Thus, at an application rate of 1 lb a.e./acre or approximately 0.0112 mg/cm², the estimated dislodgeable residue will be 0.0001 mg/cm².

Unlike the human health risk assessment, however, no transfer rates are available for wildlife species. As discussed in Durkin et al. (1995), the transfer rates for humans are based on brief (e.g., 0.5–1 hour) exposures that measure the transfer from contaminated soil to uncontaminated skin. Wildlife, compared with humans, may spend much longer periods in contact with contaminated vegetation.

It is reasonable to assume that for prolonged exposures an equilibrium may be reached between levels on the skin and levels on contaminated vegetation, although there are no available data regarding the kinetics of such a process. The available bioconcentration data on 2,4-D, discussed in section 4.1, suggest that 2,4-D is not likely to partition from the surface of contaminated vegetation to the surface of skin, feathers, or fur. Thus, a plausible partition coefficient is unity (i.e., the residue on the animal will be equal to the dislodgeable residue on the vegetation).

The exposure dose may be estimated in a manner similar to that for direct dermal exposure (section 4.2.2.1 and worksheet 26). For a 20 g mammal with a surface area of 87 cm², the exposure dose is 0.44 mg/kg

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

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

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

4.2.1.3. *Ingestion of Contaminated Vegetation or Prey* -- As in the human health risk assessment, the consumption of contaminated vegetation is a plausible route of exposure. In the human health risk assessment, residues on berries of 5 to 30 mg/kg berry associated with the application of 1 lb a.e./acre were used. Similar estimates are developed for the 20 g mammal for both acute (worksheet 27) and chronic (worksheet 28) exposure scenarios.

4.2.1.4. *Ingestion of Contaminated Water* -- Estimates of acute and chronic exposure to contaminated waters are based on assumptions that are identical to those used in the human health risk assessment but using estimates of body weight and water consumption for a 20 g mammal. These exposure assessments are detailed in worksheets 27 and 28.

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

The potential for spray drift has been investigated in several field studies that have been recently reviewed by Bird (1995). A brief synopsis of these relationships is presented in worksheet 06. In several different field monitoring studies of low-flight agricultural applications of pesticides using a variety of nozzle types under wide range of meteorological conditions, central estimates of off site drift were 0.05, 0.02, 0.01, and 0.008 of nominal application rates at distances of 100, 200, 300, and 400 feet downwind. At 400 feet down wind, deposition rates ranged from 0.002 to 0.01 of the nominal application rate. As further reviewed by Hitch et al. (1995), off-site deposition may reach 0.001 of the nominal application rate at sites that are 1000 m downwind, depending on the topography and weather conditions.

4.2.3. Soil Contamination. The herbicide concentration in soil or litter may be estimated using the Groundwater Loading Effects of Agricultural Management Systems (GLEAMS) model (Knisel et. al. 1992). GLEAMS was originally developed to predict the

vertical movement flux of pesticides within the root zone and was tested in Georgia in a soil with high hydraulic conductivity (Sichani et al 1991). For the sandy Georgia soils, the predicted concentrations of pesticides were within 2 to 3 orders of magnitude of the observed concentrations (Smith et al. 1991). In other soil types with more clay, the model appears to produce simulated pesticide concentrations that are lower than the observed concentrations (Sichani et al. 1991).

For this exposure assessment, the parameters in GLEAMS were selected to minimize the adsorption of the herbicides to organic matter and maximize their potential for loss through runoff or percolation. Only one soil type, a loamy very fine sand, was modeled. The modeling scenario used simulated terrain that sloped downhill from the site of herbicide application. Ground-based broadcast and directed application were simulated by specifying the proportion of the herbicide applied to plants. A high proportion of the herbicide applied using directed application techniques would be specifically aimed at the plant. The flexible orientation of the nozzle makes it possible to selectively treat the target plant. When the target plant has a large canopy, most of the herbicide will be intercepted by the plant. It is assumed that directed application results in 70-90% of the herbicide being applied to plants. The fixed orientation of the nozzles would make broadcast application more likely to apply herbicide to bare soil. This is especially true if the target plant has not developed a large canopy. For broadcast applications it is assumed that between 50 and 70% of the herbicide applied is actually applied to plants. It is unlikely that broadcast or directed techniques would be used to treat newly emerged plants as they would not provide a sufficient leaf area for a lethal dose of the herbicide to be translocated to the meristematic regions of the plant. Therefore it is unlikely that herbicides would be applied in situations where there is a substantial amount of bare soil compared with plant cover.

For both the dimethylamine salt and esters, the greatest concentrations occurred in the uppermost layer of the soil (0-1 cm deep) immediately after application. At modeled application rates near to or greater than the maximum anticipated application rate of 2 lbs a.e./acre, soil levels of 2,4-D—either immediately after application or after 1- to 2-inch rain falls—did not exceed 10 mg/kg in the upper 1 cm, were below 1 mg/kg in 1- to 8-cm soil levels, and were less than 0.04 mg/kg in the 8-15 mg/kg soil layer.

4.2.4. Aquatic Organisms. For aquatic organisms, the estimates of 2,4-D in ambient water detailed in section 4.2.1.4 may be used as plausible and conservative estimates of exposure. After an accidental spill, maximum initial concentrations of 2,4-D in water can be estimated at 6 mg/L·lb applied. This concentration will diminish rapidly due to microbial degradation, binding to suspended particulate, or dispersion. Similarly, after the application of Aqua-Kleen, levels in ambient water should not exceed 0.02 mg/L. As with the spill scenario, this concentration will be applied only to estimate the consequences of short-term peak exposure. For longer-term exposures, the average concentration in water associated with an application rate of 1 lb a.e./acre is 0.002 mg/L with a range of 0.001-0.004 mg/L, as discussed in section 3.2.3.4.

4.3. DOSE-RESPONSE ASSESSMENT

4.3.1. Toxicity to Terrestrial Organisms.

4.3.1.1. Mammals--As summarized in the dose/response assessment for the human health risk assessment (see section 3.3.2.), the current RfD for 2,4-D is 0.01 mg/kg/day (U.S. EPA 1997) based on a NOAEL of 1 mg/kg/day and an uncertainty factor of 100 to account for species to species extrapolation and sensitive individuals in the human population. As detailed in risk characterization for the human health risk assessment (see section 3.1.2) and illustrated in Figure 3-1, the available data on the acute toxicity of 2,4-D to several different mammalian species weakly supports the conservative assumption that larger mammals will be more sensitive to 2,4-D than smaller mammals. Thus, the RfD of 0.01 mg/kg/day may be used as the most conservative estimate of a dose that should not be associated with any adverse effect in any mammalian species. Smaller mammals may be less sensitive to 2,4-D and the mammalian NOAEL of 1 mg/kg/day is not likely to result in adverse effects in most smaller species. Estimates of the likelihood of observing adverse or frank effects in mammalian species may be based on the categorical regression analysis presented in section 3.3.3. More qualitative assessments of dose response relationships for both large mammals (i.e., humans) as well as smaller laboratory mammals are discussed in section 3.3.3. and summarized in Table 3-10.

4.3.1.2. Birds--As noted in section 4.1.2.2 (Birds, Hazard Identification), the available oral toxicity studies suggest that birds may be somewhat less sensitive to 2,4-D than mammals. Thus, for exposure scenarios involving the ingestion of 2,4-D from either contaminated vegetation or water, the dose-response relationships for mammals may serve as conservative estimated for avian species.

As also summarized in section 4.1.2.2, there are some additional toxicity studies that may be used for the assessment of the effects of direct deposition of 2,4-D on to bird eggs. These studies suggest the direct deposition onto eggs at an application rate of 192 lbs/acre will correspond to the LC₅₀ (Hoffman and Albers 1984) but that no effects would be anticipated on bird eggs from direct deposition at an application rate of 10 lbs/acre (Somers et al. 1978).

4.3.1.3. Terrestrial Invertebrates--The available toxicity data on terrestrial invertebrates is relatively sparse. Based on reported oral LD₅₀ values, the acute oral toxicity of 2,4-D to honey bees is comparable to that for experimental mammals and birds.

Some field studies are also useful for assessing dose/response relationships in terrestrial invertebrates. As detailed in section 4.1.2.3, application rates of 2.5 lbs a.i./acre of the isooctyl ester of 2,4-D, equivalent to about 1.65 lbs a.e./acre, did not result in mortality to bees (Moffett and Morton 1971). Similar NOAELs, 1.34 and 2.68 lb/acre, have been reported for collembolan and mite populations in soil (Prasse 1979). In millipedes, increased mortality was observed after applications of 2,4-D ester at 3 lbs a.e./acre but the increase was not significantly different from the control group. At 30 lbs a.e./acre, however, the mortality rate in millipedes was statistically significant (Hoy 1985).

4.3.1.4. Terrestrial Plants (Macrophytes)--As discussed in section 4.1.2.4, 2,4-D is a contact herbicide that may be used to selectively control broadleaf vegetation.

For direct spray or drift, the relevant exposure metameter is the application rate or functional rate of deposition expressed in units of toxicant weight per unit area (e.g., lb/acre). In some respects, the product labels for 2,4-D (CPR 1997) provide useful information on effective levels of application and suggests differences in species or life-stage sensitivity. For example, the maximum broadcast application rate, about 2 lbs a.e./acre, is effective against most species and life stages of terrestrial plants, except grasses. Conversely, application levels of 0.5 to 1 lb a.e./acre are likely to damage broadleaf vegetation but less likely to impact other species of vegetation.

4.3.1.5. Terrestrial Microorganisms--Most microorganisms will be exposed to 2,4-D as soil residues. As would be expected from the phytotoxic properties of 2,4-D, soil algae are more sensitive than other types of soil microorganisms. The lowest concentration of 2,4-D reported to cause an inhibition of the growth of soil algae is 1 mg/L (Peterson et al.1994). In other assays, soil concentrations of 10^{-4} to $2 \cdot 10^{-3}$ M caused growth inhibition (Pelekis et al.1987). Using a molecular weight of 221 for 2,4-D acid, these levels correspond to 22.1 to 442 mg a.e./L. In some algal species, however, the lower end of this range may be associated with growth stimulation (Bertagnolli and Nadakavukaren 1974).

Fungi appear to be somewhat less sensitive to 2,4-D, with minimal effect levels reported at 10 ppm and substantial inhibition reported at 1000 ppm (Estok et al.1989, Ibola, 1978).

4.3.2. Aquatic Organisms.

4.3.2.1. Fish--As discussed in section 4.1.3, the ester formulations of 2,4-D—including the butoxyethyl ester found in Aqua-Kleen — are more toxic to fish than the amine formulations by a factors of about 200-1000 when toxicity is measured by acute (24- to 48-hour) LC_{50} values. This difference in toxic potency probably relates to differences in rates of absorption. For example, Kenaga (1980) has proposed the following relationship between water solubility (WS) and the bioconcentration factor (BCF)

$$\text{Log BCF} = 2.791 - 0.564 \text{ Log WS.}$$

Based on the water solubility data summarized in Table 2-3, using the solubility at pH 7 for the acid, the BCFs for 2,4-D acid and the octyl ester of 2,4-D would be about 2 [anti log of $2.791 - 0.564 \log(23180) = 2.13$] and 2800 [anti log of $2.791 - 0.564 \log(0.07) = 2769$]. This difference, about a factor of 1000, is reasonably close to the difference in observed acute lethal potency between the salt and ester forms of 2,4-D. The supposition that the differences in acute lethal potency between the salt and ester formulations are attributable to differences in rates of uptake is supported by the observation that the toxicity of 2,4-D acid is substantially enhanced by the presence of emulsifiers.

The presumed basis for the increased toxicity of the ester formulations of 2,4-D has a substantial impact in the interpretation of the acute toxicity data for this risk assessment. The esters of 2,4-D, while chemically stable, have a relatively short half-time in natural water due to biological degradation. As summarized in Table 2-3, the biodegradation half-times of 2,4-D esters in natural waters range from about 1 hour to 1 day. Thus, in natural waters, the rate of conversion of the 2,4-D esters to 2,4-D acid would be about 0.02-0.07 hour⁻¹ and the proportion of 2,4-D ester remaining after 24 hours would range from about 0.04-0.5. In other words, after 1 day, half to nearly all of the 2,4-D ester would likely be broken down to the 2,4-D acid.

Acute laboratory bioassays in fish are typically conducted in relatively large amounts of water (i.e., relative to the biomass of the fish) that contain no sediments and a low concentration of microorganisms. Because the esters of 2,4-D are chemically stable in water, it is reasonable to suppose the esters would be much more rapidly absorbed and thus more toxic in such laboratory bioassays. In natural waters that have relatively high microbial or algal populations, however, it is likely that the 2,4-D esters would break down relatively rapidly to the 2,4-D acid.

For this risk assessment, 1 mg a.e./L will be used as a conservative estimate of the exposure level to 2,4-D esters that would likely be associated with mortality in fish. This value is somewhat lower than the lowest reported LC₅₀ for the esters of 2,4-D encountered in the literature. The corresponding value for 2,4-D salts will be taken at 100 mg a.e./L. These values will be applied to acute exposure scenarios, recognizing that the lower value for the 2,4-D esters may be conservative in natural waters in which the breakdown of the 2,4-D esters to the acid form is very rapid.

For chronic exposures, it is not reasonable to assume that any substantial amount of 2,4-D esters will remain in natural waters. Even in the lower range of the decay rate for the esters (i.e., a half-time of 1 day) only about 0.01 of the original concentration of the ester would remain after 1 week and only about 1·10⁻⁹ would remain after 30 days. For longer-term exposures, a NOEL of 10 mg/L will be used based on the observation by Bettoli and Clark (1992) that levels of 11 mg/L of 2,4-D dimethylamine were not associated with any adverse effects [i.e., no changes in the reproductive behavior (nest guarding)] in red ear or bluegill sunfish.

In characterizing the likelihood of fish being exposed to relatively high concentrations of 2,4-D after a spill, it is worth noting that fish will avoid concentrations of 2,4-D in water at levels as low as 1 mg/L (Sassaman et al. 1984).

4.3.2.2. Amphibians--The toxicity of 2,4-D to amphibians has not been investigated as thoroughly as the toxicity of 2,4-D to fish but the available data suggest the 2,4-D may be more toxic to some amphibians than to fish.

In general, the acute LC₅₀ values for amphibians are comparable although at the lower range of the LC₅₀ values for fish (i.e. about 200 mg/L) (Pyle 1995). The report by Vardia et al. (1984), however, suggests that at least some species of amphibians (i.e., *Bufo melanostictus*) have LC₅₀ values for 2,4-D acid that are much lower (i.e., a 96-hour LC₅₀ of about 8 mg/L). No data have

been encountered on the toxicity of 2,4-D esters to amphibians. By analogy to the data on fish, the acute toxicity of 2,4-D ester is likely to be greater than the acute toxicity of 2,4-D acid or salts.

For this risk assessment, a level of 10 mg a.e./L will be used as an estimate of possible lethal exposures for at least some species of amphibians based on the data of Vardia et al. (1984). By analogy to the data on fish, 2,4-D esters could be 100 to 1000 times more toxic than 2,4-D acid or salts. For this risk assessment, a level of 0.1 mg a.e./L will be used as an estimate of possible lethal acute exposures to 2,4-D esters.

A chronic NOAEL for the effects of 2,4-D on amphibians has not been encountered in the literature.

4.3.2.3. Aquatic Invertebrates--As discussed in section 4.1.3.3, the acute toxicity of 2,4-D to some species of aquatic invertebrates may be somewhat greater than the toxicity to fish. While most LC₅₀ values for aquatic invertebrates are at or near the lower range of LC₅₀ values in fish (i.e., about 100-400 mg/L) acute LC₅₀ values in aquatic invertebrates are reported to be as low as 25-36.5 mg/L for 2,4-D acid (Alexander et al.1985). It is possible, however, that this apparently higher toxicity was due to changes in water pH, since a bioassay of the dimethylamine salt of 2,4-D by the same investigators resulted in 24- and 48-hour LC₅₀ values of 406 and 184 mg/L, respectively. The lowest reported acute LC₅₀ is 2.89 mg/L, for the zoeae stage of the Dungeness crab (Caldwell et al.1979). Juvenile and adult stages of the crab have LC₅₀ values comparable to those for fish (i.e., about 2000-3000 mg/L).

The chronic toxicity of 2,4-D is relatively well characterized in some aquatic invertebrates and again appears to be somewhat greater than the chronic toxicity to fish, particularly for small invertebrates. In the Dungeness crab, exposure to 2,4-D for 4 weeks decreased the LC₅₀ values from 2000 to 3000 mg/L (96-hour exposures) to >50 for adults and 30.36 mg/L for juveniles (Rodriguez et al.1992). In daphnids, 21-day reproduction studies with 2,4-D yielded an EC₅₀ of 1.25 mg/L (Claus 1976).

For this risk assessment, a level of 1 mg a.e./L will be used as an estimate of possible lethal exposures to at least some aquatic invertebrates, specifically small free swimming organism. Larger aquatic invertebrates appear to be no more sensitive to 2,4-D than fish. By analogy to the data on fish, 2,4-D esters could be much more toxic than 2,4-D acid or salts. The limited data on the toxicity of 2,4-D esters and salts to daphnids suggest that the esters are more toxic by a factor of about 10 (see Table 4-2).

For chronic reproductive effects, a NOAEL has not been identified. A concentration of about 1 mg/L is clearly toxic [i.e., an EC₅₀ in the study by Claus (1976)].

4.3.2.4. Aquatic Plants (Macrophytes and algae)--Although 2,4-D is a herbicide registered for use on aquatic vegetation, the toxicity of 2,4-D to aquatic plants does not appear to be remarkably greater than the toxicity of this compound to sensitive invertebrate and amphibian

species. Based on the studies summarized in section 4.1.3.4, the NOEC (no-observed effect concentration) for most unicellular algal species appears to be above 1 mg/L. EC₅₀ values for growth inhibition are typically in the lower range of LC₅₀ values for fish, invertebrates, or amphibians: 10 to about 200 mg/L.

Aquatic macrophytes appear to be more sensitive than unicellular algae, with EC₅₀ values at about 0.3 mg/L for a reduction in chlorophyll concentrations. As noted above, this is only somewhat below the concentration of 1 mg/L reported as the EC₅₀ for reproductive impairment in daphnids.

4.3.2.5. Other Aquatic Microorganisms--There are substantial differences in the sensitivity of different groups of aquatic non-algal microorganisms to 2,4-D. The most sensitive group appears to be the nitrogen-fixing cyanobacteria that evidence growth inhibition at concentrations as low as 1 mg/L (Pandey and Tiwari 1986). No adverse effects and occasionally a stimulation of growth are apparent at lower levels (i.e., 0.3-0.5 mg/L) (Pandey and Tiwari 1986, Swain et al.1994). For protozoa and heterotrophic bacteria communities in fish ponds, adverse effects are reported in the range of concentrations that may be toxic to fish.

4.4. RISK CHARACTERIZATION

4.4.1. Terrestrial Animals. As in the human health risk assessment, the quantitative risk characterization for terrestrial animals is expressed as the hazard quotient, the ratio of anticipated exposure divided by some dose estimate that can serve as an index for assessing the consequences of exposure. If the hazard index is well below unity, there is little cause for concern. As the hazard index approaches or exceeds unity, concern increases. While hazard quotients or analogous values such as margins of safety are commonly used in risk assessments to characterize risk numerically, they seldom express fully the consequences of the exposure or uncertainties in the assessment. Thus, while a quantitative summary of the risk characterization for terrestrial animals is summarized in Table 4-4, the verbal interpretation of each of these hazard quotients, provided in the following paragraphs, is necessary to appreciate the meaning and limitations that are inherent in this risk assessment. In plain language, the words are more important than the numbers.

For the direct spray scenarios, absorption rates of 0.012(0.004 to 0.062) day⁻¹ are used as plausible estimates for most species (section 3.1.8), and 100% absorption is used as a conservative upper limit intended to account for the effects of grooming or unusually high skin permeability (section 4.2.1.1.). As might be expected, the exposure scenario involving direct dermal spray results in high levels of exposure for which the endpoint of concern is lethality. In other words, this is an accidental exposure scenario and is not likely to occur frequently in the same area. Thus, it is not reasonable to evaluate the potential hazard of a one-time direct spray with a subchronic NOEL. Consequently, for the direct spray scenarios, the relevant hazard quotients are based on a dose estimate that is not likely to cause death after acute exposure. As discussed in section 3.3, this dose cannot be precisely defined. Given the scatter in the data (Figure 3-10) and the results of the categorical regression analysis (Figure 3-11), a dose of 10 mg/kg is selected as the acute non-lethal dose. At this level, the categorical regression analysis suggests that there is less than a 5% probability of observing any adverse effect.

All of the dermal exposure scenarios are conservative. The assumption of 100% dermal absorption is only marginally plausible. Although grooming is a reasonable concern, there is no evidence in the literature to suggest that grooming will substantially increase exposure to 2,4-D among wildlife species or experimental mammals. Furthermore, the study by Gaines (1969) suggests that grooming is not significant in the toxic response of small mammals.

Based on the estimated first-order dermal absorption rates for 2,4-D, even the upper limit of the likely absorbed dose is well below a level of concern for a mammal. For this and all other dermal absorption scenarios, note that all exposure assessments are based on a small mammal. This approach will overestimate the dose for a large mammal. Conversely, the dose/response assessment is based on a large mammal because larger mammals appear to be more sensitive to 2,4-D than smaller mammals (section 3.1.2). Thus, this risk characterization is based on an extremely conservative use of the available data.

