SERA MD-2014-02b

Preparation of Environmental Documentation and Risk Assessments for the USDA/Forest Service

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REVISION NOTES

(Most recent first)

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This is a minor revision to SERA MD-2014-02a dated May 1, 2014. This updates the worker exposure rates in Table 6 based on post-peer review final report on worker exposure, which is cited in this document as SERA (2014). Other minor revisions involve replacing references to the peer review draft, which had been cited as SERA (2013), with references to SERA (2014). As with the previous revision, the PDF of the current document is 508 compliant.

A PDF version of this document is available at www.SERA-INC.com and should also be available at http://www.fs.fed.us/foresthealth/pesticide/risk.shtml.

May 1, 2014, SERA MD-2014-02a

This is an update and revision to modification to SERA MD-2011-01a, Preparation of Environmental Documentation and Risk Assessments for the USDA/Forest Service, dated September 8, 2011.

This revised document updates the discussion of worker exposure based on SERA TR-052-30-03a, Revised and Corrected Reassessment of Worker Exposure Rates, Final Report, dated October 13, 2013. The new worker exposure methods have been in used in Forest Service risk assessments since November, 2013. It should be noted that the external peer review of the newer worker exposure rates has not been completed and these rates are not yet incorporated in WorksheetMaker, Version 6 (SERA 2011a). It is anticipated that the new worker exposure rates will be incorporated into a new release of WorksheetMaker sometime in 2014 or 2015.

In addition, this document includes details of the methods used in WorksheetMaker 6 to calculate maximum time-weighted averages for longer-term exposure scenarios involving the consumption of contaminated vegetation following multiple applications of a pesticide. These methods are detailed in Appendix 1.

Lastly, this document has been modified for compliance with Section 508 of the Rehabilitation Act of 1973 as amended by the Workforce Investment Act of 1998. The compliance report is attached to the PDF version of this document.

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ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
AChE	acetylcholinesterase
AEL	adverse-effect level
a.i.	active ingredient
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
bw	body weight
calc	calculated value
CBI	confidential business information
ChE	cholinesterase
CI	confidence interval
cm	centimeter
CNS	central nervous system
DAA	days after application
DAT	days after treatment
DER	data evaluation record
d.f.	degrees of freedom
EC_x	concentration causing X% inhibition of a process
EC_{25}	concentration causing 25% inhibition of a process
EC_{50}	concentration causing 50% inhibition of a process
ED_x	dose causing X% inhibition of a process
ED_{25}	dose causing 25% inhibition of a process
EC_{50}	dose causing 50% inhibition of a process
EFED	Environmental Fate and Effects Division (U.S. EPA/OPP)
ERA	Ecological Risk Assessment
ExToxNet	Extension Toxicology Network
F	female
FH	Forest Health
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FQPA	Food Quality Protection Act
g	gram
GLP	Good Laboratory Practices
ha	hectare
HED	Health Effects Division (U.S. EPA/OPP)
HHRA	Human Health Risk Assessment
HQ	hazard quotient
IARC	International Agency for Research on Cancer
IRED	Interim Reregistration Eligibility Decision
IRIS	Integrated Risk Information System
k_a	absorption coefficient
k_e	elimination coefficient
kg	kilogram
$K_{o/c}$	organic carbon partition coefficient
$K_{o/w}$	octanol-water partition coefficient
K_p	skin permeability coefficient
L	liter
lb	pound

LC_{50}	lethal concentration, 50% kill
LD_{50}	lethal dose, 50% kill
LOAEL	lowest-observed-adverse-effect level
LOC	level of concern
m	meter
M	male
MEI	most exposed individual
MFO	mixed-function oxidases
mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
mM	millimole
mPa	millipascal, (0.001 Pa)
MOS	margin of safety
MRID	Master Record Identification Number
MSDS	material safety data sheet
MSMA	monosodium methanearsonate
MW	molecular weight
NAWQA	USGS National Water Quality Assessment
NCI	National Cancer Institute
NCOD	National Drinking Water Contaminant Occurrence Database
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NOS	not otherwise specified
NRC	National Research Council
NTP	National Toxicology Program
OM	organic matter
OPP	Office of Pesticide Programs
OPPTS	Office of Pesticide Planning and Toxic Substances
OSHA	Occupational Safety and Health Administration
Pa	Pascal
PBPK	physiologically-based kinetic
ppm	parts per million
RBC	red blood cells
RED	re-registration eligibility decision
RfC	reference concentration
RfD	reference dose
SERA	Syracuse Environmental Research Associates
TEP	typical end-use product
T.G.I.A.	Technical grade active ingredient
TIPA	Triisopropanolamine
TRED	Tolerance Reassessment Eligibility Decision
UF	uncertainty factor
U.S.	United States
USDA	U.S. Department of Agriculture

U.S. EPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
WHO	World Health Organization

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

To convert ...	Into ...	Multiply by ...
acres	hectares (ha)	0.4047
acres	square meters (m^2)	4,047
atmospheres	millimeters of mercury	760
centigrade	Fahrenheit	1.8℃+32
centimeters	inches	0.3937
cubic meters (m^3)	liters (L)	1,000
Fahrenheit	centigrade	0.556℉-17.8
feet per second (ft/sec)	miles/hour (mi/hr)	0.6818
gallons (gal)	liters (L)	3.785
gallons per acre (gal/acre)	liters per hectare (L/ha)	9.34
grams (g)	ounces, (oz)	0.03527
grams (g)	pounds, (oz)	0.002205
hectares (ha)	acres	2.471
inches (in)	centimeters (cm)	2.540
kilograms (kg)	ounces, (oz)	35.274
kilograms (kg)	pounds, (lb)	2.2046
kilograms per hectare (hg/ha)	pounds per acre (lb/acre)	0.892
kilometers (km)	miles (mi)	0.6214
liters (L)	cubic centimeters (cm^3)	1,000
liters (L)	gallons (gal)	0.2642
liters (L)	ounces, fluid (oz)	33.814
miles (mi)	kilometers (km)	1.609
miles per hour (mi/hr)	cm/sec	44.70
milligrams (mg)	ounces (oz)	0.000035
meters (m)	feet	3.281
ounces (oz)	grams (g)	28.3495
ounces per acre (oz/acre)	grams per hectare (g/ha)	70.1
ounces per acre (oz/acre)	kilograms per hectare (kg/ha)	0.0701
ounces fluid	cubic centimeters (cm^3)	29.5735
pounds (lb)	grams (g)	453.6
pounds (lb)	kilograms (kg)	0.4536
pounds per acre (lb/acre)	kilograms per hectare (kg/ha)	1.121
pounds per acre (lb/acre)	mg/square meter (mg/m^2)	112.1
pounds per acre (lb/acre)	µg/square centimeter ($\mu g/cm^2$)	11.21
pounds per gallon (lb/gal)	grams per liter (g/L)	119.8
square centimeters (cm^2)	square inches (in^2)	0.155
square centimeters (cm^2)	square meters (m^2)	0.0001
square meters (m^2)	square centimeters (cm^2)	10,000
yards	meters	0.9144

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

CONVERSION OF SCIENTIFIC NOTATION

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

1. INTRODUCTION

1.1. Scope and Intent

SERA, Inc. has prepared risk assessments for the USDA Forest Service, Office of Forest Health, since 1995 (USDA/FS Contract No. 53-3187-5-12). In addition, SERA, Inc. has prepared various other risk assessments for both the Forest Service and USDA/APHIS since 1990. During this period, the methods used to conduct these risk assessments evolved and changed substantially. The purpose of this document is to describe in detail the methods currently used by SERA, Inc. in the conduct of these risk assessments. Specifically, this document is an update to the methods described in SERA (2007a).

The risk assessments prepared by SERA consist of analyses of both human-health effects and ecological effects to support an assessment of the environmental consequences of the use of various chemicals in Forest Service programs. In this context, support does not imply that any attempt is made to bias analyses toward making the chemicals look safe. To the contrary, the Forest Service has accepted and often insisted on the use of very conservative methods both in the assessment of exposures as well as consequences. These methods are detailed in the current document.

Although the risk assessments are technical support documents and typically address specialized technical areas, an effort is made to ensure that the document can be understood by individuals who do not have specialized training in the chemical and biological sciences. At the same time, the risk assessments must be sufficiently detailed and technical to allow for review by individuals with substantial and often highly specialized expertise in various areas of environmental science. As a consequence, some of the discussions and calculations presented in each risk assessment may be very complicated. These discussions are presented as necessary in the major sections of each risk assessment. Nonetheless, each of the major sections is preceded by an *Overview* section that is intended to be readily understood by readers who do not have training in the sciences.

The basic philosophy for preparing the risk assessments is that each risk assessment must be totally transparent. If a risk assessment is to be properly reviewed, understood, critiqued, and used, the source of all numbers, the calculations used in generating the numbers, and the assumptions used in manipulating the numbers must be outlined clearly. In some respects, the transparency of a risk assessment is more important than the specific methods or calculations used to prepare it. Risk assessment is a form of analysis that relies on scientific method but is not itself a science. Reasonable individuals may disagree over which of the numerous methods, tools, and approaches should be used to prepare a risk assessment. Often, available information is not sufficient to support one analytical approach over another. Professional judgment must then be used to select the method; in which case, the risk assessment must clearly state which assumptions are used and why. As long as the assumptions are made clear, the quality of the risk assessment may be reviewed and the risk assessment may be critiqued as appropriate and improved in review.

As part of this transparency, this current document details the methods that are used to prepare risk assessments for the USDA Forest Service. To the extent possible, the

organization of this document parallels the organization of the risk assessments. This introductory chapter presents the basic conceptual framework for the risk assessment process, briefly discusses the organization of the risk assessment, and describes the methods used to identify and screen information for inclusion into the risk assessments. The subsequent sections of this document further parallel those of each risk assessment: Program Description (Section 2), Human Health Risk Assessment (Section 3), and Ecological Risk Assessment (Section 4). As with each of the risk assessments, various types of supporting information are included in appendices. The organization of all of these chapters is very similar to the organization of the corresponding chapters in each risk assessment.

1.2. Risk Assessment Framework

1.2.1. Basic (NAS) Approach

An overview of the risk assessment process use by SERA in Forest Service risk assessments is given in Figure 1. This process is based on the National Research Council of the National Academy of Sciences (NRC 1983) report which recommended a four step process: hazard identification, exposure assessment, dose-response assessment, and risk characterization. Each of the two risk assessment chapters (human health and ecological effects) are organized in this manner. As a corollary, the fundamental principle in all SERA risk assessments is that:

> *Risk cannot be characterized quantitatively unless a hazard can be identified, exposures can be quantitatively estimated, and a dose-response relationship can be expressed quantitatively.*

Each of the basic steps illustrate in Figure 1 are summarized in the following subsections. Details of these steps are given in the appropriate sections of methodology for human health risk assessments (Section 3) and ecological risk assessments (Section 4).

1.2.1.1. Hazard Identification

Hazard identification is the process of identifying what, if any, effects a compound is likely to have on an exposed population. Hazard identification is the first and most critical step in any risk assessment. Unless a biological effect can be demonstrated, the nature of the subsequent dose-response assessment and risk characterization is extremely limited. Both the human health and ecological risk assessments are prepared based on *in vivo* and *in vitro* data from experimental animal studies. Additional sources of information like epidemiology studies, case reports, and clinical investigations are used to prepare human health risk assessment. Studies on various model nontarget test species (e.g., ducks, quail, fish, aquatic invertebrates, plants, and terrestrial invertebrates) are commonly available to strengthen an ecological risk assessment. In addition, available field studies on nontarget species are used in ecological risk assessments in much the same way epidemiology studies are used in human health risk assessments. The hazard identification is based on a review of the toxicological and pharmacokinetics data and is arranged to focus on the dose-response and dose-severity relationships. Of these two relationships, the dose-severity relationship is generally more relevant for non-carcinogenic effects in humans and nontarget species.

The severity scale used to conduct the risk assessment typically employs five levels of severity, which are defined in Table 1. The terminology used in human health and ecological risk assessments is somewhat different, but the concepts are virtually identical. In human health risk assessment, severity is typically defined by the consequences of different levels of exposure. These include the no-observed-effect level (NOEL), no-observed-adverse-effect level (NOAEL), adverse-effect level (AEL), and frank-effect level (FEL). An additional term, lowest-observed-adverse-effect level (LOAEL) is sometimes used to designate the lowest AEL. This scale, with minor differences in nomenclature, is used by many government agencies to classify the toxicological effects observed in experimental or epidemiology studies. In the ecotoxicology literature, the term NOEC—no observed effect concentration—is sometimes used rather than the term NOEL.

The hazard identification process involves making judgments about which effects are most relevant to the assessment of human health or nontarget species. During this process, studies may be eliminated from consideration because they are inherently flawed, or because they are grossly inconsistent with the preponderance of other studies.

Although hazard identification results in a qualitative determination, quantitative methods are usually required as in most other assessments of causality. For instance, the process of hazard identification often hinges on a statistical assessment of exposure-response or dose-response relationships. Furthermore, hazard identification must also consider fundamental and qualitative differences among species. Depending on the chemical of concern, hazard identification also may include the use of quantitative or qualitative structure activity relationships or differences in pharmacokinetics.

1.2.1.2. Exposure Assessment

The exposure scenarios considered in a risk assessment involving pesticide exposure are determined by the application method and the chemical and toxicological properties of the compound. Depending on the properties of the chemical and the application method, the risk assessment may consider acute, subchronic, or chronic durations of oral, dermal, inhalation or combined routes of exposure to the pesticide.

1.2.1.2.1. Human Health

Exposure scenarios are developed for workers and members of the general public. For each group, two types of exposure scenarios are generally taken into consideration: general exposure and accidental/incidental exposure.

The term general exposure refers to human exposure resulting from the normal use of the chemical. For workers, general exposure involves the handling and application of the compound. These general exposure scenarios can be interpreted relatively easily and objectively. The exposure estimates are calculated from the amount of the chemical handled/day and the exposure rates for the worker group. Although each of the specific exposure assessments for workers involves degrees of uncertainty, the exposure estimates are objective in that they are based on empirical relationships of absorbed dose to pesticide use. For the general public the general exposure scenarios are somewhat more arbitrary and may be less plausible. For each pesticide, at least three general exposure scenarios are considered, including walking through a contaminated area shortly after treatment, the consumption of

ambient water from a contaminated watershed, and the consumption of contaminated vegetation. These three scenarios are consistently used because one of them usually leads to the highest estimates of exposure. Additional scenarios discussed below may be considered for each of the individual compounds as warranted by the available data and the nature of the program activities.

Some, if not all, of these general exposure scenarios for the general public may seem implausible or at least extremely conservative. For example, in many cases compounds are applied in relatively remote areas and so it is not likely that members of the general public would be exposed to plants shortly after treatment. Similarly, the estimates of longer-term consumption of contaminated water are based on estimated application rates and monitoring studies that can be used to relate levels in ambient water to treatment rates in a watershed; however, in most pesticide applications, substantial portions of a watershed are not likely to be treated. Finally, the exposure scenarios based on longer-term consumption of contaminated vegetation assume that an area of edible plants is inadvertently sprayed and that these plants are consumed by an individual over a 90-day period. While such inadvertent contamination might occur, it is extremely unlikely to happen as a result of directed applications (e.g., backpack applications). Even in the case of boom spray operations, the spray is directed at target vegetation and the possibility of inadvertent contamination of cultivated or edible vegetation would be low. In addition, for herbicides and other phytotoxic compounds, it is likely that the contaminated plants would show obvious signs of damage over a relatively short period of time and would therefore not be consumed.

All of the factors discussed above concerning general exposure scenarios for the general public have merit and must be considered in the interpretation of the risk characterization (Section 3.4). Thus, the typical hazard to the general public may often be negligible because significant levels of exposure are not likely. For the general public, the general exposures may be regarded as extreme in that they are based on very conservative exposure assessments and/or very implausible events. Nonetheless, these general exposure assessments are included because the risk assessment is intended to be extremely conservative with respect to potential effects on the general public, and to provide estimates regarding the likelihood and nature of effects after human exposure to pesticides.

Accidental/incidental exposure scenarios describe specific examples of gross over-exposure associated with mischance or mishandling of a chemical. All of these exposure scenarios are arbitrary in that the nature and duration of the exposure is fixed. For example, the worker exposure scenario involving immersion of the hands is based on a 1-minute period of exposure but could just as easily be based on an exposure period of 5 seconds or 5 minutes. Similarly, the consequences of wearing contaminated gloves could be evaluated at 4 hours rather than at 1 hour. These scenarios are intended to provide an indication of relative hazard among different pesticides and different events in a manner that facilitates conversion or extrapolation to other exposure conditions.

Like the general exposure scenarios, the accidental exposures for the general public may be regarded as more extreme than those for workers. Three scenarios are included in each

exposure assessment. They include direct spray, the consumption of contaminated water shortly after a spill, and the consumption of contaminated vegetation shortly after treatment. The direct spray scenario is clearly extreme. It assumes that a naked child is sprayed directly with a pesticide as it is being applied and that no steps are taken to remove the pesticide from the child for 1 hour. There are no reports of such incidents in the literature, and the likelihood of such an incident occurring appears to be remote. Nonetheless, this scenario and others like it are useful not only as a uniform comparison among pesticides but also as a simplifying step in the risk assessment. If the 'naked child' scenario indicates no basis for concern, other dermal spray scenarios will not suggest a potential hazard and need not be explored. If there is a potential hazard, other more plausible exposure scenarios may need to be considered. The other two accidental scenarios are similarly intended to serve as uniform comparisons among chemicals as well as a means of evaluating the need to explore additional exposure scenarios.

Typically, the level of exposure is directly proportional to the exposure parameters. The exposure associated with wearing gloves for 4 hours is 4 times the exposure associated with wearing contaminated gloves for 1 hour. Similarly, the general exposure scenarios for workers are based on an 8-hour work day. If a 4-hour application period were used, the hazard indices would be reduced by a factor of two. As another example, general exposure scenarios for both workers and the general public are linearly related to the application rate. Consequently, if the application rate were to double or vary by some other factor, the estimated exposure would double or vary by the same factor. Thus, the specific exposure parameters used in the risk assessment are selected to allow for relatively simple extrapolation to greater or lesser degrees of exposure.

Additional variability is taken into consideration by estimating exposure doses or absorbed doses for individuals of different age groups (i.e., adults, young children, toddlers, and infants). Children may behave in ways that increase their exposure to applied pesticides (e.g., long periods of outdoor play, pica, or imprudent consumption of contaminated media or materials). In addition, anatomical and physiological factors, such as body surface area, and breathing rates and consumption rates for food and water, are not linearly related to body weight and age. Consequently, the models used to estimate the exposure dose (e.g., mg/kg body weight/day) based on chemical concentrations in environmental media (e.g., ppm in air, water, or food) indicate that children, compared with individuals of different age groups, are generally exposed to the highest doses of chemicals for a given environmental concentration.

1.2.1.2.2. Ecological Effects

The exposure assessments for ecological effects are conceptually similar to those conducted in the human health risk assessment, and for many terrestrial organisms the exposure assessments are parallel to those used in the human health risk assessment. Similarly, exposures of aquatic species are typically based on the same estimates of concentrations of the chemical in water that are used in the human health risk assessment.

Terrestrial animals might be exposed to any applied pesticide from direct spray, the ingestion of contaminated media (vegetation, prey species, or water), grooming activities, or indirect contact with contaminated vegetation. Estimates of oral exposure are expressed in the same units as the available toxicity data. As in the human health risk assessment, these units are

usually expressed as mg of agent per kg of body weight and abbreviated as mg/kg body weight. For dermal exposure, the units of measure usually are expressed in mg of agent per cm^2 of surface area of the organism and abbreviated as mg/cm^2. In estimating dose, however, a distinction is made between the exposure dose and the absorbed dose. The exposure dose is the amount of material on the organism (i.e., the product of the residue level in mg/cm^2 and the amount of surface area exposed), which can be expressed either as mg/organism or mg/kg body weight. The absorbed dose is the proportion of the exposure dose that is actually taken in or absorbed by the animal.

For the exposure assessments discussed below, general allometric relationships are used to model exposure (e.g., Nagy 1987, 1994, 2001, 2005; Nagy and Obst 1991; Nagy et al. 1999). These relationships dictate that for a fixed level of exposure (e.g., concentrations 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. Details of the species typically considered in Forest Service risk assessments are discussed further in Section 4.2.

1.2.1.3. Dose-Response Assessment

The purpose of the dose-response assessment is to describe the degree or severity of risk as a function of dose. In classical toxicology, dose-response assessments are usually expressed as linear or non-linear equations, such as probit analysis and the multistage model, respectively. Using these methods, the prevalence or magnitude of a response can be estimated for any dose level. In regulatory toxicology, this approach is the exception rather than the rule.

Most dose-response assessments in regulatory toxicology, as discussed below, result in point estimates. Although some methods in regulatory toxicology use dose-response models, the regulatory value actually used in the risk assessment is a point estimate. For example, U.S. EPA cancer risk assessments usually employ a form of the multistage model or some other linear dose-response relationship that provide measures of variability or error. The estimate used in setting exposure criteria, however, is typically a point estimate that is a single value rather than a range of values (U.S. EPA/RAF 2005). The results of other commonly used dose-response assessments, such as RfDs, and RfCs, are point estimates of doses that are not believed to be associated with any adverse effect and that are not directly related to a dose-response model.

The practice of relying on point estimates in regulatory toxicology is grounded in the history of this discipline (Dourson and Stara 1983). From its inception, the focus of regulatory toxicology has been the development of criteria (i.e., levels of exposure that are defined as safe). Consequently, the methods used in regulatory toxicology are conservative.

Consistent with the recommendation of NRC (1983) that various groups within the federal government adopt common risk assessment methodologies, standard dose-response assessments are generally based on reference values, like RfDs, derived by other government agencies. This approach avoids a duplication of effort, capitalizes on the expertise of other organizations, and decreases the size, complexity, and cost of risk assessments.

In most respects, dose-response assessments for ecological effects are conceptually similar to the methods employed in the human health risk assessments, with one major exception.

Human health risk assessments focus on protecting the individual. This is why uncertainty factors (sometimes very large uncertainty factors) are used to derive RfD values and why cancer risk is estimated using very conservative assumptions. In ecological risk assessment, the focus is on a population or community rather than an individual. Thus, the use of uncertainty factors is less common and the general methods for dose-response assessment are less conservative.

For terrestrial mammals, the dose-response assessment generally is based on the same data used to derive the acute and chronic RfDs in the human health risk assessment: an NOAEL from an acute or chronic exposure study. The data on other terrestrial animals, both birds and invertebrates, are often not as detailed as the available information on experimental mammals. Fewer toxicological endpoints are examined, and, at least for vertebrates, lifetime or chronic studies are seldom available.

For some aquatic species as well as other groups of organisms, sensitive life-stage studies are often available. Such studies include egg-and-fry studies in fish and life-cycle toxicity studies in *Daphnia magna*, both of which are typically required by the U.S. EPA for the registration of herbicides. The studies are obtained and assessed following the same criteria applied to studies for the human health risk assessment. The principal difference is that NOEL, NOEC, or other toxicity values are used directly rather than RfD values that involve the application of uncertainty factors.

Nonetheless, dose-response assessments for some nontarget species considered in a risk assessment can be complicated (Section 4.3). As in the human health dose-response assessment, the nature of the available data as well as the potential risk may dictate the use of relatively complex dose-response analyses.

1.2.1.4. Risk Characterization

Conceptually, risk characterization is simply the process of comparing the exposure assessment to the dose-response assessment. In this process, risk is characterized quantitatively either as a ratio or as an incidence of response or a defined risk level – i.e., a risk of 5%.

Because the risk characterization flows directly from the exposure and dose-response assessments, the complexity and clarity of the risk characterization will be dependent on complexity and clarity of both the exposure and dose-response assessments. In most cases, risk will be quantitatively characterized as a ratio: a level of exposure divided by some defined effect level. In the human health risk assessment, the defined effect level is almost always the reference dose (RfD), and the ratio of the exposure to the reference dose is referred to as the hazard quotient (HQ). In the ecological risk assessments, the defined effect level is may be an NOEC or a risk level. The risk level, in turn, may be a lethal dose (e.g., LD_{50} or some other response level such as an LD_{25}) or a dose causing some risk of a non-lethal effect (e.g., an ED_{50} or ED_{25}). For aquatic organisms and for some terrestrial organisms for which exposure is characterized by a concentration rather than a dose, the defined risk levels may be expressed as a lethal concentration (LC_{50} or some other response level) or a sublethal concentration that leads to some effect (e.g., an EC_{50}). In general, the Forest Service prefers to use NOAEL or NOEC values in risk characterizations. If NOAEL

or NOEC values are not available, a sublethal effective dose at some response rate (e.g., ED_X or EC_X where X is some level of response) may be used to approximate an NOAEL or NOAEL as detailed further in Section 4.3.

If sufficient data are available and if the HQs suggest some level of concern, dose-response or dose-severity relationships may be used to characterize risk. Dose-response relationships most often involve explicit dose-response functions that lead to an explicit estimate of risk (e.g., a response rate of 13.2% for some effect or an 8% decrease in some biological function). Dose-severity relationships are typically less quantitative and lead to some assessment of what effects might be observed in a population at various levels of exposure. A fuller discussion of the hazard quotient method as well as the dose-response and dose-severity relationships are given in Section 3.4 (Human Health Effects) and Section 4.4 (Ecological Effects).

1.2.2. Elaborations

1.2.2.1. Probabilistic Risk Assessment

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. In general, variability and uncertainty can be distinguished from each other depending on the state of knowledge or information. Variability reflects the knowledge of how things may change. By acquiring more knowledge or information, better estimates of variability may be obtained but the variability itself will not decrease – i.e., it is inherent in the population or system being considered. Differences in human body weights are a good example of variability. Uncertainty reflects a lack of knowledge and uncertainty can be reduced by acquiring information. For example, while the toxicity of herbicides has been tested in the honey bee, very little information is available on the toxicity of most herbicides to other nontarget terrestrial insects. This leads to uncertainty (in terms of how representative the honey bee is for other insects) but this uncertainty can be reduced by conducting experiments on the toxicity of the herbicide to other insects.

Variability may take several forms. For this risk assessment, three types of variability are distinguished: statistical, situational, and arbitrary. Statistical variability reflects 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 an 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 on to 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.

In order to quantitatively address both variability and uncertainty, risk assessment methods generically referred to as probabilistic risk assessment have been and continue to be developed. The general approach for probabilistic risk assessment, particularly with respect to ecological species, has been articulated by Ecological Committee on FIFRA Risk Assessment Methods (ECOFRAM 1999a,b). The basic approach given in ECOFRAM (1999a,b) involves a tiered risk assessment process:

Tier 1: Very conservative screening methods involving worse case assumptions in terms of both exposure and dose-response. Risk is typically expressed as a point estimate such as an HQ or RQ.

Tier 2: Typically elaborates or refines the exposure assessment to include more realistic estimates of exposures and may elaborate the dose-response assessment to include the use of full dose-response curves. Risk may be expressed in terms of probabilities rather than point estimates.

Tier 3: An extension of a Tier 2 approach that may involve the inclusion of data on additional species (e.g., species sensitivity distributions) and more sophisticated exposure models.

Tier 4: Is the most complex risk assessment and may involve experimental or monitoring programs designed to definitively characterize either exposure and toxicity and the use of all available data including microcosm, mesocosm, and field studies.

As implied by the term Tier, probabilistic risk assessments under the general ECOFRAM model are designed to be conducted in stages going from the most conservative or worst-case approach (Tier 1) to less extreme and presumably more realistic assessments. Because this staged approach typically results in progressively lessened perceptions of risk, probabilistic risk assessments have been criticized as simply mechanisms to make risk disappear by mathematical manipulations. This criticism is addressed in ECOFRAM (1999) and is largely unfounded. While any risk assessment, probabilistic or otherwise, can be manipulated to distort risk (either upward or downward), the proper application of probabilistic risk assessment typically results not in conflicting risk characterizations at the different tiers but rather in more fully elaborated and refined risk assessments.

The nomenclature of probabilistic risk assessments, particularly as embodied in ECOFRAM (1999a,b) is somewhat different from that of NAS (1983) but the concepts are essentially the same. The first stage of a probabilistic risk assessment is typically referred to as the Problem Formulation. This is similar to the Hazard Identification as defined by NAS (1983) but focuses on identifying which organisms are likely to be at greatest risk. The other stages of the risk assessment process defined by ECOFRAM (1999a,b) are exposure characterization, effects characterization, and risk characterization and correspond closely to more general definitions given by NAS (1983) for the exposure assessment, dose-response assessment, and risk characterization.

In the higher tiered risk assessments, the probabilistic approach is based on more sophisticated methods of handling data and expressing both variability and uncertainty. A central feature of many higher tiered probabilistic risk assessments is Monte Carlo Analysis. Monte Carlo Analysis is a general term for any simulation that uses probability distributions rather than point estimates to represent and approximate the variability in a system model. The method was originally developed in the 1940's, shortly after the development of computers, to make probabilistic approximations to the solutions of mathematical equations or models that could not be solved analytically (U.S. EPA/RAF, 1997).

Monte Carlo Analyses can be relatively simple or very complicated depending on the simplicity or complexity of the model. As a simple example, take a situation in which we knew that a population of individuals will be exposed each day to up to 200 mg of a chemical. In this population, the smallest individual will have a body weight of about 52 kg. Thus, the maximum daily dose is about 3.8 mg/kg body weight. In addition, we knew that the RfD for the general population is 3.5 mg/kg. Taking a standard ratio approach using point estimates (Section 1.2.1.4), the hazard quotient would be about 1.1, somewhat above the level of concern. This would be a standard point-estimate worst-case approach and the risk assessment would conclude that some unspecified number of individuals could be subject to exposures that would not be generally considered acceptable.

Suppose, however, that the average body weight was 70 kg and the body weights in the population evidenced a normal distribution with a standard deviation of 10 kg. In addition, suppose that we knew that not all individuals would be exposed to the same amount of the chemical but that the amount could vary from 50 mg/day to 200 mg/day. Lastly, while the RfD was 3.5 mg/kg/day, we also knew that some individuals could be more sensitive and might respond with an adverse effect at a dose above 2 mg/kg/day, but that other individuals would not respond adversely until the dose reached 10 mg/kg/day. This sort of variability could be modeled in a Monte Carlo Analysis with the following assumptions:

Parameter	Distribution
Body weight	Normal distribution with a mean of 70 kg and a standard deviation of 10 kg
Exposure	Uniform distribution with a range of 50 mg/day to 200 mg/day.
RfD	Triangular with a mode of 3.5 mg/kg/day, a lower limit of 2 mg/kg/day and an upper limit of 10 mg/kg/day

An illustration of the results of a Monte Carlo Analysis of this simple model is given in Figure 2. Under the conditions of the simulation, the hazard quotient would be greater than unity (the level of concern for this scenario) for about 5% of the population.

Note that the use of a Monte Carlo simulation does not necessarily change the conclusions risk assessment. In the above example, the simulation is consistent with the worst-case point

estimate approach: some people will be at risk. The Monte Carlo simulation, however, does incorporate more information into the assessment and allows the risk assessor to better characterize the consequences – i.e., about 5% of the individuals may be exposed to more of the agent than would be generally considered acceptable.

Most practical Monte Carlo simulations are much more complicated and may involve quantitative considerations of differences in sensitivity among different species (e.g., Posthuma et al. 2002) as well as very complex applications of environmental fate models (e.g., Randall et al. 2003). Also, although elementary Monte Carlo Analyses can be conducted in commonly available software programs like EXCEL, most Monte Carlo analyses require relatively specialized software. The above example was conducted using an EXCEL add-in called Crystal Ball (Decisioneering 2004) that is commonly used in probabilistic risk assessments conducted by or for the U.S. EPA's Office of Pesticides, Environmental Fate and Effects Division. Other packages designed for Monte Carlo analyses include acslXtreme (AEgis Technologies Group 2004), ModelMaker (Cherwell Scientific 2000), and Mathematica (Wolfram Research, 1996, 2005).

1.2.2.2. Extreme Value Risk Assessment

The USDA Forest Service has not adopted probabilistic risk assessment methods. Historically, the Forest Service has developed different scenarios that have been referred to as typical and worst-case (e.g., USDA/FS 1989a,b,c). With the advent of the SERA risk assessments, a somewhat different approach was taken in which almost no values used in a risk estimate are presented as a single number. Instead, most numbers used in calculating risk values are expressed as a central estimate and a range, which is sometimes very large. The central estimate would generally correspond to the typical value and the upper value in the range (or more specifically the upper or lower bound that leads to the highest estimate of risk) would generally correspond to what used to be called the "worst-case" value. The other end of the range (the upper or lower bound that leads to the lowest estimate of risk) might be termed the "best case" value. The best case assessment is made simply because an unacceptable level of risk from a best case would lead to the clear conclusion that the use of the agent under any circumstances would likely result in some adverse effect.

As with a probabilistic risk assessment, an attempt is often made to apply the extreme value approach both to the exposure assessment as well as to the dose-response assessment. Applications of the exposure assessment are relatively simple and may involve various assumptions concerning animal weight, food consumption, water consumption, rainfall and so forth. Many of the specific assumptions are detailed in Section 3.2 (Human Health) and Section 4.2 (Ecological Effects). In terms of the dose-response assessment, the extreme value approach most often involves the identification of both tolerant and sensitive species, typically in the ecological risk assessment (Section 4.3). In the human health risk assessment (Section 3.3), different RfD values may be derived for sensitive subgroups – e.g., children or women of child-bearing age.