Adding the additional conservative assumption of 100% dermal absorption or its functional equivalent due to extensive grooming, the direct spray scenario leads to a central estimate of exposure that is a factor of about 2 above the estimated non-lethal acute dose of 10 mg/kg/day. Based on the categorical regression analysis (summarized in section 3.3. and illustrated in Figure 3.11), the probability that this dose would be associated with frank signs of toxicity is about 0.05. The probability of covert toxic effects would be substantial (>0.80). The verbal interpretation of this numeric expression of hazard is somewhat ambiguous. On the one hand, it could be argued that the highly conservative series of assumptions involved in the calculations for this scenario is so extreme that the results are meaningless. On the other hand, the limitations in the nature of the data used in the quantitative assessment must be appreciated. In this and in most ecological risk assessments, an attempt is made to assess potential effects on a very large number of free-living species based on data from relatively few species of experimental mammals in a very artificial environment. In balance, a reasonable verbal interpretation of the direct spray scenarios is that it is unlikely that signs of frank toxicity would be evident but subclinical effects could result in some species.

The direct spray scenario for the bee is less ambiguous. It is a simple matter to calculate the deposited dose on a bee if the bee is directly sprayed with an amount of 2,4-D that is equivalent to an application rate of 1 lb a.e./acre (worksheet 26). The toxicity data on bees involve contact bioassays in which known amounts of a compound are applied to groups of bees and mortality is observed as the endpoint. As detailed in section 4.1.2.4, such contact toxicity tests in bees indicate a range of LD₅₀ values of 124-1129 mg/kg. While these values are not remarkably different from LD₅₀ values in experimental mammals, the small size of the bee leads to dose estimates after a direct spray that are much higher than those for mammals. For the bee, uncertainties concerning dermal absorption rates are a lesser issue than for mammals because the acute toxicity studies on bees involve dermal exposure. Thus, if a bee is sprayed at an application rate of 1 lb/acre, the amount deposited on the bee will be 163 mg/kg. This is greater than the lower range of the LD₅₀ but a factor of about 7 less than the upper range. A reasonable interpretation is that some populations of bees subject to a direct spray could evidence substantial mortality. Other populations would not. An additional consideration, of course, involves

interception of the 2,4-D by vegetation. This would tend to reduce the level of exposure but the magnitude of the reduction would depend on the proportion of the 2,4-D that is intercepted prior to contacting the bee. While this cannot be well quantified in general, it may account for the failure of some field studies to note toxicity in bees after the application of 2,4-D (Moffett and Morton 1971).

Neither of the drinking water scenarios lead to hazard quotients that reach a level of concern. For the longer-term drinking water scenario, the anticipated exposures are far below a level of concern. The acute scenario leads to an upper limit of hazard quotient that approaches a level of concern (i.e., 0.3 in Table 4-4) and is considered further in the risk characterization for multi-route exposures.

As in the characterization of risk for potential human health effects, both the acute and longer term exposures of a small mammal to vegetation contaminated with 2,4-D lead to hazard quotients above unity. As summarized in Table 4-3, the dose estimates for the acute exposure scenario for the small mammal are about a factor of 10 above those for the longer term exposure scenario. Since risk for the shorter-term exposure scenario is characterized by a 10-fold higher dose (i.e., an acute non-lethal dose of 10 mg/kg rather than the chronic NOAEL of 1 mg/kg/day) the resulting hazard quotients are virtually identical. The numerical risk characterizations for both scenarios are based on the assumption that 100% of the diet is contaminated. This may be a very reasonable assumption for the acute exposure scenario. It will probably be less reasonable for longer-term exposure scenarios unless the animal tends to stay in the contaminated area. Similar to the corresponding characterization of risk for human health, the likelihood of an animal consuming contaminated vegetation for a prolonged period may be limited by damage to the vegetation or an objectionable taste in the vegetation. These considerations cannot be numerically expressed with objectivity. Nonetheless, given the conservative nature of the assumption that 100% of the diet is contaminated as well as the marginal nature of the hazard quotients (i.e., 0.5-2) it seems reasonable to assume that, at least in some and perhaps most instances, actual exposures would be below and sometimes far below a level of concern. Nonetheless, if contaminated vegetation is the sole diet of the animal, some subclinical toxic effects could occur. No frank signs of toxicity, however, are likely.

All of the hazard quotients summarized in Table 4-4 apply to application rates of 1 lb a.e./acre. The relationship of these hazard quotients to application rates is linear. The reciprocal of the highest anticipated application rate, 2 lbs a.e./acre, is 0.5. Thus, hazard quotients ≥ 0.5 given in Table 4-4 would be a marginal cause for concern. The only hazard quotients approaching this level is that for the consumption of contaminated vegetation, a scenario that also lead to levels of concern at an application rate of 1 lb a.e./acre. Thus, given the relationships of application rates to levels of exposure and anticipated toxic effects, the relatively modest differences in application rates anticipated by the Forest Service ($\leq 1-2$ lbs a.e./acre) do not have a substantial impact on the characterization of risk.

Table 4-4: Summary of quantitative risk characterization for terrestrial animals.

Scenario	Dose (mg/kg/day)		
	Typical	Lower	Upper
Acute/Accidental Exposures^a			
Direct spray, small mammal, first-order absorption	0.03	0.01	0.2
Direct spray, small animal, 100% absorption	2	1	5
Direct spray, bee, 100% absorption ^b	1	0.7	3
Consumption of contaminated vegetation, acute exposure	0.5	0.2	2
Consumption of contaminated water, acute exposure	0.1	0.09	0.3
Longer Term Exposures^c			
Consumption of contaminated vegetation, 90 days	1	0.2	2
Consumption of contaminated water, longer term	0.001	0.0003	0.001

^a Hazard quotients for acute exposures of mammals are based on the estimated acute non-lethal dose of 10 mg/kg.

^b Hazard quotient for bee is based on LD₅₀ of 124 mg/kg. Upper range of LC₅₀ is 1129 mg/kg.

^c Hazard quotients for chronic mammalian exposures are calculated as the estimated exposure divided by the chronic rat NOAEL of 1 mg/kg/day and then rounded to one significant decimal or digit.

In the environment, organisms are exposed to compounds by more than one pathway. Taking the central estimates for the acute scenarios for a small mammal given in Table 4-3 results in a dose estimate of about 30.4 mg/kg [24+5+1.4] for the 20 g mammal. Taking the upper range of each scenario leads to a dose estimate of 70.8 mg/kg [49+19+2.8]. This estimated is based on the assumption that the animal is sprayed directly, that 100% of dermal dose is absorbed, that the animal eats nothing but contaminated vegetation during the day of exposure, and that the animal drinks nothing but water from a small pond after an accidental spill of 200 gallons of 2,4-D solution. These assumptions may seem absurd; they are intended, however, to characterize the likelihood of observing frank signs of toxicity during or shortly after an application. The estimated doses of 30-70 mg/kg approach the lower range of the estimated LD₅₀ of 100 mg/kg for large mammals but is well below the LD₅₀ for small mammals. Based on the categorical regression analysis, the probability that this dose would be associated with a frank toxic effect is approximately 5-15%. The only effect that might be observed is frank signs of neurotoxicity. These doses are in the range of doses that could be associated with reproductive effects but such effects would probably not be observable over a short period. The probability of adverse but

covert toxic effects such as degenerative changes in several organs (kidney, liver, and thyroid) is approximately 80%.

This multi-route worst-case exposure scenario is not proposed as a likely or typical outcome. It does, however, support a concern for potential although perhaps isolated effects on terrestrial vertebrates. The dose-response assessment on which this hazard characterization is based is most clearly relevant to mammalian species. Nevertheless, because the dose-response assessment encompasses more sensitive species (i.e., large mammals) and the exposure assessment is based on a small (20 g) mammal, the assessment is inherently conservative.

Although the data on avian species are not as extensive as those for mammals, acute toxicity studies in birds (see section 4.1.2.2.) suggest that avian species are somewhat less sensitive than mammals. In addition, the available studies on the effects of 2,4-D on avian eggs (see section 4.3.2.2.) suggest that no effects would be anticipated from a direct spray of avian eggs at application rates of up to 10 lbs/acre (Somers et al. 1978), a rate that is far in excess of those anticipated by the Forest Service (1-2 lbs/acre).

As summarized in section 4.3.2.3, studies conducted on 4 orders of insects suggest that application rates in the range of 1-2 lbs a.e./acre are not likely to be associated with insect mortality. At a 15- to 30-fold higher application rate, a significant increase in mortality has been observed in millipedes (Hoy 1985).

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

The extent of drift will depend on conditions during application, such as wind speed, wind direction, topography, the distance from the ground at which the herbicide is applied, and the droplet size of the herbicide spray. Nonetheless, as detailed in section 4.2.2, off-site deposition at 100 feet (~30 m) downwind is likely to be about 0.05 of the nominal application rate. Thus, at the high range of application contemplated by the Forest Service, 2 lbs a.e./acre, the deposition at 30 m would be ~0.1 lb a.e./acre. This application is below the application rate recommended for the control of even very sensitive broadleaf species.

Because 2,4-D is an effective herbicide, it is used to control the growth of various broadleaves and other undesirable plant species. When the growth of such plants is inhibited, secondary ecological effects occur due to changes in habitat, food supply, lighting, and other conditions (e.g., Freemark and Boutin 1995, Hurlbert 1975). Such changes are due to differences in vegetation and are not specific to 2,4-D. Similar effects would be induced if the undesirable vegetation were removed by any other herbicide or non-herbicide vegetation management practice.

4.4.3. Effects of Soil Contamination. As summarized in section 4.2.3, soil levels of 2,4-D in the upper 1 cm of the soil horizon are not expected to exceed 10 mg/kg. In the 1-8 cm soil level, concentrations should be below 1 mg/kg. As discussed in section 4.3.2.5, these levels of 2,4-D should be associated with minimal if any effects in soil fungi but may inhibit the growth of some soil algae.

4.4.4. Aquatic Organisms. As in the human health risk assessment, three sets of 2,4-D concentrations in water are used to roughly characterize exposures to aquatic species: levels associated with ambient concentrations resulting from the application of 2,4-D to a water shed, levels associated with the application of 2,4-D as Aqua-Kleen to bodies of water for the control of aquatic vegetation, and levels in water after an accidental spill. The methods and data used to estimate each of these levels are discussed in section 3.2.3.4, and are briefly summarized here.

The estimate of ambient levels is based on the monitoring study of Waite et al. (1992) in which 2,4-D was monitored in ground water, pond water, and runoff in a watershed in which a known amount of 2,4-D had been applied over a 3-year period. Based on this study, the concentration of 2,4-D that could occur in ambient water is estimated at 0.002 (0.001-0.004) mg/L per lb a.e. of 2,4-D applied per acre (see section 3.2.3.4). In that the Forest Service programs will seldom if ever involve the treatment of an entire water shed, these estimates are likely to be conservative.

The estimates of levels of 2,4-D in water after treatment with Aqua-Kleen is also based on a monitoring study (Hoeppel and Westerdahl 1983). As noted in section 3.2.3.4, concentration of 2,4-D in the water 24 hours after the application of Aqua-Kleen were essentially identical at application rates of 20 or 40 lbs a.e./acre: 0.58 (0.51-0.65) mg/L and 0.54 (0.28-0.68) mg a.e./L, respectively. For the risk characterization, these values will be rounded to 0.6 (0.5-0.7) mg/L.

The levels of 2,4-D after an accidental spill will, of course, be dependent on the amount of the spill and the volume of the body of water. For this exposure assessment as well as the corresponding assessment for human health effects, it is assumed that 200 gallons of a field solution is spilled into a small (about one-quarter acre pond) with an average depth of about 3 feet. The resulting concentration of 2,4-D in the water is estimated at 6 (4-11) mg a.e./L.

4.4.4.1. Ambient Contamination--As discussed in section 4.3.3, the aquatic macrophytes appear to be the most sensitive to concentrations of 2,4-D in water, with EC₅₀ value for the inhibition of chlorophyll levels at about 0.3 mg/L. This inhibitory concentration is about a factor of 150 above the central estimate of ambient levels of 2,4-D in watersheds that are completely treated with 2,4-D at 1 lb a.e./acre (i.e., 0.002 mg/L). This ambient concentration is also a factor of over 200 below the EC₅₀ for any fish species (i.e., 0.452 mg/L) (see Table 4-1) or aquatic invertebrate (0.440 mg/L) (see Table 4-2). Thus, even if local differences in topography, climate, or other factors were to result in an order of magnitude difference in ambient concentrations of 2,4-D in water, there is no indication that mortality in any aquatic species would be observed or plausible. The relatively modest differences in application rates likely to be used by the Forest Service (i.e., up to 2 lbs a.e./acre) are inconsequential to the risk characterization. Thus, under any foreseeable

set of conditions, no impact is anticipated in any aquatic species from the general use of 2,4-D in a watershed.

4.4.4.2. Aquatic Application--The direct application of Aqua-Kleen to bodies of water for the control of undesirable vegetation may lead to concentrations on the order of 0.5-0.7 mg/L. At this concentration, mortality might be expected in some sensitive fish or invertebrate species, particularly over the first day or two when a substantial proportion of 2,4-D could exist in the ester form. On the other hand, at least some fish species will avoid 2,4-D in water at subtoxic levels (i.e., 1 mg/L) (Folmar 1976, 1979). Thus, the presence of toxic levels of 2,4-D in treated bodies of water would not necessarily lead to fish kills or other overt toxic effects. In any event, the treated area of a lake or pond will undoubtedly evidence ecological changes associated with a change in vegetation (i.e., the destruction of the undesirable aquatic macrophytes). As with the secondary effects of vegetation management on terrestrial systems (see section 4.4.2), the extent to which these changes are regarded as adverse may be largely subjective.

4.4.4.3. Accidental Spills--The consequences of an accidental spill will depend not on the application rate but on the amount spilled into the water and the amount of water contaminated. As with other accidental exposure scenarios covered by this risk assessment, any number of more or less severe exposure scenarios could be developed. For this risk assessment, as discussed in section 3.2.3.4. and detailed in worksheet 20, it is assumed that 200 gallons of a 2,4-D solution is released into a shallow pond that has an average depth of 1 m and a surface area of about one-quarter of an acre (1000 m²). The resulting concentrations of 2,4-D in water cover a range from about 1-6 mg/L. These values are rounded from worksheet 20.

The consequences of this exposure scenario will greatly depend on the formulation of 2,4-D that is spilled and the presence or absence of emulsifying agents.

At a concentration of 1-6 mg/L, 2,4-D amine alone would not be likely to result in fish kills. Given the size of the body of water used in this scenario [1,000,000 L], 100,000,000 mg (100 million mg that is equal to 100 kg or about 220 lbs) a.e. of 2,4-D would have to be spilled in order to reach concentrations in the lower range of fish LC₅₀ values (100 mg/L). While amphibians may be more sensitive to 2,4-D than fish, the concentration of 1 mg/L from the spill scenario is a factor of 10 below the estimate of possible lethal exposures for at least some species of amphibians. Nonetheless, some mortality could be expected in some sensitive species of invertebrates and some species of aquatic plants.

In the case of a spill of a 2,4-D ester, some fish mortality might be anticipated. This would depend on specific local conditions that would influence the rate of dispersion and/or the rate of conversion of the 2,4-D ester to the free acid. Similarly, there is at least some evidence for 2,4-D that the presence of emulsifiers with 2,4-D amine will substantially enhance the toxicity of the 2,4-D amine (Sassaman et al. 1984). As with a spill of 2,4-D amine, effects on sensitive species or life stages of aquatic invertebrates as well as effects on aquatic plants would be likely.

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6. GLOSSARY

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

Active transport – An energy-expending mechanism by which a cell moves a chemical across a cell membrane from a point of lower concentration to a point of higher concentration, against the diffusion gradient.

Acid equivalent (a.e.) – The amount of active ingredient expressed in terms of the parent acid.

Active ingredient (a.i.) – An ingredient in a formulated pesticide product, which will prevent, destroy, repel, or mitigate any pest.

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

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

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

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

Adsorption coefficient (K_{oc}) – A measure of a material's tendency to adsorb to soil particles. High K_{oc} values indicate a tendency for the material to be adsorbed by soil particles rather than remain dissolved in the soil solution. Strongly adsorbed molecules will not leach or move unless the soil particle to which they are adsorbed moves (as in erosion). K_{oc} values of less than 500 indicate little or no adsorption and a potential for leaching.

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

Aerobes -- Organisms that require oxygen.

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

Anaerobes -- Organisms that do not require oxygen.

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

Aquatic invertebrates – Organisms that do not have a spinal column and live in water. These species include crayfish, mites, etc.

Aqueous – Watery; pertaining to water.

Atrophy – The wasting away or reduction in the size of a cell, tissue, or organ(s).

Bioaccumulation – The absorption, via breathing, eating drinking, or active uptake, and concentration of a substance in plants or animals.

Bioconcentration – The accumulation of a chemical in tissues of an organism (such as fish) to levels that are greater than the level in the medium (such as water) in which the organism resides; Movement of a substance such as a pesticide from the surrounding environment (abiotic) into living organisms, especially via absorption.

Bioconcentration factor (BCF) -- A measure of the tendency for a chemical to accumulate. The ratio of the concentration of a substance in a living organism (mg/kg) to the concentration of that substance in the surrounding environment (mg/L for aquatic systems).

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

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

Carcinogen -- Any substance capable of producing cancer, or a chemical that causes or induces cancer.

Carcinoma -- A malignant tumor of epithelial origin.

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

CAS No. – Chemical Abstracts Service Registry Number. The CAS No. is assigned to a specific compound and is used for cross-referencing chemical names which refer to the same compound.

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

Clay – A soil component consisting of very fine particles (<0.002 mm diameter). Clay particles provide ample surface area for the adsorption of molecules. Clay soils provide the most resistance to leaching. Soil texture and many other soil characteristics are determined by the relative amounts of sand, silt, clay and loam in a soil.

Concentration – The amount of active ingredient or pesticide equivalent in a quantity of diluent, expressed as lb/gallon, mL/L, etc.

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

Conjugate – A compound resulting from the bonding of two other compounds. Examples include glucoside conjugates formed from pesticides in plants and glucuronide conjugates formed from pesticides in animals.

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

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

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

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

Degradation – A chemical alteration to a pesticide. Chemical or biological breakdown of a complex compound into simpler compounds.

Dermal -- Of the skin: through or by the skin.

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

Dose – A measure of exposure. Dose is often expressed in milligrams per kilogram (mg/kg) or parts per million (ppm).

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

EC₅₀ -- A concentration that causes 50% inhibition or reduction. As used in this document, this value refers to a 50% inhibition of growth.

EC₁₀₀ -- A concentration that causes complete inhibition or reduction. As used in this document, this value refers to a complete inhibition of growth.

Endpoint – A biological effect used as an index of the effect of a chemical or organism.

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

Environmental fate – The destiny of a chemical after release to the environment: involves considerations such as transport through air, soil, and water, bioconcentration, degradation, volatilization, etc.

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

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

Exposure – Contact with a chemical. Some common routes of exposure are dermal (skin), oral (by mouth) and inhalation (breathing).

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

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

Fetotoxicity – A compound-induced toxic effect on the fetus during the latter phase of pregnancy.

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

Frank effects -- Obvious signs of toxicity.

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

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

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

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

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

Granular formulation – A dry, ready-to-use pesticide product that consists of an active ingredient mixed with or impregnated into a carrier, such as coarse particles of clay.

Groundwater – Water located in saturated zones below the soil surface. Many wells and springs are fed by groundwater.

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

Hazard – The potential that the use of a product would result in an adverse effect on man or the environment in a given situation.

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

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

Hematological -- Pertaining to the blood.

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

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

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

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

Histology – The study of the structure of cells and tissues; usually involves microscopic examination of tissue slices.

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

Hormone – A chemical substance secreted in one part of an organism and transported to another part of that organism where it has a specific effect.

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

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

Inert ingredient – An ingredient in a formulated pesticide product that will not prevent, destroy, repel, or mitigate any pest and which is intentionally included in the product. Includes carriers and materials that dilute the active ingredient.

Inhalation – Drawing of air into the lungs (breathing).

In vivo -- Occurring in the living organism.

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

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

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

Intraperitoneal -- Injection into the abdominal cavity.

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

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

Isomers -- Two or more chemical compounds that have the same structure but different properties.

K_{oc} -- See “Adsorption coefficient”

K_{ow} -- See “Octanol-water partition coefficient”

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

Lethal Concentration₅₀ (LC₅₀) -- A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose₅₀ (LD₅₀) -- The dose of a chemical calculated to cause death in 50% of a defined experimental animal population over a specified observation period. The observation period is typically 14 days.

Loam -- A soil of intermediate texture containing moderate amounts of sand, silt, and clay. Soil texture and many other soil characteristics are determined by the relative amounts of sand, silt, clay, and loam in a soil.

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

Malignant -- Cancerous.

Mammals -- The class of organisms that have backbones (vertebrates): includes all animals that have hair and suckle their young.

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

Metabolism -- The process of chemical change by which energy is provided in living cells.

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

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

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

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

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

Modeling – Use of mathematical equations to simulate and predict real events and processes.

Monitoring – Measuring concentrations of substances in environmental media or in human or other biological tissues.

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

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

Mutagen – An agent that causes a permanent genetic change in a cell other than that which occurs during normal genetic recombination.

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

Mutation – An alteration in genetic structure that is passed from one generation to the next.

Myeloma -- primary tumor of the bone marrow.

Myotonic -- pertaining to muscle spasms.

Neuropathy -- Damage to the peripheral nervous system.

Neurotoxicity – The ability of a substance to destroy nerve tissues or affect behavior.

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

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

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

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

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

Octanol-Water Partition Coefficient (K_{ow}) – A measure of how a chemical is distributed at equilibrium between octanol and water. It is an important parameter and is used often in the assessment of environmental fate and transport for organic chemicals. Additionally, K_{ow} is a key variable used to estimate other properties.

Ocular -- Pertaining to the eye.

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

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

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

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

Pesticide – The U.S. EPA define a pesticide as “... any substance or micture of substances intended for preventing, destroying, repelling, or mitigating any pest and any substance or mixture of substances intended for use as a plant regulator, defoliant, or dessicant.”

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

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

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

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

Potency – The measure of the relative strength of a chemical.

Potentiatio – The ability of a substance to increase the toxic effect(s) of another compound.

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

Qualitative – Descriptive of size, magnitude, or degree.

Rate – The amount of active ingredient or acid equivalent applied per unit area or other treatment unit.

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

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

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

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

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

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

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

Risk assessment – A qualitative or quantitative evaluation of the environmental and /or health risk resulting from exposure to a chemical or physical agent (pollutant); combines exposure assessment results with toxicity assessment results to estimate risk.

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

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

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

Sensitization – The development of a hypersensitive or allergic reaction upon reexposure to a substance. The reaction may be immediate or delayed and may be of short-term or chronic duration.

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

Solubility – The concentration of a substance that dissolves in a given solvent.

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

Statistically significant – Probably caused by something other than mere chance.

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

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

Surface water – Water at the soil surface in open bodies such as streams, rivers, ponds, lakes, and oceans.

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

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

Target – The target species is the organism that the pesticide is intended to control. Conversely, the non-target species are those that because they are either beneficial or harmless, are not intended to be injured or killed by the pesticide.

Terata – Birth defects.

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

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

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

Thyroid – An endocrine gland that lies in front of the trachea or wind pipe.

Tissue – A group of similar cells.

TLV (Threshold limit value) – The highest allowable air concentration of a chemical in which workers may work for many years (8 hours/day, 40 hours/week) without negative health effects. Expressed in milligrams (mg) per cubic meter of air (mg/m^3). The level to which persons may be exposed for an 8-hour workday without adverse effects.

TLV-TWA – Threshold limit value-time weighted average. The time-weighted average concentration for a normal 8-hour workday and a 40-hour work week, to which nearly all workers may be repeatedly exposed without adverse effects.

Toxic – Harmful; poisonous.

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

Translocation – Transport of a substance through a plant from the site of absorption to other parts of the plant.

TWA (Time-weighted average) – The time-weighted average concentration is the average exposure concentration based on the duration of exposure to airborne concentration as it varies during an 8-hour workday.

Uncertainty – A lack of knowledge or information. Contrasted with *variability*.

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

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

Variability – knowledge or at least an explicit assumption about how a measure or estimate may change or be distributed in a population.

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

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

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

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

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APPENDICES

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

Appendix 2. Mutagenicity of 2,4-D.

Appendix 3. Subchronic toxicity and neurotoxicity of 2,4-D to mammals and chickens.

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

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 n: 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. Reproductive effects and teratogenicity of 2,4-D to mammals

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 at 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.
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

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

Reference	Type of 2,4-D	Species	Exposure/ Duration	Effects
Nemec et al. 1983	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
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

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

Reference	Type of 2,4-D	Species	Exposure/ Duration	Effects
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	isooctyl 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	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

Appendix 2. Mutagenicity of 2,4-D

Assay	Exposure Dose	Response ^a	With or Without Metabolic Activation ^b	Reference
<i>In vivo</i> micronucleus test in mouse bone marrow erythro- poietic cells with Lilly/Miller Envy 2,4-D	20 mg/kg	–	–	SanSebastian 1994 (MRID No. 43374801)
	100 mg/kg	–	–	
	200 mg/kg	–	–	
Ames/ <i>Salmonella</i> plate incorporation (<i>Salmonella</i> <i>typhimurium</i> TA1535, TA1537, TA1538, TA98, and TA100) with Lilly/Miller Envy 2,4-D	50 µg/plate	–	±	Stankowski 1994a (MRID No. 43418101)
	167 µg/plate	–	±	
	500 µg/plate	–	±	
	1670 µg/plate	–	±	
	5000 µg/plate	–	±	
AS52/XPRT mammalian cell forward gene mutation in Chinese hamster ovary cells with Lily/Miller Envy 2,4-D	10 µg/mL	–	±	Stankowski 1994b (MRID No. 43429801)
	50 µg/mL	–	±	
	100 µg/mL	+	±	
	500 µg/mL	+	–	
	1670 µg/mL	+	–	
	1830 µg/mL	+	–	
	2000 µg/mL	+	±	
	2500 µg/mL	+	±	
	3000 µg/mL	+	±	
	3500 µg/mL	+	±	
4000 µg/mL	+	±		
AS52/XPRT mammalian cell forward gene mutation in Chinese hamster ovary cells with Lily/Miller Envy 2,4-D	50 µg/mL	+	+	Stankowski 1994b (MRID No. 43429801)
	100 µg/mL	+	+	
	500 µg/mL	+	+	
	1670 µg/mL	+	+	
	1830 µg/mL	+	+	
	2000 µg/mL	+	+	
	2250 µg/mL	+	+	
	2500 µg/mL	+	+	
	2750 µg/mL	+	+	
	3000 µg/mL	+	+	
3500 µg/mL	+	+		
4000 µg/mL	+	+		

^a“–” = negative response; “+” = positive response

^b“–” = without metabolic activation; “+” = with metabolic activation; “±” = with or without metabolic activation

Appendix 3. Subchronic toxicity and neurotoxicity of 2,4-D to mammals and chickens

Reference	2,4-D Species	Animal Species/ Strain/Sex	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 cerebroside 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.
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

Appendix 3. Subchronic toxicity and neurotoxicity of 2,4-D to mammals and chickens

Reference	2,4-D Species	Animal Species/ Strain/Sex	Exposure/ Duration	Effects
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 intraperitoneally 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
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 intraperitoneally 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 concentrations in most other tissues; liver and kidney showed the greatest accumulation of 2,4-D, but levels in the kidney were decreased by pretreatment.
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 3. Subchronic toxicity and neurotoxicity of 2,4-D to mammals and chickens

Reference	2,4-D Species	Animal Species/ Strain/Sex	Exposure/ Duration	Effects
Elo and Ylitalo 1979	radiolabeled 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 the brain and cerebrospinal fluid were 7-fold and 22-fold greater, compared with concentrations reported for rats that were not pretreated with unlabeled 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 dimethylamine (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 dimethylamine (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-hydroxyindoleacetic 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 dimethylamine (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-hydroxyindoleacetic acid in the brain stem but had no effect on serotonin levels

Appendix 3. Subchronic toxicity and neurotoxicity of 2,4-D to mammals and chickens

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
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 injection 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

WORKSHEETS FOR

2,4-D

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GENERAL ASSUMPTIONS, VALUES, and MODELS

Worksheet 01: Constants and conversion factors used in calculations [CONST]		
Conversion	ID	Value
mg/lb	mg_lb	453,600
mL/gallon	ml_gal	3,785
lb/gallon to mg/mL	lbg_mgml	119.8
lb/acre to $\mu\text{g}/\text{cm}^2$	lbac_ugcm	11.21
lb/acre to mg/cm^2	lbac_mgcm	0.01121
gallons to liters	gal_lit	3.785

Worksheet 02: General Assumptions Used in Worker Exposure Assessments [STD]				
Parameter	Code	Value	Units	Reference
Body Weight (General)	BW	70	kg	ICRP (1975), p. 13
Surface area of hands	Hands	840	cm^2	U.S. EPA 1992a
Surface area of lower legs	LLegs	2070	cm^2	U.S. EPA 1992a
Weight of liquid adhering to surface of skin after a spill	Liq	0.008	mg/cm^2	Mason and Johnson 1987

Worksheet 03a: Directed Ground Sprays (includes backpack, cut surface, and streamline applications) - General Assumptions Used in Worker Exposure Assessments [BACKPACK]

Parameter/Assumption	Code	Value	Units	Reference
Hours of application per day				
Central estimate		7	hours	USDA/FS 1989a,b,c
Lower estimate		6		
Upper estimate		8		
Acres treated per hour				
Central estimate		0.625	acres/hour	USDA/FS 1989a,b,c
Lower estimate		0.25		
Upper estimate		1		
Acres treated per day				
Central estimate	ACREC	4.375	acres/day	N/A ¹
Lower estimate	ACREL	1.5		
Upper estimate	ACREU	8		
Absorbed dose rate				
Central estimate	RATEC	0.003	(mg agent/kg bw) ÷ (lbs agent handled per day)	Rubin et al. 1998, Table 5
Lower estimate	RATEL	0.0003		
Upper estimate	RATEU	0.01		
<p>¹ Calculated as the product of the number of hours of application and the number of acres treated per hour for each category - i.e., central estimate, lower estimate, and upper estimate.</p> <p>² “Agent” refers to the material being handled and may be expressed in units of a.i. or a.e. Depending on the agent under consideration, additional exposure conversions may be made in the exposure assessment and dose response assessment. For the risk assessment, the only important point is that the exposure and dose/response assessments must use the same units - that is, a.i., a.e., etc. - or the units must be converted to some equivalent form in the risk characterization.</p>				

Worksheet 03b: Hydraulic/Broadcast Ground Sprays - General Assumptions Used in Worker Exposure Assessments [HYDSPRAY]

Parameter/Assumption	Code	Value	Units	Reference
Hours of application per day				
Central estimate		7	hours	USDA/FS 1989a,b,c
Lower estimate		6		
Upper estimate		8		
Acres treated per hour				
Central estimate		16	acres/hour	USDA/FS 1989a,b,c
Lower estimate		11		
Upper estimate		21		
Acres treated per day				
Central estimate	ACREC	112	acres/day	N/A ¹
Lower estimate	ACREL	66		
Upper estimate	ACREU	168		
Absorbed dose rate				
Central estimate	RATEC	0.0002	(mg agent/kg bw) ÷ (lbs agent handled per day) ²	Rubin et al. 1988, Table 5
Lower estimate	RATEL	0.00001		
Upper estimate	RATEU	0.0009		
<p>¹ Calculated as the product of the number of hours of application and the number of acres treated per hour for each category - i.e., central estimate, lower estimate, and upper estimate.</p> <p>² “Agent” refers to the material being handled and may be expressed in units of a.i. or a.e. Depending on the agent under consideration, additional exposure conversions may be made in the exposure assessment and dose response assessment. For the risk assessment, the only important point is that the exposure and dose/response assessments must use the same units - that is, a.i., a.e., etc. - or the units must be converted to some equivalent form in the risk characterization.</p>				

Worksheet 03c: Aerial Broadcast Sprays (includes pilots, mixers, and loaders) - General Assumptions Used in Worker Exposure Assessments. [AERIAL]

Parameter/Assumption	Code	Value	Units	Reference
Hours of application per day				
Central estimate		7	hours	USDA/FS 1989a,b,c
Lower estimate		6		
Upper estimate		8		
Acres treated per hour				
Central estimate		70	acres/hour	USDA/FS 1989a,b,c
Lower estimate		40		
Upper estimate		100		
Acres treated per day				
Central estimate	ACREC	490	acres/day	N/A ¹
Lower estimate	ACREL	240		
Upper estimate	ACREU	800		
Absorbed dose rate				
Central estimate	RATEC	0.00003	(mg agent/kg bw) ÷ (lbs agent handled per day)	Rubin et al. 1998, Table 5
Lower estimate	RATEL	0.000001		
Upper estimate	RATEU	0.0001		
<p>¹ Calculated as the product of the number of hours of application and the number of acres treated per hour for each category - i.e., central estimate, lower estimate, and upper estimate.</p> <p>² “Agent” refers to the material being handled and may be expressed in units of a.i. or a.e. Depending on the agent under consideration, additional exposure conversions may be made in the exposure assessment and dose response assessment. For the risk assessment, the only important point is that the exposure and dose/response assessments must use the same units - that is, a.i., a.e., etc. - or the units must be converted to some equivalent form in the risk characterization.</p>				

Worksheet 04: General Assumptions Used in Exposure Assessments for the General Public
[PUBL]

Narrative: This table contains various values used in the exposure assessments for the general public. Three general groups of individuals are considered: adult male, adult female, and a 2 year old child. Values are specified for body weight, surface areas for various parts of the body, water intake, fish consumption, and the consumption of fruits or vegetables. Not all types of value are specified for each group. The only values specified are those generally used in the risk assessment.

Description	ID	Value	Units	Reference
Body Weights				
Male, Adult	BWM	70	kg	ICRP (1975), p. 13.
Female, Adult	BWF	64	kg	Burnmaster 1998; U.S. EPA 1985 ¹
Child, 2-3 years old	BWC	13.3	kg	U.S. EPA, 1996, page 7-1, Table 7-2
Body Surface Areas				
Female, feet and lower legs	SAF1	2915	cm ²	U.S. EPA, 1992a, p. 8-11, Table 8-3, total for feet and lower legs
Female, exposed skin when wearing shorts and a T-shirt	SAF2	5300	cm ²	U.S. EPA, 1992a, p. 8-11, Table 8-3, total for arms, hands, lower legs, and feet.
Child, male, 2-3 years old, total body surface area	SAC	6030	cm ²	U.S. EPA, 1996, p. 6-15, Table 6-6, 50 th percentile.

Worksheet 04: General Assumptions Used in Exposure Assessments for the General Public
[PUBL]

Narrative: This table contains various values used in the exposure assessments for the general public. Three general groups of individuals are considered: adult male, adult female, and a 2 year old child. Values are specified for body weight, surface areas for various parts of the body, water intake, fish consumption, and the consumption of fruits or vegetables. Not all types of value are specified for each group. The only values specified are those generally used in the risk assessment.

Description	ID	Value	Units	Reference
Water Intake				
Adult				
typical	WCAT	2	L/day	U.S. EPA, 1996, p. 3-28, Table 3-30, midpoint of mean (1.4 L/day and 90 th percentile (2.4 L/day) rounded to one significant place.
lower range for exposure assessment	WCAL	1.4	L/day	U.S. EPA, 1996, p. 3-28, Table 3-30, mean
upper range	WCAH	2.4	L/day	U.S. EPA, 1996, p. 3-28, Table 3-30, 90 th percentile
Child, <3 years old				
typical	WCT	1	L/day	U.S. EPA, 1996, p. 3-28, Table 3-30, midpoint of mean (0.61L/day and 90 th percentile (1.5 L/day) rounded to one significant place.
lower range for exposure assessment	WCL	0.61	L/day	U.S. EPA, 1996, p. 3-28, Table 3-30, mean
upper range	WCH	1.50	L/day	U.S. EPA, 1996, p. 3-28, Table 3-30, 90 th percentile
Fish Consumption				
Freshwater anglers, typical intake per day over a prolonged period	FAT	0.010	kg/day	U.S. EPA, 1996, p. 10-51, average of means from four studies
Freshwater anglers, maximum consumption for a single day	FAU	0.158	kg/day	Ruffle et al. 1994
Native American subsistence populations, typical intake per day	FNT	0.081	kg/day	U.S. EPA, 1996, p. 10-51, median value of 94 individuals
Native American subsistence populations, maximum for a single day	FNU	0.770	kg/day	U.S. EPA, 1996, p. 10-51, highest value of 94 individuals

Worksheet 04: General Assumptions Used in Exposure Assessments for the General Public
[PUBL]

Narrative: This table contains various values used in the exposure assessments for the general public. Three general groups of individuals are considered: adult male, adult female, and a 2 year old child. Values are specified for body weight, surface areas for various parts of the body, water intake, fish consumption, and the consumption of fruits or vegetables. Not all types of value are specified for each group. The only values specified are those generally used in the risk assessment.

Description	ID	Value	Units	Reference
Consumption of Fruits or Vegetables				
Amount of food consumed per kg bw per day for longer term exposures scenarios. <i>[Note that these are in units of g food/kg bw/day. Separate sex or age specific values in units of g food/person/day have not been developed.]</i>				
Typical	VT	0.0043	kg food/kg bw/day	U.S. EPA, 1996, Table 9-21, p. 9-39, mean intake of vegetables
Upper	VU	0.01	kg food/kg bw/day	U.S. EPA, 1996, Table 9-21, p. 9-39, 95 th percentile for intake of vegetables
Worst-case scenario for consumption in a single day, acute exposure scenario only.	VAcute	0.454	kg food	1 lb. The approximate mid range of the above typical and upper limits based on the 64 kg body weight.
Miscellaneous				
Estimate of dislodgeable residue as a proportion of application rate shortly after application.	DisL	0.1	none	Harris and Solomon 1992, data on 2,4-D
¹ This is the average value (63.79 kg), rounded to the nearest kg for 3 different groups of women between 15-49 year old: control (62.07 kg), pregnant (65.90 kg), and lactating (63.48 kg). See Burnmaster 1998, p.218, Table III., Risk Analysis. 18(2): 215-219. This is identical to the body weight for females, 45-55 years old, 50 th percentile from U.S. EPA, 1985, page 5, Table 2-2, rounded to nearest kilogram.				

Worksheet 05a: Estimated concentrations of pesticides on or in various types of vegetation shortly after application at 1 lb a.i./acre [from Hoerger and Kenaga (1972), Table 9, p. 22]. [HK]

Type of Vegetation	Concentration (mg chemical/kg vegetation)			
	Typical		Upper Limit	
	ID	Value	ID	Value
Range grass	RGT	125	RGU	240
Grass	GST	92	GSU	110
Leaves and leafy crops	LVT	35	LVU	125
Forage crops	FCT	33	FCU	58
Pods containing seeds	PDT	3	PDU	12
Grain	GNT	3	GNU	10
Fruit	FRT	1.5	FRU	7

Worksheet 05b: Concentrations of chemical on spheres (berries) at the specified application rate. [FRUIT]

Diameter (cm)	Planar Surface Area (<i>PA</i> in cm ²) ^a	Amount deposited (<i>A</i> in mg) ^b	Weight of sphere in kg ^c	Concentration (C) in mg/kg ^d
1	0.7853981634	0.008796459	0.0005236	16.8
5	19.6349540849	0.21991148575	0.065449847	3.36
10	78.5398163397	0.87964594301	0.5235987756	1.68
Application rate		1 lb/acre =	0.0112	mg/cm ²

- a Planar surface area of a sphere = $\pi r^2 = A$ (cm²) where r is the radius in cm.
- b Amount deposited is calculated as the application rate in mg/cm² multiplies by the planar surface area.
- c Assumes a density of 1 g/cm³ for the fruit. The volume of a sphere is $(\pi/6) \times d^3$ where d is the diameter in cm. Assuming a density of 1 g/cm³, the weight of the sphere in kg is equal to:
- $$\text{kg} = (\pi/6) \times d^3 \div 1000$$
- d Amount of chemical in mg divided by the weight of the sphere in kg.

Worksheet 06: Central estimates of off-site drift associated with aerial application of pesticides (from Bird 1995, p. 205) [OFFSITE]

Distance Down Wind ()	ID	Drift as a proportion of application rate
100	DRFT100	0.05
200	DRFT200	0.02
300	DRFT300	0.01
400	DRFT400	0.008

Worksheet 07a: Estimate of first-order absorption rate (k_a in hours⁻¹) and 95% confidence intervals (from Durkin et al. 1998). [KAMODEL]

Model parameters	ID	Value	
Coefficient for $k_{o/w}$	C_KOW	0.233255	
Coefficient for MW	C_MW	0.005657	
Model Constant	WS01	1.49615	
Number of data points	DP	29	
Degrees of Freedom (d.f.)	DF	26	
Critical value of $t_{0.025}$ with 26 d.f. ¹	CRIT	2.056	
Standard error of the estimate	SEE		
Mean square error or model variance	MDLV	0	
Standard deviation of model (s)	MSD	0	MDLV ^{0.5}
X'X, cross products matrix		0.307537	0.00822769
		-0.00103089	-0.0000944359
		0.0082	0.0085286

¹ Mendenhall and Scheaffer, 1973, Appendix 3, 4, p. A31.

Central (maximum likelihood) estimate:

$$\log_{10} k_a = 0.233255 \log_{10}(k_{o/w}) - 0.005657 \text{ MW} - 1.49615$$

95% Confidence intervals for $\log_{10} k_a$

$$\log_{10} k_a \pm t_{0.025} \times s \times (\mathbf{a}'\mathbf{X}'\mathbf{X}\mathbf{a})^{0.5}$$

where \mathbf{a} is a column vector of {1, MW, $\log_{10}(k_{o/w})$ }.

NB: Although the equation for the central estimate is presented with $k_{o/w}$ appearing before MW to be consistent with the way a similar equation is presented by EPA, MW must appear first in column vector \mathbf{a} because of the way the statistical analysis was conducted to derive $\mathbf{X}'\mathbf{X}$.

See following page for details of calculating $\mathbf{a}'\mathbf{X}'\mathbf{X}\mathbf{a}$ without using matrix arithmetic.

Worksheet Worksheet 07a (continued)
Details of calculating $\mathbf{a}'\mathbf{X}'\mathbf{X}\mathbf{a}$

The term $\mathbf{a}'\cdot(\mathbf{X}'\mathbf{X})^{-1}\cdot\mathbf{a}$ requires matrix multiplication. While this is most easily accomplished using a program that does matrix arithmetic, the calculation can be done with a standard calculator.