The extreme value approach has some but not all of the benefits of probabilistic risk assessment. For example, it can and often does indicate that a particular use of an agent might not cause any adverse effects under some circumstances but could cause adverse effects under other circumstances. To the extent that the circumstances are clearly defined,

this may serve as a guide to using the agent in a manner that will minimize the potential for adverse effects. While probabilistic risk assessments may be used by the Forest Service at some point in the future, probabilistic risk assessments generally take longer to conduct and involve the commitment of greater resources.

1.3. Literature Search

1.3.1. Open Literature

There are many commercial databases that can be used to search the published literature. Initially, SERA conducts on-line searches of TOXLINE (including PubMed) and AGRICOLA. These two data bases usually identify most of the relevant published literature. Other supplemental searches may be conducted using other commercial data bases as detailed below.

TOXLINE (Toxicology Literature Online) is a bibliographical database constructed by the U.S. National Library of Medicine (NLM). The database is available at: http://toxnet.nlm.nih.gov/. The database covers the pharmacological, biochemical, physiological, and toxicological effects of agricultural and industrial chemicals, drugs, and several other classes of specialty chemicals. TOXLINE is a collection of databases derived from BIOSIS (up to 2002), National Library of Medicine, American Society of Hospital Pharmacists, National Institute for Occupational Safety and Health, Environmental Mutagen Information Center, Environmental Teratology Information Center, and U.S. Environmental Protection Agency. Sources for the BIOSIS sub-file include journal articles, reviews, reports, and meeting papers. The sources of information in TOXLINE include journal articles, letters, meeting abstracts, monographs, research and project summaries, technical reports, theses, and unpublished materials.

1.3.2. FIFRA/CBI Studies

For many pesticides, particularly those developed only in the past decade, the most relevant and critical information is found in unpublished studies submitted by the registrant of the pesticide to the U.S. EPA as part of the registration package. These studies are classified as "Confidential Business Information" and cannot be accessed without special clearance from the U.S. EPA. Summaries of these studies in the form of Date Evaluation Records (DERs) usually are available through a Freedom of Information Act (FOIA) request or at http://www.epa.gov/pesticides/foia/reviews.htm, a web site maintained by the U.S. EPA/OPP. While the nature and complexity of DERs will vary with the nature and complexity of the studies, each DER involves an independent assessment of the study by the U.S. EPA to ensure that the EPA Guidelines are followed. In addition, each DER undergoes internal review within the EPA (and sometimes several layers of internal review). Often, the U.S. EPA/OPP conducts detailed statistical reanalyses of the raw data. Consequently, well-prepared DERs (which are typical of DERs prepared since the 1990s) are often as valuable as full studies.

Sometimes, summaries of certain CBI studies are published by the U.S. EPA in Federal Register notices, Reregistration Eligibility Decision (RED) documents, or other Agency publications such as Science Chapters prepared by Health Effects Division or the Environmental Fate and Effects Division of the Office of Pesticides.

Although SERA uses DERs and other U.S. EPA summaries to reflect the views of the U.S. EPA, SERA prefers not to rely totally on these summaries and will attempt to acquire full studies from registrants (i.e., the chemical companies that supply the pesticide). If the full studies can be obtained, SERA summarizes as much of this information as possible – i.e., within the limits of the FIFRA statue – in appendices that accompany all full risk assessments.

A major advantage of the FIFRA studies submitted for pesticide registration is that they follow a relatively uniform set of guidelines or study protocols. Some of the specific components of these guidelines have evolved over time and continue to be modified as needed. A summary of recent guidelines is given by U.S. Environmental Protection Agency's Office of Prevention, Pesticides, and Toxic Substances and are available at http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm. These guidelines are intended to constitute a consistent set of study standards that are used by both the Office of Pesticide Programs (OPP) as well as Office of Pollution Prevention and Toxics (OPPT). A very large number of guidelines are available in ten different areas:

The OPPTS harmonized guidelines are organized in the following 10 series:

810 - Product Performance Test Guidelines
830 - Product Properties Test Guidelines
835 - Fate, Transport and Transformation Test Guidelines
840 - Spray Drift Test Guidelines
850 - Ecological Effects Test Guidelines
860 - Residue Chemistry Test Guidelines
870 - Health Effects Test Guidelines
875 - Occupational and Residential Exposure Test Guidelines
880 - Biochemicals Test Guidelines
885 - Microbial Pesticide Test Guidelines

Forest Service risk assessments primarily involve Health Effects Test Guidelines (Series 870), Ecological Effects Test Guidelines (Series 850) and Fate, Transport and Transformation Test Guidelines (Series 835).

While it is beyond the scope of the current document to discuss all of these guidelines in detail, a summary of U.S. EPA/OPP guideline studies is given in Table 2 with the most relevant studies indicated in bold font. Specific guidelines are discussed further in the current document as necessary and referenced to U.S. EPA/OPPTS (2011). For example, the guideline for acute oral toxicity tests (discussed in Section 3.1.4 and Section 4.1.2.1) is entitled Health Effects Test Guidelines OPPTS 870.1100 Acute Oral Toxicity with the EPA report designation of EPA 712–C–96–190. In the current document, this is referred to simply as OPPTS 870.1100.

1.3.3. Credible Reviews

For the most part, the risk assessments are based on primary literature, either from the open or published literature (Section 1.3.1.1) or the FIFRA files (Section 1.3.1.2). In some cases, however, credible reviews may be used directly as both a source of information and as the

basis for the risk assessment. This may be done for some impurities or adjuvants for which there is a very large body of literature or for abbreviated risk assessments. Conducting a full risk assessment on some of these agents may not be feasible based on resource limitations. In addition, some of these agents may be the subject of extensive reviews and risk assessments by other agencies or organizations and it simply would not make sense to duplicate the effort. In general, credible reviews are limited to groups such as the U.S. EPA, the World Health Organization (WHO), and the Agency for Toxic Substances and Disease Registry (ATSDR).

The U.S. EPA has conducted a very large number of reviews on pesticides (e.g., http://www.epa.gov/pesticides/reregistration/status.htm) and other chemicals that may be used in pesticide formulations (e.g., http://www.epa.gov/IRIS/). Because of the unique role and legislative mandate of the U.S. EPA in the conduct of risk assessments that are typically subject to extensive review and deliberation, the Forest Service will often defer to the U.S. EPA on evaluation and selection of studies used in the dose-response assessment for both human health (Section 3.3) and ecological effects (Section 4.3). This allows Forest Service risk assessments to focus the analysis of uses of the agent that are specific to the program activities of the Forest Service.

ATSDR is part of the Centers for Disease Control (CDC) and was created by Congress to provide public health-related analyses specifically related to hazardous wastes and environmental spills of hazardous substances. Part of these activities include the preparation of toxicological profiles for hazardous substances (i.e., http://www.atsdr.cdc.gov/). While many of the compounds reviewed by ATSDR are industrial compounds rather than pesticides, some compounds reviewed by ATSDR are pesticide contaminants. As an example, ATSDR has an extensive review of hexachlorobenzene, which is a contaminant in two herbicides, picloram and clopyralid. In both of these Forest Service risk assessments, the ATSDR review of hexachlorobenzene was used extensively (http://www.fs.fed.us/foresthealth/pesticide/risk.shtml).

The World Health Organization has conducted a very large number of reviews on both pesticides and industrial chemicals under the Programme on Chemical Safety (IPCS). Environmental Health Monographs on a large number of compounds are available at: http://www.inchem.org/pages/ehc.html. The preparation of these monographs typically involves several primary authors from academia or government and the process is reviewed by a larger group of individuals with expertise on the particular agent. A major benefit of WHO reviews is that they often contain summaries of unpublished studies from Europe that were submitted to the WHO in support of the monograph preparation. Unlike documents prepared by U.S. EPA and ATSDR, the Forest Service risk assessments will typically use WHO reviews only as an information source and does not typically use risk assessment values derived in WHO documents directly in the risk assessment.

1.3.4. Other Secondary Sources and Gray Literature

The above discussion of credible reviews by ATSDR, U.S. EPA, and WHO is not intended to be exclusive. There are many other agencies and organizations in and outside of the U.S. government that prepare credible reviews and these reviews are consulted. In addition, both pesticide manufacturers as well as organization that are generally opposed to the use of

pesticides will conduct reviews on pesticides. These reviews are also consulted and if reasonable analyses are made, these analyses may be cited and discussed in SERA risk assessments. Otherwise, reviews are referenced and used simply as a check on SERA's literature search (Section 1.3.1). SERA uses discretion in identifying reliable sources of information and clearly identifies those sources in the risk assessment.

1.4. Other Considerations

1.4.1. EXCEL Workbooks

Forest Service risk assessments are closely linked to the EXCEL workbooks that accompany most Forest Service risk assessments. Most Forest Service risk assessments use a relatively standard set of exposure scenarios and, for many years, the calculations involved in these exposure scenarios have been isolated from the body of the risk assessment by worksheets. The worksheets were originally developed by SERA in WordPerfect (Versions 1 and 2) and later ported to EXCEL (Version 3). In Versions 1 through 3, the worksheets were developed manually for each risk assessment.

Since Version 4, the further development of the worksheets has been directly supported by the Forest Service and Forest Service personnel have substantially contributed to the design, quality assurance, and further development of the worksheets. With Version 4 and in all subsequent versions, the worksheets have been prepared by WorksheetMaker, a program developed in EXCEL which creates EXCEL workbooks that are used in and released with Forest Service risk assessments.

The EXCEL workbooks are intended to isolate the large number of calculations that are involved in a Forest Service risk assessment from the discussion of the rationale for assessing the data, which is given in the text of Forest Service risk assessments. This approach is intended to lead to clearer and briefer discussions in the risk assessment and to allow the reader of the risk assessment to clearly understand the details of the calculations as presented in the worksheets.

The current methodology document mentions specific worksheets in these EXCEL workbooks, particularly in discussions of the exposure assessments (Sections 3.2 and 4.2). These discussions refer to the EXCEL workbooks created with WorksheetMaker Version 6 (SERA 2011a). As full discussion of the structure of the EXCEL workbooks, however, is not repeated in the current document and the interested reader is referred to the most current documentation for WorksheetMaker (SERA 2011a).

Occasionally, Forest Service risk assessments will not be accompanied by EXCEL workbooks. This is sometimes the case with pheromones or other semiochemicals. In these cases, the exposure assessments that are conducted may differ substantially from those detailed in the current document. Nonetheless, the exposure scenarios and rationale for the exposure scenarios will be fully detailed in the risk assessment. Similarly, some pesticides may involve atypically application methods or present other unusual technical issues that require custom workbooks – i.e., workbooks not created with WorksheetMaker or workbooks that are substantially modified from those created by WorksheetMaker. In these cases, the general structure of the exposure scenarios will be similar to those discussed below and the

differences from standard WorksheetMaker workbooks will be addressed in the pesticide risk assessment.

1.4.2. Appendices (Organization and Function)

Most Forest Service risk assessments are accompanied by appendices. The number and nature of the appendices will vary with different pesticides but a typical listing of appendices is given below:

- Appendix 1: Toxicity to Mammals
- Appendix 2: Toxicity to Birds
- Appendix 3: Toxicity to Terrestrial Plants
- Appendix 4: Toxicity to Fish
- Appendix 5: Toxicity to Aquatic Invertebrates
- Appendix 6: Toxicity to Aquatic Plants
- Appendix 7: Results of Gleams-Driver Modeling

With the exception of the appendix containing the results of the Gleams-Driver model (discussed further in Section 3.2.3.4.3), each of the appendices consists of a series of tables. Each table will typically contain four columns labeled Species, Exposure, Response, and Reference. The number of tables will vary according to the complexity of the available studies but at least two tables are typically in each appendix: acute toxicity and chronic toxicity. The appendix on mammalian toxicity is usually the most complex and a typical listing of tables in the mammalian appendix is given below:

- Table A1-1: MSDS Summary of Selected Formulations
- Table A1-2: Acute Oral Toxicity
- Table A1-3: Acute and Subchronic Dermal Toxicity
- Table A1-4: Acute and Subchronic Inhalation Studies
- Table A1-5: Skin Irritation Studies
- Table A1-6: Skin Sensitization Studies
- Table A1-7: Eye Irritation Studies
- Table A1-8: Developmental Toxicity Studies
- Table A1-9: Reproductive Toxicity Studies
- Table A1-10: Subchronic and Chronic Oral Toxicity Studies

At the start of all appendices, the specific listing the tables in the appendix is given.

The appendices contain a relatively detailed summary of the data in each toxicity study discussed in the risk assessment. In many ways, the function of the appendices is analogous to the function of the EXCEL workbooks. Where the workbooks isolated the detailed calculations from the discussion in the risk assessment, the appendices isolate the experimental detail of each study from the more focused and comparative discussion given in the body of the risk assessment. The details in the appendices are not repeated in the body of the risk assessment except in the case of the most critical studies. For critical discussions, the body of the risk assessment may contain a more detailed discussion of the data in the appendices, including statistical reanalyses as necessary. Most often, these more detailed

discussions are focused on a comparative evaluation and synthesis of several studies. Studies not critical to the conclusions of risk assessment are still summarized in the appendices for the sake of documentation but these studies may receive only a cursory mention in the body of the risk assessment. Again and as with the worksheets, this approach is intended to allow the discussions in the body of the risk assessment to be a focused, brief, and clear as possible.

1.4.3. References (Structure and Function)

References cited in the risk assessment are contained in Section 5 of every Forest Service risk assessment. Individual references are given as in the following examples:

> {Alabaster 1969} Alabaster JS. 1969. Survival of Fish in 164 Herbicides, Insecticides, Fungicides, Wetting Agents and Miscellaneous Substances. Int Pest Control. 11(2): 29-35. [Set02]

> {Alexander and Batchelder 1965} Alexander HC; Batchelder TL. 1965. Results of a Study on the Acute Toxicity of Tordon^(R) Herbicide to Three Species of Fish. (Unpublished study received Nov 6, 1967 under 0F0863; submitted by Dow Chemical U.S.A., Midland, Mich.; CDL:094524-H) MRID 00041475. [MRID03R]

> {Anthony et al. 1996} Anthony DC; Montine TJ; Graham DG. 1996. Toxic Responses of the Nervous System. In: Casarett and Doull's Toxicology: The Basic Science of Poisons. 5th Edition. McGraw-Hill, Health Professions Division, New York, NY. pp. 463-486. [Std]

The initial citation in curly brackets, {}, give the citation as it is given in the text or appendices of the risk assessment. The full reference, following the general format used by TOXLINE (Section 1.3.1), is given following the curly brackets. Lastly, a further designation is given in square brackets, []. This designation refers to the source of the information. For example, [Set02] refers to a search from the open literature and [MRID03R] refers to a full study that was submitted to the U.S. EPA/OPP in support of the registration of the pesticide.

Each reference will start with a key that identifies the information given in the square brackets. The above examples are taken from a risk assessment on picloram that is currently under peer review. A typical key for a full reference list is given below:

ClrRev	**Cleared reviews at http://www.epa.gov/pesticides/foia/reviews.htm.**
E-Docket	**Selected documents (out of a total of 456) from www.regulations.gov.**
MCS	**Papers on Multiple Chemical Sensitivity**
MRID03	**Registrant studies from 2003 RA.**
MRID11	**Registrant studies received for 2011 RA.**
RA 2003	**Summary from 2003 Forest Service risk assessment**
RA 2003r	**2003 Forest Service risk assessment, reordered**
Sec	**Studies taken from secondary sources.**
Set00	**Preliminary scoping and related risk assessments.**
Set01	**TOXLINE update.**
Set02	**ECOTOX.**
Set03	**Anion transport and sundry additional citations.**
Set04	**Hexachlorobenzene in vegetation.**
Std	**Standard references used in most Forest Service risk assessments.**

The intent of this key is to allow the reader of the risk assessment additional insights into the data bases that were searched, the number of searches that were conducted, and whether or not the risk assessment had access to full studies that were submitted to the U.S. EPA/OPP. These full studies and only the full studies are designated with MRID designations. While a general summary of the literature review is always given in the introduction to each risk assessment, the elaborations given in Section 5 allow a fuller explanation, by citation, of the information sources that were used in the risk assessment.

1.4.4. Acronyms and Terminology/Jargon

Common acronyms, such as U.S. EPA (U.S. Environmental Protection Agency), are used commonly in Forest Service risk assessments. Less common acronyms are avoid. Whether or not an acronym is common or uncommon/unclear is often a editorial judgment influenced strongly by the background of the individual. Forest Service risk assessments will or at least attempt to use acronyms sparingly. All acronyms, however, are spelled out in full the first time that they are used. In addition, each risk assessment has a list of acronyms and abbreviations in the front matter of the risk assessment. A representative list of acronyms and abbreviations is given in the front matter of the current document.

Jargon is also avoided in Forest Service risk assessments. As with *common* versus *uncommon* acronyms, whether a particular word is viewed as reasonable terminology or as jargon is often influenced by the background of the individual. While an attempt is made to avoid terms that may be viewed as jargon, some readers may view the use of certain relatively common technical terms are jargon. For example, the terms *allelopathy* or *allelopathic effect* is clear to most foresters involved in herbicide applications. The term *allelopathy* can be used to refer to translocation of a herbicide to the roots of plants and subsequent loss from the roots to the surrounding soil, possibly posing a risk to neighboring vegetation. To many other people, this perfectly reasonable and concise term is unfamiliar and may be viewed as jargon.

The balance between concise language and jargon is difficult. Editorial discretion is used in the development of Forest Service risk assessments to use terms that will be understood by most readers. When potentially unfamiliar terms are used in a risk assessment, the terms are defined when they are first used. In addition, SERA has prepared a glossary of terms that may be unfamiliar to some readers and could be viewed as jargon. This glossary is maintained as separate document available at www.sera-inc.com. A large number of excellent and specialized glossaries are maintained by many organizations and are available on the Internet. A listing of some of these glossaries is given at the start of the SERA glossary.

2. PROGRAM DESCRIPTION

2.1. Overview

Program descriptions are relatively brief discussions about the pesticide under review and how the Forest Service plans to use the pesticide. The information summarized in the program description includes the identity of the pesticide and its commercial formulations, as well as the identity of the inerts, adjuvants, and contaminants in the commercial formulations. SERA contacts one or more individuals in the Forest Service to obtain information about how the pesticide will be used in Forest Service Programs. Typically, a draft of the program description is prepared and reviewed by Forest Service personnel prior to the preparation of the rest of the risk assessment to ensure that the risk assessment is based on Forest Service practices. The program description may include additional information about the use of the pesticide by other organizations, which can be useful in assessing the extent to which the application of the pesticide by the Forest Service contributes to the environmental levels of the compound.

2.2. Chemical Description and Commercial Formulations

In the program description, the identity of the pesticide (i.e., active ingredient) is summarized followed by a brief discussion of the commercial formulations. The discussion of the commercial formulations includes information about the proportion or concentration of the active ingredient in each formulation as well as a general description of the formulation(s) (e.g., physical state—liquid, dispersible granules, etc.—and type of carrier or binding matrix).

Physical and chemical properties that are environmentally significant and probably of greatest relevance to most risk assessments include the vapor pressure, ionization constants (pKa), water and lipid solubility, and adsorption properties (e.g., Kd, Koc, and Kow). SERA obtains most of the information regarding the physical and chemical characteristics of a compound from the U.S. EPA/CBI files and standard reference sources like the Merck Index (e.g., Budavari 1989) or the USDA ARS Pesticide Database (USDA/ARS 2011). In some instances, quantitative structure-activity relationships, such as the U.S. EPA's EPI-Suite program (Clements et al. 1996; EPI Suite 2011; Meylan and Howard 1998) may be used to estimate physical and chemical properties for which experimental data are not available. Data regarding chemical reactivity (e.g., rates of hydrolysis, biodegradation, photodegradation, etc.) and monitored rates of environmental dissipation of the pesticide also are included in this section of the program description. In addition, SERA conducts supplemental literature searches (e.g., Hazardous Substances Database and ChemIDPlus available online via the National Library of Medicine at http://toxnet.nlm.nih.gov) as necessary to obtain information about chemical structures and nomenclature.

The chemical and physical properties of a pesticide are summarized in a table that also includes the name of the compound, synonyms, the CAS number(s), and U.S. EPA registration number. If necessary, the table also indicates the conditions under which certain measurements were made. For example, the solubility of weak acids in water is highly dependent on the pH of the water. Similarly, soil-water partition coefficients vary substantially for different soil types such as clay, loam, and sand. Generally, the program

description does not include a detailed discussion of the chemical or physical properties of an agent. When necessary, those kinds of discussions may be incorporated into the exposure assessment. As discussed further in Section 3.2.3.4.3, an additional table defining the chemical and physical properties used in the model is included in the document if Gleams-Driver modeling is conducted.

The program description also addresses the issue of inerts in commercial pesticide formulations, which are regulated by the U.S. EPA and these regulations affect pesticide labeling and testing requirements. Most chemical manufacturers consider the identity of inert ingredients proprietary information. Inert compounds classified as hazardous by the U.S. EPA must be specified on the MSDS when they are present at a concentration greater than 0.1%. A lack of disclosure means that none of the inert ingredients present at concentrations greater than 0.1% in the formulation are classified as hazardous. As noted by Levine (1996) and discussed further in Section 3.1.14, the testing requirements for inerts are less rigorous than the testing requirements for active ingredients. The identity of the inerts is always disclosed to the U.S. EPA as part of the registration process.

2.3. Application Methods

The use of herbicides in silviculture and the various methods of herbicide application are described in detail in the general literature (e.g., Cantrell and Hyland 1985), and in environmental impact statements conducted by the Forest Service (e.g., USDA 1989a,b,c). No attempt is made to summarize this information again in the risk assessment. Instead, SERA discusses information relevant to the exposure assessments for application methods that the Forest Service uses or may consider using.

Generally, consideration is given to three conventional application methods, including directed foliar applications, broadcast ground applications, and aerial applications. The rationale for selecting these basic application methods is discussed in SERA (1998). Sometimes, as with the application of granules (e.g., hexazinone) or the application of a compound directly to water (e.g., 2,4-D), additional application methods are described. Other applications such as cut surface treatments may also be considered.

For each application method, this section of the risk assessment focuses on the number of acres that an individual worker might handle in a single work day and any special precautions that may be employed routinely. SERA obtains this information from descriptions of pesticide applications provided by the Forest Service (e.g., USDA 1989b, p 2-9 to 2-10) and any chemical-specific or site-specific information provided by the Forest Service.

For example, 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 exposure, 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.

Boom spray or broadcast ground applications are used primarily in rights-of-way management. Spray equipment mounted on tractors or trucks is used to apply the herbicide on either side of the roadway. Usually, about 8 acres are treated in a 45-minute period (approximately 11 acres/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)

Aerial applications are made with helicopters or fixed wing aircraft. The compound is applied under pressure through specially designed spray nozzles and booms. The nozzles are designed to minimize turbulence and maintain a large droplet size, both of which contribute to a reduction in spray drift. In aerial applications, approximately 40-100 acres may be treated per hour.

2.4. Mixing and Application Rates

This section of the program description provides a discussion of information provided by the Forest Service regarding proposed application rates and pesticide concentrations in field solutions.

The specific application rates used in ground or aerial applications vary according to local conditions and the nature of the target vegetation. SERA does not derive the application rates, but refers to the product labels to ensure that the proposed application rates do not exceed the labeled rate for a particular use. This kind of information is obtained either directly from literature released by the manufacturer or from C&P Press at http://www.greenbook.net. SERA's primary concern with application rates involves identifying a range of application rates that may be used in Forest Service programs. Product labels may be very detailed, are often modified, and the labeled uses may vary in different regions of the country. The label summaries in SERA risk assessments are not intended to serve as a substitute for reviewing current product labels with care.

In most risk assessments, only a single application rate is explicitly considered in the exposure assessments (Section 3.2 and 4.2). This is based either on information obtained from the Forest Service on planned used or past records of Forest Service use (Section 2.4). The consequences of varying application rates within the range of rates that the Forest Service may use are considered in the risk characterization for human health (Section 3.4) and ecological effects (Section 4.4).

Usually, pesticides are diluted prior to field applications. This detail is referred to in the risk assessment as field dilution. For example, the recommended range of mixing volumes for many liquid pesticide formulations is about 5-25 gallons of water per acre for aerial applications and about 10-100 gallons of water per acre for ground applications.

For the risk assessment, the extent to which a formulation is diluted prior to application primarily influences dermal and direct spray scenarios, both of which depend on the field dilution. As the concentration of pesticide in the applied solution increases, exposure and potential risk also increase. Like application rates, field dilutions are generally expressed as a range with a central or average value.

It should be noted that the selection of a specific application rate and dilution volume in a risk assessment is intended to simply reflect typical or central estimates as well as plausible lower and upper bounds. In the assessment of specific program activities, the Forest Service may use program specific application rates in the worksheets that are included with each risk assessment to assess any potential risks for a proposed application.

2.5. Use Statistics

Forest Service risk assessments attempt to characterize the use of an herbicide or other pesticide in Forest Service programs relative to the use of the herbicide or other pesticide in agricultural applications. Forest Service pesticide use reports up to the year 2004 are available on the Forest Service web site (http://www.fs.fed.us/foresthealth/pesticide/reports.shtml). Information on agricultural use is compiled by the U.S. Geological Survey (http://water.usgs.gov/nawqa/pnsp/usage/maps/). In addition, detailed pesticide use statistics compiled by the state of California (http://www.calepa.ca.gov/).

The USDA Forest Service tracks and reports pesticide use by geographical areas referred to as "Regions". The Forest Service classification divides the U.S. into nine regions designated from Region 1 (Northern) to Region 10 (Alaska). [Note: There is no Region 7 in the Forest Service system.] An overview of the Forest Service regions is given in Figure 3.

Although neither the statistics pertaining to pesticide use by the Forest Service nor the statistics pertaining to total national or regional pesticide use have a direct impact on the risk assessment, they can be useful in interpreting and better understanding the results of the risk assessment in determining whether Forest Service uses are likely to substantially impact exposures to the pesticide in different regions of the country.

3.1. HAZARD IDENTIFICATION

3.1.1. Overview

The hazard identification process involves making judgments about which effects are most relevant to the assessment of human health. During this process, studies may be eliminated from consideration because they are inherently flawed or because they are grossly inconsistent with the preponderance of other studies. Although hazard identification results in a qualitative determination, quantitative methods are usually required as in most other assessments of causality. For instance, the process of hazard identification often hinges on a statistical assessment of exposure-response or dose-response relationships. Furthermore, hazard identification must also consider fundamental and qualitative differences among species. Depending on the chemical of concern, hazard identification also may include the use of quantitative or qualitative structure-activity relationships or differences in pharmacokinetics.

The hazard identification may cover any number of endpoints, depending on the chemical under assessment. The following topics are generally considered explicitly in each hazard identification:

- Mechanism of Action
- Pharmacokinetics and Metabolism
- Acute Oral Toxicity
- Subchronic or Chronic Systemic Toxic Effects
- Effects on the Nervous System
- Effects on the Immune System
- Effects on the Endocrine System
- Reproductive and Teratogenic Effects
- Carcinogenicity and Mutagenicity
- Irritation and Sensitization of the Skin and Eyes
- Systemic Toxic Effects from Dermal Exposures
- Systemic Toxic Effects from Inhalation Exposures
- Inerts and Adjuvants
- Impurities and Metabolites
- Toxicologic Interactions

Additional effects may be discussed, depending on the nature of the available information on the chemical. Most standard texts in toxicology provide overviews of the diverse nature of the effects on different organs (e.g., Klaassen 1996, Haschek and Rousseaux 1991).

3.1.2. Mechanism of Action

Mechanism of action is a rather general term that refers to our understanding of how a particular chemical is likely to affect humans or other organisms. To the extent that the mechanism of action is understood, confidence in a risk assessment is enhanced. If the mechanism is not understood, extrapolations or suppositions concerning levels of exposure that may or may not cause an adverse effect are less certain.

The mechanism of action can be described at many levels of biological organization: molecular, biochemical, sub-cellular (e.g., effects on organelles such as mitochondria), cellular, organ, organ system or whole animal. This section of the risk assessment attempts to summarize this information in a manner that focuses attention on the following sections of hazard identification. Thus, even if the mechanism of action is not clearly defined at the molecular level, some attempt is made to characterize the general types of effects that are most often seen and to suggest whether or not these observations might be related to a plausible mechanistic assumption.

For many pesticides considered in Forest Service risk assessments, the mechanisms of action may be very well understood in the target species (e.g., the effect of an herbicide on a plant or the effect of an insecticide on an insect) but these mechanisms may have very little to do with potential human health effects. For example, many sulfonylurea herbicides inhibit acetolactate synthase (ALS), an enzyme that catalyzes the biosynthesis of three branched-chain amino acids (valine, leucine, and isoleucine), all of which are essential for plant growth. In terms of potential effects in humans, all of these amino acids are essential amino acids – i.e., amino acids that are not produced by humans and must be obtained from the consumption of plants. Thus, the mechanism of action of the sulfonylurea herbicides in plants has no direct relevance to the human health risk assessment.

3.1.3. Pharmacokinetics and Metabolism

Pharmacokinetics refers to the quantitative study of how chemicals may be absorbed, distributed, altered (metabolized), and excreted. From a practical perspective, this section of the risk assessment focuses on what is known about metabolism, absorption (particularly dermal absorption), and excretion.

3.1.3.1. General Considerations

Consideration of metabolism focuses on similarities between metabolism in humans and metabolism in experimental animals. This is a very important issue to most risk assessments, because toxicity studies in experimental animals are typically used to derive acceptable levels of exposure in humans (Section 3.3). Implicit in this practice is the assumption that studies on whole animals, such as those used to derive acceptable levels of exposure in humans, will encompass the toxicity of both the parent compound as well as any metabolites that formed *in vivo*. For chemicals that are extensively metabolized and chemicals whose metabolites are known to be more toxic than the parent compound, this assumption can be supported by information showing that the metabolic pathways in humans and experimental mammals are similar. To the extent that the metabolic pathways in humans and experimental mammals differ, confidence in use of data on experimental mammals and subsequent confidence in the risk assessment itself may be diminished.

In any risk assessment, a major distinction is made between *in vivo* and environmental metabolites. *In vivo* metabolites, as discussed in this section, refer to the compounds that are formed within the animal after the agent has been absorbed. Environmental metabolites refer to compounds that may be formed in the environment by a number of different biological or chemical processes including breakdown in soil or water or breakdown by sunlight (photolysis). In many cases, environmental metabolites will be less biologically active than the parent compound. Thus, environmental metabolism is regarded as a detoxification

mechanism and the metabolites are not quantitatively considered in the risk assessment. In other cases, such as the formation of 3,5,6-trichloro-2-pyridinol (TCP) from triclopyr, the metabolites may be as toxic as or more toxic than the parent compound. In such cases, the metabolite or metabolites may need to be treated in the same manner as the parent compound – i.e., a full exposure assessment and dose-response assessment is required (SERA 2011c).

A major uncertainty in this type of assessment on metabolites involves the kind of toxicity data available on metabolites. The example of TCP as a metabolite of triclopyr is exceptional in that information is available on both the acute and chronic toxicity of TCP. For most pesticide metabolites either no toxicity data are available or the toxicity data are limited to acute toxicity data (Section 3.1.4). As discussed in Section 1.2.1, a fundamental principle in the Forest Service risk assessments is that risk cannot be quantitatively characterization unless both toxicity and exposure can be characterized. Thus, in cases in which inadequate toxicity data are available to quantify the risks associated with one or more metabolites, the metabolites are not quantitatively considered in the risk assessment and any attendant uncertainties are discussed qualitatively in the risk characterization (Section 3.4).

3.1.3.2. Dermal Absorption

In most cases, chemicals may be absorbed by oral, dermal, and inhalation routes. From a practical perspective, only dermal exposures are quantitatively considered in this section of the risk assessment. This approach is taken because route-to-route extrapolations are made in the risk assessments only for dermal exposures. In other words, oral exposures are estimated in units such as mg/kg/day (Section 3.2) and these exposures are compared to toxicity values based on oral exposures (Sections 3.1.4 and 3.1.5). While inhalation exposures are not typically important for most pesticides (e.g., Ecobichon 1998; van Hemmen 1992), any relevant inhalation exposures are typically compared to inhalation toxicity studies (Section 3.1.13).

In Forest Service risk assessments, however, the potential effects of dermal exposures are typically assessed using oral toxicity data. This is one area in which Forest Service risk assessments tend to differ from some of those conducted by the U.S. EPA's Office of Pesticides (OPP). The U.S. EPA/OPP will sometimes use dermal toxicity data to assess the potential for adverse effects in humans. Thus, as with the oral and inhalation exposures discussed above, no route-to-route extrapolation is required. Forest Service risk assessments, on the other hand, will generally take a dermal exposure and estimate an absorbed dose. This absorbed dose is then compared to oral toxicity values in making the risk characterization. The risk assessments conducted by the Forest Service take this approach because the dermal toxicity data on most chemicals, including herbicides and other pesticides, is much more limited than the data from oral toxicity studies. For example, reproductive effects are of critical concern to the Forest Service but very few reproductive studies (Section 3.1.9) are conducted using dermal exposures. While dermal toxicity data is considered in Forest Service risk assessments (Section 3.1.12), they are most often used as a check on estimates of dermal absorption rates (see below) rather than as the basis for a risk characterization.