Letting

$$\mathbf{a} = \{a_1, a_2, a_3\}$$

and

$$(\mathbf{X}'\mathbf{X})^{-1} = \begin{Bmatrix} \{b_1, b_2, b_3\}, \\ \{c_1, c_2, c_3\}, \\ \{d_1, d_2, d_3\} \\ \} \end{Bmatrix}$$

$\mathbf{a}'\cdot(\mathbf{X}'\mathbf{X})^{-1}\cdot\mathbf{a}$ is equal to

$$\begin{aligned} \text{Term 1: } & \{a_1 \times ([a_1 \times b_1] + [a_2 \times c_1] + [a_3 \times d_1])\} + \\ \text{Term 2: } & \{a_2 \times ([a_1 \times b_2] + [a_2 \times c_2] + [a_3 \times d_2])\} + \\ \text{Term 3: } & \{a_3 \times ([a_1 \times b_3] + [a_2 \times c_3] + [a_3 \times d_3])\}. \end{aligned}$$

Worksheet 07b: Estimate of dermal permeability (K_p in cm/hr) and 95% confidence intervals (data from U.S. EPA 1992). [PKMODEL]

Model parameters	ID	Value	
Coefficient for $k_{o/w}$	C_KOW	0.706648	
Coefficient for MW	C_MW	0.006151	
Model Constant	WS01	2.72576	
Number of data points	DP	90	
Degrees of Freedom (d.f.)	DF	87	
Critical value of $t_{0.025}$ with 87 d.f. ¹	CRIT	1.96	
Standard error of the estimate	SEE	45.9983	
Mean square error or model variance	MDLV	0.528716	
Standard deviation of model (s)	MSD	0.727129	MDLV ^{0.5}
X'X, cross products matrix		0.0550931	-0.0000941546
		-0.0000941546	0.0000005978
		-0.0103443	-0.0000222508
		-0.0103443	0.00740677

¹ Mendenhall and Scheaffer, 1973, Appendix 3, Table 4, p. A31.

NOTE: The data for this analysis is taken from U.S. EPA (1992, Dermal Exposure Assessment: Principles and Applications, EPA/600/8-91/011B, Table 5-4, pp. 5-15 through 5-19). The EPA report, however, does not provide sufficient information for the calculation of confidence intervals. The synopsis of the above analysis was conducted in STATGRAPHICS Plus for Windows, Version 3.1 (Manugistics, 1995) as well as Mathematica, Version 3.0.1.1 (Wolfram Research, 1997). Although not explicitly stated in the EPA report, 3 of the 93 data points are censored from the analysis because they are statistical outliers: [Hydrocortisone-21-yl]-hemipimelate, n-nonanol, and n-propanol. The model parameters reported above are consistent with those reported by U.S. EPA but are carried out to greater number of decimal places to reduce rounding errors when calculating the confidence intervals. See notes to Worksheet 08 for details of calculating maximum likelihood estimates and confidence intervals.

CHEMICAL SPECIFIC VALUES

Worksheet 08: Anticipated Application Rates of 2,4-D and the Concentrations of 2,4-D in Acid and Ester Formulations [APPL]

Item	Code	Value	Units	Reference/Source
Typical application rate	Typ	1	lb a.e./acre	Table 2-3
Lowest application rate	Low	0.5	lb a.e./acre	Table 2-3
Highest application rate	Hi	2.0	lb a.e./acre	Table 2-3
Concentration in solution, amine	ConcAM	455	mg a.e./mL	Table 2-1
Concentration in solutions, ester	ConcEst	670	mg a.e./mL	Table 2-1

Recommended Field Dilutions					
Acid	Lower	LDilA	5	gal./acre	CPR, 1997, p. 1940
	Typical	TDilA	20	gal./acre	CPR, 1997, p. 1940
	Higher	HDilA	100	gal./acre	CPR, 1997, p. 1940
Ester	Lower	LDilE	8	gal./acre	CPR, 1997, p. 1227
	Typical	TDilE	16	gal./acre	CPR, 1997, p. 1227
	Higher	HDilE	25	gal./acre	CPR, 1997, p. 1227

Worksheet 08 (Continued)

Calculations of concentrations in Field Solutions.

Acid Formulations

Typical concentration in applied solution:

Typical application rate divided by the typical dilution, converted to mg/mL, and rounded to two significant places after the decimal.

$$1 \text{ lb/acre} \div 20 \text{ gal/acre} \times 119.8 \text{ (mg/mL)/(lb/gal)} = 5.99 \text{ mg/mL [TypDrA]}$$

Lower range of concentration in applied solution:

Typical application rate divided by the upper dilution, converted to mg/mL, and rounded to two significant places after the decimal.

$$1 \text{ lb/acre} \div 100 \text{ gal/acre} \times 119.8 \text{ (mg/mL)/(lb/gal)} = 1.2 \text{ mg/mL [LowDrA]}$$

Upper range of concentration in applied solution:

Typical application rate divided by the lower dilution, converted to mg/mL, and rounded to two significant places after the decimal.

$$1 \text{ lb/acre} \div 5 \text{ gal/acre} \times 119.8 \text{ (mg/mL)/(lb/gal)} = 23.96 \text{ mg/mL [HI_DrA]}$$

Ester Formulations

Typical concentration in applied solution:

Typical application rate divided by the typical dilution, converted to mg/mL, and rounded to two significant places after the decimal.

$$1 \text{ lb/acre} \div 16 \text{ gal/acre} \times 119.8 \text{ (mg/mL)/(lb/gal)} = 7.49 \text{ mg/mL [TypDrE]}$$

Lower range of concentration in applied solution:

Typical application rate divided by the upper dilution, converted to mg/mL, and rounded to two significant places after the decimal.

$$1 \text{ lb/acre} \div 25 \text{ gal/acre} \times 119.8 \text{ (mg/mL)/(lb/gal)} = 4.79 \text{ mg/mL [LowDrE]}$$

Upper range of concentration in applied solution:

Typical application rate divided by the lower dilution, converted to mg/mL, and rounded to two significant places after the decimal.

$$1 \text{ lb/acre} \div 8 \text{ gal/acre} \times 119.8 \text{ (mg/mL)/(lb/gal)} = 14.98 \text{ mg/mL [HI_DrE]}$$

Worksheet 09: Chemical specific values used for 2,4-D in exposure assessment worksheets.
[CHEM]

Parameter	ID	Value	Units	Source/Reference
Molecular weight, acid	MW	221	grams/mole	Table 2-3
Water Solubility, pH 7	WS	23108	mg/L	Table 2-3
K_{ow} , pH 7	K_{ow}	0.177	unitless	Table 2-3
Foliar half-time ($t_{1/2}$)	FT12	14	days	Section 3.2.3.6
Bioconcentration factor, Lower range ($BCF_{(kg\ fish/L)}$)	BCFL	10	kg fish/L	Section 3.2.3.5.
Bioconcentration factor, Upper range ($BCF_{(kg\ fish/L)}$)	BCFU	40	kg fish/L	Section 3.2.3.5.
Conc. in ambient water per lb a.i./acre	AMBWAT	0.002	mg/L per lb a.e. applied	Section 3.2.3.4
U.S. EPA RfD	RfDP	0.01	mg/kg bw	Section 3.3.3

Worksheet 10: Calculation of dermal permeability rate (K_p) in cm/hour for 2,4-D, acid. [KP_CHEM]							
Parameters	Value	Units			Reference		
Molecular weight	221	g/mole					
$K_{o/w}$ at pH 7	0.177	unitless					
$\log_{10} K_{o/w}$	-0.75202673364						
Column vector \mathbf{a} for calculating confidence intervals (see Worksheet 08 for definitions.)							
a_1	1						
a_2	221						
a_3	-0.75202673364						
Calculation of $\mathbf{a}' \cdot (\mathbf{X}'\mathbf{X})^{-1} \cdot \mathbf{a}$ - see Worksheet 07b for details of calculation.							
Term 1	0.0420641235						
Term 2	0.0120870196						
Term 3	0.0156660824						
$\mathbf{a}' \cdot (\mathbf{X}'\mathbf{X})^{-1} \cdot \mathbf{a}$	0.0698						
$\log_{10} k_a = 0.706648 \log_{10}(k_{o/w}) - 0.006151 MW - 2.72576$					Worksheet 07b		
\log_{10} of K_p							
Central estimate	-4.61654918727	\pm	$t_{0.025}$	\times	s	\times	$\mathbf{a}' \cdot (\mathbf{X}'\mathbf{X})^{-1} \cdot \mathbf{a}^{0.5}$
Lower limit	-4.99307542825	-	1.9600	\times	0.727129	\times	0.2641968963
Upper limit	-4.24002294629	+	1.9600	\times	0.727129	\times	0.2641968963
K_p							
Central estimate	0.0000242	cm/hour					
Lower limit	0.0000102	cm/hour					
Upper limit	0.0000575	cm/hour					

Worksheet 11: Summary of chemical specific dermal absorption values used for 2,4-D dermal absorption. [DERM]				
Description	Code	Value	Units	Reference/Source
Zero-order absorption (K_p)				
Central estimate	K_{pC}	0.0000242	cm/hour	Worksheet 10, values rounded to two significant figures
Lower limit	K_{pL}	0.0000102	cm/hour	
Upper limit	K_{pU}	0.000058	cm/hour	
First-order absorption rates (k_a)				
Central estimate	$AbsC$	0.0005	hour ⁻¹	Section 3.1.8.
Lower limit	$AbsL$	0.00017	hour ⁻¹	
Upper limit	$AbsU$	0.0026	hour ⁻¹	

WORKER EXPOSURE ASSESSMENTS

Worksheet 12a: Worker exposure estimates for directed foliar (backpack) applications of 2,4-D [WKBKEXP01]				
NOTE: The upper and lower estimates of dose are based on the typical application rate. Variability is encompassed by differences in the number of acres treated and the absorbed dose rate.				
Parameter/Assumption	Code	Value	Units	Source/Designation
Application rates				
Central estimate	APPLC	1	lbs a.i./day	WS10.TYP
Lower estimate	APPLL	0.5	lbs a.i./day	WS10.LOW
Upper estimate	APPLU	2	lbs a.i./day	WS10.HI
Acres treated per day				
Central estimate	ACREC	4.375	acres/day	WS03.ACREC
Lower estimate	ACREL	1.5	acres/day	WS03.ACREL
Upper estimate	ACREU	8	acres/day	WS03.ACREU
Amount handled per day (product of application rate and acres treated per day)				
Central estimate	HANDLC	4.375	lb/day	N/A ¹
Lower estimate	HANDLL	1.5	lb/day	
Upper estimate	HANDLU	8	lb/day	
Absorbed dose rate				
Central estimate	RATEC	0.003	(mg agent/kg bw) ÷ (lbs agent handled per day) ²	WS03.RATEC
Lower estimate	RATEL	0.0003		WS03.RATEL
Upper estimate	RATEU	0.01		WS03.RATEU
Absorbed dose (product of amount handled and absorbed dose rate)				
Central estimate	DOSEC	0.01313	mg/kg bw	N/A
Lower estimate	DOSEL	0.000450		
Upper estimate	DOSEU	0.08		
<p>¹ Calculated as the product of the number of hours of application and the number of acres treated per hour for each category - i.e., central estimate, lower estimate, and upper estimate.</p> <p>² “Agent” refers to the material being handled and may be expressed in units of a.i. or a.e. Depending on the agent under consideration, additional exposure conversions may be made in the exposure assessment and dose response assessment. For the risk assessment, the only important point is that the exposure and dose/response assessments must use the same units - that is, a.i., a.e., etc. - or the units must be converted to some equivalent form in the risk characterization.</p>				

Worksheet 12b: Worker exposure estimates for boom spray (hydraulic ground spray) applications of 2,4-D [WKHYEXP01]

NOTE: The central, upper, and lower estimates of dose are based on the typical application rate. Variability is encompassed by differences in the number of acres treated and the absorbed dose rate.

Parameter/Assumption	Code	Value	Units	Source/Designation
Application rates				
Central estimate	WS10C	1	lbs a.i./day	APPL.TYP
Lower estimate	WS10L	0.5	lbs a.i./day	APPL.LOW
Upper estimate	WS10U	2	lbs a.i./day	APPL.HI
Acres treated per day				
Central estimate	ACREC	112	acres/day	HYDSPRAY.ACRC
Lower estimate	ACREL	66	acres/day	HYDSPRAY.ACRL
Upper estimate	ACREU	168	acres/day	HYDSPRAY.ACRCU
Amount handled per day (product of application rate and acres treated per day)				
Central estimate	HANDLC	112	lb/day	N/A ¹
Lower estimate	HANDLL	66	lb/day	
Upper estimate	HANDLU	168	lb/day	
Absorbed dose rate				
Central estimate	RATEC	0.00020	(mg agent/kg bw) ÷ (lbs agent handled per day)	HYDSPRAY.RATEC
Lower estimate	RATEL	0.00001		HYDSPRAY.RATEL
Upper estimate	RATEU	0.00090		HYDSPRAY.RATEU
Absorbed dose (product of amount handled and absorbed dose rate)				
Central estimate	DOSEC	0.0224	mg/kg bw	N/A
Lower estimate	DOSEL	0.000660		
Upper estimate	DOSEU	0.1512		

¹ Calculated as the product of the number of hours of application and the number of acres treated per hour for each category - i.e., central estimate, lower estimate, and upper estimate.

² “Agent” refers to the material being handled and may be expressed in units of a.i. or a.e. Depending on the agent under consideration, additional exposure conversions may be made in the exposure assessment and dose response assessment. For the risk assessment, the only important point is that the exposure and dose/response assessments must use the same units - that is, a.i., a.e., etc. - or the units must be converted to some equivalent form in the risk characterization.

Worksheet 12c: Worker exposure estimates for aerial applications of 2,4-D [WKAREXP01]

NOTE: The upper and lower estimates of dose are based on the typical application rate. Variability is encompassed by differences in the number of acres treated and the absorbed dose rate.

Parameter/Assumption	Code	Value	Units	Source/Designation
Application rates				
Central estimate	WS10C	1	lbs a.i./day	APPL.TYP
Lower estimate	WS10L	0.5	lbs a.i./day	APPL.LOW
Upper estimate	WS10U	2	lbs a.i./day	APPL.HI
Acres treated per day				
Central estimate	ACREC	490	acres/day	AERIAL.ACREC
Lower estimate	ACREL	240	acres/day	AERIAL.ACREL
Upper estimate	ACREU	800	acres/day	AERIAL.ACREU
Amount handled per day (product of application rate and acres treated per day)				
Central estimate	HANDLC	490	lb/day	N/A ¹
Lower estimate	HANDLL	240	lb/day	
Upper estimate	HANDLU	800	lb/day	
Absorbed dose rate				
Central estimate	RATEC	0.00003	(mg agent/kg bw) ÷ (lbs agent handled per day) ²	AERIAL.RATEC
Lower estimate	RATEL	0.000001		AERIAL.RATEL
Upper estimate	RATEU	0		AERIAL.RATEU
Absorbed dose (product of amount handled and absorbed dose rate)				
Central estimate	DOSEC	0.0147	mg/kg bw	N/A
Lower estimate	DOSEL	0.00024		
Upper estimate	DOSEU	0.08		

¹ Calculated as the product of the number of hours of application and the number of acres treated per hour for each category - i.e., central estimate, lower estimate, and upper estimate.

² “Agent” refers to the material being handled and may be expressed in units of a.i. or a.e. Depending on the agent under consideration, additional exposure conversions may be made in the exposure assessment and dose response assessment. For the risk assessment, the only important point is that the exposure and dose/response assessments must use the same units - that is, a.i., a.e., etc. - or the units must be converted to some equivalent form in the risk characterization.

Worksheet 12d: Analysis of worker exposure rates in the aquatic application of 2,4-D [NIGGSTAMP]

Study Description: Nigg and Stamper (1983) monitored the exposure of four workers in the application of a liquid formulation of 2,4-D amine for the control of water hyacinths using airboat handguns. Each worker applied 3 to 4 tanks, 50 gallons/tank, of a 2,4-D solution containing 0.0046 kg a.i./L or 0.0038 kg a.e./L (0.0046×0.861). Taking 3.5 tanks as the average, each worker thus handled 2.52 kg a.e. 2,4-D [3.5 tanks × 50 gallons/tank × 3.785 L/gallon × 0.0038 kg a.e./L] or **5.6 lbs a.e. 2,4-D** [2.52 kg × 2.2046 lbs/kg]. Absorbed dose was monitored as total urinary elimination of 2,4-D over a 24 hour period.

	Worker 1	Worker 2	Worker 3	Worker 4
Body Weight (kg) ^a	70.4	74.9	68.1	74.9
Mean 24 hour urinary 2,4-D (mg) ^b	0.190	0.645	0.315	0.402
Dose rate (mg/kg bw) ^c	0.0027	0.0086	0.0046	0.0054
Occupational exposure rate (mg/kg bw per lb a.e. applied) ^d	0.00048	0.00154	0.00082	0.00096
Average Exposure rate (95% C.I.) ⁵		9×10 ⁻⁴ (4×10 ⁻⁴ -2×10 ⁻³)		

^a Nigg and Stamper (1983), Table 1, p. 209.

^b Nigg and Stamper (1983), Table 4, p. 213.

^c Mean 24 hour 2,4-D in mg divided by body weight in kg.

^d Dose rate divided by 5.6 lbs a.e. See description of study.

⁵ Based on log-normal distribution.

Worksheet 12e: Worker exposure estimates for aquatic applications of 2,4-D [WKAQEXP01]

NOTE: The upper and lower estimates of dose are based on the typical application rate. Variability is encompassed by differences in the number of acres treated and the absorbed dose rate.

Parameter/Assumption	Code	Value	Units	Source/Designation
Application rates				
Central estimate	WS10C	19	lbs a.e./day	Section 3.2.2.1.
Lower estimate	WS10L	1	lbs a.e./day	Section 3.2.2.1.
Upper estimate	WS10U	1	lbs a.e./day	Section 3.2.2.1.
Acres treated per day				
Central estimate	ACREC	1	acres/day	Section 3.2.2.1.
Lower estimate	ACREL	1	acres/day	Section 3.2.2.1.
Upper estimate	ACREU	1	acres/day	Section 3.2.2.1.
Amount handled per day (product of application rate and acres treated per day)				
Central estimate	HANDLC	19	lb/day	N/A ¹
Lower estimate	HANDLL	19	lb/day	
Upper estimate	HANDLU	19	lb/day	
Absorbed dose rate				
Central estimate	RATEC	0.0009	(mg agent/kg bw) ÷ (lbs agent handled per day) ²	WS12d
Lower estimate	RATEL	0.0004		WS12d
Upper estimate	RATEU	0.002		WS12d
Absorbed dose (product of amount handled and absorbed dose rate)				
Central estimate	DOSEC	0.0171	mg/kg bw	N/A
Lower estimate	DOSEL	0.0076		
Upper estimate	DOSEU	0.038		

¹ Calculated as the product of the number of hours of application and the number of acres treated per hour for each category - i.e., central estimate, lower estimate, and upper estimate.

² “Agent” refers to the material being handled and may be expressed in units of a.i. or a.e. Depending on the agent under consideration, additional exposure conversions may be made in the exposure assessment and dose response assessment. For the risk assessment, the only important point is that the exposure and dose/response assessments must use the same units - that is, a.i., a.e., etc. - or the units must be converted to some equivalent form in the risk characterization.

Worksheet 13a: Workers: 2,4-D acid, Dermal Exposure Assessments Using Zero-Order Absorption [WZA]			
Parameter	Value	Units	Source
Body weight (W)	70	kg	STD.BW
Surface Area of hands (S)	840	cm ²	STD.Hands
Dermal permeability (K _p , cm/hour) [see Worksheet 10]			
Typical	0.0000242	cm/hour	DERM.KpC
Lower	0.0000102	cm/hour	DERM.KpL
Upper	0.0000580	cm/hour	DERM.KpU
Concentration in solution (C)	455	mg/mL	APPL.CONCAM

Note that 1 mL is equal to 1 cm³ and thus mg/mL = mg/cm³.

Details of calculations for worker zero-order dermal absorption scenarios.

Equation (U.S. EPA 1992a)

$$K_p \cdot C \cdot Time(hr) \cdot S \cdot \div W = Dose(mg/kg)$$

where:

C = concentration in mg/cm³ or mg/mL.

S = Surface area of skin in cm²

W = Body weight in kg.

Immersion of Hands or Wearing Contaminated Gloves for One-Minute

Typical Value: Use central estimate of K_p.

$$0.0000242 \text{ cm/hr} \times 455 \text{ mg/cm}^3 \times 1/60 \text{ hr} \times 840 \text{ cm}^2 \div 70 \text{ kg} = 2.2\text{e-}03 \text{ mg/kg [WZAT1M]}$$

Lower Estimate: Use lower limit of K_p.

$$0.0000102 \text{ cm/hr} \times 455 \text{ mg/cm}^3 \times 1/60 \text{ hr} \times 840 \text{ cm}^2 \div 70 \text{ kg} = 9.2\text{e-}04 \text{ mg/kg [WZAL1M]}$$

Upper Estimate: Use upper limit of K_p.

$$0.0000580 \text{ cm/hr} \times 455 \text{ mg/cm}^3 \times 1/60 \text{ hr} \times 840 \text{ cm}^2 \div 70 \text{ kg} = 5.3\text{e-}03 \text{ mg/kg [WZAU1M]}$$

Wearing Contaminated Gloves for One-Hour

Typical Value: Use typical concentration and central estimate of K_p.

$$0.0000242 \text{ cm/hr} \times 455 \text{ mg/cm}^3 \times 1 \text{ hr} \times 840 \text{ cm}^2 \div 70 \text{ kg} = 1.3\text{e-}01 \text{ mg/kg [WZAT1H]}$$

Lower Estimate: Use lower range of estimated concentration and lower limit of K_p.

$$0.0000102 \text{ cm/hr} \times 455 \text{ mg/cm}^3 \times 1 \text{ hr} \times 840 \text{ cm}^2 \div 70 \text{ kg} = 5.5\text{e-}02 \text{ mg/kg [WZAL1H]}$$

Upper Estimate: Use upper range of estimated concentration and upper limit of K_p.

$$0.0000580 \text{ cm/hr} \times 455 \text{ mg/cm}^3 \times 1 \text{ hr} \times 840 \text{ cm}^2 \div 70 \text{ kg} = 3.2\text{e-}01 \text{ mg/kg [WZAU1H]}$$

Worksheet 13b: Workers - 2,4-D ester, Dermal Exposure Assessments Using Zero-Order Absorption [WZB]			
Parameter	Value	Units	Source
Body weight (W)	70	kg	STD.BW
Surface Area of hands (S)	840	cm ²	STD.Hands
Dermal permeability (K _p , cm/hour) [see Worksheet 10]			
Typical	0.0000242	cm/hour	DERM.KpC
Lower	0.0000102	cm/hour	DERM.KpL
Upper	0.0000580	cm/hour	DERM.KpU
Concentration in solution (C)	670	mg/mL	APPL.CONCAM

Note that 1 mL is equal to 1 cm³ and thus mg/mL = mg/cm³.

Details of calculations for worker zero-order dermal absorption scenarios.

Equation (U.S. EPA 1992a)

$$K_p \cdot C \cdot \text{Time}(\text{hr}) \cdot S \cdot \div W = \text{Dose}(\text{mg/kg})$$

where:

C = concentration in mg/cm³ or mg/mL.

S = Surface area of skin in cm²

W = Body weight in kg.

Immersion of Hands or Wearing Contaminated Gloves for One-Minute

Typical Value: Use central estimate of K_p.

$$0.0000242 \text{ cm/hr} \times 670 \text{ mg/cm}^3 \times 1/60 \text{ hr} \times 840 \text{ cm}^2 \div 70 \text{ kg} = 3.2\text{e-}03 \text{ mg/kg [WZBT1M]}$$

Lower Estimate: Use lower limit of K_p.

$$0.0000102 \text{ cm/hr} \times 670 \text{ mg/cm}^3 \times 1/60 \text{ hr} \times 840 \text{ cm}^2 \div 70 \text{ kg} = 1.4\text{e-}03 \text{ mg/kg [WZBL1M]}$$

Upper Estimate: Use upper limit of K_p.

$$0.0000580 \text{ cm/hr} \times 670 \text{ mg/cm}^3 \times 1/60 \text{ hr} \times 840 \text{ cm}^2 \div 70 \text{ kg} = 7.8\text{e-}03 \text{ mg/kg [WZBU1M]}$$

Wearing Contaminated Gloves for One-Hour

Typical Value: Use typical concentration and central estimate of K_p.

$$0.0000242 \text{ cm/hr} \times 670 \text{ mg/cm}^3 \times 1 \text{ hr} \times 840 \text{ cm}^2 \div 70 \text{ kg} = 1.9\text{e-}01 \text{ mg/kg [WZBT1H]}$$

Lower Estimate: Use lower range of estimated concentration and lower limit of K_p.

$$0.0000102 \text{ cm/hr} \times 670 \text{ mg/cm}^3 \times 1 \text{ hr} \times 840 \text{ cm}^2 \div 70 \text{ kg} = 8.2\text{e-}02 \text{ mg/kg [WZBL1H]}$$

Upper Estimate: Use upper range of estimated concentration and upper limit of K_p.

$$0.0000580 \text{ cm/hr} \times 670 \text{ mg/cm}^3 \times 1 \text{ hr} \times 840 \text{ cm}^2 \div 70 \text{ kg} = 4.7\text{e-}01 \text{ mg/kg [WZBU1H]}$$

Worksheet 14a: Worker - 2,4-D acid - Accidental Spill Based on the Assumption of First-Order Absorption [WFA]				
Parameter	Code	Value	Units	Source
Liquid adhering to skin after a spill (<i>L</i>)	L	0.008	mg/mL	PUBL.Liq
Body weight (<i>W</i>)	W	70	kg	STD.BW
Surface Areas (<i>A</i>)				
Hands	Ah	840	cm ²	STD.Hands
Lower legs	Al	2070	cm ²	STD.LLegs
First-order dermal absorption rates (<i>k_a</i>)				
Central Estimate	Kac	0.00050	hour ⁻¹	DERM.ABSC
Lower limit of range	Kal	0.000170	hour ⁻¹	DERM.ABSL
Upper limit of range	Kau	0.00260	hour ⁻¹	DERM.ABSU
Concentration in solution (<i>C</i>)	Cc	455	mg/mL	CHEM..CONCAM

Details of calculations.

Equation (from Durkin et al. 1995)

$$Dose_{(mg/kg\ bw)} = k_a_{(1/hours)} \times L_{(mg/cmsq)} \times C_{(mg/mL)} \times T_{(hours)} \times A_{(cm\ sq)} \div W_{(kg)}$$

where *T* is the duration of exposure in hours and other terms are defined as above.

Lower Legs: Spill with 1 Hour (*T*) Exposure Period

Typical Value

$$0.0005000\ h^{-1} \times 0.008\ mL/cm \times 455\ mg/cm^3 \times 1\ hr \times 2070\ cm^2 \div 70\ kg = 5.4e-02\ mg/kg\ [WFATL1]$$

Lower range

$$0.0001700\ h^{-1} \times 0.008\ mL/cm \times 455\ mg/cm^3 \times 1\ hr \times 2070\ cm^2 \div 70\ kg = 1.8e-02\ mg/kg\ [WFALL1]$$

Upper range

$$0.0026000\ h^{-1} \times 0.008\ mL/cm \times 455\ mg/cm^3 \times 1\ hr \times 2070\ cm^2 \div 70\ kg = 2.8e-01\ mg/kg\ [WFAUL1]$$

Hands: Spill with 1 Hour (*T*) Exposure Period

Typical Value

$$0.0005000\ h^{-1} \times 0.008\ mL/cm \times 455\ mg/cm^3 \times 1\ hr \times 840\ cm^2 \div 70\ kg = 2.2e-02\ mg/kg\ [WFATH1]$$

Lower range

$$0.0001700\ h^{-1} \times 0.008\ mL/cm \times 455\ mg/cm^3 \times 1\ hr \times 840\ cm^2 \div 70\ kg = 7.4e-03\ mg/kg\ [WFALH1]$$

Upper range

$$0.0026000\ h^{-1} \times 0.008\ mL/cm \times 455\ mg/cm^3 \times 1\ hr \times 840\ cm^2 \div 70\ kg = 1.1e-01\ mg/kg\ [WFAUH1]$$

Worksheet 14b: Worker - 2,4-D ester - Accidental Spill Based on the Assumption of First-Order Absorption [WFB]				
Parameter	Code	Value	Units	Source
Liquid adhering to skin after a spill (<i>L</i>)	L	0.008	mg/mL	PUBL.Liq
Body weight (<i>W</i>)	W	70	kg	STD.BW
Surface Areas (<i>A</i>)				
Hands	Ah	840	cm ²	STD.Hands
Lower legs	Al	2070	cm ²	STD.LLegs
First-order dermal absorption rates (<i>k_a</i>)				
Central Estimate	Kac	0.00050	hour ⁻¹	DERM.ABSC
Lower limit of range	Kal	0.000170	hour ⁻¹	DERM.ABSL
Upper limit of range	Kau	0.00260	hour ⁻¹	DERM.ABSU
Concentration in solution (<i>C</i>)	Cc	670	mg/mL	CHEM..CONCAM

Details of calculations.

Equation (from Durkin et al. 1995)

$$Dose_{(mg/kg\ bw)} = k_a_{(1/hours)} \times L_{(mg/cm^2)} \times C_{(mg/mL)} \times T_{(hours)} \times A_{(cm\ sq)} \div W_{(kg)}$$

where *T* is the duration of exposure in hours and other terms are defined as above.

Lower Legs: Spill with 1 Hour (*T*) Exposure Period

Typical Value

$$0.0005000\ h^{-1} \times 0.008\ mL/cm \times 670\ mg/cm^3 \times 1\ hr \times 2070\ cm^2 \div 70\ kg = 7.9e-02\ mg/kg\ [WFBTL1]$$

Lower range

$$0.0001700\ h^{-1} \times 0.008\ mL/cm \times 670\ mg/cm^3 \times 1\ hr \times 2070\ cm^2 \div 70\ kg = 2.7e-02\ mg/kg\ [WFBLL1]$$

Upper range

$$0.0026000\ h^{-1} \times 0.008\ mL/cm \times 670\ mg/cm^3 \times 1\ hr \times 2070\ cm^2 \div 70\ kg = 4.1e-01\ mg/kg\ [WFBUL1]$$

Hands: Spill with 1 Hour (*T*) Exposure Period

Typical Value

$$0.0005000\ h^{-1} \times 0.008\ mL/cm \times 670\ mg/cm^3 \times 1\ hr \times 840\ cm^2 \div 70\ kg = 3.2e-02\ mg/kg\ [WFBTH1]$$

Lower range

$$0.0001700\ h^{-1} \times 0.008\ mL/cm \times 670\ mg/cm^3 \times 1\ hr \times 840\ cm^2 \div 70\ kg = 1.1e-02\ mg/kg\ [WFBTH1]$$

Upper range

$$0.0026000\ h^{-1} \times 0.008\ mL/cm \times 670\ mg/cm^3 \times 1\ hr \times 840\ cm^2 \div 70\ kg = 1.7e-01\ mg/kg\ [WFBTH1]$$

EXPOSURE ASSESSMENTS for the GENERAL PUBLIC

Worksheet 15a: Direct spray of child with 2,4-D acid. [DSCA]			
<i>Verbal Description: A naked child is accidentally sprayed over the entire body surface with a field dilution as it is being applied. The child is effectively washed - i.e., all of the compound is removed - after 1 hour. The absorbed dose is estimated using the assumption of first-order dermal absorption.</i>			
Parameter/Assumption	Value	Units	Source/Reference
Period of exposure (<i>T</i>)	1	hour	N/A
Body weight (<i>W</i>)	13.3	kg	PUBL.BWC
Exposed surface area (<i>A</i>)	6030	cm ²	PUBL.SAC
Liquid adhering to skin per cm ² of exposed skin (<i>L</i>)	0.008	mL/cm ²	STD.LIQ
Concentrations in solution (<i>C</i>)			
Typical/Central	5.99	mg/mL	TYPDrA
Low	1.2	mg/mL	LOWDrA
High	23.96	mg/mL	HI_DrA
First-order dermal absorption rate (<i>k_a</i>)			
Central	0.0005	hour ⁻¹	DERM.AbsC
Low	0.000170	hour ⁻¹	DERM.AbsL
High	0.0026	hour ⁻¹	DERM.AbsU
Estimated Absorbed Doses (<i>D</i>) - <i>see calculations below.</i>			
Central	0.01086	mg/kg	DSCAC
Low	0.000740	mg/kg	DSCAL
High	0.226	mg/kg	DSCAH

Details of calculations

Equation: $L \times C \times A \times k_a \times T \div W$

Central Estimate [DSCAC]:

$$0.008 \text{ mg/mL} \times 5.99 \text{ mg/mL} \times 6030 \text{ cm}^2 \times 0.0005 \text{ h}^{-1} \times 1 \text{ h} \div 13.3 \text{ kg} = 0.01086 \text{ mg/kg}$$

Lower Range of Estimate [DSCALL]:

$$0.008 \text{ mg/mL} \times 1.2 \text{ mg/mL} \times 6030 \text{ cm}^2 \times 0.00017 \text{ h}^{-1} \times 1 \text{ h} \div 13.3 \text{ kg} = 0.00074 \text{ mg/kg}$$

Upper Range of Estimate [DSCAH]:

$$0.008 \text{ mg/mL} \times 23.96 \text{ mg/mL} \times 6030 \text{ cm}^2 \times 0.0026 \text{ h}^{-1} \times 1 \text{ h} \div 13.3 \text{ kg} = 0.226 \text{ mg/kg}$$

Worksheet 15b: Direct spray of child with 2,4-D ester. [DSCE]			
<i>Verbal Description: A naked child is accidentally sprayed over the entire body surface with a field dilution as it is being applied. The child is effectively washed - i.e., all of the compound is removed - after 1 hour. The absorbed dose is estimated using the assumption of first-order dermal absorption.</i>			
Parameter/Assumption	Value	Units	Source/Reference
Period of exposure (<i>T</i>)	1	hour	N/A
Body weight (<i>W</i>)	13.3	kg	PUBL.BWC
Exposed surface area (<i>A</i>)	6030	cm ²	PUBL.SAC
Liquid adhering to skin per cm ² of exposed skin (<i>L</i>)	0.008	mL/cm ²	STD.LIQ
Concentrations in solution (<i>C</i>)			
Typical/Central	7.49	mg/mL	TYPDrE
Low	4.79	mg/mL	LOWDrE
High	14.98	mg/mL	HI_DrE
First-order dermal absorption rate (<i>k_a</i>)			
Central	0.0005	hour ⁻¹	DERM.AbsC
Low	0.000170	hour ⁻¹	DERM.AbsL
High	0.0026	hour ⁻¹	DERM.AbsU
Estimated Absorbed Doses (<i>D</i>) - see calculations below.			
Central	0.01358	mg/kg	DSCEC
Low	0.002954	mg/kg	DSCEL
High	0.141	mg/kg	DSCEH

Details of calculations

Equation: $L \times C \times A \times k_a \times T \div W$

Central Estimate [DSCEC]:

$$0.008 \text{ mg/mL} \times 7.49 \text{ mg/mL} \times 6030 \text{ cm}^2 \times 0.0005 \text{ h}^{-1} \times 1 \text{ h} \div 13.3 \text{ kg} = 0.01358 \text{ mg/kg}$$

Lower Range of Estimate [DSCEL]:

$$0.008 \text{ mg/mL} \times 4.79 \text{ mg/mL} \times 6030 \text{ cm}^2 \times 0.00017 \text{ h}^{-1} \times 1 \text{ h} \div 13.3 \text{ kg} = 0.002954 \text{ mg/kg}$$

Upper Range of Estimate [DSCEH]:

$$0.008 \text{ mg/mL} \times 14.98 \text{ mg/mL} \times 6030 \text{ cm}^2 \times 0.0026 \text{ h}^{-1} \times 1 \text{ h} \div 13.3 \text{ kg} = 0.141 \text{ mg/kg}$$

Worksheet 16a: Direct spray of woman with 2,4-D acid. [DSWA]			
<i>Verbal Description: A woman is accidentally sprayed over the feet and legs with a field dilution as it is being applied. The woman washes and removes all of the compound after 1 hour. The absorbed dose is estimated using the assumption of first-order dermal absorption..</i>			
Parameter/Assumption	Value	Units	Source/Reference
Period of exposure (<i>T</i>)	1	hour	N/A
Body weight (<i>W</i>)	64	kg	PUBL . BWF
Exposed surface area (<i>A</i>)	2915	cm ²	PUBL . SAF1
Liquid adhering to skin per cm ² of exposed skin. (<i>L</i>)	0.008	mL/cm ²	STD . LIQ
Concentrations in solution (<i>C</i>)			
Typical/Central	5.99	mg/mL	TYPDrA
Low	1.2	mg/mL	LOWDrA
High	23.96	mg/mL	HI_DrA
First-order dermal absorption rate (<i>k_a</i>)			
Central	0.0005	hour ⁻¹	DERM . AbsC
Low	0.000170	hour ⁻¹	DERM . AbsL
High	0.0026	hour ⁻¹	DERM . AbsU
Estimated Absorbed Doses (<i>D</i>) - <i>see calculations below.</i>			
Central	0.00110	mg/kg	DSWAC
Low	0.000074	mg/kg	DSWAL
High	0.023	mg/kg	DSWAH

Details of calculations

Equation: $L \times C \times A \times k_a \times T \div W$

Central Estimate [DSWAC]:

$$0.008 \text{ mg/mL} \times 5.99 \text{ mg/mL} \times 2915 \text{ cm}^2 \times 0.0005 \text{ h}^{-1} \times 1 \text{ h} \div 64 \text{ kg} = 0.0011 \text{ mg/kg}$$

Lower Range of Estimate [DSWAL]:

$$0.008 \text{ mg/mL} \times 1.2 \text{ mg/mL} \times 2915 \text{ cm}^2 \times 0.00017 \text{ h}^{-1} \times 1 \text{ h} \div 64 \text{ kg} = 0.000074 \text{ mg/kg}$$

Upper Range of Estimate [DSWAH]:

$$0.008 \text{ mg/mL} \times 23.96 \text{ mg/mL} \times 2915 \text{ cm}^2 \times 0.0026 \text{ h}^{-1} \times 1 \text{ h} \div 64 \text{ kg} = 0.023 \text{ mg/kg}$$

Worksheet 16b: Direct spray of woman with 2,4-D ester. [DSWE]			
<i>Verbal Description: A woman is accidentally sprayed over the feet and legs with a field dilution as it is being applied. The woman washes and removes all of the compound after 1 hour. The absorbed dose is estimated using the assumption of first-order dermal absorption..</i>			
Parameter/Assumption	Value	Units	Source/Reference
Period of exposure (<i>T</i>)	1	hour	N/A
Body weight (<i>W</i>)	64	kg	PUBL.BWF
Exposed surface area (<i>A</i>)	2915	cm ²	PUBL.SAF1
Liquid adhering to skin per cm ² of exposed skin. (<i>L</i>)	0.008	mL/cm ²	STD.LIQ
Concentrations in solution (<i>C</i>)			
Typical/Central	7.49	mg/mL	TYPDrE
Low	4.79	mg/mL	LOWDrE
High	14.98	mg/mL	HI_DrE
First-order dermal absorption rate (<i>k_a</i>)			
Central	0.0005	hour ⁻¹	DERM.AbsC
Low	0.000170	hour ⁻¹	DERM.AbsL
High	0.0026	hour ⁻¹	DERM.AbsU
Estimated Absorbed Doses (<i>D</i>) - see calculations below.			
Central	0.0014	mg/kg	DSWEC
Low	0.0003	mg/kg	DSWEL
High	0.014	mg/kg	DSWEH

Details of calculations

Equation: $L \times C \times A \times k_a \times T \div W$

Central Estimate [DSWEC]:

$$0.008 \text{ mg/mL} \times 7.49 \text{ mg/mL} \times 2915 \text{ cm}^2 \times 0.0005 \text{ h}^{-1} \times 1 \text{ h} \div 64 \text{ kg} = 0.0014 \text{ mg/kg}$$

Lower Range of Estimate [DSWEL]:

$$0.008 \text{ mg/mL} \times 1.2 \text{ mg/mL} \times 2915 \text{ cm}^2 \times 0.00017 \text{ h}^{-1} \times 1 \text{ h} \div 64 \text{ kg} = 0.0003 \text{ mg/kg}$$

Upper Range of Estimate [DSWEH]:

$$0.008 \text{ mg/mL} \times 14.98 \text{ mg/mL} \times 2915 \text{ cm}^2 \times 0.0026 \text{ h}^{-1} \times 1 \text{ h} \div 64 \text{ kg} = 0.014 \text{ mg/kg}$$

Worksheet 17: Dermal contact with contaminated vegetation. [VEGDWA]				
<i>Verbal Description: A woman wearing shorts and a short sleeved shirt is in contact with contaminated vegetation for 1 hour shortly after application of the compound - i.e. no dissipation or degradation is considered. The chemical is effectively removed from the surface of the skin - i.e., washing - after 24 hours.</i>				
Parameter/Assumption	Code	Value	Units	Source/Reference
Contact time (T_c)	T_c	1	hour	N/A
Exposure time (T_e)	T_e	24	hours	N/A
Body weight (W)	W	64	kg	PUBL.BWF
Exposed surface area (A)	A	5300	cm ²	PUBL.SAF2
Dislodgeable residue (Dr) as a proportion of application rate	Dr	0.1	none	PUBL.DisL
Application Rates(R)	R	1	lb a.i./acre	APPL.TYP
First-order dermal absorption rate (k_a)				
Central	K_{aC}	0.00050	hour ⁻¹	DERM.AbsC
Low	K_{aL}	0.00017	hour ⁻¹	DERM.AbsL
High	K_{aH}	0.00260	hour ⁻¹	DERM.AbsU
Estimated Absorbed Doses (D) - see calculations on next page.				
Central	VEGDWC	0.0013	mg/kg	
Low	VEGDWL	0.00043	mg/kg	
High	VEGDWH	0.0066	mg/kg	

Description of Calculations:

Step 1:

Use method of Durkin et al. (1995, p. 68, equation 4) to calculate the transfer rate (Tr) in units of $\mu\text{g}/(\text{cm}^2\cdot\text{hr})$ from the application rate and the proportion of the applied amount that is expected to be dislodgeable(Dr) after converting application rate in lb a.i./acre to units of $\mu\text{g}/\text{cm}^2$:

$$x = \log(Tr (\mu\text{g}/(\text{cm}^2\cdot\text{hr}))) = (1.09 \times \log_{10}(R \times WS01.lbac_ugcm)) + 0.05$$

$$Tr (\mu\text{g}/(\text{cm}^2\cdot\text{hr})) = 10^x$$

Step 2:

Convert Tr from units of $\mu\text{g}/(\text{cm}^2\cdot\text{hr})$ to units of $\text{mg}/(\text{cm}^2\cdot\text{hr})$ by dividing by 1000:

$$Tr(\text{mg}/(\text{cm}^2\cdot\text{hr})) = Tr(\mu\text{g}/(\text{cm}^2\cdot\text{hr}))/1000$$

Step 3:

Estimate amount ($Amnt$) transferred to skin in mg during the exposure period:

$$Amnt(\text{mg}) = Tr(\text{mg}/(\text{cm}^2\cdot\text{hr})) \times T_c (\text{hours}) \times A (\text{cm}^2)$$

Step 4:

Estimate the absorbed dose (D_{Abs}) in mg/kg bw as the product of the amount on the skin, the first-order absorption rate, and the duration of exposure divided by the body weight:

$$D_{Abs} = Amnt(\text{mg}) \times k_a (\text{hours}^{-1}) \times T_e (\text{hours}) \div W (\text{kg})$$

See next page for details of calculations

Dermal contact with contaminated vegetation -Details of calculations.