In making dermal-to-oral extrapolations, estimates of dermal absorption rates are critical. In the Forest Service risk assessments, estimates of dermal absorption rates (k_a, expressed in units of amount/unit time [zero-order] or reciprocal time [first-order]) or dermal penetration

rates (K_p expressed in units of cm/hour) are required for many of the exposure scenarios. The biological and chemical processes pertinent to these scenarios are illustrated in Figure 4. The chemical may be deposited on the skin instantaneously (e.g., as in an accidental spill) or gradually (e.g., as uptake from contaminated vegetation). In order for absorption in the systemic circulation to occur, *permeation* across the stratum corneum must occur first—at least in intact skin.

The stratum corneum and dermis are basically lipid-rich barriers that prevent water loss. Thus, compounds with higher lipid solubility are generally more permeable than more water soluble compounds. In addition, transport through the skin is inversely related to molecular size. Thus, for compounds of comparable lipophilicity, smaller compounds tend to be more permeable than larger compounds.

Classical pharmacokinetic dermal absorption rates are used to estimate the absorbed dose associated with dermal deposition scenarios. These rates (k_a) express the amount (zero-order absorption) or proportion (first-order absorption) of a chemical absorbed *into the body* per unit time. In this context, the phrase *into the body* means that the chemical will be in the blood stream and subject to metabolism or excretion and capable of interacting in other ways with viable tissue.

3.1.3.2.1. Zero-Order Dermal Absorption

As discussed in U.S. EPA (1992), most QSAR relationships for estimating dermal permeability (K_p) take these relationships into account with dermal permeability being positively related to the octanol/water partition coefficient ($K_{o/w}$) and inversely related to molecular weight (MW). U.S. EPA/ORD (1992, 2007) recommends the following equation:

$$\log_{10} K_p = -2.72 + 0.71 \log_{10} K_{ow} - 0.0061 MW \qquad \text{(Eq. 1)}$$

where K_p is in units of cm/hour. This equation is based on measured K_p values for 95 organic compounds (Flynn 1990, Table 5-4 in U.S. EPA 1992) with log $K_{o/w}$ values ranging from about -2.5 to 5.5 and molecular weights ranging from about 30 to 770. Estimates of K_p from the above equation have an error of about one order of magnitude.

As reviewed by U.S. EPA (1992), some analyses (e.g., Flynn 1990) suggest that the effects of both molecular weight and lipophilicity on permeability may be linear only within certain limits. Based on the analysis by Flynn (1990), relatively lipophobic compounds with log K_{ow} values <0.5 appear to have log K_p values of approximately -3 (MW<150) or -5 (MW>150). At the upper limit, highly lipophilic compounds with log $K_{o/w}$ values >3 and molecular weights <150 appear to have log K_p values of about -0.5. Compounds with log $K_{o/w}$ values>3.5 and molecular weights >150 appear to have log K_p values of about -1.5 (Flynn 1990).

3.1.3.2.2. First-Order Dermal Absorption

The series of studies by Feldmann and Maibach (1969, 1970, 1974) represents a unique and highly relevant source of information on *in vivo* dermal absorption in humans. As discussed in U.S. EPA (1992), however, the Feldmann and Maibach publications do not provide sufficient experimental details for the complete derivation of zero-order dermal absorption

rates. Nonetheless, as illustrated by Durkin et al. (1995), estimates of dermal absorption rates from the Feldmann and Maibach publications gave much better estimates of absorbed dose than did estimates based on Fick's first law. Thus, when exposure scenarios are best characterized by deposition on the surface of the skin – as opposed to immersion of the skin in an aqueous solution – first order dermal absorption rates are estimated either from chemical specific data or from structure activity relationships.

SERA (1997) has completed an extensive re-evaluation of these data to improve on the methods proposed by Durkin et al. (1995) in which the Feldmann and Maibach data were fit to the following equation:

$$X_t = \frac{k_a A_0 / (k_e - (k_a + k_r))}{e^{-(k_a + k_r)t} - e^{-k_e t}} \qquad \text{(Eq. 2)}$$

where k_a is the first order dermal absorption rate coefficient, k_e is the first order excretion rate coefficient, and k_r is the first order fugitive loss rate coefficient

Feldmann and Maibach (1969, 1970, 1974) did not conduct i.v. elimination studies in humans for all of the compounds. For some of the compounds i.v. studies were conducted in rats and for other compounds judgment was used to estimate k_e. Thus, in the re-analysis, only the 29 chemicals that included i.v. elimination studies in humans are included.

For each of these 29 chemicals, a spreadsheet was set up in Excel and the Excel SOLVER function was used to estimate the rate coefficients. Because the results reported in the Feldmann and Maibach publications are expressed as the proportion of applied dose eliminated over a given period, both sides of the above equation were multiplied by k_e. In all cases, the k_e values were derived from the half-times ($t_{½}$) reported in the Feldmann and Maibach publications - i.e., $k_e = \ln(2) \div t_{½}$ - and these k_e values were used as constants rather than as parameters estimated from the models. The only constraint applied to the models was that k_a and k_r both must be greater than or equal to zero.

Unlike the earlier results of Durkin et al. (1995), first-order absorption rate coefficients were best estimated based on both molecular weight and log $K_{o/w}$:

$$\log_{10} k_a = -1.49 + 0.233 \log_{10} K_{ow} - 0.00566\, MW \qquad \text{(Eq. 3)}$$

All coefficients were significant at $p<0.004$ but the squared correlation coefficients for both models were low, about 0.32. This correlation coefficient is not remarkably lower than the squared correlation coefficient of 0.43 that is obtained for the regression of log K_p on molecular weight and log $K_{o/w}$ using Table 5-7 from U.S. EPA (1992) without censoring. The fugitive loss rates (k_r) were not significantly correlated with either the molecular weight or the $K_{o/w}$. The observed fugitive loss rates fit a log normal distribution [p=0.35] with a mean of 0.032 hour^{-1} and a 95% confidence interval of 0.0028 to 0.037 hour^{-1}.

Although there is no information with which to compare the absorption of the esters of weak acids with the acids themselves, Feldmann and Maibach (1969) did assay the absorption of hydrocortisone and testosterone as well as esters of these compounds (Table 3). As indicated

in Table 3, hydrocortisone and hydrocortisone acetate show a relatively direct relationship between dermal permeability (K_p) and dermal absorption. For testosterone and its esters, however, the correspondence is poor. Although the estimated K_p for testosterone is less than that for either of its esters, testosterone is absorbed to a substantially greater extent than either of its esters. This relationship holds true whether the estimates of K_p for the esters are based on Equation 1 or the upper limit on K_p suggested by Flynn (1990). Thus, while the lipophilicity of the esters is greater than that of the parent compound for both testosterone and hydrocortisone, and the esters of both of these compounds are estimated to have a greater permeability (K_p) than the corresponding parent compound, the relationship of ester formation to dermal absorption is inconsistent.

3.1.3.2.3. Other Considerations

Many factors can influence the dermal penetration and dermal absorption of chemicals, and some of these factors may be useful in understanding the lack of a consistent relationship between dermal permeability and dermal absorption. U.S. EPA/ORD (1992) provides an overview of these factors, and additional information and analyses are presented in other reviews and books on dermal absorption (e.g., Klein-Szanto et al. 1991, Rice and Cohen 1996, Scott et al. 1989, Wang et al. 1993).

As illustrated in Figure 4, dermal absorption involves both permeation of the epidermis as well as partitioning from the dermis into capillary blood. At least to some extent, this process will be affected by the relative differences in the fat and water content of the skin and blood. As illustrated in Figure 5, whole skin tissue contains about 10% fat [260 g/2600 g] and 61% water [1600 g/2600 g] (ICRP 1992, Table 105, p. 284). The outer layer of the skin, the stratum corneum, contains almost 20% fat and 40% water (Klein-Szanto et al. 1991). Whole blood contains only about 0.65% fat [36 g/5500 g] and about 80% water [4400 g/5500 g] (ICRP 1992, Table 105, p. 280). Blood plasma contains about the same amount of fat as whole blood [23 g/3100 g or 0.74% fat] but a greater proportion of water [2900 g/3100 g or 93% water].

Because the skin, and especially the epidermis, is comprised of more lipids and less water than blood or plasma, increasing lipophilicity, which tends to increase dermal permeability or penetration, will tend to decrease partitioning from skin into blood. The binding of the chemical to endogenous protein may also complicate the relationship between estimates of dermal permeability and dermal absorption. Skin is relatively rich in protein [750 g/2600 g or about 29%] (ICRP 1992, Table 105, p. 280). Plasma contains less but still significant levels of protein [210 g/3100 g or 6.7%], as does whole blood [990 g/5500 g or 18%]. Different chemicals may bind to different proteins with varying degrees of affinity. Moreover, skin and blood consist of different and multiple kinds of proteins. Compounds that bind tightly to some skin proteins may penetrate quickly into the dermis (high K_p) but partition rather slowly into blood plasma (low k_a). Conversely, if a chemical has a high affinity for plasma proteins, the concentration in the aqueous phase of the plasma will tend to diminish, favoring the partitioning from the dermis to the blood. Thus, the net effect of protein binding on dermal penetration or dermal absorption cannot be determined in the absence of specific data on the chemical of concern. Hence, protein binding is another factor that may account for apparent discrepancies between dermal absorption rates (k_a) and dermal permeability (K_p).

Another factor affecting the rate of dermal absorption involves penetration of the chemical through the epidermis to the dermis where absorption into the blood may occur. The epidermis is relatively thin, generally about 35 to 100 micrometers for men and 20 to 65 micrometers for women. In some parts of the body, like the fingers and soles of the feet, the epidermis is much thicker, ranging from 400 to 1400 micrometers for men and from 400 to 1000 micrometers for women (ICRP 1992, Table 6, p. 49). The consequences of different skin thicknesses are variability in permeation/absorption and a lag period in apparent absorption.

Furthermore, different parts of the body may have different rates of dermal absorption. Similarly, skin thickness and/or composition in the same part of the body may differ among individuals. The variability among individuals is likely to contribute to the observed inter-individual differences in dermal absorption rates. Differences in skin composition may also influence the permeability rate (K_p) of a compound either at different anatomic sites of an individual or at the same anatomic site among individuals (Klein-Szanto et al. 1991).

The other consequence of different skin thicknesses or differences in skin composition involves the apparent lag period between dermal exposure and dermal absorption. U.S. EPA/ORD (1992, p. 4-28) indicates that the apparent lag time for penetration of the stratum corneum is proportional to the square of the thickness of the stratum corneum and inversely proportional to the diffusiveness of a chemical within the stratum corneum. Although this relationship may adequately describe permeation (K_p), the rate of absorption is not likely to change in a quantal manner (i.e., remaining zero at times less than the 'lag time' and changing to a constant value at times greater than the lag time). In other words, penetration of the stratum corneum and functional saturation of the underlying skin tissue is not instantaneous. Initially, the functional absorption rate (k_a), which is assumed to be a constant under the assumption of zero- or first-order kinetics, may actually be negligible but approach a constant value, either in terms of zero- or first-order coefficients, as permeation of the skin approaches a steady-state or pseudo-steady state.

The vehicle in which a compound is applied also may affect permeability and absorption. There is ample evidence that some vehicles enhance dermal absorption and dermal permeability of various compounds, while other vehicles retard the processes (e.g., Walters 1989, Guy et al. 1989, Williams and Barry 1989). In general, vehicles that hydrate the skin or alter the physical state of the stratum corneum (e.g., some solvents) may enhance permeability. On the other hand, highly lipophilic vehicles may retard both permeability and the subsequent absorption of lipophilic compounds by impeding the partitioning of the chemical from the vehicle into the skin. The converse is true for highly lipophobic compounds in a lipophobic vehicle. Thus, the influence of a specific vehicle on *absorption* may not be related to its effect on *permeation*. These confounding factors may need to be addressed when data regarding the effects of various vehicles on dermal absorption are not consistent.

3.1.3.3. Excretion

The excretion of a chemical from an animal is also an important factor in assessing risk. Unlike dermal absorption rates, however, data on excretion rates are typically not used

quantitatively in the risk assessment. Similar to information on metabolism, the assumption is generally made that the role of excretion is explicitly encompassed in the available toxicity data on an agent. In other words, data will generally be available on both acute toxicity (Section 3.1.4) as well as subchronic or chronic toxicity (Section 3.1.5). These data, along with information on effects of special concern (Sections 3.1.6 to 3.1.10) are then used to directly derive both acute and chronic toxicity values (Section 3.3).

Nevertheless, information on excretion rates, particularly whole-body excretion rates, can be useful in assessing the chemical-specific implications of the general terms *acute*, *subchronic*, and *chronic*. These terms do have general although somewhat vague definitions in toxicology. As discussed below (Sections 3.1.4 and 3.1.5), acute toxicity generally refers to single exposures or exposures that occur over a short period, typically 10-days or less. Subchronic toxicity is often defined somewhat circularly as 90-days because this is a period of exposure commonly used in studies that are referred to as *subchronic*. Other definitions of subchronic will sometimes define this exposure as about 10% of a life span, at least when referring to studies in rodents.

Pharmacokinetics can be used to define somewhat more biologically based characterizations of acute and chronic exposures in terms of body burden. Many compounds appear to be excreted from the body by first-order kinetics. In other words, a constant proportion of the compound is eliminated per unit time – e.g., 5% per day. The differential equation for this process is:

$$\frac{dA}{dt} = -k_e A \qquad \text{(Eq. 4)}$$

where **A** is the amount of the chemical, k_e is the first-order excretion rate constant, and ∂t is the period of time. In plain language, this equation simply states that the rate of change of the amount in the animal (∂A) is equal to some constant $-k_e$ (in units of reciprocal time), multiplied by the time interval, ∂t. At any particular time, *T*, the amount in the animal is simply the integral of Equation 4:

$$A_t = A_0 e^{-k_e t} \qquad \text{(Eq. 5)}$$

where A_0 is the amount in the animal at time zero – i.e., immediately after dosing.

Intuitively, it should be apparent that the amount of a chemical in an animal after repeated doses will tend to increase more rapidly and to a greater extent as the elimination rate (k_e) decreases. This is the case, and the mathematics of this relationship has been formalized in the *plateau principle* (e.g., Goldstein et al. 1974). This principle can be applied to a compound that is eliminated by first-order kinetics (Eq. 5) when the compound is administered repeatedly at a fixed interval (t*). In such a situation, the maximum amount of a chemical that will be in the animal after an infinite time (X_{Inf}) can be calculated as:

$$X_{Inf} = \frac{X_0}{1 - e^{-k_e t^*}} \qquad \text{(Eq. 6)}$$

By simple algebraic rearrangement, the increase or concentration of the chemical in the body after an infinite period of time relative to the amount in the body immediately after a single dose can be calculated as:

$$\frac{X_{Inf}}{X_0} = \frac{1}{1 - e^{-k_e t^*}} \qquad \text{(Eq. 7)}$$

In the case of first-order excretion, the half-life ($t_{1/2}$) of a chemical in the body can be defined as:

$$t_{1/2} = \ln(2) \div k_e \qquad \text{(Eq. 8)}$$

And, by rearrangement of the above equation, the elimination rate (k_e) can also be calculated from the half-life:

$$k_e = \ln(2) \div t_{1/2} \qquad \text{(Eq. 9)}$$

where *ln* is the natural $\log_e$ (rather than the common log or $\log_{10}$) with ln(2) having a value of approximately 0.6931.

Thus, a compound with a whole-body halftime of 2 days has a k_e of about 0.347 $days^{-1}$. Substituting this value into Equation 7 and assuming a dosing interval of one day (t*=1), the maximum concentration of the chemical in the animal after an infinite number of doses would be 3.4. By comparison, a compound with a long halftime such as 1 year (365 days) would have a k_e of about 0.002 $days^{-1}$ and substituting this value into Equation 7 leads to a concentration of about 527. Under the assumption that the critical factor for the animal is body burden, we would expect a substantial difference between the acute and chronic toxicity of a slowly eliminated chemical (e.g., a halftime of 1 year) but a lesser difference for a compound that is more rapidly eliminated (e.g., a halftime of 2 days).

The plateau principle can be extended to calculating the fraction (*f*) of the eventual steady-state condition that is reached after a certain period of time after *n* doses at a fixed interval:

$$f = 1 - e^{-k_e t^* n} \qquad \text{(Eq. 10)}$$

Thus, for a compound with a whole-body halftime of 2 days (k_e = 0.347 $days^{-1}$), a 90 day exposure to doses administered each day (t* = 1 day) would result in a fraction of virtually 1. For a compound with a halftime of 1 year (ke = 0.002 $days^{-1}$), the fraction of the steady-state value would be only about 0.16. After a period of 2 years (730 days), the fractional value for the slowly eliminated compound would be about 0.76 or about 4.75 times higher than the 90-day value [0.76/0.16 = 4.75]. Again assuming that body burden is the critical factor, there would be a greater concern for the slowly eliminated compound compared to the rapidly eliminated compound that a subchronic study might underestimate the toxicity that could be seen in a full chronic study.

A rearrangement of Equation 10 may be used to express the time (or more properly the number of doses) to a given fraction of steady-state:

$$n = \frac{\ln(1-f)}{-k_e t^*} \qquad \text{(Eq. 11)}$$

Going back to the example of the rapidly eliminated compound (k_e = 0.347 days^{-1}) and slowly eliminated compound (k_e = 0.002 days^{-1}), Equation 11 can be used to calculate the number of daily doses that would be required to reach 0.9 (90%) of the eventual steady-state value – i.e., about 6.6 days for the rapidly eliminated compound and about 1151 days (or about 3.2 years) for the slowly eliminated compound.

While the above relationships are not typically used in any quantitative manner in deriving toxicity values, these equations may be useful in discussing temporal relationships in the dose-response assessment (Section 3.3). For many rapidly eliminated compounds, there is a very weak temporal relationship in dose-response and dose-severity relationships and these may sometimes be explained or at least rationalized using the plateau principle. Conversely, if large differences are noted in short-term and longer-term toxicity values for a rapidly eliminated chemical, this suggests that cumulative damage (i.e., damage for which the rate of repair is very slow rather than an accumulation of the chemical itself) may be occurring.

3.1.4. Acute Oral Toxicity

Acute oral toxicity studies are among the most commonly available types of information on chemicals. For pesticides, acute oral toxicity studies are typically available on the purified active ingredient, the technical grade active ingredient, and at least some formulations. If information is available on the toxicity of metabolites, this information is often limited to acute oral toxicity studies. Thus, these types of studies are often the only kind of information that is available to assess the toxicological importance of inerts and adjuvants (Section 3.1.14) as well as impurities and metabolites (Section 3.1.15).

Two types of acute oral toxicity studies are available: gavage exposure (e.g., OPPTS 870.1100) and dietary exposure (e.g., OPPTS 850-2400). Both types of studies involve administering various doses or concentrations of the substance being tested to groups of test animals (typically rats) and observing the animals for a period of time after exposure (typically 14 days). Gavage studies are most common. In a gavage study, the test substance is administered by a stomach tube and a fixed amount of the material is placed into the stomach of the animal. For some chemicals, a vehicle must be used – i.e., the chemical is dissolved in a toxicologically inert compound such as water or corn oil prior to administration. In dietary studies, the compound is mixed into the normal diet of the animals (typically laboratory chow). Vehicles may also be used in dietary studies to facilitate the handling or mixing of the compound. Typically, at least five animals of each sex are used per dose or concentration and at least five doses or concentrations are used in a full study. The spacing between doses or concentrations is variable. In an oral gavage study, typical doses might be 1, 2, 4, 8, or 16 mg/kg/day in a closely spaced study. In a more widely spaced study, doses might be 1, 3, 10, 30, or 100 mg/kg/day. The idea behind the dose

spacing is to obtain fractional mortality at several doses or concentrations. Without fractional mortality, statistical analyses of the data is limited (Section 3.3.5.).

Both gavage and dietary studies may involve either range finding studies or limit tests. Range-finding studies typically involve fewer numbers of animals and fewer but more widely spaced doses – often a factor of 10. Limit tests are conducted when it is suspected that a test substance is not very toxic. These are conducted at only a single dose or concentration. For the acute oral gavage study in rats used by OPP, the limit dose is 2000 mg/kg bw (U.S. EPA/OPPTS 2011, OPPTS 870.1100).

The results of acute toxicity studies are usually expressed as time-specific LD_{50} or LC_{50} values (doses or concentrations of a toxicant that result in 50% mortality of the test species during a specified exposure or observation period). The statistic methods used to compute these values are discussed in Section 3.3.5. In some studies, the results do not permit the calculation of LD_{50} or LC_{50} values. For example, if a limit test is conducted at a dose of 2000 mg/kg and no mortality is seen, no LD_{50} can be calculated. In such a case, the results might be expressed as an LD_{50} of >2000 mg/kg/day. This type of reporting can lead to confusion. For example, a full study in which the doses were 125, 250, 500, 1000, and 2000 mg/kg, and mortality was observed only at 2000 mg/kg/day and the response was 40% mortality could also express the results as an LD_{50} of >2000 mg/kg/day. In SERA risk assessments, an attempt is made to avoid this sort of ambiguity by reporting time-specific LD_{50} or LC_{50} values along with NOEL or NOEC values in addition to fractional mortality if appropriate. Thus, the two types of LC_{50} values of >2000 mg/kg would be reported as either a NOEC for mortality of 2000 mg/kg or an LC_{50} value of >2000 mg/kg with 40% mortality at 2000 mg/kg.

LD_{50} and LC_{50} studies may also involve oral, dermal, or inhalation exposures of mammals, birds, and some invertebrates like the honey bee. These types of studies are conceptually similar to the gavage LD_{50} and dietary LC_{50} studies conducted in this section and are discussed in lesser detail in other sections of this document as appropriate. All of these different types of data may be used to categorize the toxicity of a chemical.

In terms of human health risk assessments, the categories that may be discussed include those used by U.S. EPA for determining the use of signal words on labels that must accompany pesticide formulations (U.S. EPA/OPP 2010). These toxicity categories are summarized in Table 4 for oral and inhalation LC_{50} values, dermal LD_{50} values, and effects on the eye and skin.

For acute oral toxicity, the most toxic category is designated as Category I and involves compounds with oral LD_{50} values of less than 50 mg/kg bw. The least toxic compounds fall into Category IV, defined as compounds for which LD_{50} values are greater than 5000 mg/kg bw. These categories are used only in the hazard identification to reflect how the U.S. EPA would classify the chemical. As discussed in Section 1.2.1.4, the risk characterization must be based on both an exposure and a dose-response assessment. Toxicity categories for other endpoints are discussed below in the appropriate subsections and similar toxicity categories used in the ecological risk assessment are discussed in Section 4.1.

3.1.5. Subchronic or Chronic Systemic Toxic Effects

3.1.5.1. General Considerations

Subchronic and chronic toxicity studies form the basis of most quantitative values used in risk assessments. The quantitative use of these types of studies typically involves identifying an NOEL (no observed effect level) or NOAEL (no observed adverse effect level) and dividing this value by one or more uncertainty factors. This quantitative use is detailed further in Section 3.3.2 (Chronic RfD). This section focuses on the kinds of data that are available and the factors that go into evaluating these data.

As with acute toxicity studies, the U.S. EPA (U.S. EPA/OPPTS 2011) has developed a number of very specific protocols or guidelines for subchronic and chronic toxicity studies. These include 28-day repeated dosing studies in rodents (OPPTS 870.3050), 90-day subchronic studies in rodents (OPPTS 870.3100) and non-rodents (OPPTS 870.3150), chronic toxicity studies in rodents (OPPTS 870.4100) and carcinogenicity studies in rodents (OPPTS 870.4200). For the 28-day and 90-day studies, the rodents are typically some strain of rat and the non-rodent assay typically involves beagle dogs. For the chronic rodent studies, both mice and rats are often included. While chronic toxicity studies and carcinogenicity studies have separate protocols and may sometimes be conducted separately, these studies are often combined into a single chronic toxicity/carcinogenicity study (OPPTS 870.4300). All of these studies usually entail some sort of preliminary range finding study, conceptually similar to the range finding studies conducted in acute toxicity studies. Chronic studies typically involve only 2 doses (although some may have up to 5 doses) with groups of about 50 animals per sex per dose. The subchronic studies will typically have a greater number of doses but may involve fewer numbers of animals per dose.

3.1.5.2. Statistical Considerations

While the number of doses and number of animals per dose level are not strictly defined, the criteria for an *acceptable study*, at least in terms of acceptability for pesticide registration, is that the study identifies both an NOEL or NOAEL as well as an adverse effect level (LOAEL). The method of determining an NOAEL or LOAEL typically involves comparisons between the control group and one of the dose groups. A commonly used statistical test is called the Fisher Exact Test which is used to determine if the incidence in a dose group is significantly higher than the incidence in the control group (Uitenbroek 1997). As a convention, a significance level of 0.05 (often express as $p<0.05$) is used as a criterion for statistical significance. This level of significance indicates that the probability that the difference in incidence between the control group and the dose group occurred by chance is less than 5 percent.

In some cases, multiple comparisons must be made – i.e., the incidence of several different effects between dose groups. In dealing with multiple comparisons, however, the use of the standard *p*-value of 0.05 may overestimate the number of significant associations. For example, if 100 sets of comparisons are made within the same population—i.e., there are by definition no differences because there is only one population—some comparisons may appear to be statistically significant only because of random differences in the sampling. To address this issue, one standard approach is to divide the pre-determined significance level, typically taken as 0.05, by the number of comparisons being made. This is referred to as

Bonferroni's correction (e.g., Curtin and Schulz 1998). A large number of other statistical methods – ranging from common *t*-tests to more sophisticated methods to determine whether a dose-response relationship is significant – may be used. These are discussed and referenced as necessary in each risk assessment.

3.1.5.3. Definition of Adversity

While statistical considerations are important in defining whether or not a particular effect is associated with a particular level of exposure, statistics do not address whether or not a particular effect should or should not be classified as adverse. Central to any risk assessment is the interpretation of the effects that are seen and the classification of these effects as incidental, adaptive, or adverse.

The identification of adverse health effects is, in some cases, trivial. Death is obviously an adverse health effect. In other cases, however, the classification of a particular effect as adverse may involve objective analysis, professional judgment, and even subjective or ethical considerations. For example, an extreme position would be that any effect caused by a chemical is adverse. At the other extreme is the position that only gross and obvious signs of toxicity should be regarded as adverse. Most organizations involved in risk assessments have adopted, at least implicitly, a central position. Some effects are classified as *adverse* and others are not, and judgments are usually based on the clinical significance of the magnitude or intensity of an adverse effect.

In general, any deviation from the state of health can be considered an adverse effect. For the purposes of defining an adverse health effect, the definition of health can be given as:

> ***health*** *- the condition of an organism or one of its parts in which it performs its functions at a level which is at least adequate for the normal activity of the whole organism over the normal lifetime of the organism.*

The term health may be applied either to the whole organism or its component parts. This definition explicitly incorporates the relative nature of health, which can be described by three categories—adequate, normal, and optimal—recognizing that these are general states in a graded series. These categories are intended to have a clinical interpretation in terms of the severity of the effect. A deviation from *adequate health* would constitute an obvious and frank disease state. At the other extreme, a deviation from *optimal heath* would be characterized as a detectable and statistically significant decrement in function with the magnitude of the decrement remaining within the normal range of clinical variation. A deviation from *normal health* would be considered as any decrement in function outside the normal clinical range that does not result in a frank disease state.

The whole organism must be able to function in an at least a normal manner. At any lower level of observation, a sub-normal functioning is classified as *unhealthy* to the extent that the reduction in normal activity of the subsystem is affected.

Based on this definition of health, an adverse effect may be defined as:

adverse effect *- any effect that decreases the capacity of an organism or a component of the organism to function in a normal manner, or that leads to a frank disease state.*

This definition intentionally omits any distinction between signs (objective and measurable indices of an effect) and symptoms (subjective reports) of toxicity. Subjective reports of effects such as headache, nausea, and dizziness often must be addressed in a risk assessment. In some instances, these symptoms may be associated with anxiety rather than exposure. In assessing the toxicity of an individual chemical, the issue of attribution is critical, but this does not affect the definition of an adverse effect. If the symptom is consistent with the above definition of an adverse effect, then the symptom is considered an adverse effect whether or not it is attributable to chemical exposure.

Another very explicit and intentional limitation of this definition is that it does not consider **severity** and does not limit the definition of an adverse effect to any particular level of observation. In other words, any effect on the function of the whole organism or any of its component parts at any biological level is considered adverse.

For example, at the molecular level, the formation of methemoglobin incapacitates the hemoglobin molecule just as the reaction of organophosphates or carbamates inactivates acetylcholinesterase. Thus, the formation of methemoglobin or the inhibition of an enzyme such as acetylcholinesterase is regarded as an adverse effect regardless of the magnitude of the effect because such interactions reduce the ability of the organism to respond to further stress (i.e., agents that induce methemoglobin or inhibit acetylcholinesterase).

Structural alterations, albeit secondary to functional changes, can be an indicator of impairment. The relationship between structural and functional effects is not always clear. As detailed by Ruben and Rousseaux (1991), some pathological changes are clear indices of functional impairment while other changes may have a more complex interpretation or be of questionable toxicological significance. Consequently, general guidelines for classifying a particular type of lesion as adverse or not adverse cannot be given. As detailed in the companion report, the significance of a pathological lesion depends on the nature and site of the lesion as well as other information regarding the health of the organism.

3.1.5.4. Some Key Endpoints

NOAEL or LOAEL values may be based on virtually any organ or organ system in the body. Some effects are of central concern for a variety of reasons. Such effects include effects on the nervous system, immune function, the endocrine system, reproductive and teratogenic effects, and carcinogenic and mutagenic effects. Each of these categories of effects are discussed in subsequent subsections of this document and are included as separate and corresponding subsections in each risk assessment. Nonetheless, there are a larger number of other organs or organ systems that may not be linked directly to any of these effects of special concern. While it is beyond the scope of this document to cover all effects that might be observed, two classes of effects – effects on body weight or organ weight and effects on the liver – are discussed below.

3.1.5.4.1. Body and Organ Weight

All organisms grow and develop in regular patterns. Normal body weights and organ weights for humans have been relatively well documented (ICRP 1992; Snyder et al. 1975), and similar data are available on a variety of other species (e.g., U.S. EPA 1989a,b,1993). Furthermore, a variety of methods have been developed to quantify and model animal growth and development (e.g. Karlberg 1987; Moore 1985).

There is no doubt that changes in growth rate (i.e., whole body weight) and organ weight associated with chemical exposure can be indices of toxicity. For example, dioxins cause a wasting syndrome in experimental animals that is at least partially characterized by a general decrease in body weight. Similarly, diuretics or uncouplers of oxidative phosphorylation can cause general decreases in body weight. For all of these compounds, the gross decrease in body weight can be directly related to the mechanisms of action of these compounds.

At the level of the organ, decreases or increases in weight may also be related to mechanisms of action. Perhaps the best studied example is liver enlargement associated with the induction of mixed function oxidases (MFO). Many compounds that induce MFO may cause a corresponding and profound increase in liver weight that can be related directly to the mechanism of action of these compounds. In some cases organ weight changes may reflect clear toxicological processes. For example, compounds that damage the lungs may cause edema, which will be reflected as an increase in absolute or relative lung weights. Toxicological damage also may be reflected as a decrease in organ weight, such as the effects of some phthalates on the testicles.

In all of these examples the interpretation of the significance of changes in whole body or organ weights is linked to an understanding of how the particular chemical influences the organism or organ. Notwithstanding these examples, changes in whole body weight or organ weights, in the absence of any information on the mechanism of action or clear signs of toxicity, are marginal kinds of information for classifying a particular exposure as adverse or not adverse. Nonetheless, these effects may be used by groups such as the U.S. EPA as a conservative/protective basis for defining an NOAEL and it is not uncommon for RfD's and similar values to be based solely on changes in body weight or organ weight in the absence of data indicating that these effects are not toxicologically significant. If a decrease in whole body weight can be related to a decrease in food consumption, the effect may simply reflect an organoleptic property (i.e., the compound may make the food unpalatable to the organism). A decrease in food consumption, however, may also reflect an underlying pathology that suppresses the appetite of the organism. Thus, a decrease in whole body weight associated with a decrease in food consumption does not necessarily indicate the absence of an adverse effect.

3.1.5.4.2. Liver

The liver is an extremely common target tissue in toxicology and is discussed in detail in standard toxicology texts (e.g., Moslen 1996); effects on the liver commonly serve as a basis for many RfDs derived by the U.S. EPA. The liver is important not only in the activation or detoxification of xenobiotics but also in various normal physiological functions, including several functions affecting the blood (clotting factors) and the metabolism, synthesis, and/or regulation of a variety of endogenous substance, such as various sugars, fatty acids and fat

catabolism and anabolism, amino acid and protein synthesis, the removal of toxic metabolites such as ammonia, the storage of iron and copper, the synthesis of vitamin A and the storage of vitamins A, D, and B_{12}, and the secretion of bile into the gastrointestinal tract via the gall bladder.