Central Estimate: Use typical application rate with central estimate of first-order dermal absorption rate.

Step 1:

$$\log_{10}(Tr (\mu\text{g}/(\text{cm}^2\cdot\text{hr}))) = (1.09 \times \log_{10}(1 \times 0.1 \times 11.21)) + 0.05 = 0.104 \mu\text{g}/(\text{cm}^2\cdot\text{hr})$$

$$Tr (\mu\text{g}/(\text{cm}^2\cdot\text{hr})) = 10^{0.104} = 1.27 \mu\text{g}/(\text{cm}^2\cdot\text{hr})$$

Step 2:

$$Tr (\text{mg}/(\text{cm}^2\cdot\text{hr})) = 1.27 \mu\text{g}/(\text{cm}^2\cdot\text{hr}) \div 1000 \mu\text{g}/\text{mg} = 0.00127 \text{mg}/(\text{cm}^2\cdot\text{hr})$$

Step 3:

$$Amnt(\text{mg}) = 0.00127 \text{mg}/(\text{cm}^2\cdot\text{hr}) \times 1 \text{ hr} \times 5300 \text{ cm}^2 = 6.73 \text{ mg}$$

Step 4:

$$D_{Abs} (\text{mg}/\text{kg bw}) = 6.73 \text{ mg} \times 0.0005 \text{ hr}^{-1} \times 24 \text{ hours} \div 64 \text{ kg} = 0.0013 \quad [\text{VDWAC}]$$

Lower Estimate: Use typical application rate with lower estimate of first-order dermal absorption rate.

Step 1:

$$\log_{10}(Tr (\mu\text{g}/(\text{cm}^2\cdot\text{hr}))) = (1.09 \times \log_{10}(1 \times 0.1 \times 11.21)) + 0.05 = 0.104 \mu\text{g}/(\text{cm}^2\cdot\text{hr})$$

$$Tr (\mu\text{g}/(\text{cm}^2\cdot\text{hr})) = 10^{0.104} = 1.27 \mu\text{g}/(\text{cm}^2\cdot\text{hr})$$

Step 2:

$$Tr (\text{mg}/(\text{cm}^2\cdot\text{hr})) = 1.27 \mu\text{g}/(\text{cm}^2\cdot\text{hr}) \div 1000 \mu\text{g}/\text{mg} = 0.00127 \text{mg}/(\text{cm}^2\cdot\text{hr})$$

Step 3:

$$Amnt(\text{mg}) = 0.00127 \text{mg}/(\text{cm}^2\cdot\text{hr}) \times 1 \text{ hr} \times 5300 \text{ cm}^2 = 6.73 \text{ mg}$$

Step 4:

$$D_{Abs} (\text{mg}/\text{kg bw}) = 6.73 \text{ mg} \times 0.00017 \text{ hr}^{-1} \times 24 \text{ hours} \div 64 \text{ kg} = 0.00043 \quad [\text{VDWAL}]$$

Upper Estimate: Use typical application rate with upper estimate of first-order dermal absorption rate.

Step 1:

$$\log_{10}(Tr (\mu\text{g}/(\text{cm}^2\cdot\text{hr}))) = (1.09 \times \log_{10}(1 \times 0.1 \times 11.21)) + 0.05 = 0.104 \mu\text{g}/(\text{cm}^2\cdot\text{hr})$$

$$Tr (\mu\text{g}/(\text{cm}^2\cdot\text{hr})) = 10^{0.104} = 1.27 \mu\text{g}/(\text{cm}^2\cdot\text{hr})$$

Step 2:

$$Tr (\text{mg}/(\text{cm}^2\cdot\text{hr})) = 1.27 \mu\text{g}/(\text{cm}^2\cdot\text{hr}) \div 1000 \mu\text{g}/\text{mg} = 0.00127 \text{mg}/(\text{cm}^2\cdot\text{hr})$$

Step 3:

$$Amnt(\text{mg}) = 0.00127 \text{mg}/(\text{cm}^2\cdot\text{hr}) \times 1 \text{ hr} \times 5300 \text{ cm}^2 = 6.73 \text{ mg}$$

Step 4:

$$D_{Abs} (\text{mg}/\text{kg bw}) = 6.73 \text{ mg} \times 0.0026 \text{ hr}^{-1} \times 24 \text{ hours} \div 64 \text{ kg} = 0.0066 \quad [\text{VDWAH}]$$

Worksheet 18: Consumption of contaminated fruit, acute exposure scenario. [VEGCWA]				
<i>Verbal Description: A woman consumes 1 lb (0.4536 kg) of contaminated fruit shortly after application of the chemical - i.e. no dissipation or degradation is considered. Residue estimates based on relationships from Hoerger and Kenaga (1972) summarized in Worksheet 05 as well as monitoring data.</i>				
Parameters/Assumptions	Value	Units	Source/Reference	
Body weight (<i>W</i>)	64	kg	PUBL.BWF	
Amount of fruit consumed (<i>A</i>)	0.454	kg	N/A	
Typical Application rates (<i>R</i>) ²				
Central	1	lb a.i./acre	APPL.TYP	
Lower	1	lb a.i./acre	APPL.TYP	
Upper	1	lb a.i./acre	APPL.TYP	
Residue rates (<i>rr</i>)	Typical	5	RUD ¹	Section 3.2.3.6
	Lower	1	RUD ¹	Section 3.2.3.6
	Upper	30	RUD ¹	Section 3.2.3.6
Dose estimates (<i>D</i>) - see details of calculations below				
	Typical	0.035	mg/kg bw	VEGCWAT
	Lower	0.0071	mg/kg bw	VEGCWAL
	Upper	0.21	mg/kg bw	VEGCWAU
¹ RUD: Residue Unit Dosage, term used by Hoerger and Kenaga (1972) for anticipated concentration on vegetation (mg chemical per kg of vegetation) for each 1 lb a.i./acre applied.				
² This is currently set up to use only the typical application rate for all estimates - i.e., lower, typical, and upper.				

Equation (terms defined in above table):

$$D \text{ (mg/kg bw)} = A \text{ (kg)} \times R \text{ (lb a.i./acre)} \times rr \text{ (mg/kg} \div \text{lb a.i./acre)} \div W \text{ (kg bw)}$$

Details of Calculations

Typical: Use typical application rate and typical RUD.

$$D = 0.454 \text{ kg} \times 1 \text{ lb a.i./acre} \times 5 \text{ mg/kg} \div \text{lb a.i./acre} \div 64 \text{ kg} = 0.035 \text{ mg/kg bw}$$

Lower: Use typical application rate and lower estimate of RUD.

$$D = 0.454 \text{ kg} \times 1 \text{ lb a.i./acre} \times 1 \text{ mg/kg} \div \text{lb a.i./acre} \div 64 \text{ kg} = 0.0071 \text{ mg/kg bw}$$

Upper: Use typical application rate and upper estimate of RUD.

$$D = 0.454 \text{ kg} \times 1 \text{ lb a.i./acre} \times 30 \text{ mg/kg} \div \text{lb a.i./acre} \div 64 \text{ kg} = 0.21 \text{ mg/kg bw}$$

Worksheet 19: Consumption of contaminated fruit, chronic exposure scenario. [VEGCWC]

Verbal Description: A woman consumes contaminated fruit for a 90 day period starting shortly after application of the chemical. Initial residue estimates are based on estimates detailed in section 3.2.3.6.. The foliar half-time is used to estimate the concentration on vegetation after 90 days. The geometric mean of the initial and 90 day concentrations is used as a central estimate of effective dose over the 90 day period. This table is currently set up to use only the typical applications rate. Variability is expressed by differences in the amount of vegetation consumed and differences in the estimated levels of 2,4-D on the fruit.

Parameters/Assumptions	Value	Units	Source/Reference
Foliar half-time ($t_{1/2}$)	14	days	CHEM..FT12
Duration of exposure (t)	90	days	N/A
Body weight (W)	64	kg	PUBL.BWF
Amount of vegetation consumed per unit body weight(A)			
Typical	0.0043	kg veg./kg bw	PUBL.VT
Upper	0.01	kg veg./kg bw	PUBL.VU
Application rates (R)			
Typical	1	lb a.i./acre	APPL.Type
Lower	1	lb a.i./acre	APPL.Type
Upper	1	lb a.i./acre	APPL.Type
Residue rates (rr)			
Typical	5	RUD ¹	Section 3.2.3.6.
Lower	1	RUD ¹	Section 3.2.3.6.
Upper	30	RUD ¹	Section 3.2.3.6.
Dose estimates (D) - see details of calculations on next page			
Typical	0.0023	mg/kg bw	VEGCWCT
Lower	0.00046	mg/kg bw	VEGCWCL
Upper	0.032	mg/kg bw	VEGCWCU
¹ RUD: Residue Unit Dosage, term used by Hoerger and Kenaga (1972) for anticipated concentration on fruit (mg chemical per kg of vegetation) for each 1 lb a.i./acre applied.			

Equations (terms defined below or in above table:

Step 1: Calculate C_0 , concentration in vegetation on Day 0 - i.e., day of application.

$$C_0 \text{ (mg/kg)} = R \text{ (lb a.i./acre)} \times rr \text{ (mg/kg} \div \text{lb a.i./acre)}$$

Step 2: Calculate C_{90} , concentration in vegetation on Day 90 ($t=90$ days) based on dissipation coefficient (k) derived from foliar half-life ($t_{1/2}$).

$$k \text{ (days}^{-1}\text{)} = \ln(2) \div t_{1/2} \text{ (days)}$$

$$C_{90} \text{ (mg/kg)} = C_0 \text{ (mg/kg)} \times e^{-tk}$$

Step 3: Use the geometric mean of C_0 and C_{90} to get a central estimate of concentration in vegetation (mg/kg veg.) and multiply this value by the vegetation consumption (kg veg/kg bw) to calculate the daily dose (mg/kg bw) over the exposure period.

$$D \text{ (mg/kg bw)} = (C_0 \times C_{90})^{0.5} \text{ (mg/kg veg.)} \times A \text{ kg veg./kg bw} \times W \text{ kg bw} \div B \text{ (kg bw)}$$

$$= (C_0 \times C_{90})^{0.5} \text{ (mg/kg veg.)} \times A \text{ kg veg./kg bw}$$

Details of calculations on next page

Subchronic consumption of vegetation: Details of calculations

Central Estimate:

Use the typical application rate, the typical vegetation consumption rate, and the typical residue rate along with the estimate of foliar half-time.

Step 1:

$$C_0 = 1 \text{ lb a.i./acre} \times 5 \text{ mg/kg veg.} = 5 \text{ mg/kg veg.}$$

Step 2:

$$k = \ln(2) \div 14 \text{ days}^{-1} = 0.0495$$

$$C_{90} = 5 \text{ mg/kg} \times e^{-0.0495 \times 90} = 0.0581 \text{ mg/kg veg.}$$

Step 3:

$$D \text{ (mg/kg bw/day)} = (5 \times 0.0581)^{0.5} \text{ (mg/kg veg.)} \times 0.0043 \text{ (kg veg/kg bw)} = 0.0023 \text{ mg/kg bw}$$

Lower Estimate:

Use the lower range of the residue rates along with the typical application rate and the estimate of foliar half-time. Also the typical vegetation consumption rate because a lower limit on this value is not available.

Step 1:

$$C_0 = 1 \text{ lb a.i./acre} \times 1 \text{ mg/kg veg.} = 1 \text{ mg/kg veg.}$$

Step 2:

$$k = \ln(2) \div 14 \text{ days}^{-1} = 0.0495$$

$$C_{90} = 1 \text{ mg/kg} \times e^{-0.0495 \times 90} = 0.01162 \text{ mg/kg veg.}$$

Step 3:

$$D \text{ (mg/kg bw)} = (1 \times 0.01162)^{0.5} \text{ (mg/kg veg.)} \times 0.0043 \text{ (kg veg/kg bw)} = 0.00046 \text{ (mg/kg bw)}$$

Upper Estimate:

Use the upper range of the residue rates and upper range of the vegetation consumption rate along with the typical application rate and the estimate of foliar half-time.

Step 1:

$$C_0 = 1 \text{ lb a.i./acre} \times 30 \text{ mg/kg veg.} = 30 \text{ mg/kg veg.}$$

Step 2:

$$k = \ln(2) \div 14 \text{ days}^{-1} = 0.0495$$

$$C_{90} = 30 \text{ mg/kg} \times e^{-0.0495 \times 90} = 0.3486 \text{ mg/kg veg.}$$

Step 3:

$$D \text{ (mg/kg bw)} = (30 \times 0.3486)^{0.5} \text{ (mg/kg veg.)} \times 0.01 \text{ (kg veg/kg bw)} = 0.032 \text{ (mg/kg bw)}$$

Worksheet 20: Consumption of contaminated water, acute exposure scenario. [WATCCA]

Verbal Description: A young child (2-3 years old) consumes 1 liter of contaminated water shortly after an accidental spill of 200 gallons of a field solution of ester into a pond that has an average depth of 1 m and a surface area of 1000 m² or about one-quarter acre . No dissipation or degradation is considered.

Parameters/Assumptions	Value	Units	Source/Reference
Surface area of pond [SA]	1000	m ²	N/A
Average depth [DPTH]	1	m	N/A
Volume of pond in cubic meters [VM]	1000	m ³	N/A
Volume of pond in Liters [VL]	1000000	L	1 m ³ = 1,000 L
Volume of spill [VS]	200	gallons	N/A
Concentrations in solution ($C_{(mg/L)}$) ¹			
Central	7490	mg/L	TYPDRE×1000
Low	4790	mg/L	LOWDRE×1000
High	14980	mg/L	HI_DRE×1000
Body weight (W)	13.3	kg	PUBL .BWC
Amount of water consumed (A)			
Typical	1	L/day	PUBL .WCT
Lower	0.61	L/day	PUBL .WCL
Upper	1.5	L/day	PUBL .WCH
Dose estimates (D) - see details of calculations on next page.			
Typical	0.426	mg/kg bw	WATCCAT
Lower	0.1663	mg/kg bw	WATCCAL
Upper	1.28	mg/kg bw	WATCCAU

¹ As indicated in Worksheet 08, the field concentrations are calculated in units of mg/mL. Thus, to get units of mg/L, these values must be multiplied by 1000.

Details of calculations on next page

Acute Consumption of Contaminated Water from an Accidental Spill

Details of calculations

Equations (terms defined below or in table on previous page)

Step 1: Calculate the concentration in the pond based on the concentration in the spilled solution, the volume spilled and the volume of the pond, assuming instantaneous mixing.

$$\text{Conc. (mg/L)} = VS_{(\text{gal.})} \times 3.785 \text{ L/gal} \times C_{(\text{mg/L})} \div VL_{(\text{liters})}$$

Step 2: Calculate the dose based on the concentration in the water, the amount of water consumed, and the body weight.

$$D_{(\text{mg/kg bw})} = \text{Conc. (mg/L)} \times A_{(\text{L})} \div W_{(\text{kg})}$$

Calculations

Central Estimate:

Use the typical field dilution, and the typical water consumption.

Step 1:

$$\text{Conc. (mg/L)} = 200_{(\text{gal.})} \times 3.785 \text{ L/gal} \times 7490_{(\text{mg/L})} \div 1000000_{(\text{liters})} = 5.67_{(\text{mg/L})}$$

Step 2:

$$D_{(\text{mg/kg bw})} = 5.67_{(\text{mg/L})} \times 1_{(\text{L})} \div 13.3_{(\text{kg})} = 0.426_{(\text{mg/kg bw})}$$

Lower Estimate:

Use the lowest estimated field dilution and the lower range of water consumption.

Step 1:

$$\text{Conc. (mg/L)} = 200_{(\text{gal.})} \times 3.785 \text{ L/gal} \times 4790_{(\text{mg/L})} \div 1000000_{(\text{liters})} = 3.626_{(\text{mg/L})}$$

Step 2:

$$D_{(\text{mg/kg bw})} = 3.626_{(\text{mg/L})} \times 0.61_{(\text{L})} \div 13.3_{(\text{kg})} = 0.1663_{(\text{mg/kg bw})}$$

Upper Estimate:

Use the highest estimated field concentration and the upper range of water consumption.

Step 1:

$$\text{Conc. (mg/L)} = 200_{(\text{gal.})} \times 3.785 \text{ L/gal} \times 14980_{(\text{mg/L})} \div 1000000_{(\text{liters})} = 11.34_{(\text{mg/L})}$$

Step 2:

$$D_{(\text{mg/kg bw})} = 11.34_{(\text{mg/L})} \times 1.5_{(\text{L})} \div 13.3_{(\text{kg})} = 1.28_{(\text{mg/kg bw})}$$

Worksheet 21: Consumption of contaminated water, chronic exposure scenario. [WATCMC]			
<i>Verbal Description: An adult (70 kg male) consumes contaminated ambient water for a lifetime. The levels in water are estimated from monitoring data and thus dissipation, degradation and other environmental processes are implicitly considered. This table only considers the typical application rate. Other application rates are considered in the risk characterization.</i>			
Parameters/Assumptions	Value	Units	Source/Reference
Application Rates ($R_{(lb\ a.i./acre)}$)			
Central	1	lb a.i./gal	APPL.TYP
Low	1		APPL.TYP
High	1		APPL.TYP
Water Contamination Rate (WCR)($C_{(mg/L)} \div R_{(lb\ a.i./gal)}$)			
Central	0.002	mg/L/lb a.i./acre	Section 3.2.3.4.
Low	0.001		Section 3.2.3.4.
High	0.004		Section 3.2.3.4.
Body weight (W)	70	kg	PUBL.BWM
Amount of water consumed ($A_{(L/day)}$)			
Typical	2	L/day	PUBL.WCAT
Lower	1.4	L/day	PUBL.WCAL
Upper	2.4	L/day	PUBL.WCAH
Dose estimates (D) - see details of calculations on next page.			
Typical	0.000057	mg/kg bw	WS27T
Lower	0.00002	mg/kg bw	WS27L
Upper	0.00014	mg/kg bw	WS27U

Details of calculations on next page

Chronic Consumption of Contaminated Ambient Water

Details of calculations

Equations (terms defined in table on previous page)

Verbal Description: Multiply the application rate (R (lb a.i./acre)) by the water contamination rate (WCR ((mg/L)×(lb a.i./gal))) to get the concentration in ambient water. This product is in turn multiplied by the amount of water consumed per day (A (L/day)) and then divided by the body weight (W (kg)) to get the estimate of the absorbed dose (D (mg/kg bw)).

$$D_{(\text{mg/kg bw})} = R_{(\text{lb a.i./acre})} \times WCR_{((\text{mg/L}) \times (\text{lb a.i./gal}))} \times A_{(\text{L/day})} \div W_{(\text{kg})}$$

Central Estimate: [WATCMCT]

Use the typical application rate, typical contamination rate (WCR), and the typical water consumption.

$$D_{(\text{mg/kg bw})} = 1_{(\text{lb a.i./acre})} \times 0.002_{((\text{mg/L}) \times (\text{lb a.i./gal}))} \times 2_{(\text{L/day})} \div 70_{(\text{kg bw})} = 0.000057_{(\text{mg/kg bw})}$$

Lower Range of Estimate: [WATCMCL]

Use the typical application rate, the low end of the range of the water contamination rate (WCR), and the low end of the range for water consumption.

$$D_{(\text{mg/kg bw})} = 1_{(\text{lb a.i./acre})} \times 0.001_{((\text{mg/L}) \times (\text{lb a.i./gal}))} \times 1.4_{(\text{L/day})} \div 70_{(\text{kg bw})} = 0.000020_{(\text{mg/kg bw})}$$

Upper range of Estimate: [WATCMCU]

Use the typical application rate, the low end of the range of the water contamination rate (WCR), and the low end of the range for water consumption.

$$D_{(\text{mg/kg bw})} = 1_{(\text{lb a.i./acre})} \times 0.004_{((\text{mg/L}) \times (\text{lb a.i./gal}))} \times 2.4_{(\text{L/day})} \div 70_{(\text{kg bw})} = 0.00014_{(\text{mg/kg bw})}$$

Worksheet 22: Consumption of contaminated fish, acute exposure scenario. [FISHAM]

Verbal Description: An adult angler consumes fish taken from contaminated water shortly after an accidental spill of 200 gallons of a field solution into a pond that has an average depth of 1 m and a surface area of 1000 m² or about one-quarter acre . No dissipation or degradation is considered. Because of the available and well documented information and substantial differences in the amount of caught fish consumed by the general public and native American subsistence populations, separate exposure estimates are made for these two groups.