At the level of the whole organism, one of the most common effects of liver dysfunction is jaundice, the accumulation of bilirubin and other bile pigments in the skin and mucous membranes, which is associated with obstruction of the bile ducts (*obstructive jaundice*). Jaundice may also be caused by an increase in the destruction of red cells by the spleen (*hemolytic jaundice*). Because of the importance of the liver in a variety of metabolic processes, liver damage can lead to a great variety of other gross toxic effects.

When liver cells are damaged, various enzymes may be released into the plasma, depending on the test species and the type of damage, and may serve as very sensitive measures of liver damage. The interpretation of changes in plasma enzymes, however, is complicated by the wide tissue distribution of some enzymes as well as varying rates of enzyme synthesis and plasma clearance. Because the plasma clearance rates for most enzymes are relatively rapid, plasma enzyme levels are more commonly used in acute rather than chronic studies. Commonly used enzymes for assessing hepatocellular damage include aspartate and alanine aminotranferases as well as lactate, sorbitol, malate, and glutamate dehydrogenases (Woodman 1988). In the absence of any other signs of liver toxicity, increases in plasma levels of these enzymes may be used to classify an effect level – i.e., a dose or concentration – as adverse.

In contrast to plasma levels of liver enzymes, changes in plasma levels of endogenous proteins, which are synthesized by the liver, are generally indicators of more severe damage because they more closely reflect functional impairment. Effects on endogenous proteins include changes in serum albumin, fibrinogen, α_1 antitrypsin, haptoglobin, ceruloplasmin, transferrin, and prothrombin (Woodman 1988). Because of the longer protein half-lives and slower clearance rates of plasma proteins compared with plasma enzymes, these changes are more likely to reflect chronic damage.

Changes in plasma lipid levels usually indicate more serious effects on liver function (Woodman 1988). Increased plasma concentrations of cholesterol, low density lipoproteins, triglycerides, non-esterified fatty acids, and phospholipids, while not specific to liver damage, are suggestive of serious functional impairment. If other more specific data (e.g., effects discussed above or fatty accumulation in hepatocytes) indicate that such changes in plasma lipids are likely to be associated with liver damage, these effects should be classified as less serious or more serious but not as minimal.

One of the most common histopathological changes observed in the liver is necrosis or cell death. Classically, liver necrosis is characterized as massive, focal, centrolobular, midzonal, and periportal (Popp and Cattley 1991). As its name implies, massive necrosis involves large portions of one or more lobes of the liver and is readily apparent on gross necropsy. Because of the large reserve capacity of the liver, massive necrosis does not necessarily lead to death, and lobules with enough viable hepatocytes may regenerate. In other words, massive

necrosis can be reversible. In more severe cases, massive necrosis will lead to fibrosis, with relatively few viable hepatocytes in the lobe. In spite of the potential reversibility of this condition, massive necrosis clearly indicates a substantial decrement in organ function, which may be fatal or debilitating. At the other extreme, focal necrosis consists of relatively small (<1 mm) areas of dead hepatocytes. Although the pathogenesis of focal necrosis is poorly understood, this condition is reversible, does not lead to fibrosis, and is observed in animals that do not show clear signs of toxicity. Although this condition is clearly adverse at the cellular level, it is not clearly associated with impaired liver function.

Centrolobular, midzonal, and periportal necrosis are intermediate and variable in severity. It is difficult to generalize the level of adversity on the basis of these designations alone. Centrolobular necrosis associated with hemorrhagic lesions or involving endothelial cells may be indicative of a more serious adverse effect. Otherwise, the rapid reversibility of this condition, usually 1 week, suggests that this is a less serious adverse effect (i.e., it may have a transient effect on organ function but is not likely to lead to frank signs of toxicity). Periportal and midzonal necrosis are less common than centrolobular necrosis but are also rapidly reversible.

As discussed above, liver damage may result in increased lipid synthesis, which can lead to a condition referred to as fatty liver, fatty degeneration, or lipidosis. Moderate fatty changes do not seem to affect organ function. Other common forms of hepatocytic pathology include hydropic degeneration, glycogen accumulation, accumulation of lipofuscin pigment, and excess storage of iron and copper. All of these changes are reversible, and, in the absence of other signs of liver toxicity, are not usually associated with organ dysfunction. Thus, although these changes are classified as adverse effects, they are adverse at the cellular level but do not substantially impair the function of the organ or whole animal. Several other lesions in nonparenchymal cells or bile ducts may be associated with chemical exposure. Hepatitis and cirrhosis, like massive necrosis, can lead to profound clinical signs of toxicity and are clearly adverse effects.

3.1.5.5. Epidemiology Studies

Strictly defined, epidemiology refers to the study of disease patterns in humans. When good epidemiology data are available, they can serve as the definitive qualitative and/or quantitative estimate of potential human hazard. Occasionally, the unique nature of a chemically-induced effect (e.g., liver angiosarcoma by vinyl chloride) will lead quickly to the recognition of a human risk. More often, however, epidemiology studies in non-occupational situations are not definitive enough to establish chemical cause-and-effect relationships with certainty.

For some compounds, information may be available on toxic effects associated with accidental or normal occupational exposures. This type of information may be used to assess dose-response relationships in humans. Data from human exposure incidents must be carefully analyzed with regard to the nature of the chemical involved, the quantity of chemical present, and the duration of exposure. In addition, the possibility of synergistic and antagonistic effects of other chemicals is quite significant, especially in industrial situations. It is hoped that reports of human surveillance studies, including personal monitoring data as well as retrospective investigations in work populations, can be obtained. Because human

living conditions and lifestyles vary greatly, a detailed analysis of particular human situations involving chemical exposures can be extremely valuable in defining public health hazards. Furthermore, toxicological screens and animal model systems cannot substitute for every aspect of human living conditions and cannot duplicate the everyday exposures to which humans are subject.

Information regarding the human health effects of chemical exposure should come from human experience; however, these data are difficult to obtain. Controlled laboratory experiments in which humans are exposed to chemical substances are limited by ethical considerations. When chemicals are administered to humans under controlled conditions, the results may be inconclusive because of inter-individual variability and because of the generally small number of individuals participating in the studies. Case reports of persons with known exposure to a particular chemical generally provide qualitative evidence of a causal relationship between exposure to that chemical and a particular toxic effect, but exposure levels are seldom known and control data are not available.

Epidemiology studies are often difficult to interpret and open to criticism because these studies are very difficult to control; it is difficult to identify two virtually identical groups of individuals for which the only significant or substantial difference is exposure to the chemical. In addition, most epidemiology studies do not well-characterize exposure and the exposures themselves are not controlled. For these reasons, it is unusual for epidemiology studies to serve as the sole basis for a hazard identification, and it is very unusual for epidemiology studies to serve as the basis for a dose-response assessment. This is similar to the use of field studies in ecological risk assessment (Section 4.1). Epidemiology data are most often used in combination with standard toxicity studies in laboratory animals to assess whether or not a particular effect is plausible.

3.1.6. Effects on Nervous System

The EPA/OPPTS (2005) does have protocols for some specialized studies on effects on the nervous system. These include a general neurotoxicity screening battery (OPPTS 870.6200), an acute and a 28-day delayed neurotoxicity assay for organophosphorus substances (OPPTS 870.6100), a developmental neurotoxicity assay (OPPTS 870.6300), and an assay for peripheral nerve function (OPPTS 870.6850). It should be noted that the delayed neurotoxicity study (OPPTS 870.6100) uses hens (*Gallus gallus domesticus*) as a test species. For this particular assay, this is considered a sensitive species that is relevant to the assessment of neurotoxicity in humans.

For most pesticides, specific studies designed to detect neurotoxicity are conducted only if neurological effects are noted in more routine toxicity studies, or if the chemical belongs to a class for which there is a strong presumption that all members of the class will be neurotoxic. Typical subchronic or chronic animal bioassays rely on morphological and functional assessments to detect neurotoxicity. Morphological assessment usually consists of examination of the brain and spinal cord for visible changes at the naked-eye and light microscopic level. Structure of the terminal portions of the peripheral nervous system is evaluated as part of the morphological examination of endocrine and exocrine glands, muscles, and other tissues. In some assays, including the standard procedures used by the

National Toxicology Program (NTP), evaluation of the spinal cord and peripheral nerves (e.g., sciatic nerve) is only performed if the study finds other indications of neurotoxicity.

Behavioral assessments typically include observations of the animals in their cages for gross deficits in movement, balance, or coordination (e.g., gait, posture, visible tremor) (O'Donoghue1996). These are sometimes further supplemented with a more comprehensive functional observation battery consisting of various qualitative or quantitative tests of movement, gait, balance and coordination, muscle strength, and reflexes (Weiss1999). Beyond the realm of most typical bioassays are various, more explicit tests of motor, sensory and cognitive function that can provide a more quantitative evaluation of neurological deficits (Weiss1999). These would usually be conducted only if there were other indications of a possible direct neurotoxic effect of the agent.

Assays for neurological effects may be complicated and difficult to interpret because of the complexity of the nervous system itself. The nervous system can be subdivided anatomically into the *central nervous system* (CNS), which includes the brain and spinal cord, and the *peripheral nervous system* (PNS), which includes nerves connecting organs and tissues with the spinal cord and brain. The latter include the nerves that carry information to the CNS about sensation (*sensory neurons*), and nerves that carry information to muscles to control movement (*motor neurons*). From the perspective of mechanisms of neurotoxicity, the nervous system can be more meaningfully subdivided into the various functional components of nerve cells (*neurons*) that can be the targets of chemical agents. The structural organization of neurons reflects their principal function to process, store, and convey information about the body, either within the CNS, or between the CNS and other tissues and organs. This is accomplished by a combination of chemical signaling between neurons, and electrical potentials and currents within neurons. Neurons consist of 1) a *cell body*, containing the nucleus and other organelles that carry out synthesis and catabolism; 2) *dendrites*, elongated cellular processes that emanate from the cell body and that function to receive information, in the form of chemical signals, from other neurons and translate these signals into electrical potentials and currents within the cell body; 3) the *axon*, an elongated process (which can be more than a meter in length) that transmits information, in the form of electrical potentials and currents, from the cell body to nerve terminals; and 4) the *nerve terminal* which receives information encoded in electrical currents from the axon and communicates, in the form of chemical signals, to other neurons. In addition to neurons, the nervous system includes a variety of other types of cells that are critical to the function of the system. These include neuroglia (in the CNS), Schwann cells (in the PNS), and various specialized sensory receptors (in the PNS). Neuroglia and other supporting cells make up approximately 90% of the cells in the CNS (Jones 1988).

Neurotoxicants are chemical agents that disrupt the function of neurons, either by interacting with neurons specifically, or with supporting cells in the nervous system (e.g., neuroglia, Schwann cells, sensory receptors). The above definition is central to this discussion because it distinguishes agents that act directly on the nervous system *(direct neurotoxicants*), from those agents that might produce neurologic effects that are secondary to other forms of toxicity (*indirect neurotoxicants*) (O'Donoghue 1994). An example of the latter would be an agent that disrupts the respiratory or cardiovascular system and, thereby, deprives the brain

of oxygenated blood. Another example would be an agent that disrupts kidney function and, thereby, alters nervous system function by producing irregularities in body sodium and potassium levels.

The term *indirect neurotoxicants* may be a misnomer – or at least somewhat confusing – in that the indirect agent, by definition, does not directly damage nerve tissue. Nonetheless, the distinction between direct and indirect neurotoxicants is important because the types of biological assays needed to fully characterize these two very different causes of neurological effects will be very different. For example, an agent that disrupts kidney function may also produce, secondarily, neurological effects that are similar to those produced by agents that disrupt potassium or sodium transport in nerve cells (lethargy, stupor, muscle tremors, convulsions). However, in a typical whole animal chronic toxicity bioassay, such effects would be observed in concert with irregular serum sodium and potassium levels, and other indications of impaired kidney function. The same neurologic effects observed in the absence of indicators of impaired kidney function, or impaired function of other organ systems that might secondarily result in neurological effects, would be much more provocative evidence that the agent might be a direct neurotoxicant. However, bioassays directed at detecting specific forms and mechanisms of neurotoxic activity would be needed to confirm that the agent is a direct neurotoxicant. These might include evaluations of motor or sensory function, histopathological examination of the nervous system for assessment of exposure-related structural changes, or assessments of the toxicity of the agent using *in vitro* preparations of neurons of nervous system cells. In general, lethal exposures to toxic agents, regardless of their mechanism of toxicity, almost always give rise to neurological effects in the terminal stages of the intoxication. These effects can arise from many causes, including fluid and electrolyte imbalances, pulmonary edema, or cardiovascular collapse. Thus, very little information about the direct actions of a chemical agent on the nervous system is typically gained from studies of acute lethal, or near-lethal intoxications. The assessment of direct neurotoxic potential usually relies on studies of subchronic, chronic, or acute exposures, well below those that produce effects on other organ systems that might imperil the nervous system.

Evidence of neurotoxicity relies largely on the corroborated demonstration, usually in animal models, of a dose-related abnormality in the structure (*morphology*) of the nervous system (*histopathological change*) and/or a dose-related effect of the chemical on neurologic function, such as impaired movement, response to sensory stimuli, learning, or memory. The occurrence of both histopathological changes and functional deficits, in particular if the histopathological changes occur in regions of the nervous system thought to control the observed function, would be strong evidence for neurotoxicity.

3.1.7. Effects on Immune System

The *immune system* consists of a set of defense agents including the mononuclear phagocytic cells (macrophages in tissues, monocytes in circulation) and the natural killer cells, as well as specific lymphoid tissues dispersed throughout the body. The *lymphoid tissue* is comprised of T and B lymphocytes, epithelial cells and stromal cells, and is arranged into structurally and functionally distinct organs such as the *thymus, spleen, and lymph nodes* or accumulations of diffuse lymphoid tissue such as the *gut-associated lymphoid tissue* (GALT). All cells of the immune system derive from a pluripotent stem cell in the *bone*

marrow. *T lymphocytes* become immunologically competent (mature) in the thymus. *B lymphocytes* mature in the GALT (mammals) or bursa of Fabricius (birds). The immune system defends its host against foreign agents by utilizing both the non-specific and specific components, its mature lymphocytes with their associated cell-surface antigens (cellular immunity), special proteins in circulation (immunoglobulins), specific *antibodies* produced by the plasma cell (humoral immunity) in response to foreign *antigens* (bacterial, viral, parasites, foreign proteins etc.) and a number of other cell products known as *cytokines*. Cells of an individual are recognized as self by their cell-surface recognition antigens. Each individual has a unique signature of cell recognition antigens, known as the *major histocompatibility complex* (MHC). Changes in these signature antigens identifies a cell as foreign or abnormal, and triggers an unwanted immune response. The MHC together with other types of cell-surface antigens on lymphocytes (cluster differentiation antigens, CD) enable the immune system to recognize and respond to foreign or abnormal cells. In *autoimmune* diseases, this recognition system fails, and the immune system mounts an often destructive response against self cells and tissues. Examples of autoimmunity include Hashimoto's thyroiditis due to the production of antibodies to native thyroglobulin, which is the major iodine-containing protein; autoimmune hemolytic anemias, in which patients produce antibodies to their own red cells, and the Goodpasture's syndrome in which autoantibodies are produced to glomerular basement membrane of the kidneys leading to glomerulonephritis (kidney damage).

Immunotoxicants are chemicals that disrupt the function of the immune system. Depending on the mechanism of action, immunotoxicants can either impair immune responses (immune suppression) or stimulate the immune responses (hyperreactivity). *Immune suppression* may lead to enhanced susceptibility to infectious agents or inability to clear cancerous cells from the system. Examples of immunotoxicants include corticosteroids, which are drugs used in the treatment of inflammation, and cyclosporin, a drug used to suppress the immune response in transplantation patients (Diasio and LoBuglio1996). Environmental pollutants that are known to be immunosuppressive include benzene, PAHs, PCBs, TCDDs, certain heavy metals (e.g. lead, mercury and cadmium), and certain organophosphate and organochlorine insecticides (Burns et al.1996; Luster et al.1992, 1993; Tryphonas and Feeley, 2001). *Hyperreactivity* can lead to *allergy or hypersensitivity* in which the immune system of genetically predisposed individuals responds in an exaggerated manner to substances (allergens) such as plant pollen, cat dander, peanuts, and eggs, which do not pose a threat to other non-susceptible individuals. This type of reaction involves a sensitization phase during which the individual is subjected to repeated exposures of the allergen and a subsequent encounter with the allergen which may result in a mild reaction (skin rashes or hives, congestion, sneezing etc.) or a less frequent but severe reaction (*anaphylaxis*) leading to death. Hyperreactivity can also lead to autoimmunity in which the immune system produces antibodies to self-antigens resulting in damage of the organ or tissue involved. Only a few agents, mostly metals, have been shown to cause autoimmunity.

Evidence of immunotoxicity relies largely on the corroborated demonstration, usually in animal models, of a dose-related histopathological change in lymphoid tissue and/or a dose-related effect of the chemical on immune response to a foreign antigen. The occurrence of both histopathological changes in lymphoid tissue and abnormalities in one or more types of

immune responses, would be strong evidence for immunotoxicity. Typical subchronic or chronic animal bioassays conduct morphological assessments of the major lymphoid tissues, including bone marrow, major lymph nodes, spleen and thymus (thymus weight is usually measured as well), and blood leukocyte counts. These assessments can detect signs of inflammation or injury indicative of a direct toxic effect of the chemical on the lymphoid tissue. Changes in cellularity of lymphoid tissue and blood, indicative of a possible immune system stimulation or suppression, can also be detected.

The U.S. EPA (U.S. EPA/OPPTS 2011) has only one specific protocol for an assay of immune function, OPPTS 870.7800. This is a 28-day bioassay in which rats or mice are exposed to the test chemical. Typically, exposures are conducted by the oral route although dermal and inhalation routes can be considered for some compounds. The test assays response to a T cell dependent antigen, sheep red blood cells (SRBC). The animals are exposed to SRBCs about four days before the exposure period ends, after which assays are conducted for splenic anti-SRBC (IgM) response and/or serum anti-SRBC IgM levels. If significant depression in response to the antigen is noted, additional assays for immune function are required.

A variety of other tests have been developed to assess the effects of chemical exposures on various types of immune responses (e.g., Luster et al.1988, 1992, 1993). These include measuring the effects of chemical exposure on antibody-antigen reactions (*humoral immunity*), measuring changes in the activity of specific types of lymphoid cells when exposed to foreign antigens (*cell-mediated immunity*), and assessing changes in the susceptibility of exposed animals to resist infection from pathogens or proliferation of tumor cells (*host resistance*). Tests of immune responsiveness are not typically conducted as part of standard toxicity bioassays, unless there are other indications that the chemical may have immunologic potential. These indications might include histopathological change in lymphoid tissue, changes in blood leukocyte counts, or indications of excessive infectious disease in treatment groups.

3.1.8. Effects on Endocrine System

A variety of short-term *in vitro* and *in vivo* tests have also been described that assess whether a chemical interferes with hormone availability (e.g., synthesis, secretion, transport in the bloodstream) or with the target tissue response (e.g., hormone receptor binding or postreceptor processing). These assays can be used to assess the potential for endocrine disruption and have been proposed as screening assays for endocrine disruption (EDSTAC1998). The observation of endocrine activity of a test chemical in these short-term assays together with the observation of abnormalities in growth, development, reproduction, homeostasis, or in endocrine glands, in a multigeneration study in whole animals, would be strong evidence that a chemical is a potential endocrine disruptor.

The *endocrine system* is critical to the health of an animal because it participates in the control of metabolism and body composition, growth and development, reproduction, and many of the numerous physiological adjustments needed to maintain constancy of the internal environment (*homeostasis*). The *endocrine system* consists of *endocrine glands*, *hormones*, and *hormone receptors*. *Endocrine glands* are specialized tissues that produce and export (*secrete*) *hormones* to the bloodstream and other tissues. The major endocrine

glands in the body include the adrenal, hypothalamus, pancreas, parathyroid, pituitary, thyroid, ovary, and testis. Hormones are also produced in the gastrointestinal tract, kidney, liver, and placenta. *Hormones* are chemicals produced in endocrine glands that bind to *hormone receptors* in target tissues. Binding of a hormone to its receptor results in a process known as *postreceptor activation* which gives rise to a *hormone response* in the target tissue, usually an adjustment in metabolism or growth of the target tissue. Examples include the release of the hormone *testosterone* from the male testis, or *estrogen* from the female ovary, which act on receptors in various tissues to stimulate growth of sexual organs and development of male and female sexual characteristics. The target of a hormone can also be an endocrine gland, in which case, receptor binding may stimulate or inhibit hormone production and secretion. An example of this would be the hormone LH (luteinizing hormone), secreted from the pituitary gland, which acts on receptors in the testes to stimulate the secretion of testosterone. This system of endocrine glands, that are responsive to hormones released from other endocrine glands, provides a complex network of control systems for turning on and turning off hormone stimulation of tissues in response to physiological demands, or at appropriate stages of the life span or reproductive cycle. Examples of this are the dramatic changes in growth and development that occur as the fetus develops in the uterus and as individuals sexually mature during puberty. Repeated cycles of turning on and turning off hormone stimulation of the ovary and uterus occur approximately each month in females to produce the menstrual cycle.

An *endocrine disruptor* is an exogenous agent (from outside of the body) that produces adverse effects on an organism or population of organisms by interfering with endocrine function (Kavlock et al.1996). The endocrine system is highly regulated to achieve hormone activities in amounts needed to respond to physiological demands. *Endocrine disruption* is a state of uncontrolled hormone action, in which hormone responses are absent or insufficient when needed, or occur inappropriately when they are not needed. These can result in abnormalities in growth and development, reproduction, body composition, homeostasis, and behavior. Endocrine disruptors are not considered to be a major cause of endocrine disorders in humans. However, a variety of inherited endocrine diseases are known to be caused by abnormalities in endocrine glands, hormone transport, or hormone receptors. Certain endocrine diseases are thought to be caused by autoimmune disorders in which the body attacks and destroys its own endocrine glands.

Some important drugs are endocrine disruptors. Examples of these include thyroid blocking agents used in the treatment of hyperthyroidism (e.g., thiopropyluracil); corticosteroids used in the treatment of inflammation, and as diuretics in the treatment of edema and hypertension; estrogens used in female birth control and to manage symptoms of menopause; hypoglycemics used in the treatment of certain forms of diabetes mellitus; and various adrenergic agonists and antagonists used in the treatment of allergic reactions, asthma, heart disease, and hypertension (Hardman and Limbird1996). Endocrine-active agents are also in our diet, including iodine, needed for the production of thyroid hormone, and phytoestrogens, estrogenic compounds found in many edible plants.

Endocrine disruptors can exert effects by affecting the availability of a hormone to its target tissue(s) and/or affecting the response of target tissues to the hormone (EDSTAC1998).

These effects can enhance the action of natural hormones or diminish or abolish these actions. Effects may be transient or permanent, and may occur soon after exposure to the agent or may occur long after exposure ceases (*latent*).

Evidence of endocrine disruption relies on the corroborated demonstration, usually in animal models, of a dose-related abnormality in the structure of endocrine glands (histopathological change); a dose-related effect of the chemical on endocrine function, including hormone synthesis, secretion, transport and elimination; receptor binding; or postreceptor processes that give rise to a response in a target tissue and a demonstration that the effect on endocrine function gives rise to an adverse effect in the organism or population (EDSTAC1998). Examples of adverse effects include impairment in growth or development, reproduction, homeostasis, or behavior. This latter evidence of an adverse effect is particularly important, since it distinguishes endocrine disruptors from chemicals that are merely endocrine-active but have little or no potential for disruption of the endocrine system.

Morphological examination of the major endocrine glands for histopathological changes are usually included in well-designed subchronic or chronic rodent bioassays. However, typical rodent subchronic or chronic bioassays begin exposures after weaning, whereas, the assessment of potential adverse consequences of endocrine disruption requires the evaluation of exposures that span all of the critical stages of the lifespan at which endocrine controlled growth and development occur (EDSTAC1998). Organisms may be particularly sensitive to endocrine disruption during embryonic development and post-natal, and during growth and maturation (e.g., puberty). Disruption of the endocrine system during development may give rise to effects on the reproductive system that may be expressed only after maturation. For this reason, multigeneration exposures are recommended for toxicological assessment of suspected endocrine disruptors (EDSTAC 1998). These assays are discussed further below (Section 3.1.9).

Dose-response relationships for endocrine disruptors may be complex; the response may increase or decrease over intervals of a dose range of a given agent. For example, testosterone can stimulate sperm production at low doses and inhibit sperm production at high doses (EDSTAC1998). As a result, assays conducted at a high dose range may not be predictive of responses at a lower dose. Dose ranging studies are recommended to ensure that the assays include a dose range of adequate width to include a clearly toxic dose (maximum tolerated dose) and to capture possible *low-dose effects*. If these types of assays examine an adequately wide dose range below and including the maximum tolerated dose, they can be expected to detect adverse consequences, including latent consequences, of endocrine disruption. However, they cannot be expected to provide definitive conclusions about whether the observed abnormalities do in fact result from endocrine disruption. Other studies directed at identifying endocrine mechanisms underlying the abnormalities would be needed for this purpose.

3.1.9. Reproductive and Developmental Effects

3.1.9.1. Developmental Studies

Chemically-induced reproductive impairment is an important response parameter in human and ecological risk assessments. In human risk assessment, teratogenicity, sterility, or

decreased reproductive capacity can serve as endpoints in establishing NOELs from chronic exposure. However, the threshold for adverse reproductive effects in mammals is often above the threshold for more general toxic effects (e.g., decreased total body weight gain or altered organ weights). Furthermore, many mammalian teratology studies involve single short-term exposures and are difficult to apply directly to estimating the risks from longer-term environmental exposures.

Teratogenicity studies typically entail gavage administration to pregnant rats or rabbits on specific days of gestation. Teratology assays as well as studies on reproductive function (Section 3.1.9.2) are typically required for the registration of pesticides. Protocols for developmental studies have been established by U.S. EPA/OPPTS (2011) – i.e., OPPTS 870-3500 and 870.3700. Typically, the compound is administered by gavage and at least three doses are used; exposure may occasionally be dietary or inhalation. The compound is administered daily to pregnant female animals over a specified period during gestation. The dams are observed for signs of toxicity and the offspring are observed for signs of abnormal development. Other end-points that are examined include signs of pre-implantation losses and resorptions.

Developmental toxicity relates specifically to effects on the embryo or fetus and not to the pregnant female. Although this area of study was traditionally concerned with compounds that resulted in the birth of grossly abnormal offspring, it has been expanded to encompass those dose-related effects resulting in death of the embryo or fetus, functional impairment and altered growth and/or developmental patterns. The physiological processes that produce abnormal development are the same cellular mechanisms associated with chemical toxicity in the adult, including inflammation, degeneration, necrosis, cell differentiation, and proliferation. Nonetheless, the embryo and fetus are viewed as a uniquely susceptible target, due to the occurrence of unusually rapid proliferation and differentiation during fetal development.

Teratology studies may be used to derive acute RfDs (Section3.3.3). The rationale for this use is that most teratology studies involve relatively short-term exposures. In addition, many teratogenic effects are very time-specific and it is possible that only a single exposure or dose occurring on a single critical day during development could account for the observed effect.

3.1.9.2. Reproduction Studies

Another type of reproduction study involves exposing one or more generations of a test animal to a compound. Protocols for reproduction studies have been established by U.S. EPA/OPPTS (2011) – i.e., OPPTS 870-3800. Typically, the compound is administered at 3 or more dose levels. Dietary administration is the most common route although drinking water or gavage studies are sometimes conducted. These tests are almost always conducted on rats. The general experimental method involves dosing the parental (P) generation (i.e., the male and female animals used at the start of the study) to the test substance prior to, during mating, after mating, and through weaning of the offspring (F_1). Typically, this procedure is repeated with male and female offspring from the F_1 generation to produce another set of offspring (F_2). During the study, standard observations for gross signs of toxicity are made. Additional observations often include the length of the estrous cycle, assays on sperm and other reproductive tissue, and number and viability of offspring.

This is a very important type of study for many risk assessments. As discussed in Section 3.1.8, multigeneration reproduction studies are often the most relevant of the commonly available studies for assessing the effects of a compound on endocrine effects relating to reproduction. In addition, multigeneration reproduction studies may be used by U.S. EPA to derive chronic RfDs if the NOAEL for reproduction studies is below the NOAELs for standard chronic toxicity studies (Section 3.3.3).

3.1.9.3. Target Organ Toxicity

As part of most standard acute and chronic toxicity studies, observations are often made on reproductive tissue – e.g., ovaries and testes. This type of information is often included in this section (and may be repeated from previous sections for emphasis). This type of information can be used to assess concern for potential reproductive effects and to supplement information from developmental or reproduction studies.

3.1.10. Carcinogenicity and Mutagenicity

Three kinds of data are commonly used to assess potential carcinogenic hazard: epidemiology studies; bioassays on mammals; and tests for genetic toxicity, including mutagenicity. The general protocols for carcinogenicity studies are similar to those of chronic toxicity studies and chronic toxicity studies and bioassays for carcinogenicity are typically combined.

Epidemiology studies involve the comparison of the cancer incidence in two or more populations with varying degrees of exposure to the chemical under study. They are of limited use because many important variables—like quantitative estimates of exposure to the test chemical, differences in diet, and exposure to other potential carcinogens—are not adequately controlled or characterized. Nevertheless, data from well-designed epidemiology studies are the only data accepted as unequivocal proof that a chemical is a human carcinogen. The problems in precisely defining exposure levels and other factors merely inhibit the use of these studies in quantitative risk assessment.

Bioassays on mammals involve the controlled exposure of experimental animals, usually rats or mice, to defined levels of the test substance. In carcinogenicity bioassays, an attempt is made to expose the organism for a significant portion of its life span to the test substances, or at least to observe the organism for a significant portion of its life span. This protocol is necessary because many tumors appear only late in the life of the organism; thus, premature sacrifice may lead to false negative results. Furthermore, in terms of environmental toxicology, the major concern is the incremental increase in the incidence of cancer attributable to lifetime exposures. Another important element in the design of mammalian bioassays is the proper selection of dose levels. Since for practical and economic reasons, only limited numbers of animals (usually 20-50) are used at each dose level, it is necessary to use elevated dose levels in order to elicit a detectable response. Because excessively high doses that result in overt signs of toxicity may alter the physiology of the animal so that it is no longer a reasonable model for projected human exposures, attempts are made to ensure that doses below the maximum tolerated level are used. In addition, excessively high doses can cause premature mortality, which may mask carcinogenic activity. At the end of the experimental period, all animals are sacrificed and subjected to extensive histopathological

analyses. A positive response is usually defined as a significant dose-related increase in the incidence of malignant tumors at a given site in exposed animals.

Because carcinogenicity bioassays are time consuming and expensive, it is becoming common to use several mutagenicity screening tests to detect potential carcinogenicity. Mutagenicity studies include tests with microorganisms (e.g., Ames assay), tests for genetic damage in cultured mammalian cells (e.g., unscheduled DNA repair synthesis, sister chromatid exchange, point mutations), and tests for *in vitro* transformation of rodent cell lines. Although the tests are extremely valuable for detecting chemicals requiring further study (i.e., animal bioassay and/or epidemiology), they are not capable of detecting all potential carcinogens or indicating the relative potency of the carcinogens in humans.

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

Studies on effects of pesticides and pesticide formulations are relatively standardized and include assays for acute eye irritation (OPPTS 870.2400), acute dermal irritation (OPPTS 870.2500), and skin sensitization (OPPTS 870.2600).

The acute eye and skin irritation studies typically involve rabbits. The test material is applied either to one eye of the animal or to an area of the skin (intact or abraded). In the eye irritation studies, the untreated eye of the animal typically serves as the control. In the dermal studies, an untreated area of the skin typically serves as a control. As summarized in Table 3-2, both eye and skin irritations studies are used to classify pesticides (corrosive to non-irritant) and these classifications reflect how the pesticide or pesticide formulations must be labeled.

Quantitative risk assessments for irritation are not derived in risk assessments conducted for the Forest Service or in risk assessments conducted by other organizations such as the U.S. EPA. Nonetheless, from a practical perspective, effects on the eyes or skin are overt effects that are frequently seen as a consequence of mishandling pesticides. These effects, however, can typically be avoided by proper industrial hygiene practices – e.g., wearing gloves or protective goggles to avoid or minimize exposure.

Eye and skin irritation studies may also be important in a risk assessment in evaluating the potential role of inerts or adjuvants (Section 3.1.14). Along with acute oral toxicity studies (Section 3.1.4) and dermal toxicity studies (Section 3.1.12), studies on eye and skin irritation are often available on both the active ingredient as well as at least some commercial formulations. While comparisons between studies on the active ingredient and commercial formulations are not generally used quantitatively, substantial differences in the activity (e.g., irritant effects or other signs of toxicity) of the active ingredient and commercial formulation can be used to suggest that adjuvants or inerts may or may not have a toxicologically significant effect.

3.1.12. Systemic Toxic Effects from Dermal Exposure

Most of the occupational exposure scenarios and many of the exposure scenarios for the general public involve the dermal route of exposure. As discussed in Section 3.1.3.2, dermal absorption is estimated and compared to an estimated acceptable level of oral exposure based on subchronic or chronic toxicity studies. Nonetheless, any studies that are available on the

dermal toxicity of the chemical are included in this section and an attempt is made to evaluate the estimates of dermal absorption rates that are derived in Section 3.1.3.2.