Parameters/Assumptions	Value	Units	Source/Reference
Surface area of pond [SA]	1000	m ²	N/A
Average depth [DPTH]	1	m	N/A
Volume of pond in cubic meters [VM]	1000	m ³	N/A
Volume of pond in Liters [VL]	1000000	L	1 m ³ = 1,000 L
Volume of spill [VS]	200	gallons	N/A
Concentrations in spilled solution ($C_{(mg/L)}$)			
Central	7490	mg/L	TYPDRE×1000
Low	4790	mg/L	LOWDRE×1000
High	14980	mg/L	HI_DRE×1000
Body weight (W)	70	kg	PUBL.BWM
Amount of fish consumed (A)			
General Population	0.158	kg/day	PUBL.FAU
Native American subsistence populations	0.77	kg/day	PUBL.FNU
Bioconcentration factor ($BCF_{(kg\ fish/L)}$)	25	kg fish/L	Average of range from WS09
Dose estimates (D) - see details of calculations on next page.			
General Population			
Typical	0.32	mg/kg bw	FISHAMGPT
Lower	0.20	mg/kg bw	FISHAMGPL
Upper	0.64	mg/kg bw	FISHAMGPU
Native American subsistence populations			
Typical	1.6	mg/kg bw	FISHAMNAT
Lower	1.0	mg/kg bw	FISHAMNAL
Upper	3.1	mg/kg bw	FISHAMNAU

Details of calculations on next page

Acute Consumption of Contaminated Fish after an Accidental Spill

Details of calculations

Equations (terms defined below or in table on previous page)

Step 1: As in the acute drinking water scenario, calculate the concentration in the pond based on the concentration in the spilled solution, the volume spilled and the volume of the pond, assuming instantaneous mixing.

$$\text{Conc.}_{(\text{mg/L})} = VS_{(\text{gal.})} \times 3.785_{\text{L/gal}} \times C_{(\text{mg/L})} \div VL_{(\text{liters})}$$

Step 2: Calculate the dose based on the concentration in the water, the bioconcentration factor, the amount of fish consumed, and the body weight.

$$D_{(\text{mg/kg bw})} = \text{Conc.}_{(\text{mg/L})} \times BCF_{(\text{kg fish/L})} \times A_{(\text{kg fish})} \div W_{(\text{kg bw})}$$

General Public

Central Estimate:

Use the typical field dilution as well as the experimental BCF and upper range of daily fish consumption for the general public.

Step 1:

$$\text{Conc.}_{(\text{mg/L})} = 200_{(\text{gal.})} \times 3.785_{\text{L/gal}} \times 7490_{(\text{mg/L})} \div 1000000_{(\text{liters})} = 5.67_{(\text{mg/L})}$$

Step 2:

$$D_{(\text{mg/kg bw})} = 5.67_{(\text{mg/L})} \times 25_{(\text{L/kg})} \times 0.158_{(\text{kg fish})} \div 70_{(\text{kg})} = 0.32_{(\text{mg/kg bw})}$$

Lower End of Range for the Estimate:

Use the lower field dilution as well as the experimental BCF and upper range of daily fish consumption for the general public.

Step 1:

$$\text{Conc.}_{(\text{mg/L})} = 200_{(\text{gal.})} \times 3.785_{\text{L/gal}} \times 4790_{(\text{mg/L})} \div 1000000_{(\text{liters})} = 3.626_{(\text{mg/L})}$$

Step 2:

$$D_{(\text{mg/kg bw})} = 3.626_{(\text{mg/L})} \times 25_{(\text{L/kg})} \times 0.158_{(\text{kg fish})} \div 70_{(\text{kg})} = 0.2_{(\text{mg/kg bw})}$$

Upper End of Range for the Estimate:

Use the upper field dilution as well as the experimental BCF and upper range of daily fish consumption for the general public.

Step 1:

$$\text{Conc.}_{(\text{mg/L})} = 200_{(\text{gal.})} \times 3.785_{\text{L/gal}} \times 14980_{(\text{mg/L})} \div 1000000_{(\text{liters})} = 11.34_{(\text{mg/L})}$$

Step 2:

$$D_{(\text{mg/kg bw})} = 11.34_{(\text{mg/L})} \times 25_{(\text{L/kg})} \times 0.158_{(\text{kg fish})} \div 70_{(\text{kg})} = 0.64_{(\text{mg/kg bw})}$$

(continued on next page)

Acute Consumption of Contaminated Fish after an Accidental Spill ***Details of calculations*** (continued)

Native American Subsistence Populations

Central Estimate:

Use the typical field dilution as well as the experimental BCF and upper range of daily fish consumption for the native American subsistence populations.

Step 1:

$$\text{Conc. (mg/L)} = 200_{(\text{gal.})} \times 3.785_{\text{L/gal}} \times 7490_{(\text{mg/L})} \div 1000000_{(\text{liters})} = 5.67_{(\text{mg/L})}$$

Step 2:

$$D_{(\text{mg/kg bw})} = 5.67_{(\text{mg/L})} \times 25_{(\text{L/kg})} \times 0.77_{(\text{kg fish})} \div 70_{(\text{kg})} = 1.6_{(\text{mg/kg bw})}$$

Estimate of Lower End of Range:

Use the lower field dilution as well as the experimental BCF and upper range of daily fish consumption for the native American subsistence populations.

Step 1:

$$\text{Conc. (mg/L)} = 200_{(\text{gal.})} \times 3.785_{\text{L/gal}} \times 4790_{(\text{mg/L})} \div 1000000_{(\text{liters})} = 3.630_{(\text{mg/L})}$$

Step 2:

$$D_{(\text{mg/kg bw})} = 3.63_{(\text{mg/L})} \times 25_{(\text{L/kg})} \times 0.77_{(\text{kg fish})} \div 70_{(\text{kg})} = 1.0_{(\text{mg/kg bw})}$$

Estimate of Upper End of Range:

Use the upper field dilution as well as the experimental BCF and upper range of daily fish consumption for the native American subsistence populations.

Step 1:

$$\text{Conc. (mg/L)} = 200_{(\text{gal.})} \times 3.785_{\text{L/gal}} \times 14980_{(\text{mg/L})} \div 1000000_{(\text{liters})} = 11.340_{(\text{mg/L})}$$

Step 2:

$$D_{(\text{mg/kg bw})} = 11.34_{(\text{mg/L})} \times 25_{(\text{L/kg})} \times 0.77_{(\text{kg fish})} \div 70_{(\text{kg})} = 3.1_{(\text{mg/kg bw})}$$

Worksheet 23: Consumption of contaminated fish, chronic exposure scenario. [FISHMC]			
<i>Verbal Description: An adult (70 kg male) consumes fish taken from contaminated ambient water for a lifetime. The levels in water are estimated from monitoring data and thus dissipation, degradation and other environmental processes are implicitly considered. For these calculations, only the typical application rate is considered.</i>			
Parameters/Assumptions	Value	Units	Source/Reference
Application Rates ($R_{(lb\ a.i./acre)}$)			
AppC	1	lb a.i./gal	APPL.Typ
AppL	1		APPL.Typ
AppU	1		APPL.Typ
Water Contamination Rate (WCR)($C_{(mg/L)} \div R_{(lb\ a.i./gal)}$)			
WCRC	0.002	mg/L/lb a.i./acre	Section 3.2.3.4.
WCRL	0.001		Section 3.2.3.4.
WCRU	0.004		Section 3.2.3.4.
Bioconcentration factor ($BCF_{(kg\ fish/L)}$)	25	kg fish/L	Average of range from WS08
Body weight (W)	70	kg	PUBL.BWM
Amount of fish consumed (A)			
General Population typical	0.01	kg/day	PUBL.FAT
upper limit	0.158	kg/day	PUBL.FAU
Native American subsistence populations typical	0.081	kg/day	PUBL.FNT
upper limit	0.77	kg/day	PUBL.FNU
Dose estimates (D) - see details of calculations on next page.			
General Public			
Typical	0.000007	mg/kg bw	FISHMCT
Lower	0.0000036	mg/kg bw	FISHMCL
Upper	0.00023	mg/kg bw	FISHMCU
General Public			
Typical	0.000058	mg/kg bw	FISHNMCT
Lower	0.000029	mg/kg bw	FISHNMCL
Upper	0.0011	mg/kg bw	FISHNMCU

Details of calculations on next page

Chronic Consumption of Contaminated Fish, Details of calculations

Equations (terms defined below or in table on previous page)

Verbal Description: Multiply the application rate ($R_{(\text{lb a.i./acre})}$) by the water contamination rate ($WCR_{((\text{mg/L}) \times (\text{lb a.i./gal}))}$) to get the concentration in ambient water. This product is in turn multiplied by the bioconcentration factor ($BCF_{(\text{kg fish/L})}$) and the amount of fish consumed per day ($A_{(\text{kg fish/day})}$) and then divided by the body weight ($W_{(\text{kg bw})}$) to get the estimate of the absorbed dose ($D_{(\text{mg/kg bw})}$).

$$D_{(\text{mg/kg bw})} = R_{(\text{lb a.i./acre})} \times WCR_{((\text{mg/L}) \times (\text{lb a.i./gal}))} \times A_{(\text{kg/day})} \times BCF_{(\text{kg fish/L})} \div W_{(\text{kg})}$$

General Public

Central Estimate:

Use the typical application rate, typical contamination rate (WCR), the typical fish consumption, the measured bioconcentration factor, and standard body weight.

$$D_{(\text{mg/kg bw})} = 1_{(\text{lb a.i./acre})} \times 0.002_{((\text{mg/L}) \times (\text{lb a.i./gal}))} \times 25_{(\text{kg fish/L})} \times 0.01_{(\text{kg fish/day})} \div 70_{(\text{kg bw})} = 0.0000071_{(\text{mg/kg bw})}$$

Lower Range of Estimate:

Use the anticipated application rate, lower range of contamination rate (WCR), the typical fish consumption, the measured bioconcentration factor, and standard body weight. Typical fish consumption is used because there is no published lower estimate.

$$D_{(\text{mg/kg bw})} = 1_{(\text{lb a.i./acre})} \times 0.001_{((\text{mg/L}) \times (\text{lb a.i./gal}))} \times 25_{(\text{kg fish/L})} \times 0.01_{(\text{kg fish/day})} \div 70_{(\text{kg bw})} = 0.0000036_{(\text{mg/kg bw})}$$

Upper Range of Estimate:

Use the highest labelled application rate, upper range of contamination rate (WCR), the maximum 1 fish consumption, the measured bioconcentration factor, and standard body weight.

$$D_{(\text{mg/kg bw})} = 1_{(\text{lb a.i./acre})} \times 0.004_{((\text{mg/L}) \times (\text{lb a.i./gal}))} \times 25_{(\text{kg fish/L})} \times 0.158_{(\text{kg fish/day})} \div 70_{(\text{kg bw})} = 0.00023_{(\text{mg/kg bw})}$$

Chronic Consumption of Contaminated Fish ***Details of calculations*** (continued)

Native American Subsistence Populations

Central Estimate:

Use the typical application rate, typical contamination rate (WCR), the typical fish consumption for native American subsistence populations, the measured bioconcentration factor, and standard body weight.

$$D_{(\text{mg/kg bw})} = 1_{(\text{lb a.i./acre})} \times 0.002_{((\text{mg/L}) \times (\text{lb a.i./gal}))} \times 25_{(\text{kg fish/L})} \times 0.081_{(\text{kg fush/day})} \div 70_{(\text{kg bw})} = 0.000058_{(\text{mg/kg bw})}$$

Lower Range of Estimate:

Use the lowest anticipated application rate, lower range of contamination rate (WCR), the typical fish consumption for native American subsistence populations, the measured bioconcentration factor, and standard body weight. Typical fish consumption is used because there is no published lower estimate.

$$D_{(\text{mg/kg bw})} = 1_{(\text{lb a.i./acre})} \times 0.001_{((\text{mg/L}) \times (\text{lb a.i./gal}))} \times 25_{(\text{kg fish/L})} \times 0.081_{(\text{kg fush/day})} \div 70_{(\text{kg bw})} = 0.000029_{(\text{mg/kg bw})}$$

Upper Range of Estimate:

Use the highest labelled application rate, upper range of contamination rate (WCR), the maximum 1 fish consumption for native American subsistence populations, the measured bioconcentration factor, and standard body weight.

$$D_{(\text{mg/kg bw})} = 1_{(\text{lb a.i./acre})} \times 0.004_{((\text{mg/L}) \times (\text{lb a.i./gal}))} \times 25_{(\text{kg fish/L})} \times 0.77_{(\text{kg fush/day})} \div 70_{(\text{kg bw})} = 0.0011_{(\text{mg/kg bw})}$$

EXPOSURE ASSESSMENTS for Terrestrial Species

Worksheet 24: Direct spray of small mammal assuming first order absorption kinetics. [SMDS]			
<p>Verbal Description: A 20 g mammal is directly sprayed over one half of the body surface as the chemical is being applied. The absorbed dose over the first day - i.e., a 24 hour period) is estimated using the assumption of first-order dermal absorption. In the absence of any data on dermal absorption in a small mammal, the estimated absorption rate for humans is used. An empirical relationship between body weight and surface area (Boxenbaum and D'Souza 1990) is used to estimate the surface area of the animal. Note: Uncertainty in this analysis is encompassed only by estimated differences in dermal absorption rates using the anticipated typical application rate. The effect of varying the application rate is considered in the risk characterization.</p>			
Parameter/Assumption	Value	Units	Source/Reference
Period of exposure (<i>T</i>)	24	hour	N/A
Body weight (<i>W</i>)	0.020	kg	Section 4.2.1.
Exposed surface area (<i>A</i>)	$\text{cm}^2 = 1110 \times \text{BW}(\text{kg})^{0.65}$		Boxenbaum and D'Souza 1990
	87.69	cm^2	
Application rate (<i>R</i>)			
Typical	1	lb a.i. /acre	WS08.TYP
Low	1		WS08.LOW
High	1		WS08.HI
Conversion Factor (<i>F</i>) for lb/acre to mg/cm^2	0.01121		WS01.LBAC_MGCM
First-order dermal absorption rate (<i>k_a</i>)			
Central	0.00050	hour^{-1}	WS15.AbsC
Low	0.000170	hour^{-1}	WS15.AbsL
High	0.0026	hour^{-1}	WS15.AbsU
Estimated Absorbed Doses (<i>D</i>) - see calculations below.			
Central	0.3	mg/kg	SMDSDC
Low	0.1	mg/kg	SMDSDL
High	1.5	mg/kg	SMDSDH

Details of calculations on next page.

Direct Spray of Small Mammal, first-order absorption, Details of calculations

Equation: $0.05 \times F \times R \times A \times 1^{-ka \times T} \div W$

Verbal Description: Multiply by 0.5 because only one half of the body surface is assumed to be sprayed. Calculate the amount deposited on the animal as the product of the application rate converted to mg/cm² and the surface area of the animal in cm². Get the proportion of the amount that is absorbed using the assumption of first order absorption kinetics. Divide by the body weight.

Central Estimate [SMDSDC]: Use the central estimate of the application rate and dermal absorption rate,

$$0.5 \times 0.01121 \text{ (mg/cm}^2\text{-lb/acre)} \times 1 \text{ lb/acre} \times 87.69 \text{ cm}^2 \\ \times 1 - e^{-0.0005/\text{h} \times 24\text{h}} \div 0.02 \text{ kg} = 0.3 \text{ mg/kg}$$

Lower Range of Estimate [CSPRYL]: Use the lowest anticipated application rate and lower 95% limit of the estimated dermal absorption rate,

$$0.5 \times 0.01121 \text{ (mg/cm}^2\text{-lb/acre)} \times 1 \text{ lb/acre} \times 87.69 \text{ cm}^2 \\ \times 1 - e^{-0.00017/\text{h} \times 24\text{h}} \div 0.02 \text{ kg} = 0.1 \text{ mg/kg}$$

Upper Range of Estimate [CSPRYH]: Use the highest anticipated application rate and upper 95% limit of the estimated dermal absorption rate,

$$0.5 \times 0.01121 \text{ (mg/cm}^2\text{-lb/acre)} \times 1 \text{ lb/acre} \times 87.69 \text{ cm}^2 \\ \times 0.0026/\text{h} \times 24 \text{ h} \div 0.02 \text{ kg} = 1.5 \text{ mg/kg}$$

Worksheet 25: Direct spray of small mammal assuming 100% absorption over the first 24 hour period. [SMDS2]

Verbal Description: A 20 g mammal is directly sprayed over one half of the body surface as the chemical is being applied. The deposited dose is assumed to be completely absorbed during the first day. An empirical relationship between body weight and surface area (Boxenbaum and D'Souza 1990) is used to estimate the surface area of the animal. The only source of variability in this exposure assessment is the application rate.

Parameter/Assumption	Value	Units	Source/Reference
Period of exposure (<i>T</i>)	24	hour	N/A
Body weight (<i>W</i>)	0.020	kg	Section 4.2.1.
Exposed surface area (<i>A</i>)	$\text{cm}^2=1110 \times \text{BW}(\text{kg})^{0.65}$		Boxenbaum and D'Souza 1990
	87	cm^2	
Application rate (<i>R</i>)			
Typical/Central	1	lb a.e. /acre	WS08.TYP
Low	0.5		WS08.LOW
High	2		WS08.HI
Conversion Factor (<i>F</i>) for lb/acre to mg/cm^2	0.01121		WS01.LBAC_MGCM
Estimated Absorbed Doses (<i>D</i>) - <i>see calculations below.</i>			
Central	24	mg/kg	SMDS2DC
Low	12	mg/kg	SMDS2DL
High	49	mg/kg	SMDS2DH

Details of calculations on next page.

Direct Spray of Small Mammal, Complete absorption, Details of calculations

Equation: $0.5 \times F \times R \times A \div W$

Verbal Description: Multiply by 0.5 because only one half of the body surface is assumed to be sprayed. Calculate the amount deposited on the animal as the product of the application rate converted to mg/cm² and the surface area of the animal in cm². Divide by the body weight.

Central Estimate [SMDS2DC]: Use the central estimate of the application rate,
 $0.5 \times 0.01121 \text{ (mg/cm}^2\div\text{lb/acre)} \times 1 \text{ lb/acre} \times 87.69 \text{ cm}^2 \div 0.02 \text{ kg} = 24 \text{ mg/kg}$

Lower Range of Estimate [SMDS2DL]: Use the lowest anticipated application rate,
 $0.5 \times 0.01121 \text{ (mg/cm}^2\div\text{lb/acre)} \times 0.5 \text{ lb/acre} \times 87.69 \text{ cm}^2 \div 0.02 \text{ kg} = 12 \text{ mg/kg}$

Upper Range of Estimate [SMDS2DH]: Use the highest anticipated application rate,
 $0.5 \times 0.01121 \text{ (mg/cm}^2\div\text{lb/acre)} \times 2 \text{ lb/acre} \times 87.69 \text{ cm}^2 \div 0.02 \text{ kg} = 49 \text{ mg/kg}$

Worksheet 26: Direct spray of bee assuming 100% absorption over the first 24 hour period. [BEEDS2]			
<i>Verbal Description: A 0.093 g bee is directly sprayed over one half of the body surface as the chemical is being applied. The deposited dose is assumed to be completely absorbed during the first day. An empirical relationship between body weight and surface area (Boxenbaum and D'Souze 1990) is used to estimate the surface area of the animal.</i>			
Parameter/Assumption	Value	Units	Source/Reference
Period of exposure (<i>T</i>)	24	hour	N/A
Body weight (<i>W</i>)	0.000093	kg	Section 4.2.1.
Exposed surface area (<i>A</i>)	$\text{cm}^2=1110 \times \text{BW}(\text{kg})^{0.65}$		Boxenbaum and D'Souza 1990
	2.7	cm^2	
Application rate (<i>R</i>)			
Central	1	lb a.i. /acre	WS08.TYP
Low	0.5		WS08.LOW
High	2		WS08.HI
Conversion Factor (<i>F</i>) for lb/acre to mg/cm^2	0.01121		WS01.LBAC_MGCM
Estimated Absorbed Doses (<i>D</i>) - <i>see calculations below.</i>			
Central	163	mg/kg	BEES2DC
Low	81	mg/kg	BEES2DL
High	325	mg/kg	BEES2DH

Direct Spray of Bee, Complete absorption, Details of calculations

Equation: $0.5 \times F \times R \times A \div W$

Verbal Description: Multiply by 0.5 because only one half of the body surface is assumed to be sprayed. Calculate the amount deposited on the animal as the product of the application rate converted to mg/cm^2 and the surface area of the animal in cm^2 . Divide by the body weight.

Central Estimate [SMDS2DC]: Use the central estimate of the application rate,
 $0.5 \times 0.01121 (\text{mg}/\text{cm}^2 \div \text{lb}/\text{acre}) \times 1 \text{ lb}/\text{acre} \times 2.7 \text{ cm}^2 \div 0.000093 \text{ kg} = 163 \text{ mg}/\text{kg}$

Lower Range of Estimate [SMDS2DL]: Use the lowest anticipated application rate,
 $0.5 \times 0.01121 (\text{mg}/\text{cm}^2 \div \text{lb}/\text{acre}) \times 0.5 \text{ lb}/\text{acre} \times 2.7 \text{ cm}^2 \div 0.000093 \text{ kg} = 81 \text{ mg}/\text{kg}$

Upper Range of Estimate [SMDS2DH]: Use the highest anticipated application rate,
 $0.5 \times 0.01121 (\text{mg}/\text{cm}^2 \div \text{lb}/\text{acre}) \times 2 \text{ lb}/\text{acre} \times 2.7 \text{ cm}^2 \div 0.000093 \text{ kg} = 325 \text{ mg}/\text{kg}$

Worksheet 27: Consumption of contaminated fruit by a small mammal, acute exposure scenario. [VGCSMA]				
<i>Verbal Description: A 20 g mammal consumes vegetation shortly after application of the chemical - i.e. no dissipation or degradation is considered. The contaminated vegetation accounts for 100% of the diet. Residue estimates based on relationships for leafy and leafy vegetables from Hoerger and Kenaga (1972) summarized in Worksheet 07a. NOTE: As specified below, this exposure assessment uses the typical application rate. Uncertainty is encompassed by estimated differences in the vegetation residue rate</i>				
Parameters/Assumptions	Value	Units	Source/Reference	
Body weight (W)	0.020	kg	N/A	
Food consumed per day (A)	0.003	kg	U.S. EPA 1989a	
Duration of exposure (D)	1	day	N/A	
Application rates (R)				
	Typical	1	lb a.i./acre	WS08.Typ
	Lower	1	lb a.i./acre	WS08.Low
	Upper	1	lb a.i./acre	WS08.Hi
Residue rates (rr)				
	Typical	35	RUD ¹	WS05a.LVT
	Lower	10	RUD ¹	WS05a.LVL
	Upper	125	RUD ¹	WS05a.LVU
Dose estimates (D) - see details of calculations below				
	Typical	5	mg/kg bw	VGCSMAC
	Lower	1.5	mg/kg bw	VGCSMAL
	Upper	19	mg/kg bw	VGCSMAU
¹ RUD: Residue Unit Dosage, term used by Hoerger and Kenaga (1972) for anticipated concentration on vegetation (mg chemical per kg of vegetation) for each 1 lb a.i./acre applied.				