Most studies on the dermal toxicity of pesticides involve acute (single application) exposures and follow relatively standard protocols – e.g., acute dermal irritation assay given in OPPTS 870.2500. Occasionally, longer term subchronic studies such as the 28-day study given in OPPTS 870.2500 or the 90-day study given in OPPTS 870.3250 may be available but these are uncommon. As noted in previous sections, the dermal route may be used in some specialized studies – e.g., reproductive effects – but these are not typically available for pesticides covered in Forest Service risk assessments. The general design and criteria for evaluating dermal studies are very similar to those for the corresponding oral studies and both range finding and limits tests may be conducted.

3.1.13. Inhalation Exposure

Inhalation toxicity studies can be as complex and varied as those involving oral exposure (Kennedy 1989; Klaassen et al. 1996; Wang et al. 1993). These may include standard acute toxicity studies observing gross signs of toxicity and gross tissue damage (OPPTS 870.1300), acute studies in which more detailed histopathological examinations are made (OPPTS 870.1350), subchronic studies (OPPTS 870.3465), developmental studies (OPPTS 870.3600), and pharmacokinetic studies (OPPTS 870.8340). For most pesticides, particularly those covered in Forest Service risk assessments, inhalation is not a significant or substantial route of exposure (e.g., Ecobichon 1998; van Hemmen 1992).

The most commonly available study for pesticides reviewed in Forest Service risk assessments is the basic acute toxicity study (OPPTS 870.1300). Except for the route of exposure, 4-hours to a concentration of the chemical in air, the design of this study is generally similar to the acute oral toxicity study and range-finding studies as well as limit tests may be used. As indicated in Table 4, the limit test typically involves exposures to 2 mg/L.

3.1.14. Adjuvants and Other Ingredients

U.S. EPA is responsible for regulating both the active ingredients (a.i.) in pesticide formulations as well as any other chemicals that may be added to the formulation. As implemented, these regulations affect only pesticide labeling and testing requirements. The term *inert* was used to designate compounds that are not classified as active ingredient on the product label. While the term *inert* is codified in FIFRA, some inerts can be toxic, and the U.S. EPA now uses the term *Other Ingredients* rather than *inerts* (http://www.epa.gov/opprd001/inerts/). For brevity, the following discussion uses the term *inert*, recognizing that *inerts* may be biologically active and potentially hazardous components.

Inerts cover an extremely broad range of compounds including carriers, stabilizers, sticking agents, or other materials added to facilitate handling or application. However, these inerts may be toxic to humans or other nontarget species. The U.S. EPA is responsible for the regulation of inerts and adjuvants in pesticide formulations. As implemented, these regulations affect only pesticide labeling and testing requirements. As part of this regulatory activity, U.S. EPA had classified inerts into four lists based on the available toxicity

information: toxic (List 1), potentially toxic (List 2), unclassifiable (List 3), and non-toxic (List 4). List 4 was subdivided into two categories, 4A and 4B. List 4A constituted inerts for which there was adequate information to indicate a minimal concern. List 4B constituted inerts for which the use patterns and toxicity data indicated that use of the compound as an inert is not likely to pose a risk. While the U.S. EPA/OPP no longer actively maintains these lists, references to this classification system is encountered in some of the older literature and these lists may be mentioned in some Forest Service risk assessments.

Any compound classified by U.S. EPA as toxic or potentially toxic must be identified on the product label if the compound is present at a level of 1% or greater in the formulation. All such compounds are considered explicitly in the risk assessment. If the compounds are not classified toxic, all information on the inert ingredients in pesticide formulations is considered proprietary under Section 10(a) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). In that case, the formulators of the pesticide need not and typically do not disclose the identity of the inert or adjuvant.

Even if the identities of the inerts are known, the toxicity data available on inerts are often very limited. As discussed by Levine (1996), the testing requirements for inerts are less rigorous than the testing requirements for the pesticides (i.e., the active ingredients). Some standard sources are typically consulted for information on inerts, including the classifications on the U.S. EPA's inerts lists, discussed above. In addition, many inert ingredients are also approved food additives and the listing of approved food additives compiled by Clydesdale (1997) is also consulted. If an inert is on List 4 (nontoxic), concern for the inert is reduced. Similarly, if the inert is an approved food additive, concern is also reduced, particularly if the compound is classified as GRAS (generally recognized as safe). Some inerts that are potentially toxic have been reviewed and evaluated by other governmental groups. As discussed in Section 1.3.3, these reviews may be consulted to elaborate on the potential effects of the inert.

The potential risks associated with inerts may also be assessed by comparing any available toxicity data on the active ingredient – i.e., the pesticide alone without any added adjuvants or inerts – to the toxicity of the formulated product. All pesticide formulations must identify the percent active ingredient in the formulation. As noted above, toxicity studies are often available on both the formulation and the active ingredient for acute oral exposures (Section 3.1.4) and acute dermal exposures (Section 3.1.12). Taking P as the proportion of the active ingredient in the formulation and L_X as a toxicity value such as an LD_{50} or LC_{50}, the toxicity value for the formulation (LX_F) may be converted to the toxicity value in terms of the active ingredient (LX_A) by the simple formula:

$$LX_A = LX_F \times P \qquad \text{(Eq. 12)}$$

The toxicity value in terms of the active ingredient (LX_A) is then compared to experimental toxicity values on the active ingredient – i.e., LD_{50} or LC_{50}. For example, if the experimental LD_{50} for the active ingredient is substantially higher than the LX_A, this suggests that some components in the formulation may be contributing substantially to the toxicity of the formulation. Conversely, if the experimental LD_{50} for the active ingredient is substantially

lower than the LX_A, this suggests that some components in the formulation may be antagonizing the toxicity of the active ingredient. While this sort of analysis is limited, it is often the only type of quantitative information that can be used to assess the toxicity of the formulated product.

In some rare cases – e.g., the Roundup formulations of glyphosate as discussed in SERA (2011b) – very detailed information may be available on both the toxicity of the active ingredient, the toxicity of specific adjuvants, and the toxicity of the formulation. In such cases, very detailed chemical specific analyses can be and are conducted based on an assessment of toxicologic interactions (Section 3.1.16).

3.1.15. Impurities and Metabolites

In many respects, impurities and metabolites are much less difficult issues than inerts and adjuvants. Impurities often occur in pesticides. Virtually no chemical synthesis yields a totally pure product. Thus, any technical grade pesticides will contain some impurities. To some extent, concern for impurities in technical grade pesticides is reduced by the fact that the toxicity studies on pesticides are often conducted with the technical grade product. Thus, if toxic impurities are present in the technical grade product, they are likely to be encompassed by the available toxicity studies on the technical grade product.

An exception to this general rule involves carcinogens, most of which are presumed to act by non-threshold mechanisms. Because of the non-threshold assumption, any amount of a carcinogen in an otherwise non-carcinogenic mixture may pose a carcinogenic risk. An example of this is the occurrence of hexachlorobenzene in two herbicides used by the Forest Service, clopyralid and picloram. For these herbicides, the risk assessments each included a full dose-response assessment, exposure assessment, and risk characterization for the potential carcinogenic effects of hexachlorobenzene.

As discussed in Section 3.1.3.1, the assumption is generally made that studies on whole animals, such as those used to derive acceptable levels of exposure in humans, will encompass the toxicity of both the parent compound as well as any metabolites that formed *in vivo*. As also discussed in Section 3.1.3.1, however, this does not apply to toxic metabolites that are formed in the environment. In such a case, the toxicity of the metabolite as well as exposures to the metabolite may need to be quantitatively addressed in the risk assessment. Whether or not such steps are needed is discussed in this section of the risk assessment.

3.1.16. Toxicological Interactions

Pesticides may sometimes be used in mixtures and the consequences of using mixtures may need to be assessed either from information on mechanism of action or direct toxicity data on the specific mixture. While Forest Service risk assessments do not directly deal with mixtures – i.e., risk assessment on pesticide mixtures are not explicitly considered in the exposure assessment, dose-response assessment, or risk characterization – any available information on toxicologic interactions is summarized in this section. As assessment of chemical interactions can be extremely complex and is data intensive (e.g., Mumtaz et al. 1994). The type of mechanistic information used to suggest potential interactions could include an effect of one chemical on the pharmacokinetics of another chemical (e.g.,

absorption, metabolism, distribution, or excretion) or direct receptor interactions. For most pesticides, direct information on chemical interactions is very limited. When available, this information is discussed in this section of the risk assessment and may also be used in the assessment of connected actions in the risk characterization (Section 3.4.5).

3.2. EXPOSURE ASSESSMENT

3.2.1. Overview

Typically, Forest Service risk assessments will present exposure assessments for both workers and members of the general public. For workers and the general public, two types of exposure scenarios are generally taken into consideration: *general exposures* and *accidental/incidental exposures*. The term *general exposure* refers to human exposure resulting from the normal use of the chemical.

For workers, general exposures involve the handling and application of the compound. The general exposure scenarios can be interpreted relatively easily and objectively. The exposure estimates are calculated from the amount of the chemical handled/day and the exposure rates for the worker group. Although each of the specific exposure assessments for workers involves degrees of uncertainty, the exposure estimates are objective in that they are based on empirical relationships of absorbed dose to pesticide use.

For the general public, the general exposure scenarios are somewhat more arbitrary and at least some may be less plausible. For each pesticide, at least three general exposures scenarios are considered, including walking through a contaminated area shortly after treatment, the consumption of ambient water from a contaminated watershed, and the consumption of contaminated vegetation. These three scenarios are consistently used because one of these three scenarios usually leads to the highest estimates of exposure. Additional scenarios discussed below may be considered for each of the individual compounds as warranted by the available data and the nature of the program activities. Additional variability is taken into consideration by estimating exposure doses or absorbed doses for individuals of different age groups (i.e., adults, young children, toddlers, and infants). Children may behave in ways that increase the exposure to applied pesticides. In addition, anatomical and physiological factors such as body surface area, breathing rates, and consumption rates for food and water, are not linearly related to body weight and age. Consequently, the models used to estimate the exposure dose (e.g., mg/kg body weight/day) based on chemical concentrations in environmental media (e.g., ppm in air, water, or food) indicate that children, compared with individuals of different age groups, are generally exposed to the highest doses of chemicals for a given environmental concentration.

3.2.2. Workers

The potential exposures of and health effects in pesticide applicators is a major focus in such USDA risk assessments. The concern for worker exposure is motivated by obvious ethical considerations as well as the understanding that pesticide applicators are likely to be the individuals who are most exposed to the pesticide during the application process.

A major and substantial difference in risk assessments conducted by the U.S. EPA/OPP and risk assessments conducted for the Forest Service involves the methods used to estimate worker exposure. Two general types of methods can be considered for worker exposure modeling, deposition-based and absorption-based. The U.S. EPA/OPP uses a deposition-based approach. The Forest Service uses an absorption-based approach. An overview of the two approaches is given in Table 5.

While the U.S. EPA/OPP generally uses deposition-based methods for worker exposures, the Agency has expressed a preference for biomonitoring data, as indicated in the following quotation for the Reregistration Eligibility Decision for alachlor.

> *Generally, biomonitoring data are preferable to passive-dosimetry data. The use of a dermal absorption factor is not necessary for biomonitoring data. Biomonitoring data can give a more accurate estimate of absorbed dose.*
>
> U.S. EPA/OPP 1998b, p. 65

The USDA Forest Service has generally used absorption-based models in which the amount of chemical absorbed is estimated from the amount of chemical handled (e.g., USDA/FS 1989a,b,c). Absorption-based models rather than deposition-based models have been used by the Forest Service because of two common observations from field studies. First, as discussed in the review by van Hemmen (1992), most studies that attempt to differentiate occupational exposure by route of exposure indicate that dermal exposure is much greater than inhalation exposure for pesticide workers. Second, most studies of pesticide exposure that monitored both dermal deposition and chemical absorption or some other method of biomonitoring noted a very poor correlation between the two values (e.g., Cowell et al. 1991; Franklin et al. 1981; Lavy et al. 1982). In USDA Forest Service exposure assessments for workers, the primary goal is to estimate absorbed dose so that the absorbed dose estimate can be compared with available information on the dose-response relationships for the chemical of concern. Thus, if dermal deposition does not correlate well with absorbed dose and if the inhalation route is not a substantial factor in worker exposure, the absorption-based approach may have some advantages when compared to the deposition-based approach.

Both the deposition method used by the U.S. EPA (PHED Task Force 1995) as well as the general estimates of worker exposure rates currently used in risk assessments for the Forest Service may be viewed as relatively crude approximations. As better data become available and methods to use this data are refined, additional methods may be employed. For example, Durkin et al. (2004) have developed a physiologically based pharmacokinetic model for 2,4-D, and have demonstrated that the model can be used to fit the variability in worker exposure to 2,4-D from the study by Lavy et al. (1982). This analysis involved a combination of both deposition data from PHED data base and measurements of dermal absorption rates. Using this approach, the central estimates of risk to workers were virtually identical to the estimates obtained from the general approach detailed below. As kinetic models are developed for other pesticides, they may be used in place of either the deposition or simple absorption rate estimates that are currently used.

3.2.2.1. General Exposures

The absorption based methods used in Forest Service risk assessments have recently been updated (SERA 2014) and substantially elaborated from the previous methods (SERA 1998). The update is based on new studies as well as some unpublished studies that had not been identified in the earlier report. The elaborations include an expansion of the different types of applications for which discrete exposure rates are defined for directed ground applications

– i.e., backpack directed foliar, hack-and-squirt, and basal bark – as well as aquatic broadcast applications.

An additional elaboration in the worker exposure rates developed in SERA (2014) is the explicit consideration of dermal absorption rates for directed ground applications. Both the earlier and more recent reports (SERA 1998, 2014), examined the relationship between worker exposure rates from studies on several pesticides and the dermal absorption rates for these pesticides. As discussed in SERA (1998), the data reviewed in the earlier report noted no relationship between worker exposure rates and dermal absorption rates. While this is not intuitive, the SERA (1998) recommended no adjustments of worker exposure rates based on differences in dermal absorption rate for the pesticide under consideration (ka_p) and the dermal absorption rate for the reference pesticide on which the worker exposure rate is based (ka_{Ref}). Based on the expanded set of studies in SERA (2014), this approach has been changed for directed ground applications and the following adjustment to the worker exposure rate is now recommended:

$$ExpRt_P = \frac{ka_P}{ka_{\mathrm{Re}f}} \times ExpRt_{\mathrm{Re}f} \qquad \text{(Eq. 13)}$$

As with the previous methods (SERA 1998), the data on ground broadcast applications do not support the use of this adjustment. For aerial and aquatic broadcast applications, the available data are insufficient to support an assessment of the adjustment in worker exposure rates and the decision concerning whether or not to use the above adjustment is made on the case-by-case based in the specific risk assessment of the subject pesticide. A summary of the worker exposure rates derived in SERA (2014) is given in Table 6, which specifies the application method, the worker exposure rate, and the reference chemical on which the worker exposure rate is based.

A final elaboration, as summarized in Table 6, involves the consideration of variability in worker exposure rates. Both the earlier and more recent reports (SERA 1998, 2014) note the substantial variability in worker exposure rates both among and within studies. In the earlier report, this variability is encompassed with estimates of the 95% confidence interval for the worker exposure rates. While this is a useful expression of variability, the 95% confidence interval reflects variability in the mean worker exposure rate and this interval will often not encompass the extremes of the worker exposure rates within is single study. To consider the greater dispersion of exposures for individual workers, the 95% prediction intervals are used. As illustrated in Table 6, 95% prediction intervals are wider, often much wider, than 95% confidence intervals and the 95% prediction intervals may better reflect the bounds of exposures for individual workers.

While the Forest Service prefers absorption-based methods for estimating worker exposures, SERA risk assessments will sometimes use deposition based methods either for comparison or because the PHED database supports a much larger number of application variables than does the current absorption-based method summarized in Table 6.

PHED Version 1.1, the version currently used in U.S. EPA/OPP risk assessments, is implemented as both a database and a DOS computer program. U.S. EPA/OPP (1998c) has summarized surrogate exposures from PHED for 37 types of exposures, involving mixer-loaders, flaggers, and applicators, for several different types of formulations (e.g., liquid, granular, and wettable powders) applied with ground or aerial equipment. Using the estimates of deposited dose and concentration of the pesticide in air, the absorbed dose for workers can be calculated if estimates are available on absorption rates for inhalation and dermal exposure. As summarized in Keigwin (1998), dermal exposures are much greater than exposures associated with inhalation. An overview of the standard PHED exposure rates, adopted from Keigwin (1998), is given in Table 7.

Another benefit of the PHED method is that the U.S. EPA is routinely able to present different exposure scenarios for workers using different levels of personal protective equipment (PPE) based on studies in PHED. In EPA risk assessments such as those used to support reregistration of a pesticide, the risks associated with different levels of PPE may be used to set regulatory requirements for the use of PPE during pesticide applications. Forest Service risk assessments will consider PPE only when PPE is required by EPA. This is most often the case with some insecticides. When PPE is considered in Forest Service risk assessments, estimates of the effectiveness of PPE are based on chemical-specific studies, if available. Otherwise, estimates of the effectiveness of PPE are taken from EPA assessments of the specific chemical or are developed from the PHED database.

Regardless of whether a deposition-based or absorption-based model is used to estimate worker exposure, the general algorithms for estimating worker exposure (*Exp*) are similar and are calculated generally, as the product of the exposure rate (*ExpRate*) and the amount of the pesticide that is handled by the worker (*Amnt*):

$$Exp = Amnt \times ExpRate \qquad \text{(Eq. 14)}$$

Note that in the absorption-based application, the exposure rate (*ExpRate*) may be adjusted as discussed above in the application of Eq. 13.

Typically, the amount of pesticide handled is calculated as the product of the application rate (*ApRt* in lbs/acre) and the number of acres treated per day:

$$Amnt = ApRt_{lbs/acre} \times Acres / day \qquad \text{(Eq. 15)}$$

While this basic algorithm is used in Forest Service and EPA risk assessments, the number of acres treated per day for a particular application method differs among Forest Service and EPA risk assessments. The values used in Forest Service risk assessments are generally based on estimates from field crews performing typical Forest Service applications (e.g., USDA/FS 1989a,b,c) as summarized in Table 6. The values used in EPA risk assessments vary according to the risk assessment. Generally, these values reflect information from registrants as well as judgments made by the EPA. The EPA's Science Advisory Council for Exposure Policy (ExpoSAC 2001) has proposed standard values for daily acres treated in agriculture and these guidelines are cited in some EPA risk assessments. In some cases in

which standard Forest Service values do not appear to be applicable, the number of acres treated per day may be taken from ExpoSAC (2001).

3.2.2.2. Accidental Exposures

The skin surface and eyes of workers are most likely to be affected by accidental spills or splashes of pesticide solutions. Quantitative exposure scenarios for eye exposures are not developed Forest Service risk assessments. Potential effects on the eyes are considered qualitatively in the risk characterization (Section 3.4.2) based on information covered in Section 3.1.11 (Ocular Effects).

Generally, dermal exposure is the predominant route of exposure for pesticide applicators (Ecobichon 1998; van Hemmen 1992), and accidental dermal exposures are considered quantitatively in all Forest Service risk assessments. The two types of dermal exposures modeled in the risk assessments include direct contact with a pesticide solution and accidental spills of the pesticide onto the surface of the skin. In addition, two exposure scenarios are developed for each of the two types of dermal exposure, and the estimated absorbed dose for each scenario is expressed in units of mg chemical/kg body weight. Both sets of exposure scenarios are summarized in Worksheet E01 of the EXCEL workbooks that accompany Forest Service risk assessments. Additionally, Worksheet E01 references other worksheets in which the calculations of each exposure assessment are detailed.

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

For both scenarios (hand immersion and contaminated gloves), the assumption of zero-order absorption kinetics is appropriate. For these types of exposures, the rate of absorption is estimated based on zero-order kinetics using the dermal permeability coefficient (K_p). In the absence of experimental measurements, the Kp can be estimated as discussed in Section 3.1.3.2.1.

The details of the accidental dermal exposure scenarios for workers consist of spilling a chemical solution on to the lower legs as well as spilling a chemical solution on to the hands with at least some of chemical adhering to the skin. The absorbed dose is then calculated as the product of the amount of chemical on the skin surface (i.e., the amount of liquid per unit surface area multiplied by the surface area of the skin over which the spill occurs and the chemical concentration in the liquid), the first-order absorption rate, and the duration of exposure. In the absence of experimental data, the first-order absorption rate coefficient (k_a) can be estimated as discussed in Section 3.1.3.2.2.

3.2.3. General Public

3.2.3.1. General Considerations

3.2.3.1.1. Likelihood and Magnitude of Exposure

Depending on the use and application method of the pesticide under review, members of the general public may or may not likely be exposed to the pesticide. For example, exposures are likely and virtually certain for chemicals that are applied by broadcast aerial spray in inhabited areas. Such chemicals could be insecticides that are used to control insect pests. On the other hand, exposures are less likely for some herbicides that are used only in spot applications. 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. Risk assessments prepared for the Forest Service typically include several sets of scenarios that are intended to characterize exposure in a consistent manner that allows for comparisons among different pesticides that the Forest Service might consider.

The exposure assessments developed in Forest Service risk assessments are based on *Extreme Values* rather than a single value. Extreme value exposure assessments, as the name implies, bracket the most plausible estimate of exposure (referred to statistically as the central or maximum likelihood estimate) with lower and upper bounds of credible exposure levels. This Extreme Value approach is essentially an elaboration on the concept of the *Most Exposed Individual* (MEI), sometime referred to as the M*aximum Exposed Individual*. As this name implies, exposure assessments that use the MEI approach attempt to characterize the extreme but still plausible upper limit on exposure. This common approach to exposure assessment is used by U. S. EPA, other government agencies, and the International Commission on Radiological Protection (e.g., ATSDR 2002; ICRP 2005; Payne-Sturges et al. 2004). In most Forest Service risk assessments, upper bounds on exposure are intended to encompass exposures to the MEI.

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

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

3.2.3.1.2. Summary of Assessments

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

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

3.2.3.2. Direct Spray

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

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

The exposure scenario involving the young woman (Worksheet D01b) is somewhat less extreme. In this scenario, it is assumed that the woman is accidentally sprayed over the feet and lower legs. The preference for using a young woman rather than an adult male in many of the exposure assessments relates to concerns for both the *Most Exposed Individual* (MEI) as well as the most sensitive individual. Based on general allometric considerations, the smaller the individual, the greater will be the chemical doses per unit body weight (e.g., Boxenbaum and D'Souza. 1990). In general, the body size of a female is smaller than that of males. Thus, in direct spray exposure scenarios, females are subject to somewhat higher doses than males. More significantly, reproductive effects are a major concern in all Forest Service risk assessments. Consequently, exposure levels for a young woman of reproductive

age are used in order to better assess the potential for adverse effects in the population at risk from potential reproductive effects—i.e., the most exposed and the most sensitive individual.

For this exposure scenario, assumptions are made regarding the surface area of the skin and the body weight of the individual, as detailed in Worksheet A03. The rationale for and sources of the specific values used in these and other exposure scenarios are given in the documentation for the worksheets (SERA 2011a). The first-order absorption dermal absorption rates are identical to those used in the similar worker exposure scenarios (Section 3.2.2.2).

3.2.3.3. Dermal Exposure from Contaminated Vegetation

The exposure scenario involving contaminated vegetation assumes that the herbicide is sprayed at a given application rate and that a young woman comes in contact with the sprayed vegetation or with other contaminated surfaces on the same day (Worksheet D02). This exposure scenario depends on estimates of dislodgeable residue (the estimated amount of the chemical which could be released from the vegetation, expressed in units of pesticide mass/surface area of vegetation), and dermal transfer rates (i.e., the rate at which the chemical is transferred from the contaminated vegetation to the surface of the skin). Dermal transfer rates are reasonably consistent for a number of pesticides (Durkin et al.1995).

Dislodgeable residues may vary according to the pesticide, the formulation, and the site-specific conditions. In the absence of chemical-specific data, dislodgeable residues are taken as 10% of the nominal application rate in most Forest Service risk assessments.

This exposure scenario assumes both a contact period of 1hour and that the chemical is not effectively removed by washing within 24 hours of exposure. Other estimates used in this exposure scenario involve estimates of body weight, skin surface area, and first-order dermal absorption rates. The specific values for each of these estimates are provided in Worksheet D02 together with the references for each value.

3.2.3.4. Contaminated Water

3.2.3.4.1. Accidental Spill

The accidental spill scenario assumes that a young child consumes contaminated water shortly after an accidental spill of a field solution into a small pond. The specifics of this scenario are given in Worksheet D05. Because this scenario is based on the assumption that exposure occurs shortly after the spill, no dissipation or degradation is considered. Since this exposure scenario is based on assumptions that are somewhat arbitrary and highly variable, it may overestimate exposure. The actual chemical concentrations in the water will vary according to the amount of compound spilled, the size of the water body into which the chemical is spilled, the time at which water consumption occurs relative to the time of the spill, and the amount of contaminated water consumption. To reflect the variability inherent in this exposure scenario, a spill volume of 100 gallons (range of 20-200 gallons) is used to reflect plausible spill events. The concentrations of the pesticide in the field solution are also varied to reflect the likely range of concentrations in field solutions—i.e., the material that might be spilled—using the same values as in the accidental exposure scenarios for workers (Section 3.2.2.2).

3.2.3.4.2. Accidental Direct Spray of or Drift to a Pond or Stream

Scenarios involving direct spray or drift are less severe but more plausible than the accidental spill scenario described in the previous subsection. The concentrations of the pesticide in a small pond (Worksheet 10a) and a small stream (Worksheet D10b) are based on standard estimates of drift adapted from AgDrift and separate estimates of drift may be presented for up to four application methods: aerial, high boom ground broadcast, low boom ground broadcast and backpack applications. As discussed in SERA (2011a), the estimates of drift are based on Tier 1 analyses for aerial and ground broadcast applications using AgDRIFT. Details of the pond scenario are given in Worksheet D10a and details of the stream scenario are given in Worksheet D10b.

AgDRIFT permits very detailed modeling of drift based on the chemical and physical properties of the applied product, the configuration of the aircraft, wind speed, and temperature for aerial applications. The generic estimates used in the Forest Service risk assessment are intended to be conservative, and more refined estimates of drift would be appropriate in any site-specific application.

3.2.3.4.3. GLEAMS Modeling

The Forest Service has developed a program, Gleams-Driver, to estimate expected peak and longer-term pesticide concentrations in surface water. Gleams-Driver serves as a preprocessor and postprocessor for GLEAMS (Knisel and Davis 2000). GLEAMS (Groundwater Loading Effects of Agricultural Management Systems) is a field scale model developed by the USDA/ARS and has been used for many years in Forest Service and other USDA risk assessments. Gleams-Driver offers the option of conducting general exposure assessments using site-specific weather files from Cligen, a climate generator program developed and maintained by the USDA Agricultural Research Service (http://horizon.nserl.purdue .edu/Cligen). Details concerning the use of Gleams-Driver are given in SERA (2010a).

3.2.3.4.3.1. Inputs to Gleams-Driver

The generic site parameters used in the Gleams-Driver runs are summarized in Table 8, and additional details are available in the documentation for Gleams-Driver (SERA 2010a). For each site modeled, simulations were conducted using clay (high runoff, low leaching potential), loam (moderate runoff and leaching potential), and sand (low runoff, high leaching potential) soil textures. Neither GLEAMS nor PRZM/EXAMS (discussed further in Section 3.2.3.4.4) explicitly accommodate buffers. Consequently, Gleams-Driver simulations do not incorporate buffers.

The locations of the generic sites selected for modeling include a total of nine sites, as summarized in Table 9. As discussed in SERA (2010a), these locations are standard sites for the application of Gleams-Driver in Forest Service risk assessments and are intended to represent combinations of precipitation (dry, average, and wet) and temperature (hot, temperate, and cool). For each site, Gleams-Driver is used to simulate 100 applications at a unit application rate of 1 lb/acre, and each of the simulations was followed for a period of about 1½ years after application.

In addition to site parameters and locations, each risk assessment will include a table summarizing the chemical input parameters used in the Gleams-Driver modeling. A sample table of chemical-specific input parameters taken from the SERA (2011c) risk assessment on triclopyr is given in Table 10. The chemical input tables specify each parameter used in the Gleams-Driver modeling and each parameter is accompanied by a note detailing the source of and rationale for selecting the parameter. As necessary, a more detailed discussion of the parameter selection will be given in the text of the risk assessment. Also as necessary, metabolites may be modeled. As illustrated in Table 10, the triclopyr risk assessment included modeling of triclopyr BEE, triclopyr acid, and TCP (3,5,6-trichloro-2-pyridinol), a metabolite of triclopyr.

Gleams-Driver is capable of Monte Carlo simulations (SERA 2010a). In Table 10, some parameters are given as ranges or as central estimates accompanied by ranges. Parameters specified as ranges are normally modeled using a uniform distribution and parameters specified as central estimates with ranges are normally modeled using a triangular distribution.

3.2.3.4.3.2. Output Gleams-Driver

An overview of the output from Gleams-Driver modeling is typically presented in a summary table in the body of the risk assessment which includes additional information on the accidental spill scenario (Section 3.2.3.4.1), the direct spray and drift scenarios for a small pond and small stream (Section 3.2.3.4.2), other modeling efforts (Section 3.2.3.4.4), and any available monitoring data (3.2.3.4.5). An example output table, again taken from SERA's risk assessment on triclopyr (SERA 2011c) is given in Table 11.

In addition to the output summary, an appendix is provided for each of the agents modeled in the risk assessment. Each appendix will contain eight tables as listed below:

- Table 1: Effective Offsite Application Rate (lb/acre)
- Table 2: Concentration in Top 12 Inches of Soil (ppm)
- Table 3: Concentration in Top 60 Inches of Soil (ppm)
- Table 4: Maximum Penetration into Soil Column (inches)
- Table 5: Stream, Maximum Peak Concentration in Surface Water (µg/L or ppb)
- Table 6: Stream, Annual Average Concentration in Surface Water (µg/L or ppb)
- Table 7: Pond, Maximum Peak Concentration in Surface Water (µg/L or ppb)
- Table 8: Pond, Annual Average Concentration in Surface Water (µg/L or ppb)

Each of the above tables will summarize information on the 9 locations modeled with each of the soil textures – i.e., clay, loam, and sand. Each value for a site/soil texture is expressed as the median value with approximate 95% empirical limits. In other words, the two extreme lower and upper values from the 100 simulations at each site are dropped, and the lowest and highest remaining values are used for the lower and upper bound estimates.

3.2.3.4.4. Other Modeling Efforts

Forest Service risk assessments will always discussion other modeling efforts for the pesticide or pesticide metabolites under consideration. Typically, this will involve surface water modeling developed by the U.S. EPA. The U.S. EPA uses different models to estimate

pesticide concentrations in water, according to the specific types of risk assessments. For Tier 1 assessments, the EPA uses GENEEC (Generic Estimated Environmental Concentrations) (GENEEC 2001), a straightforward model that estimates pesticide concentrations in a small pond. For more refined risk assessments, the EPA uses PRZM/EXAMS (Burns 2006). PRZM (Pesticide Root Zone Model) is another root zone model which was developed and is used by the EPA. EXAMS (Exposure Analysis Modeling System) is essentially a post-processor for PRZM (just as Gleams-Driver is a post-processor for GLEAMS) that uses output from PRZM to estimate pesticide concentrations in surface water. Because of the crop-specific focus of EPA risk assessments, the EPA typically conducts PRZM/EXAMS runs for different combinations of crop and location. For human health risk assessments, the EPA uses an Index Reservoir rather than a farm pond. The index reservoir is 13 acres in surface area with a depth of 9 feet and a drainage area of 427 acres.

3.2.3.4.5. Monitoring Data

If relevant monitoring data are available, these data are discussed in this section and are used to evaluate the modeled estimates. In this context, relevant monitoring data include any studies that report concentrations in a stream or pond at known intervals after a defined application of the compound. To the extent that meteorological or other site-specific data are available, these data may be used to more fully evaluate the modeled estimates. If the modeling data and monitoring data are not concordant, the exposure estimates used in the risk assessment may be based on either modeled values or monitoring data, as discussed further in Section 3.2.3.4.6.

3.2.3.4.6. Concentrations in Water Used for Risk Assessment

This section of the risk assessment will specify the concentrations of the pesticide in water that are used in the risk assessment. This discussion will typically focus on a summary table that gives Water Contamination Rates (WCRs) – i.e., the concentration of the compound in water in units of mg/L normalized for an application rate of 1 lb/acre. An example summary table, again taken from the SERA (2011c) risk assessment on triclopyr is given in Table 12. The summary table will specify WCR values for the pesticide specified as acid equivalents (a.e.) for weak acids or active ingredient (a.i.) for other compounds. In addition, the table may include separate WCR values for different application methods as well as WCR values for any metabolites that must be considered quantitatively.

The selection of specific WCR values may be based on modeling, either Gleams-Driver or PRZM/EXAMS, estimates of concentrations associated with drift, monitoring data, or a combination of these estimates. This section will discuss the rationale for the values that are used in as much details as necessary.

The WCR values are entered in Worksheet B04Rt in each of the EXCEL workbooks that accompany the risk assessment. Worksheet B04Rt is the only worksheet that uses an exposure rate, in this case the mg/L in surface water per lb a.i./acre or lb a.e./acre (for weak acid pesticides) that is applied. If this worksheet is present, it will precede the other B04 worksheets and will be used in Worksheets B04a to calculate expected environmental concentrations based on the application rate specified in Worksheet A01. In other cases, such as aquatic applications, Gleams-Driver or other comparable models are not used to estimate concentrations in surface water and a different worksheet template will be used for

Worksheet B04a. In all workbooks, however, B04a will contain the expected concentrations of the pesticide in surface water regardless of the method that is used to calculate these concentrations.

3.2.3.5. Oral Exposure from Contaminated Fish

Many chemicals may be concentrated or partitioned from water into the tissues of animals or plants in the water. This process is referred to as bioconcentration. The concentration of the pesticide in fish (C_F) is taken as the product of the concentration of the chemical in water (C_W) and the bioconcentration factor (BCF):

$$C_{Fish_{mg/Kg}} = C_{W\,mg/L} \times BCF_{L/kg} \qquad \text{(Eq. 16)}$$

Bioconcentration is measured as the ratio of the concentration in the organism to the concentration in the water. For example, if the concentration in the organism is 5 mg/kg and the concentration in the water is 1 mg/L, the BCF is 5 L/kg [5 mg/kg ÷ 1 mg/L]. As with most absorption processes, bioconcentration depends initially on the duration of exposure but eventually reaches steady state. Details regarding the relationship of bioconcentration factor to standard pharmacokinetic principles are provided in Calabrese and Baldwin (1993).

The potential for accumulation of a pesticide or other chemical in fish is typically studied in bluegill sunfish, trout, minnows, or occasionally carp. The U.S. EPA/OPP has a general protocol for this type of study, OPPTS 850.1730. The fish are typically exposed to at least two concentrations of the radiolabelled (e.g., ^{14}C) chemical in water for periods of 28 to 60 days. At steady state, the bioconcentration factor (BCFs) is calculated as the ratio of the concentration in the fish (C_F) to that in the water (C_W). For most water soluble chemicals, steady state will typically be reached during the 28 to 60 day exposure period. If this is not the case, kinetic analyses may be used to calculate the BCF.

When data are available, two sets of BCF values are given in each risk assessment, one for acute (24 hour) exposures and the other for longer-term (steady-state) exposures. For many water soluble chemicals, these two estimates will not differ substantially. For highly lipophilic compounds – i.e., compounds that will partition into fat and other lipids – the values will differ substantially. In addition, separate BCF values are typically given for whole fish and the edible portion of fish.

Risk assessments will include three sets of exposure scenarios for the consumption of contaminated fish, and each set includes separate estimates for the general population and subsistence populations. These exposure scenarios consist of one set for acute exposures following an accidental spill (Worksheets D08a and D08b), another set for acute exposures based on expected peak concentrations (Worksheets D09c and D09d), and the third set for chronic exposures based on estimates of longer-term concentrations in water (Worksheets D09a and D09b). The two worksheets in each of these three sets are intended to account for different rates of wild-caught fish consumption in both general and subsistence populations.

3.2.3.6. Dermal Exposure from Swimming in Contaminated Water

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

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

In Forest Service risk assessments, the ingestion of water during swimming is not considered explicitly. U.S. EPA/OPP (2003) uses a model for swimming exposures based on essentially the same approach to dermal absorption used in Worksheet D11. The EPA model, however, incorporates the assumption that an adult will consume water while swimming at a rate of 50 mL/hour. This assumption is based on data from ingestion rates in swimming pools. Based on more recent studies of water ingestion while swimming in pools (Dorevitch et al. 2010; Dufour et al. 2006), the EPA assumption of 50 mL/hour is a plausible upper bound.

3.2.3.7. Oral Exposure from Contaminated Vegetation

Applications of triclopyr associated with Forest Service programs will not involve crop treatment. Under normal circumstances and in most types of applications, it is extremely unlikely that humans will consume substantial amounts of vegetation contaminated with triclopyr. Nonetheless, any number of accidental or incidental scenarios could be developed involving either spraying of crops, gardens, or edible wild vegetation. Again, in most instances and particularly for longer-term scenarios following herbicide treatment, treated vegetation would probably show signs of damage, thereby reducing the likelihood of consumption which might lead to significant levels of human exposure.

Notwithstanding the above reservations, all forest service risk assessments involving foliar applications currently include two sets of standard exposure scenarios: one for the consumption of contaminated fruit and the other for the consumption of contaminated broadleaf vegetation. These scenarios are detailed in EXCEL workbooks for foliar applications in Worksheets D03a (fruit) and D03b (broadleaf vegetation) for acute exposure and Worksheets D04a (fruit) and D04b (broadleaf vegetation) for longer-term exposure. This is a change in procedure from the previous Forest Service risk assessments which considered only exposure scenarios for the consumption of contaminated fruit (SERA 2007a).

The pesticide concentration on contaminated fruit and vegetation is typically estimated using the empirical relationships between application rate and concentration on different types of vegetation (Fletcher et al. 1994). The rates provided by Fletcher et al. (1994) are based on a reanalysis of data originally compiled by Hoerger and Kenaga (1972) and represent estimates of pesticide concentration in different types of vegetation (mg chemical/kg vegetation) after a normalized application rate of 1 lb/acre. Although the human health risk assessments conducted by the EPA do not consider this exposure scenario, the residue rates recommended by Fletcher et al. (1994) are typically used by U.S. EPA/OPP in ecological risk assessments (U.S. EPA/OPPTS, 2004, p. 59; U.S. EPA/EFED 2001, p. 44).

The residue rates recommended by Fletcher et al. (1994) are given in the upper portion of Table 13. Fletcher et al. (1994) and Hoerger and Kenaga (1972) provide only central and upper bound estimates of residue rates. Accordingly, the lower bound estimates in Table 13 are made under the assumption that the ratio of the central estimate to the upper bound estimate is identical to the ratio of the lower bound estimate to the central estimate (i.e., the variability is log-symmetrical).

The residue rates developed by Fletcher et al. (1994) are applicable only to applications of liquid formulations. For granular applications, no systematic analyses of residues on vegetation have been identified. The Forest Service risk assessment on hexazinone (SERA 2005) reviewed a study on an application of a granular formulation of hexazinone (Michael 1992). In this study, the residue rates on vegetation were a factor of about 25 below residues following a liquid application. In the absence of other data, this relationship is used to estimate concentrations of pesticides on vegetation following granular applications. The specific residue rates are given in the bottom portion of Table 13.

For the acute exposure scenario involving only a single application, the estimated residue level is taken as the product of the application rate and the residue rate from Table 13. For multiple applications, the peak concentration on fruit or other vegetation will occur immediately after the last application. This concentration can be calculated based on the initial concentration after the first application ($\boldsymbol{C_0}$), the number of applications ($\boldsymbol{n}$), and the first-order decay coefficient ($\boldsymbol{k}$), which can be calculated from the half-life ($\boldsymbol{t_{50}}$) [$\boldsymbol{k}$=ln(2) ÷ $\boldsymbol{t_{50}}$]. Assuming a first-order decrease in concentrations in contaminated vegetation, the concentration in the vegetation at time t after the first application (Ct), can be calculated as:

$$C_t = C_0\, e^{-k_e t} \qquad \text{(Eq. 17)}$$

Using the plateau principle (Section 3.1.3.3), defining t* as the interval between applications, and defining e^{-kt^*} as $\boldsymbol{p}$ to simplify notation, the concentration immediately after the nth application ($\boldsymbol{C_n}$) can be calculated as:

$$C_n = \frac{C_0\,(1-p^n)}{1-p} \qquad \text{(Eq. 18)}$$

This algorithm is used to calculate the maximum concentration on vegetation after multiple applications at the specified interval.

For the longer-term exposure scenarios, a duration of 90 days is typically used. Although the duration of exposure of 90 days is somewhat arbitrary, this duration is intended to represent the consumption of contaminated fruit that might be available over one season. Longer durations could be used for certain kinds of vegetation but doing so would lower the estimated dose (i.e., would reduce the estimate of risk).

Estimates of halftimes on vegetation can come from either field studies or greenhouse studies and may be highly variable. Substantial variability is not uncommon in field measurements of halftimes of vegetation, which are substantially impacted by site and situational differences such as rainfall, temperature, wind velocity, and vegetation type.

For the longer-term exposure scenarios, the time-weighted average concentration on vegetation or fruit is calculated from the equation for first-order dissipation. For a single application, the time-weighted average concentration (***C_{TWA}***) over time ***t*** can be calculated as the integral of Equation 16 divided by the duration (***t***):

$$C_{TWA} = \frac{C_0\,(1 - e^{-k\,t})}{k\,t} \qquad \text{(Eq. 19)}$$

This equation is used to estimate the time-weighted average concentration on vegetation after a single application (e.g., De Sapio 1976, p. 281 ff).

The calculation of the time-weighted average following multiple applications is conceptually similar but much more complex mathematically. A detailed derivation for these calculations for maximum time-weighted average concentrations associated with multiple applications is given in Appendix 1 of the current document.

3.3. DOSE-RESPONSE ASSESSMENT

3.3.1. Overview

The purpose of the dose-response assessment is to describe the degree or severity of risk as a function of dose. Most dose-response assessments used in Forest Service risk assessments are in the form of RfDs or Reference Doses. These are point estimates (single numbers rather than ranges) of doses that are not believed to be associated with any adverse effect and that are not directly related to a dose-response model. The practice of relying on point estimates in regulatory toxicology is grounded in the history of this discipline (Dourson and Stara 1983). From its inception, the focus of regulatory toxicology has been the development of criteria (i.e., levels of exposure) that are defined as *safe*. Consequently, the methods used in regulatory toxicology are conservative. Consistent with the recommendation of NRC (1983) that various groups within the federal government adopt common risk assessment methodologies, standard dose-response assessments are generally based on reference values, like RfDs, derived by other government agencies, most commonly the U.S. EPA. This approach avoids a duplication of effort, capitalizes on the expertise of other organizations, and decreases the size, complexity, and cost of risk assessments.

In classical toxicology, dose-response assessments are usually expressed as linear or non-linear equations such as probit analysis and the multistage model, respectively. Using these methods, the prevalence or magnitude of a response can be estimated for any dose level. This kind of approach is being used more in risk assessments as a method of more fully using the available data, as well as a method of incorporating estimates of variability. The most commonly used method involves the calculation of benchmark doses which may be used as a surrogate for NOAEL values. This sort of method is also used in many cancer risk assessments. For example, U.S. EPA cancer risk assessments usually employ a form of the multistage model or some other linear dose-response relationship that provide measures of variability or error.

In cases for which these standard approaches yield evidence of potential risk, attempts are made to characterize the types of effects that might be seen. This type of analysis is usually qualitative and involves a description of the nature and severity of responses that might be seen at different levels of exposure.

3.3.2. Chronic RfD

Chronic RfDs are used to characterize risks associated with chronic or longer-term exposures. These values are typically based on NOAEL values from chronic or subchronic toxicity studies (Section 3.1.5) or on multigeneration reproductive studies (Section 3.1.9.2). The selection of the specific NOAEL value depends on which endpoint appears to be the most sensitive – i.e., the NOAEL associated with the LOAEL; the lowest observed adverse effect level is identified. This effect is viewed as the *critical effect*—i.e., if this effect is prevented, no other adverse effects are anticipated. The critical effect may be defined by any systemic toxic effect or any effect on reproduction. If there is a NOAEL associated with the LOAEL (a NOAEL from the same study), then this NOAEL is used as the basis for the RfD. If no NOAEL is available, the LOAEL may be used with an additional uncertainty factor as discussed below.

Chronic RfD values are intended to estimate dose levels associated with a negligible or at least defined level of risk over a lifetime of exposure. *RfD* is a term used by U.S. EPA to designate a *Reference Dose* for use in risk characterization and most RfD values used in Forest Service risk assessments are those that are derived by U.S. EPA. Occasionally, RfD values are derived by other organizations or RfD values are derived in the risk assessment itself. These situations arise primarily in cases in which the U.S. EPA has not derived an RfD. To avoid confusion, any RfD value that is not derived by the U.S. EPA is generally referred to as a *surrogate RfD* in a Forest Service risk assessment. Specific examples of these types of dose-response assessments, including RfDs and similar values, conducted by various governmental organizations, are provided in Table 14.

The risk assessment may use acute or chronic RfDs. The definitions of acute, subchronic, and chronic exposure are vague, and to some extent, chemical specific, as discussed in Section 3.1.3. If 1-day, 10-day, or longer-term health advisories (HAs) are available (see Table 14), these values may be used to derive acute or subchronic RfDs. Acute RfD values are conceptually similar to chronic RfD values and the major difference is in the type of study used to derive an acute RfD. This is discussed further in Section 3.3.3. RfD values are used primarily for non-carcinogenic chemicals. Quantitative dose-response assessments for carcinogenicity are discussed in Section 3.3.5.

Non-carcinogenic effects are assumed to have population thresholds (i.e., levels below which no adverse effects are expected for a given exposure route and duration). RfDs for non-carcinogenic effects are intended to be estimates of exposure levels at or below the threshold level. The basic equation for deriving an RfD is very simple and can be expressed as:

$$RfD = \frac{TV}{UF} \qquad \text{(Eq. 20)}$$

where TV is an experimental toxicity value such as an NOAEL or LOAEL and UF is the uncertainty factor. Although the computations are simple, the toxicological judgments involved in deriving a reference value may be complex. To derive the RfD, the experimental threshold or NOAEL is divided by the product of a series of uncertainty factors intended to account for differences between the experimental exposure and the conditions for which the reference value is derived. The uncertainty factors used by the U.S. EPA and ATSDR are presented in Table 15.

For oral RfDs, units of dose usually are expressed as mg/kg/day. For inhalation exposures, the term RfC (Reference Concentration) is typically used and the value is expressed as mg/m^3. Data on certain species may be censored from the analysis because they are atypical and do not serve as good animal models for effects in humans. An attempt is then made to determine the most sensitive toxicological endpoint. Usually, this is accomplished by identifying a toxicologically relevant series of effects that increase in severity as dose increases.

Generally, the risk assessment will use U.S. EPA RfDs as the toxicity value for risk characterization. U.S. EPA RfDs generally provide a level of analysis, review, and resources that far exceed those that are or can be conducted in support of most Forest Service risk assessments. In addition, it is desirable for different agencies and organizations within the federal government to use concordant risk assessment values.

Nonetheless, there are cases in which different RfDs for the same chemical are derived within the U.S. EPA and other cases in which the nature of the available data suggest the need to use alternative values to capture endpoint-specific toxicities, dose-duration relationships, or dose-severity relationships as adequately as possible. Lastly, there may be cases where new data are available – i.e., data not considered by or available to the U.S. EPA when the RfD was derived. Sometimes, the alternative values are less conservative; at other times, the alternative values are more conservative. In either case, the purpose of deviating from the U.S. EPA RfDs is to characterize risk as clearly and thoroughly as possible.

Typically, inhalation exposures to pesticides used by the Forest Service are marginal and no explicit dose-response assessment is provided for inhalation exposures. When inhalation exposures are explicitly considered, RfD values derived by U.S. EPA are used if available. As an alternative, Threshold Limit Values (TLVs) derived by American Conference of Governmental Industrial Hygienists may serve as the basis for inhalation RfDs. These may be adopted without modification as inhalation RfDs for occupational exposure. For exposure scenarios involving the general public, inhalation RfCs may be adopted without modification as inhalation RfDs for chronic exposure. When RfCs are not available, the TLV may be modified to account for the duration of daily exposure and sensitive subgroups within the general population. TLVs are designed to protect workers in occupational exposure settings during the work day (8 hours/day). Inhalation RfDs for the general public must be protective for the full 24-hour day. Consequently, the TLV will be reduced by one third (8 hours/24 hours) when applied to the general public. This adjustment is made with the assumption that exposures are equitoxic as long as the product of concentration and duration is constant (e.g., $c_1 d_1 = c_2 d_2$). This is an expression of Haber's law (Kennedy 1989) which is a reasonable approximation over limited ranges of concentration and duration. TLVs do not explicitly consider sensitive subgroups; the TLV will be adjusted for continuous exposure and further decreased by a factor of 10, according to U.S. EPA procedure, to account for sensitive subgroups.

3.3.3. Acute RfD

Acute RfDs are conceptually similar to chronic RfDs. They are intended to represent levels over exposure that will not be associated with adverse effects in any member of the exposed population. As used in Forest Service risk assessments, acute RfD values are used to characterize risks associated with exposures lasting no longer than one day.

Acute RfD values for pesticides are relatively new. While the Office of Drinking Water has been deriving 1-day health advisories for many years (U.S. EPA/ODW 1990), the Office of Pesticides started using acute NOAEL values with recommended Margins of Exposure in the reregistration of pesticides and the development of pesticide tolerances under the Food Quality Protection Act (FQPA). In Forest Service risk assessments, a surrogate acute RfD is typically derived by taking the NOAEL identified by the U.S. EPA/OPP and dividing the

NOAEL by the recommended Margin of Exposure. Most often, the acute NOAEL is based on a developmental study. As noted in Section 3.1.9.1, these studies often involve exposures during gestation. This type of study is appropriate as the basis for an acute RfD under the assumption that any adverse developmental effect could have occurred as the result of a brief period of time – i.e., following a single dose – during development.

For some chemicals, the dose-duration relationship may be very weak; there may appear to be no substantial difference in the acute and longer-term or chronic toxicity of the chemical. As discussed in Section 3.1.3.3., this may occur for compounds that are rapidly eliminated and hence reach steady-state in a very brief period of time. For such compounds, no acute RfD is typically used and the consequences of acute exposures are assessed with the chronic RfD (Section 3.4).

3.3.4. Dose-Severity Relationships

For risk assessments in which the upper ranges of plausible exposures are below a level of concern (Section 3.4), only RfDs and comparable values are derived in the risk assessment. This approach is taken in order to make the risk assessments as simple as possible while maintaining an adequate expression of risk. If very conservative exposure assessments and very conservative dose-response assessments lead to no plausible basis for asserting that risks are likely, then no elaboration of the dose-response assessment is needed.

In some cases, however, some risks may be apparent and some attempt is made to further characterize the nature and severity of these risks based on the available dose-severity data. If data are sparse, this may involve little more than comparing the anticipated levels of exposure to LOAELs as well as to NOAELs. Semi-quantitative methods do not involve the use of an explicit dose-response model. In general, this will involve estimates of both animal doses and estimated human doses that might be associated with different types of adverse effects. The purpose of this type of analysis is to provide dose estimates associated with effects of varying severity which can be used in the risk characterization (Section 3.4).

3.3.5. Cancer Potency

Risk assessments conducted for the Forest Service do not typically include cancer risk assessments because the Forest Service does not typically use any pesticides that are classified as carcinogens. Nonetheless, some pesticides have been classified as carcinogens and other pesticides contain potential carcinogens either as impurities in the technical grade active ingredient or as an impurity in an adjuvant. For example, as discussed in Section 3.1.15, hexachlorobenzene is a contaminant in two herbicides used by the Forest Service, clopyralid and picloram. In such a case, cancer potency factors derived by U.S. EPA are used to estimate cancer risk. Details of the methods used are given by U.S. EPA/RAF (2005).

Because the Forest Service defers to U.S. EPA in both the categorization of a compound as a carcinogen as well as the calculation of carcinogenic potency, the Forest Service risk assessments never directly derive cancer potency factors. These potency factors are often calculated based on the multistage model (Crump and Howe 1984), which is available in U.S. EPA's benchmark dose software (U.S. EPA/OEI 2009). If necessary, these methods

could be employed in Forest Service risk assessments, but this situation has not arisen to date.

3.4. RISK CHARACTERIZATION

Risk characterization is the process of comparing the exposure assessment with the dose-response assessment to express the level of concern regarding a specific exposure scenario or set of scenarios (NRC 1983).

For systemic toxic effects, risk characterizations have been presented typically as either a Margin of Safety (MOS) or a Hazard Quotient (HQ). The Forest Service risk assessments generally use the HQ approach although the two methods are closely related.

A *margin of safety* is simply an experimental exposure level in animals, usually one that is not associated with adverse effects (i.e., *NOEL* or *NOAEL*), divided by an estimate of exposure (E_i):

$$MOS = \frac{NOAEL}{E_i} \qquad \text{(Eq. 21)}$$

Thus, as the exposure level decreases, the margin of safety increases. This approach is typically used in EPA risk assessments, particularly for workers. The EPA will sometimes use the term *Margin of Exposure* (MOE) rather than the term *Margin of Safety* (MOS).

A *hazard quotient* is the ratio of a projected level of exposure (E_i) divided by some index of an acceptable exposure or an exposure associated with a defined risk, such as an RfD. The RfD, in turn, is an experimental exposure level (i.e., *NOEL* or *NOAEL*) divided by an uncertainty factor (*UF*) – i.e., equivalent to the level of exposure divide by the RfD:

$$HQ = \frac{E_i}{NOAEL \div UF} = \frac{E_i}{RfD} \qquad \text{(Eq. 22)}$$

Consequently, as the level of projected human exposure decreases, the hazard quotient decreases.

The obvious and trivial difference between these two methods is that they are inversely related to each other. The significant difference between the *margin of safety* and the *hazard quotient* approach, however, is that the *hazard quotient* method is based on an explicit uncertainty factor, dependent on the quality of the available data. The only time that these two methods will lead to differing interpretations of risk is when the acceptable margin of safety is set to a value other than the uncertainty factor used to derive the RfD, or when the assessments use different NOAELs. Otherwise, the two methods are equivalent.

RfDs are intended to be conservative estimates that incorporate a substantial margin between a dose that does not cause adverse effects and doses that cause adverse effects. This difference is referred to as a *'margin of protection'*. If the margin of protection is substantial, adverse effects may not be observed or even induced when the hazard index is greater than unity (i.e., exposure exceeds the *presumably safe* level). In order to assess the plausibility

and nature of inducing or observing adverse effects, the relationship of exposed dose to the severity of effects is further considered, either qualitatively or quantitatively.

As with the dose-response assessments, the distinction between AELs and FELs is central to characterizing risk. When applied to risk characterizations, however, the distinction between AELs and FELs may be subject to misinterpretation. Some, and perhaps most, of the exposure scenarios derived in a risk assessment may be associated with a low likelihood of an FEL based on the categorical regression analyses. In other words, no overt toxic effects are anticipated. This is not to be interpreted as suggesting that all of the exposure scenarios are acceptable or at least equally acceptable. Hazard indices may be exceeded by a substantial margin and may be in the region in which AELs are plausible. In such cases, humans subject to such exposures would probably be asymptomatic. Nonetheless, such individuals might experience subclinical changes that, if detected, would be regarded as justification for measures to reduce or eliminate the possibility of further exposure.

For carcinogenic effects, the risk associated with a given scenario and a single route of exposure ($\boldsymbol{P_i}$) can be expressed simply as the potency factor ($\boldsymbol{B}$) in units of (mg/kg/day^{-1}) multiplied by the exposure ($\boldsymbol{E}$) in units of mg/kg/day:

$$P_i = B\,D \qquad \text{(Eq. 23)}$$

If more than one route of exposure is associated with a carcinogenic response or more than one source of exposure needed to be considered, the risks from all routes or sources can be added. A major source of uncertainty is introduced when the exposure duration for the scenario is substantially less than lifetime. This uncertainty cannot be quantified, but it is likely to result in underestimating risk if the compound affects early stages of the carcinogenic process.

In addition to these numerical expressions, the risk characterization section, more than any other part of the risk assessment, must explain the conclusions of the risk assessment in plain language. How this is done, specifically, depends largely on the nature of the perceived risk or the apparent lack of risk.

In some cases, a risk assessment may find no objective suggestion of an adverse effect based on the currently available data. In such cases, the risk characterization must clearly make the point that: ***Absolute safety cannot be proven and the absence of risk can never be demonstrated***. No chemical or other pesticide agent is studied for all possible effects and the use of data from laboratory animals to estimate hazard or the lack of hazard to humans of other species is an uncertain process. Thus, prudence dictates that normal and reasonable care should be taken in the handling of any pesticide. In other instances, risks may be apparent and these risks must be clearly stated both qualitatively and (if possible) quantitatively.

The risk characterization will also include a discussion of *biologically sensitive* subpopulations. Biological sensitivity here refers to a group of individuals or a subpopulation which for reasons of developmental stage or some other biological condition,

are significantly more susceptible than the general population to a compound. Issues regarding individuals at greater risk because of increased exposure are addressed in the exposure assessment (Section 3.2).

Biologically sensitive subpopulations do not include individuals at the extreme lower end of a normal (unimodal) distribution of tolerances. Conceptually, the welfare of those individuals should be incorporated into a unimodal model in the dose-response assessment. Failure to differentiate among *biologically sensitive* subpopulations, sensitive individuals in a unimodal population, and individuals at increased risk due to high levels of exposure may add a substantial amount of confusion to the risk assessment.

Some individuals may be atypically sensitive to chemical exposure because of their age (e.g., Calabrese1986). Frequently, the very young or the very old are especially susceptible to the toxic effects of chemicals. Thus, everyone will belong to a high risk group at one or more times during their life span. Genetic factors, in contrast to developmental and aging factors, affect smaller subsections of the population. Some genetic conditions thought to predispose or enhance susceptibility to toxicants and the associated type of toxicant include cholinesterase variants (insecticides, cystic fibrosis), ozone and respiratory irritants, cystinosis and cystinuria (metals), glucose-6-phosphatase dehydrogenase deficiency (carbon monoxide), glutathione, glutathione peroxidase, and glutathione reductase deficiencies (lead and ozone), immunoglobulin A deficiency (respiratory irritants), immunological hypersensitivity (isocyanates), porphyrias (hexachlorobenzene, lead, barbiturates), sickle cell trait (aromatic amino and nitro compounds), and sickle cell anemia (carbon monoxide, cyanide). Furthermore, any genetic deficiency that results in altered xenobiotic metabolism may enhance susceptibility to chemicals.

Pre-existing disease states make individuals more susceptible to toxic chemicals. People with liver disease are less able than others to metabolize and detoxify foreign chemicals, and people with kidney disease are less able than others to excrete toxic chemicals. Carbon tetrachloride, PCBs, DDT, and other pesticides are among the chemicals that many people with liver disease may find more difficult to tolerate. People with kidney disease are especially sensitive to the effects of lead and other heavy metals. Asthma, chronic respiratory disease and heart disease predispose individuals to respiratory irritants, such as, nitrogen dioxide, ozone, sulfates, sulfur dioxide and carbon monoxide.

Behavioral and life style factors, such as smoking and alcohol and drug use not only increase an individual's exposure to toxic chemicals, but also increase the individual's susceptibility to other chemicals. Cigarette smoke itself contains a variety of carcinogens and other toxic chemicals. The chemicals in cigarette smoke may potentiate the toxicity of other pollutants. Alcohol and drug use enhance susceptibility to PCBs and pesticides by altering metabolizing enzyme systems in the liver. Dietary habits also may influence the toxicity of chemicals by producing changes in physiological and biochemical functions and nutritional status. Obese individuals may have atypical sensitivities to some chemicals, particularly chemicals that sequestered in fatty tissue.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview

This section of the risk assessment provides an overview of the available studies on the effects of the chemical to wildlife species. Depending on the nature of the available data, subsections for terrestrial species typically include mammals, birds, terrestrial invertebrates, terrestrial plants (macrophytes), and soil microorganisms. If justified by the amount and type of data, additional sub-groupings may be used. For aquatic species, subsections are typically provided on fish, amphibians, aquatic invertebrates, and aquatic plants (both macrophytes and algae). The hazard identification for wildlife mammals is usually based on the same information considered in the human health risk assessment, and this information is typically much more detailed than the information available on other groups because studies are often available on both lethal and sublethal effects. Data on the other groups is typically much less detailed. While information on sublethal effects is often available for some groups, much of the information consists of acute bioassays for lethality. This reflects a major conceptual difference between human health and ecological risk assessment. Human health risk assessment focuses on preventing the occurrence of any effect in any individual. Ecological risk assessment tends to focus on preventing adverse effects at the population level.

4.1.2. Terrestrial Organisms

4.1.2.1. Mammals

The hazard identification for terrestrial mammals is typically based on the discussion of the toxicity of the chemical to mammals that is given in the human health risk assessment (Section 3.1). There are two major additional considerations that may be discussed in this section: toxicity to canids (species related to dogs) and inferences on the toxicity of the chemical based on field studies.

Many of the pesticides used by the Forest Service, particularly the herbicides, are weak acids. Weak acids are often removed from the blood by the kidney, with eventual secretion in the urine. Part of this process involves active transport from the blood into kidney cells (e.g., Durkin et al. 2004). This active transport process in dogs is much less active than the active transport process in primates and other mammals (e.g., Timchalk and Nolan 1997). Consequently, dogs are less able to eliminate weak acids and may be substantially more sensitive to weak acids than other mammals. Thus, in risk assessments on weak acids, this section will emphasize any available information on the pharmacokinetics or toxicity of the compound in dogs relative to other mammalian species. If dogs appear to be more sensitive than other mammals, this may be considered further in the dose-response assessment (Section 4.3) and separate NOAEL or NOEC values may be derived for dogs and other canids. These values may then be used to characterize risks for other canid species that may be covered in the risk assessment – e.g., the consumption of a small mammal by a predator such as a coyote or wolf.

A consideration of field studies involving observations on terrestrial mammals may also be included in this section. While field studies involving mammals may be useful in the

ecological risk assessment, they are not typically considered or discussed in detail in the human health risk assessment. Field studies generally involve the application of a pesticide to a defined area and subsequent observations on wildlife populations. In this respect, field studies may be viewed as analogous to epidemiology studies that may be used in the human health risk assessment (Section 3.1.5.5). Like epidemiology studies, field studies may be difficult to interpret because the control site (an untreated area) may differ from the treated site. An additional complication with field studies is that some observed effects (e.g., changes in the composition of a population of mammals) may be due to secondary effects, such as changes in vegetation cover or changes in the availability of suitable prey species, rather than direct toxic effects. This is a common problem in the use of field studies for mammals as well as other groups, both terrestrial and aquatic, that are considered in the ecological risk assessment.

Other than considerations of canids and field studies, this section may emphasize some types of toxicity studies that are not used substantially in the human health risk assessment. The U.S. EPA/OPPTS (2011) does have a test guideline for acute dietary toxicity in wild mammals (OPPTS 850-2400). This type of bioassay is not typically required for herbicides but might be required for some other pesticides, particularly rodenticides. Unlike many of the other test guidelines given by U.S. EPA/OPPTS (2011), the OPPTS 850-2400 guideline does not recommend or require specific protocols or test species. A similar type of study is often available for rats and mice as part of standard subchronic or chronic toxicity studies (e.g., OPPTS 870.4100). In the design of these studies, shorter term dietary or drinking water range-finding studies, typically lasting 2 weeks, may be provided and may be used in assessing the consequences of acute dietary exposures.

As in the human health risk assessment, the results of various types of acute toxicity bioassays may be used to classify chemicals into various levels of toxicity – e.g., highly toxic to virtually nontoxic. The classification system currently used by the Environmental Fate and Effects Division (EFED) of U.S. EPA/OPP is summarized in Table 16. As with the corresponding classification scheme for human health effects (Table 4), Table 16 is only used in the hazard identification to categorize the pesticide and is not directly used in the risk characterization. As discussed in Section 1.2.1, all quantitative risk characterizations in Forest Service risk assessments are based on both a quantitative exposure assessment and quantitative dose-response assessment.

4.1.2.2. Birds

Information on the toxicity of pesticides to birds is typically much more limited than that for experimental mammals. While some toxicity studies on birds may be available in the open literature, most of the information is usually from studies required specifically by the U.S. EPA/OPPTS (2011) for the registration of pesticides: OPPTS 850-2100, avian acute oral toxicity; OPPTS 850-2200, avian acute dietary toxicity; and OPPTS 850-2300, avian reproductive toxicity.

The acute studies, both oral and dietary, most commonly involve tests on mallard ducks (*Anas platyrhynchos*) and northern bobwhite quail (*Colinus virginianus*). The acute oral study involves administration of the chemical either by gavage or capsule. Full studies will use at least five dose levels, and the specific doses are selected based on the results of range-

finding studies which typically use doses of 2, 20, 200, and 2,000 mg/kg bw. The birds receive a single dose and are observed for 14 days, although this period can be extended to 21-days if mortality is seen in the last 3 days of the normal 14-day observation period. Observations made during these studies include mortality, food consumption, changes in body weight, signs of toxicity and gross examination of tissues after the animals are sacrificed. Histological examinations of tissue are usually not conducted. As with the mammalian oral study (Section 3.1.4), a limit test may be conducted at a single dose of 2,000 mg/kg. If no mortality occurs, the LD_{50} value may be expressed as >2,000 mg/kg and no additional testing is required. As with the mammalian studies, the risk assessment will distinguish this type of information from studies in which some but less than 50% mortality occurred at the maximum dose.