Equation (terms defined in above table):

$$D \text{ (mg/kg bw)} = A(\text{kg}) \times R(\text{lb a.i./acre}) \times rr(\text{mg/kg veg.} \div \text{lb a.i./acre}) \div W(\text{kg bw})$$

Details of Calculations

Typical: Use typical application rate and typical RUD.

$$D = 0.003 \text{ kg} \times 1 \text{ lb a.i./acre} \times 35 \text{ mg/kg} \div \text{lb a.i./acre} \div 0.02 \text{ kg} = 5 \text{ mg/kg bw}$$

Lower: Use lowest estimated application rate. Use typical RUD because no lower estimate of the RUD is available.

$$D = 0.003 \text{ kg} \times 1 \text{ lb a.i./acre} \times 10 \text{ mg/kg} \div \text{lb a.i./acre} \div 0.02 \text{ kg} = 1.5 \text{ mg/kg bw}$$

Upper: Use highest estimated application rate and highest RUD.

$$D = 0.003 \text{ kg} \times 1 \text{ lb a.i./acre} \times 125 \text{ mg/kg} \div \text{lb a.i./acre} \div 0.02 \text{ kg} = 19 \text{ mg/kg bw}$$

Worksheet 28: Consumption of contaminated vegetation by a small mammal, chronic exposure scenario. [VGCSMC]

Verbal Description: A 20 g mammal consumes contaminated vegetation for a 90 day period starting shortly after application of the chemical. It is assumed that 100% of the diet is contaminated. Initial residue estimates are based on relationships for leaves and leafy vegetables from Hoerger and Kenaga (1972) summarized in Worksheet 07a. The foliar half-time is used to estimate the concentration on vegetation after 90 days. The geometric mean of the initial and 90 day concentrations is used as the estimate of the dose.

Parameters/Assumptions	Value	Units	Source/Reference
Duration of exposure (<i>D</i>)	90	days	N/A
Body weight (<i>W</i>)	0.02	kg	WS06.BWF
Food consumed per day (<i>A</i>)	0.003	kg	U.S. EPA 1989a
kg food consumed per kg bw	0.15	Unitless	0.003/0.02
Application rates (<i>R</i>)			
Typical	1	lb a.i./acre	WS08.Typ
Lower	1	lb a.i./acre	WS08.Low
Upper	1	lb a.i./acre	WS08.Hi
Residue rates (<i>rr</i>)			
Typical	35	RUD ¹	WS07a.LVT
Lower	10	RUD ¹	WS07a.LVL
Upper	125	RUD ¹	WS07a.LVU
Dose estimates (<i>D</i>) - see details of calculations on next page			
Typical	0.566	mg/kg bw	VGCSMCT
Lower	0.1620	mg/kg bw	VGCSMCL
Upper	2.02	mg/kg bw	VGCSMCU

¹ RUD: Residue Unit Dosage, term used by Hoerger and Kenaga (1972) for anticipated concentration on fruit (mg chemical per kg of vegetation) for each 1 lb a.i./acre applied.

Equations (terms defined below or in above table):

Step 1: Calculate C_0 , concentration in vegetation on Day 0 - i.e., day of application.

$$C_0 \text{ (mg/kg)} = R \text{ (lb a.i./acre)} \times rr \text{ (mg/kg} \div \text{lb a.i./acre)}$$

Step 2: Calculate C_{90} , concentration in vegetation on Day 90 ($t=90$ days) based on dissipation coefficient (k) derived from foliar half-life ($t_{1/2}$).

$$k \text{ (days}^{-1}\text{)} = \ln(2) \div t_{1/2} \text{ (days)}$$

$$C_{90} \text{ (mg/kg)} = C_0 \text{ (mg/kg)} \times e^{-tk}$$

Step 3: Use the geometric mean of C_0 and C_{90} to get a central estimate of concentration in vegetation (mg/kg veg.) and multiply this value by the vegetation consumption (kg veg/kg bw) to calculate the daily dose (mg/kg bw) over the exposure period.

$$D \text{ (mg/kg bw)} = (C_0 \times C_{90})^{0.5} \text{ (mg/kg veg.)} \times A \text{ kg veg./kg bw}$$

Details of calculations on next page

Subchronic consumption of vegetation by a small mammal: Details of calculations

Central Estimate:

Use the typical application rate, the typical vegetation consumption rate, and the typical residue rate along with the single available estimate of foliar half-time.

Step 1:

$$C_0 = 1 \text{ lb a.i./acre} \times 35 \text{ mg/kg veg.} = 35 \text{ mg/kg veg.}$$

Step 2:

$$k = \ln(2) \div 14 \text{ days}^{-1} = 0.0495$$

$$C_{90} = 35 \text{ mg/kg} \times e^{-0.0495 \times 90} = 0.4067 \text{ mg/kg veg.}$$

Step 3:

$$D \text{ (mg/kg bw/day)} = (35 \times 0.4067)^{0.5} \text{ (mg/kg veg.)} \times 0.15 \text{ kg veg/kg bw} = 0.566 \text{ mg/kg bw}$$

Lower Estimate:

Use the lowest anticipated application rate along with the single available estimate of foliar half-time.. Also the typical vegetation consumption rate and the typical residue rate because lower limits on these estimates are not available.

Step 1:

$$C_0 = 1 \text{ lb a.i./acre} \times 10 \text{ mg/kg veg.} = 10 \text{ mg/kg veg.}$$

Step 2:

$$k = \ln(2) \div 14 \text{ days}^{-1} = 0.0495$$

$$C_{90} = 10 \text{ mg/kg} \times e^{-0.0495 \times 90} = 0.116 \text{ mg/kg veg.}$$

Step 3:

$$D \text{ (mg/kg bw)} = (10 \times 0.116)^{0.5} \text{ (mg/kg veg.)} \times 0.15 \text{ (kg veg/kg bw)} = 0.162 \text{ (mg/kg bw)}$$

Upper Estimate:

Use the highest anticipated application rate, the upper range of the vegetation consumption rate and the upper range of the residue rate along with the single available estimate of foliar half-time.

Step 1:

$$C_0 = 1 \text{ lb a.i./acre} \times 125 \text{ mg/kg veg.} = 125 \text{ mg/kg veg.}$$

Step 2:

$$k = \ln(2) \div 14 \text{ days}^{-1} = 0.0495$$

$$C_{90} = 125 \text{ mg/kg} \times e^{-0.0495 \times 90} = 1.45 \text{ mg/kg veg.}$$

Step 3:

$$D \text{ (mg/kg bw)} = (125 \times 1.45)^{0.5} \text{ (mg/kg veg.)} \times 0.15 \text{ (kg veg/kg bw)} = 2.02 \text{ (mg/kg bw)}$$

Worksheet 29: Consumption of contaminated water by a small mammal, acute exposure scenario. [WTCSMA]

Verbal Description: A small (20g) mammal contaminated water shortly after an accidental spill of 200 gallons of a field solution into a pond that has an average depth of 1 m and a surface area of 1000 m² or about one-quarter acre . No dissipation or degradation is considered. NOTE: Concentrations in water are all based on the typical application rate and vary only with the range of recommended dilution rates. This is identical to the corresponding scenario for the human health risk assessment.

Parameters/Assumptions	Value	Units	Source/Reference
Surface area of pond [SA]	1000	m ²	N/A
Average depth [DPTH]	1	m	N/A
Volume of pond in cubic meters [VM]	1000	m ³	N/A
Volume of pond in Liters [VL]	1000000	L	1 m ³ = 1,000 L
Volume of spill [VS]	200	gallons	N/A
Concentrations in solution (C _(mg/L))			
Central	7490	mg/L	TYPDRE×1000
Low	4790	mg/L	LOWDRE×1000
High	14980	mg/L	HI_DRE×1000
Body weight (W)	0.02	kg	N/A
Amount of water consumed (A)	0.005	L/day	U.S. EPA 1989a
Dose estimates (D) - see details of calculations on next page.			
Typical	1.4	mg/kg bw	WTCSMAT
Lower	0.91	mg/kg bw	WTCSMAL
Upper	2.8	mg/kg bw	WTCSMAU

Details of calculations on next page

Acute Consumption of Contaminated Water from an Accidental Spill

Details of calculations

Equations (terms defined below or in table on previous page)

Step 1: Calculate the concentration in the pond based on the concentration in the spilled solution, the volume spilled and the volume of the pond, assuming instantaneous mixing.

$$\text{Conc. (mg/L)} = \text{VS (gal.)} \times 3.785 \text{ L/gal} \times \text{C (mg/L)} \div \text{VL (liters)}$$

Step 2: Calculate the dose based on the concentration in the water, the amount of water consumed, and the body weight.

$$\text{D (mg/kg bw)} = \text{Conc. (mg/L)} \times \text{A (L)} \div \text{W (kg)}$$

Calculations

Central Estimate:

Use the typical field dilution,

Step 1:

$$\text{Conc. (mg/L)} = 200 \text{ (gal.)} \times 3.785 \text{ L/gal} \times 7490 \text{ (mg/L)} \div 1000000 \text{ (liters)} = 5.67 \text{ (mg/L)}$$

Step 2:

$$\text{D (mg/kg bw)} = 5.67 \text{ (mg/L)} \times 0.005 \text{ (L)} \div 0.02 \text{ (kg)} = 1.4 \text{ (mg/kg bw)}$$

Lower Estimate:

Use the lowest estimated field dilution,

Step 1:

$$\text{Conc. (mg/L)} = 200 \text{ (gal.)} \times 3.785 \text{ L/gal} \times 4790 \text{ (mg/L)} \div 1000000 \text{ (liters)} = 3.63 \text{ (mg/L)}$$

Step 2:

$$\text{D (mg/kg bw)} = 3.63 \text{ (mg/L)} \times 0.005 \text{ (L)} \div 0.02 \text{ (kg)} = 0.91 \text{ (mg/kg bw)}$$

Upper Estimate:

Use the highest estimated field concentration,

Step 1:

$$\text{Conc. (mg/L)} = 200 \text{ (gal.)} \times 3.785 \text{ L/gal} \times 14980 \text{ (mg/L)} \div 1000000 \text{ (liters)} = 11.3 \text{ (mg/L)}$$

Step 2:

$$\text{D (mg/kg bw)} = 11.3 \text{ (mg/L)} \times 0.005 \text{ (L)} \div 0.02 \text{ (kg)} = 2.8 \text{ (mg/kg bw)}$$

Worksheet 30: Consumption of contaminated water by a small mammal, chronic exposure scenario. [WTCSMC]			
<i>Verbal Description: A small mammal (20 gram body weight) consumes contaminated ambient water for a lifetime. The levels in water are estimated from monitoring data and thus dissipation, degradation and other environmental processes are implicitly considered. Note: As currently set up, this scenario does not considered differences in application rates and only the typical application rate is used. The influence of application rate is discussed in the risk characterization. Variability is encompassed only by differences in estimated water contamination rates.</i>			
Parameters/Assumptions	Value	Units	Source/Reference
Application Rates ($R_{(lb\ a.i./acre)}$)			
Central	1	lb a.i./gal	WS08.Typ
Low	1		WS08Typ
High	1		WS08.Typ
Water Contamination Rate (WCR)($C_{(mg/L)} \div R_{(lb\ a.i./gal)}$)			
Central	0.002	mg/L/lb a.i./acre	Section 3.2.3.4
Low	0.001		Section 3.2.3.4
High	0.004		Section 3.2.3.4
Body weight (W)	0.02	kg	U.S. EPA 1989a
Amount of water consumed ($A_{(L/day)}$)	0.005	L/day	U.S. EPA 1989a
Dose estimates (D) - see details of calculations on next page.			
Typical	0.0005	mg/kg bw	WTCSMCT
Lower	0.00025	mg/kg bw	WTCSMCL
Upper	0.001	mg/kg bw	WTCSMCU

Details of calculations

Equations (terms defined in table on previous page)

Verbal Description: Multiply the application rate ($R_{(lb\ a.i./acre)}$) by the water contamination rate ($WCR_{((mg/L) \times (lb\ a.i./gal))}$) to get the concentration in ambient water. This product is in turn multiplied by the amount of water consumed per day ($A_{(L/day)}$) and then divided by the body weight ($W_{(kg)}$) to get the estimate of the absorbed dose ($D_{(mg/kg\ bw)}$).

$$D_{(mg/kg\ bw)} = R_{(lb\ a.i./acre)} \times WCR_{((mg/L) \times (lb\ a.i./gal))} \times A_{(L/day)} \div W_{(kg)}$$

Central Estimate:

Use the typical application rate and typical water contamination rate

$$D_{(mg/kg\ bw)} = 1_{(lb\ a.i./acre)} \times 0.002_{((mg/L) \times (lb\ a.i./gal))} \times 0.005_{(L/day)} \div 0.02_{(kg\ bw)} = 0.0005_{(mg/kg\ bw)}$$

Lower Range of Estimate:

Use the typical application rate and the low end of the range of the water contamination rate (WCR)

$$D_{(mg/kg\ bw)} = 1_{(lb\ a.i./acre)} \times 0.001_{((mg/L) \times (lb\ a.i./gal))} \times 0.005_{(L/day)} \div 0.02_{(kg\ bw)} = 0.00025_{(mg/kg\ bw)}$$

Upper range of Estimate:

Use the typical application rate and the low end of the range of the water contamination rate (WCR)

$$D_{(mg/kg\ bw)} = 1_{(lb\ a.i./acre)} \times 0.004_{((mg/L) \times (lb\ a.i./gal))} \times 0.005_{(L/day)} \div 0.02_{(kg\ bw)} = 0.001_{(mg/kg\ bw)}$$

SUMMARY TABLES

Worksheet 31a: Summary of Worker Exposure Scenarios [SUMWK01]

Scenario	Dose (mg/kg/day or event)			Worksheet
	Typical	Lower	Upper	
General Exposures (dose in mg/kg/day) [acid or ester formulations]¹				
Directed ground spray (Backpack)	1.3e-02	4.5e-04	8.0e-02	12a
Broadcast ground spray (Boom spray)	2.2e-02	6.6e-04	1.5e-01	12b
Aerial Applications	1.5e-02	2.4e-04	8.0e-02	12c
Aquatic Applications ^a	1.7e-02	7.6e-03	3.8e-02	12e
Accidental/Incidental Exposures (dose in mg/kg/event)				
Acid				
Immersion of Hands, 1 minute	2.2e-03	9.2e-04	5.3e-03	13a
Contaminated Gloves, 1 hour	1.3e-01	5.5e-02	3.2e-01	13a
Spill on lower legs, 1 hour	5.4e-02	1.8e-02	2.8e-01	14a
Spill on hands, 1 hour	2.2e-02	7.4e-03	1.1e-01	14a
Ester				
Immersion of Hands, 1 minute	3.2e-03	1.4e-03	7.8e-03	13a
Contaminated Gloves, 1 hour	1.9e-01	8.2e-02	4.7e-01	13a
Spill on lower legs, 1 hour	7.9e-02	2.7e-02	4.1e-01	14a
Spill on hands, 1 hour	3.2e-02	1.1e-02	1.7e-01	14a

¹ Based on an application rate of 1 lb a.e./acre unless otherwise specified.

^b Based on treating one acre at 19 lbs a.e./acre.

Worksheet 31b: Summary of Quantitative Risk Characterization for Workers [SUMWK01]

Scenario	Hazard Quotient			Exposure Assessment Worksheet
	Typical	Lower	Upper	
General Exposures [acid or ester formulations]: Hazard Quotients^a				
Directed ground spray (Backpack)	1	0.05	8	12a
Broadcast ground spray (Boom spray)	2	0.1	15	12b
Aerial Applications	1	0.02	8	12c
Aquatic Applications ^b	2	0.8	4	12e
Accidental/Incidental Exposures: Hazard Quotients				
Acid				
Immersion of Hands, 1 minute	0.2	0.1	0.5	13a
Contaminated Gloves, 1 hour	13	6	32	13a
Spill on lower legs, 1 hour	5	2	28	14a
Spill on hands, 1 hour	2	1	11	14a
Ester				
Immersion of Hands, 1 minute	0.3	0.1	0.8	13a
Contaminated Gloves, 1 hour	19	8	47	13a
Spill on lower legs, 1 hour	8	3	41	14a
Spill on hands, 1 hour	3	1	17	14a

^a Exposures are based on an application rate of 1 lb a.e./acre and estimates of acres covered per day unless otherwise specified. The exposures are divided by the U.S. EPA RfD of 0.01 mg/kg/day to calculate the hazard quotient.

^b Based on treating one acre at 19 lbs a.e./acre.

Worksheet 32a: Summary of Exposure Scenarios for the General Public [SUMPB01]

Scenario	Target	Dose (mg/kg/day)			Worksheet
		Typical	Lower	Upper	
Acute/Accidental Exposures					
Direct spray, acid formulation , entire body	Child	0.01086	0.00074	0.226	15a
Direct spray, ester formulation , entire body	Child	0.01358	0.002954	0.141	15b
Direct spray, acid formulation , lower legs	Woman	0.0011	0.000074	0.023	16a
Direct spray, ester formulation , lower legs	Woman	0.0014	0.0003	0.014	16b
Dermal, contaminated vegetation	Woman	0.0013	0.00043	0.0066	17
Contaminated fruit, acute exposure	Woman	0.035	0.0071	0.21	18
Contaminated water, acute exposure	Child	0.426	0.1663	1.28	20
Consumption of fish, general public	Man	0.32	0.2	0.64	22
Consumption of fish, subsistence populations	Man	1.6	1	3.1	22
Chronic/Longer Term Exposures					
Contaminated fruit	Woman	0.0023	0.00046	0.032	19
Consumption of water	Man	0.000057	0.00002	0.00014	21
Consumption of fish, general public	Man	0.000007	0.0000036	0.00023	23
Consumption of fish, subsistence populations	Man	0.000058	0.000029	0.0011	23

Worksheet 32b: Summary of Quantitative Risk Characterization for the General Public

[SUMPB02]

Scenario	Target	Hazard Quotient ^a			Exposure Assessment Worksheet
		Typical	Lower	Upper	
Acute/Accidental Exposures					
Direct spray, acid formulation , entire body	Child	1	0.07	23	15a
Direct spray, ester formulation , entire body	Child	1	0.3	14	15b
Direct spray, acid formulation , lower legs	Woman	0.1	0.01	2	16a
Direct spray, ester formulation , lower legs	Woman	0.1	0.03	1	16b
Dermal, contaminated vegetation	Woman	0.1	0.04	0.7	17
Contaminated fruit, acute exposure	Woman	4	1	21	18
Contaminated water, acute exposure	Child	43	17	128	20
Consumption of fish, general public	Man	32	20	64	22
Consumption of fish, subsistence populations	Man	160	100	310	22
Chronic/Longer Term Exposures					
Contaminated fruit	Woman	0.2	0.05	3	19
Consumption of water	Man	0.006	0.002	0.01	21
Consumption of fish, general public	Man	0.0007	0.0004	0.02	23
Consumption of fish, subsistence populations	Man	0.006	0.003	0.1	23

^a Exposures are based on an application rate of 1 lb a.e./acre. The exposures are divided by the U.S. EPA RfD of 0.01 mg/kg/day to calculate the hazard quotient.

Worksheet 33a: Summary of Exposure Scenarios for terrestrial animals [SUMTRA01]				
Scenario	Dose (mg/kg/day)			Worksheet
	Typical	Lower	Upper	
Acute/Accidental Exposures				
Direct spray, small mammal, first-order absorption	0.3	0.1	1.5	24
Direct spray, small animal, 100% absorption	24	12	49	25
Direct spray, bee, 100% absorption	163	81	325	26
Consumption of contaminated vegetation, acute exposure	5	1.5	19	27
Consumption of contaminated vegetation, chronic exposure	0.566	0.162	2.02	28
Consumption of contaminated water, acute exposure	1.4	0.91	2.8	29
Consumption of contaminated water, chronic exposure	0.0005	0.00025	0.001	30

Worksheet 33b: Summary of quantitative risk characterization for terrestrial animals. [SUMTRA02]			
Scenario	Dose (mg/kg/day)		
	Typical	Lower	Upper
Acute/Accidental Exposures¹			
Direct spray, small mammal, first-order absorption	0.03	0.01	0.2
Direct spray, small animal, 100% absorption	2	1	5
Direct spray, bee, 100% absorption ²	1	0.7	3
Consumption of contaminated vegetation, acute exposure	0.5	0.2	2
Consumption of contaminated water, acute exposure	0.1	0.09	0.3
Chronic Exposures³			
Consumption of contaminated vegetation, chronic exposure	1	0.2	2
Consumption of contaminated water, chronic exposure	0.001	0.0003	0.001
¹ Hazard quotients for acute exposures of mammals are based on the estimated acute non-lethal dose of 10 mg/kg. ³ Hazard quotient for bee is based on LD ₅₀ of 124 mg/kg. Upper range of LC ₅₀ is 1129 mg/kg. ² Hazard quotients for chronic mammalian exposures are calculated as the estimated exposure divided by the chronic rat NOAEL of 1 mg/kg/day and then rounded to one significant decimal or digit.			