The avian acute dietary toxicity study, OPPTS 850-2200, is similar to the acute oral study in general design and test species. Occasionally, however, other species may be used such as pigeon (*Columba livia*), Japanese quail (*Coturnix coturnix japonica*), ring-necked pheasant (*Phasianus colchicus*), and red-legged partridge (*Alectoris rufa*). The chemical is administered in the standard diet (laboratory chow) for a period of 5-days, and the birds are observed for an additional 3-day period. This test is sometimes referred to as a 5-day dietary or 8-dietary study, which can lead to some confusion if the duration of exposure is not clearly distinguished from the duration of observation. As with the acute oral study, the duration of observation can be increased up to 21 days if signs of toxicity are noted during the standard 3-day post-exposure observation period. As discussed further in Section 4.3, either the acute oral study or acute dietary study will often serve as the basis for an acute NOAEL or NOEC that is used in the dose-response assessment for birds.

Chronic studies in birds analogous to those conducted in mammals – i.e., studies that span a full or significant fraction of the life span of the animal – are almost never available. Typically, the consequences of longer-term exposure scenarios for birds are evaluated using the avian reproductive toxicity study, OPPTS 850-2300. These studies are generally conducted on mallard ducks or bobwhite quail. The compound is administered in the diet, and usually 3 concentrations plus a control group are used. The initial period of exposure is typically 6 to 8 weeks (42 to 56 days). After this period, egg laying is induced by manipulating the photoperiod over a 2 to 3 week period. After egg laying begins, the study is continued for an additional 8 to 10 weeks. During all three periods, dietary exposure is maintained and thus the total period of exposure is 16 weeks (112 days) to 21 weeks (147 days). If adverse effects are noted, a withdrawal period of up to 3 weeks may be incorporated into the study. Observations on adult birds include those normally done in the acute studies – food consumption, body weight changes, and signs of toxicity. Egg production, egg hatching, and the viability of offspring are also assayed. As in the acute studies, gross pathological examinations are conducted on all birds but histopathology (microscopic examination of tissue) is not typically done.

Other data on birds may include field studies. If such data are available and are relevant to the hazard identification, the data are detailed in this section. The limitations on the use of field data are generally similar to those encountered in the use of field data on experimental mammals (Section 4.1.2.3).

4.1.2.3. Reptiles and Amphibians (Terrestrial-Phase)

Data on terrestrial phase amphibians and reptiles are typically sparse. Much of the available data up to the late 1990s is summarized in the database on amphibian and reptile toxicity data maintained by the Canadian National Wildlife Research Centre (Pauli et al. 2000). This reference is always consulted in the preparation of Forest Service risk assessments. When data are available, the studies are assessed in a manner similar to that used for mammals and birds.

4.1.2.4. Terrestrial Invertebrates

There is substantial variability in the types of information that are available on terrestrial invertebrates. For herbicides, the type of pesticide on which most Forest Service risk assessments have been conducted, only relatively simple and standard bioassays may be available: the honey bee acute contact toxicity (OPPTS 850.3020), the honey bee toxicity of residues on foliage (OPPTS 850.3030), and the earthworm subchronic toxicity test (OPPTS 850.6200).

The acute contact toxicity study in honey bees is often the only kind of invertebrate toxicity study available on herbicides. This acute study is similar in design to acute toxicity studies conducted on mammals and birds but involves direct application, either by micro-applicator (topical drop) or whole body exposure. For herbicides and other compounds that are generally thought to be nontoxic to invertebrates, only a limit test may be conducted and this is typically done at a dose of 25 micrograms per bee. If mortality is observed in the limit test, then standard range-finding and full studies may be conducted.

The earthworm toxicity test (OPPTS 850.6200) involves exposing a species of earthworm, typically *Eisenia fetida*, to various concentrations of the test compound in soil for a period of 28-days. The use of limit tests is not discussed in the OPPTS protocol. Range-finding studies are conducted as 0.1, 1.0, 10, 100, 1,000 mg/kg dry weight artificial soil. This type of test is not consistently required for the registration of pesticides, particularly herbicides, and is typically a greater concern in the potential effects of industrial chemicals that may be disposed of in landfills.

Earthworms and honeybees comprise only a very small fraction of the terrestrial invertebrates that may be found in any habitat. For insecticides, there are typically a very large number of additional laboratory toxicity studies as well as field studies in both target and nontarget invertebrate species, and the summary of effects and the hazard identification for terrestrial invertebrates will be much more elaborate.

4.1.2.5. Terrestrial Plants (Macrophytes)

The available data on terrestrial plants is virtually the mirror image of the available data on terrestrial invertebrates in terms of the relative amount and complexity of the information for herbicides and insecticides (as well as other biocides such as rodenticides). For insecticides and other biocides, virtually no information may be available and there may be no plausible basis for anticipating any adverse effects in plants. For some non-herbicidal pesticides that are applied directly to plants, Tier I studies may be required by the U.S. EPA for both seedling emergence (OPPTS 850.4100) and vegetative vigor (OPPTS 850.4150).

Studies on seedling emergence and vegetative vigor are the two basic types of bioassays that are covered and used in Forest Service risk assessments. Seedling emergence studies typically involve soil exposure and vegetative vigor studies typically involve direct spray. The former are used to characterize risk associated with soil contamination by runoff, and the latter are used to characterize risks associated with direct spray or spray drift.

Seedling emergence studies, as the name implies, involves the treatment of seeds with the chemical. The test is conducted by applying the chemical directly to the soil or directly to seeds and then observing whether or not germination occurs. In the Tier 1 study, three required species are corn, soybeans, and a root crop as well as seven other species, usually tomato, cucumber, lettuce, cabbage, oat, ryegrass, and onion. The test requires six species of at least four families of dicots – e.g., herbaceous plants – and four species of at least two families of monocots – e.g., grasses. The Tier II assay (OPPTS 850.4225) requires at least 10 species of plants. A required dicot species is soybean (*Glycine max*) and a required monocot is corn (*Zea mays*). At least one other dicot must be a root crop such as carrot (*Ducas carrotta*), onion (*Allium cepa*), beet (*Beta vulgaris*), or sugar beet (*Beta vulgaris*). Other species which are commonly included in the test include tomato (*Lycopersicon esculentum*), cucumber (*Cucumis sativa*), lettuce (*Lactuca sativa*), cabbage (*Brassica oleracea*), oat (*Avena sativa*), and perennial ryegrass (*Lolium perenne*). The duration of the bioassay is typically about 14 days during which time the number of emerged seedlings, shoot heights, and signs of visual phytotoxicity are recorded as well as other endpoints such as root dry weight. Results are typically reported as NOEC (or EC_5) and LOEC values as well as estimates of the EC_{25} and EC50.

Vegetative vigor studies, either Tier 1 (OPPTS 850.4150) or Tier II (OPPTS 850.4250), involve direct foliar application 2 to 4 weeks after the plants have emerged. The species tested are similar to those used in seedling emergence studies. Also as with the seedling emergence studies, the Tier I vegetative vigor study is analogous to a limit test in which the limit is defined as 3 times the maximum labeled application rate. In the Tier II test, at least 5 application rates are tested and the endpoints reported include measures of growth such as dry shoot weight, dry root weight, shoot height, and visual signs of phytotoxicity, with an observation period of at least 14-days. In the Tier II bioassay, the results are typically reported as in the Tier II seedling emergence study – i.e., the NOEC (or EC_5) and LOEC values as well as estimates of the EC_{25} and EC50.

For herbicides, a large number of field studies as well as other more specialized laboratory bioassays may be available. The field studies have the same general limitations as those discussed in Section 4.1.2.1. Most importantly, any effective herbicide will kill at least some species of plants and this will alter both the sunlight and nutrients available to other competing plants. An attempt is made in the discussion of the risk assessment to distinguish these types of secondary effects from direct toxic effects. In addition, field studies often include studies on the effects of the herbicide on various target species. These types of studies are referred to as efficacy studies and are generally not covered in detail in the risk assessment because the focus of this section of the risk assessment is on nontarget plants.

4.1.2.6. Terrestrial Microorganisms

Studies on the toxicity of pesticides, particularly the herbicides, are commonly available in both the published literature as well as the standard studies that may be submitted to U.S. EPA in support of pesticide registration. The latter group of studies include rhizobium-legume toxicity (OPPTS 850.4600) which focus on soil microorganisms that are associated with roots of plants (i.e., legumes), more general assays of soil microbial communities (OPPTS 850.5100), and soil-core sample microcosm assays (OPPTS 850.2450). The observations made in these microbial assays typically include soil microbial populations (which may or may not be broken down by type of microorganism) as well as various assays of microbial activity such as CO_2 production and NO_3 and NH_3 concentrations. A variety of other endpoints (e.g., rates of formation or decomposition of various compounds such as cellulose, protein or starch) may be reported in the open literature. The soil microcosm assays (OPPTS 850.2450) and similar studies reported in the open literature may also include information on effects on soil invertebrates. If so, these studies are also discussed in Section 4.1.2.3.

The assays on microbial toxicity submitted directly to U.S. EPA for registration involve soil exposures as these are directly relevant to the risk assessment. Many microbial toxicity studies in the open literature involve pure cultures of microorganisms in artificial media such as agar or liquid culture. These types of assays are less directly relevant and are clearly distinguished from soil assays in the risk assessment.

4.1.3. Aquatic Organisms

4.1.3.1. Fish

Three general types of relatively standardized studies may be available on fish: acute toxicity studies (e.g., OPPTS 850-1075); egg-and-fry studies, also referred to as early life-stage studies (e.g., OPPTS 850-1400); and full life cycle studies (e.g., OPPTS 850-1500).

4.1.3.1.1. Acute Toxicity

Freshwater species that are commonly used in acute assays submitted to the U.S. EPA and preferred by the U.S. EPA include rainbow trout (*Oncorhynchus mykiss*) and bluegill sunfish (*Lepomis macrochirus*). A large number of other species may be used, including Atlantic salmon (*Salmo salar*), brook trout (*Salvelinus fontinalis*), channel catfish (*Ictalurus punctatus*), coho salmon (*Oncorhynchus kisutch*), carp (*Cyprinus carpio*), fathead minnow (*Pimephales promelas*), guppy (*Poecilia reticulata*), red killifish (*Oryzias latipes*), threespine stickleback (*Gasterosteus aculeatus*), and zebrafish (*Brachydanio rerio*). A number of saltwater species may also be tested, including Atlantic silverside (*Menidia menidia*), tidewater silverside (*Menidia penisulae*), and sheepshead minnow (*Cyprinodon variegatus*). Typically, Forest Service applications will not involve applications near salt water and exposure assessments for saltwater species are not generally conducted. Information on saltwater species, however, is included and these species may be used to identify the most sensitive species for the dose-response assessment (Section 4.3).

The design of the acute toxicity bioassays is similar to the design of other acute toxicity bioassays. Range-finding studies as well as limit assays may be used. The common limit concentration is 1000 mg/L – if less than half of the fish die at a concentration of 1000 mg/L,

further testing may not be required and the LC_{50} value may be reported as >1000 mg/L. Like all similar values, the risk assessment will distinguish such values in terms of the percent mortality observed. A full study generally involves at least five concentrations with 14 to 20 fish per concentration. As with other acute studies, partial mortality is needed in at least two concentrations in order to perform the probit analysis. LC_{50} (and sometimes other response rates) values are typically reported for 24, 48, and 96 hours, and differences between these values – progressively lower LC_{50} values as time increases – may indicate either accumulation or cumulative damage (Section 3.1.3). In Forest Service risk assessments, NOEC and LOEC values are reported if available. As discussed further in Section 4.3 and Section 4.4, the U.S. EPA will typically use an LC_{50} value for risk characterization while the Forest Service prefers to use an NOEC for sublethal effects.

4.1.3.1.2. Longer-Term Toxicity

Early life-stage studies in fish (OPPTS 850-1400) are analogous to mammalian teratology studies (Section 3.1.9.1.). The test involves exposing fertilized eggs to various concentrations of the chemical and maintaining the exposure until the fish are free-feeding. Freshwater species commonly used in this assay include rainbow trout, fathead minnow, zebra fish, and rice fish (*Oryzias latipes*). The sheepshead minnow is the only saltwater species that is typically used. Typically, five concentrations are tested along with a control group. If a vehicle is used, a vehicle control is also included. The primary observations include egg hatching, survival of fry, gross abnormalities (terata), behavior, length and weight. Results are typically reported as NOEC and LOEC values using a *p*-value of 0.05 to define the NOEC. This type of bioassay is elaborate and expensive. While these types of studies are funded by registrants as part of the registration process and while these studies may occasionally be published in the open literature, egg-and-fry studies are not typically conducted by other researchers and are not typically published. While these studies are not true chronic studies, they are often the only longer-term study available on a presumably sensitive life-stage, and these studies often serve as the basis for the longer-term dose-response assessment in fish.

Fish life cycle toxicity studies (OPPTS 850.1500) involve essentially egg-to-egg exposures. As with the early life-stage study, the life cycle study starts with fertilized eggs. The study, however, continues throughout the life of the initial generation, analogous to the P (parental) generation in a multigeneration reproduction study in mammals, and continues until the P generation produces eggs. This type of test is almost always conducted on either the fathead minnow (freshwater) or the sheepshead minnow (estuarine). This type of test is very expensive and is required only when the chemical is intended to be applied directly to water or when the ambient concentrations of the chemical are expected to be equal to or greater than one-tenth of the no-effect level in the fish early life-stage or invertebrate life-cycle test. When available, these tests are used for assessing the consequences of longer-term exposures unless egg-and-fry studies on other species appear to be more sensitive indicators of risk – i.e., have lower NOEC values.

4.1.3.1.3. Other Data

The above types of tests are the studies most commonly used in Forest Service risk assessments. Fish, however, are very common test species and a large number of different types of species and types of assays may be available in the open literature. These studies

can be highly variable in the species tested, protocols used, and endpoints examined. Studies from the open literature reporting adverse effects in fish are always included in the risk assessment, at least in the appendices. The extents to which non-standard studies may be used to quantitatively modify the dose-response assessment or risk characterization are addressed on a case-by-case basis in each risk assessment. In general, Forest Service risk assessments will use non-standard studies if they suggest a greater risk than standard studies and if the reported endpoints can be related plausibly to adverse effects that might be seen in populations of fish in the environment.

Lastly, field studies that include observations on fish are occasionally available as well as mesocosm (e.g., littoral enclosure) studies. These studies are used to the extent possible as a check on the available laboratory toxicity studies. The general limitations on field studies (Section 4.1.2.1) apply to observations from field studies that involve fish. Better controlled mesocosm studies are generally more useful in assessing the relevance of standard laboratory studies to potential hazards in the field.

4.1.3.2. Amphibians (Aquatic-Phase)

Amphibians (e.g., frogs, salamanders, and toads) are cold-blooded animals that spend time both on land and in water but breeding and development typically occur in water. Although the amount of information on the toxicity of pesticides to amphibians is increasing (e.g., Pauli et al. 2000; Sparling et al. 2000), very little toxicity data are generally available on amphibians compared to other aquatic species. The most commonly available study is the Frog Embryo Teratogenesis Assay – Xenopus (FETAX) bioassay (e.g., Fort et al. 2004). This study typically involves exposing frog embryos to the test chemical for a 96 hour period. The study is similar in design to acute toxicity study in fish in terms of the number of concentrations and reporting of results. The endpoints include observations of mortality as well as malformations.

The U.S. EPA (2011) has a general protocol (OPPTS 850.1800) for a 30-day subchronic sediment toxicity study using bullfrog tadpoles (*Rana catesbeiana*). This type of study is similar in design to other aquatic bioassays.

Because of the relative scarcity of data available on toxic effects to amphibians and the high level of concern with effects on amphibians, any available information on effects to amphibians are typically reviewed in some detail. If the data are sufficient, these data are used in the dose-response assessment (Section 4.3).

4.1.3.3. Aquatic Invertebrates

4.1.3.3.1. Acute Toxicity

Many aquatic invertebrates are relatively simple organisms to culture and test in aquatic toxicity studies, and standard acute toxicity protocols from U.S. EPA/OPPTS (2011) are available on a number of invertebrate species: daphnids (OPTTS 850.1010), gammarids (OPTTS 850.1020), oysters (OPTTS 850.1025), mysid shrimp (OPTTS 850.1035), penaeid shrimp (OPTTS 850.1045), and several species of bivalves (OPTTS 850.1055). These tests are similar in design to acute toxicity studies in fish (Section 4.1.3.1), although some may involve somewhat shorter periods of exposure – e.g., the daphnid study typically only lasts

for 48 hours. Acute toxicity studies will often be available in the open literature as well and may be conducted on a large number of different species, although the overall designs of most studies are similar to those (and often follow) standard protocols from either the U.S. EPA or the European Organization for Economic Co-operation and Development (OECD).

4.1.3.3.2. Longer-Term Toxicity

Chronic studies on invertebrates are generally limited to daphnids (OPTTS 850.1300 for a freshwater species) or mysid shrimp (OPTTS 850.1350 for a saltwater species). These are true chronic studies. Although the daphnid study is typically conducted for only 21-days, the short life span and rapid reproductive capacity of daphnids may result in exposures to several generations of young. The most common test species is *Daphnia magna* although *D. pulex* are sometimes used. The chronic daphnid study is typically the only study available on the chronic toxicity of a pesticide to freshwater invertebrates.

4.1.3.3.3. Other Data

As with fish, field studies or mesocosm studies on herbicides that include observations on invertebrates are occasionally available and these studies may be used to supplement the dose-response assessment. For insecticides, field studies may be abundant and of sufficiently high quality to justify using these studies rather than laboratory studies as the basis for the dose-response assessment. This is considered and discussed on a case-by-case basis in each risk assessment.

4.1.3.4. Aquatic Plants

Aquatic plants comprise both macrophytes (large multicelluar plants) and algae (microscopic plants). Bioassays in aquatic algae typically involve freshwater green alga (*Selenastrum capricornutum* or *Raphidocelis subcapitata*), a freshwater diatom (*Navicula pelliculosa*), a marine diatom (*Skeletonema costatum*), and a blue-green alga or cyanobacterium (*Anabaena flos-aquae*). The duration of exposure for algae is typically 48-hours. Bioassays on macrophytes typically use a species of duck weed (e.g., *Lemna gibba*) and the duration for duckweed assays is typically 7-days to 14-days.

Both types of studies measure growth (either as cell count, gross weight or length, or frond count) and express results as effective concentrations (e.g., EC_{50}) rather than lethal concentrations (e.g., LC_{50}). As with most other types of bioassays, the studies often report NOEC and LOEC values, and NOEC values are typically used in the dose-response assessment.

Field studies may be relatively abundant for some herbicides, particularly for those that are intended for aquatic weed control. These studies may be directly useful in the dose-response assessment as long as concentrations in water are reported and can be associated with NOAECs or LOAECs.

4.2. EXPOSURE ASSESSMENT

4.2.1. Overview

As in the human health risk assessment, the specific exposure scenarios that are considered in this section are determined by the application method and the chemical and toxicological properties of the compound. For foliar applications, this section will consider acute and longer-term durations of oral exposure (food or drinking water) as well as soil contamination, a direct spray, and drift. For other application methods such as soil treatment, cut surface applications, or aquatic applications, only of subset of the standard exposure scenarios for foliar applications is used. The exposure assessment for aquatic species typically relies on the estimated peak and longer-term concentrations in water that are used in the human health risk assessment as well as the exposure assessments for terrestrial wildlife from the consumption of contaminated water.

Exposures of soil organisms to a pesticide are typically based on the Gleams-Driver modeling and/or available monitoring data. Exposures to terrestrial plants are estimated both as concentrations in soil and direct foliar contamination either from direct spray or drift. For some species of terrestrial animals (typically insects), standard toxicity studies may report units that are not readily converted to mg agent/kg body weight. For example, some contact toxicity studies express exposure only in mass of agent per unit surface area – e.g., lb/acre or mg/m^2. In such a case, some dose-response assessments may be based on units of mass of agent per unit surface area and the exposure assessment is simply expressed as the application rate, or some fraction of the application rate to account for drift. In other cases, such as honeybees, body weight data may be used to convert exposures in mg/organism to mg/kg bw.

The exposure scenarios for terrestrial animals are similar in many respects to the exposure scenarios used in the human health risk assessment. Terrestrial animals might be exposed to any applied insecticide from direct spray, the ingestion of contaminated media (vegetation, prey species, or water), grooming activities, or indirect contact with contaminated vegetation. Estimates of oral exposure are expressed in the same units as the available toxicity data. As in the human health risk assessment, these units are usually expressed as mg of agent per kg of body weight and abbreviated as mg/kg for terrestrial animals. For dermal exposures to terrestrial animals, the units of measure usually are expressed in mg of agent per cm^2 of surface area of the organism and abbreviated as mg/cm^2. In estimating dose, however, a distinction is made between the exposure dose and the absorbed dose. The *exposure dose* is the amount of material on the organism (i.e., the product of the residue level in mg/cm^2 and the amount of surface area exposed), which can be expressed either as mg/organism or mg/kg body weight. The *absorbed dose* is the proportion of the exposure dose that is actually taken in or absorbed by the animal.

As with the human health exposure assessment, the computational details for each exposure assessment are presented in worksheets – i.e., one or more EXCEL workbooks created by WorksheetMaker (SERA 2011a). Exposure assessments for mammals and birds are summarized in Worksheet G01. Exposures to other nontarget species – i.e., terrestrial insects, terrestrial plants, and aquatic organisms – are summarized in other G-Series

composite worksheets containing both exposure summaries and HQs. The number of G-Series worksheets varies depending on the availability of the toxicity data and the application method under consideration.

Given the large number of species that could be exposed to pesticides and the varied diets in each of these species, a very large number of different exposure scenarios could be generated. For the generic risk assessments, an attempt is made to limit the number of exposure scenarios. The specific exposure scenarios presented in the general risk assessments are designed as conservative screening scenarios that may serve as guides for more detailed site-specific assessments by identifying the groups of organisms and routes of exposure that are of greatest concern.

4.2.2. Terrestrial Vertebrates

An overview of the mammalian and avian receptors considered in the current risk assessment is given in Table 17. The species and consumption data given in Table 17 are discussed in the following subsections. Because of the relationship of body weight to surface area as well as to the consumption of food and water, small animals will generally receive a higher dose, in terms of mg/kg body weight, relative to large animals, for a given type of exposure. The exposure assessment for mammals considers five nontarget mammals of varying sizes: small (20 g) and medium (400 g) sized omnivores, a 5 kg canid, a 70 kg herbivore, and a 70 kg carnivore. Four standard avian receptors are considered: a 10 g passerine, a 640 g predatory bird, a 2.4 kg piscivorous bird, and a 4 kg herbivorous bird. Because of differences in presumed food items that are consumed, all of the mammalian and avian receptors are not considered in all of the exposure scenarios (e.g., the 640 g predatory bird is not used in the exposure assessments for contaminated vegetation).

Toxicity data are not generally available on reptiles or terrestrial-phase amphibians (Section 4.1.2.3); accordingly, exposure assessments for these terrestrial vertebrates are typically not developed and standard values for this group of organisms are not presented in Table 17. When toxicity data are available, however, custom exposure scenarios are developed to accommodate the toxicity data.

4.2.2.1. Direct Spray

In the broadcast application of any insecticide, wildlife species may be sprayed directly. This scenario 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 amount absorbed depends on the application rate, the surface area of the organism, and the rate of absorption.

Two direct spray scenarios are conducted for foliar applications. The first spray scenario (Worksheet F01a) concerns the direct spray of half of the body surface of a 20 g mammal during pesticide application. This exposure assessment assumes first-order dermal absorption. The second exposure assessment (Worksheet F01b) assumes complete absorption over Day 1 of exposure. This assessment is included in an effort to encompass increased exposures due to grooming.

All direct spray scenarios are associated with substantial uncertainties. For example, the estimate based on first-order dermal absorption does not consider fugitive losses from the surface of the animal and may overestimate the absorbed dose. Conversely, birds, mammals, and other animals may groom frequently and grooming may contribute to the total absorbed dose by direct ingestion of the compound residing on fur or feathers. Other vertebrates, particularly amphibians, may have skin that is far more permeable than the skin of most mammals. If data are available on dermal absorption and toxicity in amphibians, direct spray scenarios may be developed in risk assessments involving foliar applications.

Direct spray scenarios are not generally given for large mammals. As indicated in Table 17, allometric relationships dictate that large mammals will be exposed to lesser amounts of a compound in any direct spray scenario than smaller mammals. However, in cases where the toxicity data indicate that large mammals are more sensitive than small mammals (see Section 4.3), direct spray scenarios for larger mammals may be given.

4.2.2.2. Dermal Contact with Contaminated Vegetation

As in the human health risk assessment (see Section 3.2.3.3), the only approach for estimating the potential significance of indirect dermal contact is to assume a relationship between the application rate and dislodgeable foliar residue. Unlike the human health risk assessment in which transfer rates for humans are available, there are generally no transfer rates available for wildlife species. As discussed in Durkin et al. (1995), the transfer rates for humans are based on brief (e.g., 0.5 to 1-hour) exposures that measure the transfer from contaminated soil to uncontaminated skin. Compared to humans, wildlife are likely to spend longer periods of time in contact with contaminated vegetation. During prolonged exposures, it may be reasonable to assume that an equilibrium is reached between levels on the skin, rates of absorption, and levels on contaminated vegetation. Nonetheless, no data regarding the kinetics of these processes have been encountered.

For highly lipophilic compounds – i.e., compounds with a low water solubility and a high $K_{o/w}$ – it is plausible that the absorbed dose resulting from contact with contaminated vegetation will be as great as those associated with comparable direct spray scenarios, and possibly larger than those associated with the consumption of contaminated vegetation. For hydrophilic compounds – i.e., compounds with high water solubility and a low $K_{o/w}$ – the compound 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 concentration of the chemical on the surface of the animal will be equal to the dislodgeable residue on the vegetation). Under these assumptions, the absorbed dose resulting from contact with contaminated vegetation might be on the order of one-tenth that associated with comparable direct spray scenarios. All of these assumptions, however, are speculative and are not generally used to quantify exposures in the risk assessments. Thus, the potential for effects from contact with contaminated vegetation is only addressed qualitatively.

For most herbicides and insecticides applied in foliar applications, the failure to quantify exposures associated with dermal contact adds relatively little uncertainty to the risk assessment, because the dominant route of exposure will be the consumption of contaminated vegetation, which is addressed in the following subsection.

4.2.2.3. Ingestion of Contaminated Vegetation or Prey

In foliar applications, the consumption of contaminated vegetation is an obvious concern. Exposure assessments for the consumption of contaminated vegetation are developed for all mammals and birds listed in Table 17, except for the large carnivorous mammal and the predatory bird. Both acute and chronic exposure scenarios are developed for the consumption of contaminated fruit (Worksheets F04a-e for acute and Worksheets F10a-e for chronic) and the consumption of short grass (Worksheets F05a-e for acute and Worksheets F11a-e for chronic).

The initial concentrations of picloram in the food items is based on the U.S. EPA/OPP adaptation of the residue rates from Fletcher et al. (1997), as summarized in Table 13. The methods of estimating the peak and time-weighted average concentrations are identical to those used in the human health risk assessment (Section 3.2.3.7). As summarized in Table 13, fruit and short grass are the food items that comprise the commodities with the lowest pesticide residue rates (fruit) and the highest pesticide residue rates (short grass). Consequently, fruit and short grass are selected to encompass the range of plausible concentrations of pesticides in vegetation that might be consumed by various mammals and birds and.

For both the acute and chronic exposure scenarios, the assumption is made that 100% of the diet is contaminated. This may not be a realistic assumption for some acute exposures and will probably be a rare event in chronic exposures—i.e., animals may move in and out of the treated areas. While estimates of the proportion of the diet that is contaminated could be incorporated into the exposure assessment, the estimates would be an essentially arbitrary set of adjustments. Because the proportion of the diet that is contaminated is linearly related to the resulting HQs, the impact of variations in the proportion of the diet that consists of contaminated food is discussed further in the risk characterization (Section 4.4.2).

The estimated food consumption rates by various species of mammals and birds are based on field metabolic rates (kcal/day), which, in turn, are based on the adaptation of estimates from Nagy (1987) by the U.S. EPA/ORD (1993). These allometric relationships account for much of the variability in food consumption among mammals and birds. There is, however, residual variability, which is remarkably constant among different groups of organism (Nagy 1987, Table 3). As discussed further by Nagy (2005), the estimates from the allometric relationships may differ from actual field metabolic rates by about ±70%. Consequently, in all worksheets involving the use of the allometric equations for field metabolic rates, the lower bound is taken as 30% of the estimate and the upper bound is taken as 170% of the estimate.

The estimates of field metabolic rates are used to calculate food consumption based on the caloric value (kcal/day dry weight) of the food items considered in this risk assessment and estimates of the water content of the various foods. Estimates of caloric content are summarized in Table 39. Most of the specific values in Table 18 are taken from Nagy (1988) and U.S. EPA/ORD (1993).

Along with the exposure scenarios for the consumption of contaminated vegetation, similar sets of exposure scenarios are provided for the consumption of small mammals by either a predatory mammal (Worksheet F08a) or a predatory bird (Worksheet F08b) as well as the consumption of contaminated insects by a small mammal, a larger (400 g) mammal, and a small bird (Worksheets F07a-c).

For aquatic applications, the consumption of contaminated vegetation is not typically considered. For soil treatments, the consumption of contaminated vegetation may be considered if compound-specific data are available on the relationship between concentrations of the pesticide in soil and the resulting concentration of the pesticide in plants.

4.2.2.4. Ingestion of Contaminated Water

The methods for estimating concentrations of pesticides in water are identical to those used in the human health risk assessment (Section 3.2.3.4). The only major differences in the estimates of exposure involve the weight of the animal and the amount of water consumed. As with the estimates of food consumption, water consumption rates are well characterized in terrestrial vertebrates. The water consumption rates are based on allometric relationships in mammals and birds, as summarized in Table 17. Based on these estimates, exposure scenarios involving the consumption of contaminated water are developed for mammals and birds for accidental spills (Worksheets F02a-e), expected peak expected concentrations (Worksheets F06a-e), and expected longer-term concentrations (Worksheets F12a-e).

As with food consumption, water consumption in birds and mammals will vary substantially with diet, season, and many other factors; however, there are no well-documented quantitative estimates regarding the variability of water consumption by birds and mammals in the available literature. Accordingly, the variability in water consumption rates of birds and mammals is not considered in the exposure assessments. Typically, the upper and lower bound estimates of concentrations in surface water vary substantially for both acute and chronic exposures. Given this variability, quantitative consideration of the variability in water consumption rates of birds and mammals would not typically have a substantial impact on the risk characterization.

4.2.2.5. Ingestion of Contaminated Fish

In addition to the consumption of contaminated vegetation, insects, and other terrestrial prey (Section 4.2.2.3), the consumption of contaminated fish by piscivorous species is a viable route of exposure to pesticides; accordingly, sets of exposure scenarios are developed for an accidental spill (Worksheets F03a-b), expected peak exposures (Worksheets F09a-c), and estimated longer-term concentrations (Worksheets F13a-c). These exposure scenarios are applied to 5 and 70 kg carnivorous mammals as well as a piscivorous bird. The 70 kg carnivorous mammal would be typical of a black bear (which does not actively hunt fish) but could be representative of a small or immature Great Plains Grizzly Bear (*Ursus arctos horribilis*), which is an endangered species that actively feeds on fish (Reid 2006).

Exposures to pesticides from the consumption of contaminated fish are dependent not only on the concentration of the pesticide in water but also on the bioconcentration factor for the pesticide. The concentrations of the pesticide in water are identical to those discussed in

Section 4.2.2.4. Bioconcentration factors are developed as discussed in Section 3.2.3.5 except that bioconcentration factors for wildlife are usually based on whole-body bioconcentration factors in fish under the assumption that mammalian or avian predators will typically consume the entire fish. If chemical and species specific data are available indicating that this is not the case, then alternative custom exposure scenarios may be developed.

4.2.3. Terrestrial Invertebrates

4.2.3.1. Direct Spray and Drift

Estimated levels of exposure associated with broadcast terrestrial applications of pesticides are detailed in Worksheet G09. For risk assessments that cover aerial, ground broadcast, and backpack applications, a custom worksheet may be used that encompasses each of these application methods.

Typically, honeybees are used as a surrogate for other terrestrial insects and honeybee exposure levels associated with broadcast applications are modeled as a simple physical process based on the application rate and surface area of the bee. The surface area of the honeybee (1.42 cm^2) is based on the algorithms suggested by Humphrey and Dykes (2008) for a bee with a body length of 1.44 cm. Doses in units of mg/bee are converted to units of mg/kg bw. Typical body weights for worker bees range from 81 to 151 mg (Winston 1987, p. 54) and the mean of this range – i.e., 116 mg – is typically used as an average body weight for a worker bee.

The amount of a pesticide deposited on a bee during or shortly after application depends on how close the bee is to the application site as well as foliar interception of the spray prior to deposition on the bee. The estimated proportions of the nominal application rate at various distances downwind given in G09 are based on Tier 1 estimates from AgDRIFT (Teske et al. 2002) for distances of 0 (direct spray) to 900 feet downwind of the treated site. Further details of the use of AgDRIFT are discussed in Section 4.2.4.2 (Off-Site Drift) with respect to nontarget vegetation.

In addition to drift, foliar interception of a pesticide may occur. The impact of foliar interception varies according to the nature of the canopy above the bee. For example, in studies investigating the deposition rate of diflubenzuron in various forest canopies, Wimmer et al. (1993) report that deposition in the lower canopy, relative to the upper canopy, generally ranged from about 10% (90% foliar interception in the upper canopy) to 90% (10% foliar inception by the upper canopy). In Worksheet G09, foliar interception rates of 0% (no interception), 50%, and 90% are used.

During broadcast applications of a pesticide, it is likely that terrestrial invertebrates other than bees will be subject to direct spray. If the available toxicity data on terrestrial invertebrates supports a dose-response assessment for other species of insects, this exposure scenario may be elaborated to consider these species.

4.2.3.2. Ingestion of Contaminated Vegetation or Prey

As with terrestrial mammals and birds, terrestrial invertebrates may be exposed to pesticides through the consumption of contaminated vegetation or contaminated prey. For foliar applications, estimates of residues on contaminated vegetation or prey are based on estimated residue rates (i.e., mg/kg residues per lb applied) from Fletcher et al. (1994), as summarized in Table 13.

An estimate of food consumption is necessary to calculate a dose level for a foraging herbivorous insect. Insect food consumption varies greatly, depending on the caloric requirements in a given life stage or activity of the insect and the caloric value of the food to be consumed. The derivation of consumption values for specific species, life stages, activities, and food items is beyond the scope of the current analysis. Nevertheless, general food consumption values, based on estimated food consumption per unit body weight, are readily available.

Reichle et al. (1973) studied the food consumption patterns of insect herbivores in a forest canopy and estimated that insect herbivores may consume vegetation at a rate of about 0.6 of their body weight per day (Reichle et al. 1973, pp. 1082 to 1083). Higher values (i.e., 1.28-2.22 in terms of fresh weight) are provided by Waldbauer (1968) for the consumption of various types of vegetation by the tobacco hornworm (Waldbauer 1968, Table II, p. 247). Typically, the risk assessment will use food consumption factors of 1.3 (0.6 to 2.2) kg food /kg bw. The lower bound of 0.6 is taken from Reichle et al. (1973), and the central estimate and upper bound are taken from the range of values provided by Waldbauer (1968).

Details concerning estimated exposure levels for the consumption of contaminated vegetation by herbivorous insects are provided in Worksheets G07a, G07b, G07c, and G07d of the EXCEL workbook for terrestrial foliar applications of picloram (Attachment 1). These levels pertain to the four food items included in the standard residue rates summarized in Table 13.

4.2.3.3. Contact with Contaminated Soil

As discussion in Section 4.1.2.4, earthworm subchronic toxicity tests (OPPTS 850.6200) are available for some pesticides. In addition, the available field studies or other studies in the open literature may provide toxicity data on terrestrial invertebrates that are based on soil exposures. In such cases, soil concentrations from Gleams-Driver and/or monitoring data are used directly in the exposure assessment. This route of exposure is particularly important in risk assessments that consider soil broadcast, soil drench, or injection applications.

4.2.3.4. Honeybees Foraging for Nectar

If sufficient data are available, Forest Service risk assessments will develop an exposure assessment on honeybees foraging for nectar. This approach is prompted by concerns raised by the Tier 1 analysis for imidacloprid conducted by the Forest Service (Appleton 2008) as well as a conceptually similar analysis of the potential impact of imidacloprid on honeybees developed for the French Ministry of Agriculture (Alix and Vergnet 2007; Halm et al. 2006; Rortais et al. 2005).

The analyses conducted for the French Ministry of Agriculture develop exposure assessments for several subgroups of honeybees (i.e., nectar foragers, pollen foragers, larvae, brood

attending bees, and winter bees) following applications of imidacloprid. Exposure assessments in Forest Service risk assessments are generally limited only to nectar foragers because this is the subgroup estimated to be exposed to the highest doses (Rortais et al. 2005, p. 73, Table 1). Analogous to the approach taken in the human health risk assessment (Section 3.2.3.1.1), a nectar forager is taken as the Most Exposed Individual (MEI).

The basic algorithm for estimating the daily dose (D) to the foraging bee, based on the nutritional requirements of the bee is:

$$Dose_{mg/kg\,BW} = C_{Nec\,mg/L} \times Am_{Nec_L} \div BW_{kg} \qquad \text{(Eq. 24)}$$

where:

C = Concentration of the pesticide in nectar in units of mg/L
Am = Amount of nectar in liters consumed by a foraging bee per day based on the nutritional requirements of the bee.
BW = Body weight of the bee in kilograms.

The amount of nectar a bee needs to consume is calculated from the nutritional requirements of the bee. Nutritional requirements for bees are generally expressed in the literature as the amount of sugar per unit time. Rortais et al. (2005) express the sugar requirement of bee during flight as 8-12 mg/hour, which is reasonably close to the value of 11.5 mg/hour cited by Winston (1987). Forest Service risk assessments will typically use a sugar requirement for flight of 10 (8 to 12) mg/hour.

The number of hours/day that a bee might spend foraging is likely to be highly variable. Rortais et al. (2005) use a range from 4 to 10.7 hours/day. This range is used with a central estimate of 6.5 hours/day, the approximate geometric mean of the lower and upper bounds from Rortais et al. (2005).

Thus, the amount(s) of sugar ($Am_{SugarFl}$) required by a bee to support flight activities during foraging is calculated as the product of the sugar requirements per hour during flight and the number of hours/day that the bee spends in flight:

$$\begin{aligned} Am_{Sugar\,FL} &= Rate_{mg/h} \times Flight_{h/day} \\ Am_{Sugar\,FL} &= 10\,(8\,to\,12)_{mg/h} \times 6.5\,(4\,to\,10.7)_{h/day} \end{aligned} \qquad \text{(Eq. 25)}$$

Using the above equation, the amount(s) of sugar required per day to support flight activities is calculated as 65.5 (32 to 128.4) mg/day.

Rortais et al. (2005) base their exposure assessment only on sugar requirements during flight. In the current Forest Service risk assessment, the estimated nutritional requirement also includes time at rest, using the value of 0.7 mg/hour from Winston (1987, p. 61). From the same equation depicted above, the sugar requirement(s) for hours other than those engaged in flight is calculated as:

$$Am_{Sugar\ Other} = 7_{mg/h} \times (24_{h/day} - 6.5(4 to 10.7)_{h/day}) \quad \text{(Eq. 26)}$$

which is equivalent to 77.25 (46 to 137.71) mg/day. Compared with the method used by Rortais et al. (2005), the inclusion of metabolic requirements during non-flight hours increases the sugar demand by about 20%.

The sugar content of nectar also varies among plants and locations. Rortais et al. (2005) uses a value of 0.4—i.e., nectar consists of 40% w/w nutritional sugars. This single value is also used Forest Service risk assessments, although refinements to this estimate may be made as needed in any particular risk assessment. When the sugar requirement(s) is divided by 0.4 (mg sugar/mg nectar), the estimated amount of nectar required per day is about 193 (115 to 344) mg/day. In the worksheets for this exposure scenario, these values are converted to units of kg nectar per day by dividing mg/day by 1,000,000 mg/kg.

Although the nectar requirements of a foraging bee are relatively well documented and simple to estimate using the general method of Rortais et al. (2005), reliable information on concentrations of pesticides in nectar are rare. This exposure assessment in only used in Forest Service risk assessments when information on the concentration of the pesticide in nectar is available or can be reasonably estimated.

The basis of the exposures assessments in Forest Service risk assessments and in Rortais et al. (2005) is the sugar demand of the honeybee; accordingly, the equivalence of nectar and honey exposures hold only if the amount of the pesticide per unit of sugar in nectar and honey are constant. Because there is more sugar in honey than in nectar, it would seem to follow that the pesticide concentration in honey would be greater than the pesticide concentration in nectar. Nonetheless, the opposite trend has been observed in at least some instances discussed in the available literature. Waller et al. (1984) note that although dimethoate was not detected in hive honey, dimethoate residues at concentrations of up to 1.4 ppm were detected in nectar. Similarly, Barker et al. (1980) report honey-to-nectar ratios of about 0.44 to 0.8 after a field application of dimethoate to alfalfa. A similar pattern was observed in a study involving seed treatment of canola with clothianidin, another neonicotinoid, in which the honey-to-nectar ratio was about 0.4 (Cutler and Scott-Dupree 2007). If the concentrations of the pesticide in honey are generally less than the concentrations in nectar, in terms of equivalent amounts of sugar, the exposure assessments based on nectar consumption could overestimate pesticide exposure from honey residue.

4.2.4. Terrestrial Plants

As noted above, most pesticides used by the Forest Service are applied directly to vegetation. Thus, terrestrial plants will certainly be exposed to these pesticides. A large number of different exposure assessments could be made for terrestrial plants – i.e., direct spray, spray drift, runoff, wind erosion and the use of contaminated irrigation water. Such exposure assessments are typically conducted for herbicides. For other pesticides, however, the development of such exposure assessments would serve no purpose because there is often no basis for asserting that adverse effects in terrestrial plants are plausible. Thus, no formal exposure assessments for non-herbicidal pesticides are typically conducted for terrestrial

plants, and the following discussion of specific exposure scenarios are typically conducted only for herbicides. For risk assessments involving foliar applications, the exposure assessments for terrestrial plants are detailed in Worksheet G04 (runoff), Worksheet G05 (direct spray and drift), Worksheet G06a (contaminated irrigation water), and Worksheet G06b (wind erosion).

4.2.4.1. Direct Spray

Unintended direct spray will result in an exposure level equivalent to the application rate. For many types of herbicide applications, it is plausible that some nontarget plants immediately adjacent to the application site could be sprayed directly. This type of scenario is modeled in the worksheets that assess off-site drift, a detailed in the following section.

4.2.4.2. Off-Site Drift

Because off-site drift is more or less a physical process that depends primarily on droplet size and meteorological conditions rather than specific properties of the compound being sprayed, estimates of off-site drift can be modeled using AgDRIFT. These estimates are summarized in Worksheet G05 of the EXCEL workbook for foliar applications. For risk assessments covering aerial, ground broadcast, and backpack applications, a custom worksheet may be used that includes all of these application methods.

The drift estimates used in the current risk assessment are based on AgDRIFT (Teske et al. 2002) using Tier 1 analyses for aerial and ground broadcast applications. The term *Tier 1* is used to designate relatively generic and simple assessments that may be viewed as plausible upper limits of drift. Aerial drift estimates are based on Tier 1 using ASAE Fine to Medium drop size distributions. Tier 1 estimates of drift for ground broadcast applications are modeled using both low boom and high boom options in AgDRIFT. For both types of applications, the values are based on Very Fine to Fine drop size distributions and the 90th percentile values from AgDRIFT.

Drift associated with backpack applications (directed foliar applications) are likely to be much less than drift from ground broadcast applications. Few studies, however, are available for quantitatively assessing drift after backpack applications. For the current risk assessment, estimates of drift from backpack applications are based on an AgDRIFT Tier 1 run of a low boom ground application using Fine to Medium/Coarse drop size distributions (rather than very fine to fine) as well as 50th percentile estimates of drift (rather than the 90th percentile used for ground broadcast applications).

The values for drift used in generic (i.e., not site-specific) risk assessments should be regarded as little more than generic estimates similar to the water concentrations modeled using GLEAMS (Section 3.2.3.4.3). Actual drift will vary according to a number of conditions—e.g., the topography, soils, weather, and the pesticide formulation. All of these factors cannot be considered in generic risk assessments.

4.2.4.3. Runoff and Soil Mobility

Exposures to terrestrial plants associated with runoff and sediment loses from the treated site to an adjacent untreated site are summarized in Worksheet G04 of EXCEL workbooks that accompany Forest Service risk assessments.

Any pesticide can be transported from the soil at the application site by runoff, sediment loss, or percolation. Runoff, sediment loss, and percolation are considered in estimating contamination of ambient water. Only runoff and sediment loss are considered in assessing off-site soil contamination. This approach is reasonable because off-site runoff and sediment transport will contaminate the off-site soil surface and could impact non-target plants. Percolation, on the other hand, represents the amount of the pesticide that is transported below the root zone and thus may impact water quality but should not affect off-site vegetation. The GLEAMS modeling used to estimate concentrations in water provides data on loss by runoff. As with the estimates of pesticides in surface water, runoff estimates are modeled for clay, loam, and sand at nine sites that are representative of different temperatures and rainfall patterns (Table 9).

The results of the standard Gleams-Driver modeling are summarized in one or more appendices in Forest Service risk assessments. Table 1 of these appendices contains the information on runoff and sediment losses. Note that the proportion of runoff as a fraction of the application rate will vary substantially with different types of climates—i.e., temperature and rainfall—as well as soils with no runoff or sediment loss anticipated in predominantly sandy soils.

4.2.4.4. Contaminated Irrigation Water

The levels of exposure associated with this scenario will depend on the pesticide concentration in the ambient water used for irrigation and the amount of irrigation water used. Concentrations in ambient water are based on the peak concentrations modeled in the human health risk assessment, as discussed in Section 3.2.3.4.6.

The amount of irrigation used will depend on the climate, soil type, topography, and plant species under cultivation. Thus, the selection of an irrigation rate is somewhat arbitrary. In the absence of any general approach for determining and expressing the variability of irrigation rates, the application of 1 inch of irrigation water with a range of 0.25 to 2 inches is used in Forest Service risk assessments. Details of the calculations used to estimate the functional application rates based on irrigation using contaminated surface water are provided in Worksheet G06a of the EXCEL workbooks that accompany Forest Service risk assessments.

The product labels for some herbicides may note that water contaminated with the herbicide should not be used for irrigation. In these cases, this standard exposure scenario is included in the herbicide risk assessment with a comment indicating that this exposure scenario is not relevant to the risk assessment except to evaluate the consequences of disregarding the labeled use restrictions.

4.2.4.5. Wind Erosion

Wind erosion can be a major transport mechanism for soil (e.g., Winegardner 1996), and wind erosion is also associated with the environmental transport of herbicides (Buser 1990). Wind erosion leading to off-site movement of pesticides is likely to be highly site-specific. The amount of a pesticide that might be transported by wind erosion depends on several factors, including application rate, depth of incorporation into the soil, persistence in the soil,

wind speed, and topographical and surface conditions of the soil. Under desirable conditions—e.g., relatively deep (10 cm) soil incorporation, low wind speed, and surface conditions which inhibit wind erosion—it is unlikely that a substantial amount of a pesticide would be transported to offsite vegetation by wind.

For Forest Service risk assessments, the potential effects of wind erosion are estimated in Worksheet G06b. In this worksheet, it is assumed that the pesticide is incorporated at a depth that is identical to the depth of incorporation used in Gleams-Driver modeling, typically 1 cm. Average soil losses are estimated to range from 1 to 10 tons/ha/year with a typical value of 5 tons/ha/year. These estimates are based on the results of agricultural field studies which found that wind erosion may account for annual soil losses ranging from 2 to 6.5 metric tons/ha (Allen and Fryrear 1977).

As noted in Worksheet G07b, the use of the above values typically results in estimates of offsite losses at about 0.014% of the application rate. Larney et al. (1999), however, report that wind erosion of other herbicides could be associated with losses up to 1.5% of the nominal application rate following soil incorporation or 4.5% following surface application. This difference appears to be a due to the much higher soil losses noted by Larney et al. (1999)—i.e., up to 56.6 metric tons/ha from a fallow field. The losses reflected in Worksheet G06b may be somewhat more realistic for forest or rangeland applications, because herbicide applications are rarely made to fallow areas. In any event, the higher offsite losses reported by Larney et al. (1999) are generally comparable to exposures associated with offsite drift at distances of about 50 feet from the application site following low boom (0.017) and high boom (0.05) ground broadcast applications (Worksheet G05). All of the estimates for wind erosion and offsite drift are likely to vary dramatically according to site conditions and weather conditions.

4.2.4.6. Volatilization

For some herbicides, off-site volatilization may be an important route of exposure for nontarget plants. General methods for estimating exposures from volatilization have not been developed. Thus, this section is included only when the chemical-specific information is adequate to support both an exposure assessment and a dose-response assessment. This is consistent with the basic approach to risk assessment discussed in Section 1.2.1.

4.2.5. Aquatic Organisms

The exposure assessment for aquatic organisms is based on the concentrations of the pesticide in surface water used in the exposure assessment for terrestrial vertebrates (Section 4.2.2.4) which is in turn equivalent to the concentrations used in human health risk assessment (Section 3.2.3.4.6).

4.3. DOSE-RESPONSE ASSESSMENT

4.3.1. Overview

Forest Service risk assessments will attempt to define dose-response relationships for all of the groups of organisms discussed in the Hazard Identification for ecological risk assessments – i.e., mammals, birds, reptiles, amphibians (terrestrial and aquatic phases), terrestrial and aquatic invertebrates and macrophytes, microorganisms, and algae. For many of these groups, the dose-response assessment is elaborated to consider both sensitive and tolerant species. If sufficient data are available, the dose-response assessments may be further elaborated to consider different groups of organisms based either on allometric or phylogenetic relationships.

As in the human health risk assessment, the Forest Service will consider, discuss, and sometimes defer to dose-response assessments developed in ecological risk assessments developed by the U.S. EPA/OPP. Also as in the human health risk assessment, this approach avoids a duplication of effort, capitalizes on the substantial expertise of U.S. EPA/OPP, and decreases the size, complexity, and cost of Forest Service risk assessments. There are, however, important differences between the approach taken by U.S. EPA/OPP and the approach preferred by the Forest Service. The Forest Service prefers to use NOEC values for both acute and chronic exposures. This differs from the U.S. EPA/OPP which will base dose-response assessments for acute exposures on LC_{50} or EC_{50} values. Nonetheless, the Forest Service assessment will adapted (slightly modify) the methods used by U.S. EPA/OPP, as detailed further below, for data sets in which only LC_{50} or EC_{50} values are available.

While the methods used in dose-response analyses in ecological risk assessments are more diverse than those typically used in human health risk assessments, the various methods used in ecological dose-response assessments can be applied to all or at least most of the groups of organisms covered in these assessment. Consequently, rather than discussing dose-response assessments for each groups of organisms separately, this section is organized by the methods that may be used in dose-response assessments in order of increasing complexity:

- Point Estimates (Section 4.3.2)
- Extreme Values (Section 4.3.3)
- Relative Potency Method (Section 4.3.4)
- Species Sensitivity Distributions (Section 4.3.5)
- Allometric Relationships (Section 4.3.6)

Limitations in the application of any of these methods to specific groups of organisms are discussed as needed in the following sections.

4.3.2. Point Estimates

As the name implies, point estimates are simply single numbers. As noted in the previous section, the Forest Service prefers to use NOAEL or NOAEC values for both acute and chronic dose-response assessment. Thus, in the simplest case, the dose response assessment for a given group of organism may simply involve the selection of an *appropriate* NOAEL or

NOAEC. NOAEL and NOAEC are essentially equivalent terms. For the following discussion, NOAEL is used.

The operative word in selecting a NOAEL, however, is *appropriate*. Different types of studies may report different types of NOAELs. It is not uncommon for an acute toxicity study to identify NOAELs for lethality. This is the case because many acute toxicity studies, at least those required by the U.S. EPA/OPP for pesticide registration, are designed to estimate acute LC_{50}s or LC_{50}s.

NOAELs for lethality are not acceptable in Forest Service risk assessments. The Forest Service has clearly indicated that the acceptable NOAELs must involve reasonably sensitive sublethal effects. Consequently, Forest Service risk assessments will use NOAELs from acute toxicity studies only if the study defines a NOAEL that involves sublethal effects. In other words, the study should define both a NOAEL and a LOAEL and there should be reasonable confidence that the NOAEL involves endpoints that would not impair the ability of the organism to function normally over a short-term period.

The above restrictions on acute NOAELs will often exclude all of the available acute NOAELs for a particular pesticide. As an alternative, the Forest Service has agreed to use a modification of the U.S. EPA/OPP risk presumption system for acute toxicity. An overview of this system is given in Table 19. As summarized in Table 19, the U.S. EPA/OPP uses variable levels of concern for acute exposures based on the ratio of exposure to the LD_{50} or LC_{50}. This ratio is referred to as the RQ or Risk Quotient. Differences between the RQ used by U.S. EPA/OPP and the HQ used by the Forest Service are discussed further in Section 4.4 (Risk Characterization). As indicated in Table 19, the method in U.S. EPA/EFED (2004) defines the level of concern somewhat differently for each group of organisms. This approach also explicitly considers differences in ecological status of the organism by using different levels of concern for endangered species.

In terms of the dose-response assessment in Forest Service risk assessments, the Forest Service has approved estimating NOAELs based on the RQ method for endangered species. In other words, the LD_{50} may be divided by 10 to estimate an NOAEL for a mammal or bird. Similarly, the LC_{50} may be divided by 20 to estimate an NOAEC for aquatic animals. It should be appreciated that this approach is applied in Forest Service risk assessments to any animal, regardless of its status as an endangered species – i.e., the Forest Service prefers to treat all species with the same level of caution that the U.S. EPA/OPP applies to endangered species.

In practice, this approach for using LD_{50} and LC_{50} values often appears to be highly conservative or protective in that the estimated acute NOAEL is below experimental subchronic or chronic NOAELs. Obviously, it would not be sensible to use an estimated acute NOAEL that is less than an experimental subchronic or chronic NOAEL. Thus, in these cases, the subchronic or chronic NOAEL is adopted and used for the acute dose-response assessment.

In some cases, NOAEL values or reasonable approaches to estimating NOAELs from lethality studies may not be available. In these cases, LOAELs may be used either directly for the risk characterization or LOAELs may be adjusted to LOAELs with the application of an uncertainty factor. These types of adjustments are always done as a last resort and the specific approaches used are discussed in the individual risk assessment – i.e., a consensus on the best way to handle these situations has not been reached and no single method for adjusting a LOAEL to approximate a NOAEL is codified.

4.3.3. Extreme Values

As discussed in Section 1.2.2.2, Forest Service risk assessments are currently based on an extreme values approach. Almost no values used in a risk estimate – either toxicity values or exposure values – are presented as a single number. As detailed in Section 3.2 and 4.2, most exposure assessments are presented as central estimates with an upper and lower bound.

As a complement to this approach, separate toxicity values are presented for *sensitive* and *tolerant* species for groups of ecological receptors whenever possible. The value for sensitive species is typically the lowest reported experimental value that is available, and the value for tolerant species is the highest reported experimental value. In some cases, considerations of data quality, data documentation, and the geographical distribution of the species may be used to censor some data from the selection of the sensitive and tolerant species. For example, take a report in a secondary source indicating that a particular species of fish native to Southeast Asia is either remarkably more or less sensitive to a particular pesticide than fish native to North America. In most cases, the data on North American fish will come from studies submitted to U.S. EPA that are very well documented or studies from the peer-reviewed open literature. In such a case, the less well documented information on the extremely sensitive or tolerant fish from Southeast Asia might not be used. These sorts of judgments are considered on a case-by-case basis in the dose-response assessment for each group of organisms considered in the risk assessment. As with the point estimate approach, experimental or estimated NOAEL or NOAEC values are used.

4.3.4. Relative Potency Method

In some instances, acute toxicity values may be available for both sensitive and tolerant species but chronic toxicity values are available for only one group – i.e., sensitive or tolerant species. In such cases, the relative potency method may be used to estimate a chronic value for the group on which experimental data are missing. This method can be used for NOAEC, LD_x, or analogous values. In general, LD_{50} or LC_{50} values are preferred because these values can be used to define, at least in theory, relative sensitivities among specie.

Defining the acute toxicity (AT) value for the sensitive species as AT_S, the acute toxicity value for the tolerant species as AT_T, and the known chronic toxicity (CT) value for the tolerant species as CT_T, the chronic toxicity value for the sensitive species (CT_S) is estimated as:

$$CT_S = CT_T \times \frac{AT_S}{AT_T} \qquad \text{(Eq. 27)}$$

Alternatively, the chronic toxicity value for the tolerant species (CT_T) might be estimated from the chronic toxicity value for the sensitive species (CT_S) as:

$$CT_T = CT_S \times \frac{AT_T}{AT_S} \qquad \text{(Eq. 28)}$$

In some instances, the chronic toxicity value may be on a species of intermediate sensitivity. So long as a corresponding acute toxicity value is available for the intermediate species (AT_I), the chronic value for the sensitive or tolerant species (CT_X) can be defined as:

$$CT_X = CT_I \times \frac{AT_X}{AT_T} \qquad \text{(Eq. 29)}$$

These types of estimations must be justified on a case-by-case basis. In many instances, the underlying assumption that acute and chronic toxic potency are correlated may be difficult to support and the use of the relative potency method can add substantial uncertainty to the risk assessment.

4.3.5. Species Sensitivity Distributions

Ecological risk assessments are concerned fundamentally with differences in sensitivity among species and groups of species. While distinguishing differences in sensitivity in terms of most sensitive and most tolerant (Section 4.3.3) is useful, data are occasionally available to suggest more refined estimates in gradations of sensitivity within and among species. One approach to defining such gradations involves *species sensitivity distributions*. As discussed by Posthuma et al. (2002), species sensitivity distributions can be used quantitatively in risk assessments (e.g., Posthuma et al. 2002) and as tools in probabilistic risk assessment. This technique is not currently used quantitatively in Forest Service risk assessments. Nonetheless, species sensitivity plots are useful for illustrating differences in sensitivity among different groups of organisms.

A simple example of a species sensitivity distribution is given in Figure 6 which gives the species sensitivity distribution of mammals following oral doses of strychnine. This example is taken from the SERA (2010b) risk assessment on strychnine. In Figure 6, the x-axis is the LD_{50} value and the y-axis is the cumulative frequency of the LD_{50} values for each of the species. The individual values for the cumulative frequency are based on the following equation:

$$Freq_i = \frac{i - 0.5}{N} \qquad \text{(Eq. 30)}$$

where $Freq_i$ is the cumulative frequency for the ith ordered value and N is the number of values in the ordered data set. For example, there are a total of 14 LD_{50} values for mammals. The lowest value is an estimated lethal dose of 0.5 mg/kg bw for bears. Thus, the frequency for the first point (i=1) is calculated as $\frac{1-0.5}{14}$ or 0.037. Similarly, the second lowest LD_{50} value (i=2) is 0.6 mg/kg, which is assigned a frequency of $\frac{2-0.5}{14}$ or 0.107. Note that the x-

axis in Figure 6 represents the LD_{50} values, and these are given on a logarithmic (base 10) scale under the standard assumption that LD_{50} values in different groups of organisms will be log-normally distributed.

Based on estimated lethal doses, sensitivity differences among mammalian species spans a factor of about 50, ranging from an approximate lethal dose of 0.5 mg/kg bw for the bear to an LD_{50} of 27 mg/kg bw for nutria.

Of equal importance, however, is that these types of plots can be used to assess subgroups of organisms that may be particularly sensitive to the agent under consideration. In the example given in Figure 6, the six most sensitive species of mammals are carnivores with sensitivity rankings as follows: bear > mink > dog > fox > cat ≈ coyote. Despite the variability and differences in the types of available data, the mean toxicity value for carnivores (1.11 mg/kg bw) is less than the mean toxicity values for other mammals (11.2 mg/kg bw) by about a factor of 10.

Whenever adequate data are available, species sensitivity distributions are used in Forest Service risk assessments to both illustrate differences among species and better define subgroups of organisms that may be particularly sensitive or tolerant. These types of analyses can be used by the Forest Service in the preparation of site-specific risk assessments or similar documents to better characterize risks for species of particular concern.

4.3.6. Allometric Relationships

Allometric relationships, which are used extensively in the exposure assessment for triclopyr, are sometimes apparent for sensitivity among species. 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:

$$Y = \alpha W^{\beta} \qquad \text{(Eq. 31)}$$

W is the weight of the animal, ***Y*** is the variable to be examined, and the model parameters are designated by alpha (***α***) and beta (***β***). If Y decreases with body weight, ***β*** is negative. If Y increases with body weight, ***β*** is positive**.** If there is no relationship of ***Y*** to body weight, ***β*** is near to or at least not significantly different from zero. Systematic differences in sensitivity with changes in body weight are most often applicable to toxicity studies in mammals, as illustrated in Figure 7.

As with the illustration of species sensitivity distributions (Figure 6 as discussed in Section 4.3.6), Figure 7 is taken from the SERA (2010b) risk assessment on strychnine. As illustrated in Figure 8, the slope (***β***) in the allometric equation is negative indicating that larger mammals are somewhat more sensitive than smaller mammals. In addition, this relationship is statistically significant (p=0.037). As discussed in the strychnine risk assessment, however, the statistical significance may be specious. More importantly, however, and as illustrated in Figure 6, the allometric relationship may simply reflect the fact that the fox is a predatory mammal. As discussed in the previous subsection on species

sensitivity distributions, predatory mammals, regardless of size, appear to be more sensitive to strychnine than other mammals.

While the above example of the use of allometric relationships is somewhat complicated by the consideration of the pattern of species sensitivity distributions, the example does serve to illustrate one additional feature concerning dose-response assessments: The more sophisticated tools that can be used in ecological dose-response assessments can only be used if adequate data are available and, in such cases, the more sophisticated tools are best used in combination.

4.4. RISK CHARACTERIZATION

Structurally, the quantitative risk characterization presented in ecological risk assessments is mathematically identical to the hazard quotient approach discussed for the human health risk assessment (Section 3.4) in that the hazard quotient (HQ) is defined as the ratio of the exposure (Ex) to the chemical divided by a toxicity value (TV) for the chemical:

$$HQ = \frac{Ex}{TV} \qquad \text{(Eq. 32)}$$

Conceptually, however, there is one substantial difference between the risk characterization for the human health risk assessment and the ecological risk assessment: the level of tolerable risk. In human health risk assessments, the fundamental concern is with the individual. The toxicity values used in human health risk assessments (i.e., RfDs and other similar estimates discussed in Section 3.3) are intended to represent population thresholds. Thus, if the level of exposure is below the RfD – i.e., the HQ is less than unity – no effects are anticipated in any individuals. In ecological risk assessment, concern is most often with populations of animals rather than individual animals. Thus, no attempt is made to derive RfD-like estimates with the application of uncertainty factors.

These conceptual differences are reflected in elaborations of the above equation for the HQ. As discussed in Section 3.3, the RfD is a toxicity value such as a NOAEL divided by an uncertainty factor:

$$RfD = \frac{NOAEL}{UF} \qquad \text{(Eq. 33)}$$

As summarized in Table 15, the magnitude of the uncertainty factor is dependent on the quality and nature of the NOAEL (or sometimes the LOAEL). Typically, the uncertainty factor is has a value of 100 – i.e., a factor of 10 for potentially sensitive individuals and a factor of 10 for extrapolating from animals to humans. Thus, the equation for the hazard quotient in the human health risk assessment (HQ_{HHRA}) is elaborated as:

$$HQ_{HHRA} = \frac{Ex}{TV} = \frac{Ex}{NOAEL \div UF} = \frac{Ex}{RfD} \qquad \text{(Eq. 34)}$$

In Forest Service risk assessments, the hazard quotients used in the ecological risk assessment (HQ_{ERA}) are based directly on NOAELs without an uncertainty factor:

$$HQ_{ERA} = \frac{Ex}{TV} = \frac{Ex}{NOAEL} \qquad \text{(Eq. 35)}$$

Thus, while HQs in both the HHRA and ERA have the same general form, they are not equivalent. Again, the more conservative approach to risk characterization that is used in the

HHRA reflects concern for individuals. The less conservative approach taken the ERA reflects concern primarily for populations.

In many cases, incidental mortality in individual organisms may occur in the application of many pesticides and this often only a minor concern. An exception, however, involves threatened and endangered species. Concern for threatened and endangered species is, by definition, greater than concern for other non-endangered species.

As discussed in Section 4.3.2 and summarized in Table 19, the greater concern for threatened and endangered species relative to other species is codified in the acute risk presumption categories use by the U.S. EPA (e.g., U.S. EPA/OPP 2004). The approach to risk characterization used by the U.S. EPA in ecological risk assessments is similar but not identical to that used by the Forest Service.

U.S. EPA/OPP bases risk characterizations for the ecological risk assessment on a risk quotient (RQ). The basic algorithm for the RQ is identical to that of the HQ – i.e., a level of exposure divided by a toxicity value. As summarized in Table 19, acute RQs may calculated based on LD_{50}, LC_{50} or EC_{50} values rather than the NOECs preferred in Forest Service risk assessments. Thus, while these RQs are similar in structure to HQs, the equations are not identical. For the sake of clarity, the lack of identity is illustrated below based on the example of an RQ derived from an LD_{50},

$$HQ = \frac{Ex}{NOAEL}$$

$$RQ = \frac{Ex}{LD_{50}} \qquad \text{(Eq. 36)}$$

$$HQ \neq RQ$$

The above inequality, however, does not imply a fundamental inconsistency between the methods used in Forest Service and EPA ecological risk assessments. To the contrary, the two risk characterization methods may often be identical and balanced by differences in how the Forest Service and the U.S. EPA define the Level of Concern (LOC).

Forest Service risk assessments always use a LOC of 1. In other words and as in the human health risk assessment, an HQ of greater than 1 indicates that the exposure is greater than the NOAEC and this raises concern that an adverse effect might be possible. As summarized in Table 19, however, the U.S. EPA/OPP uses a variable level of concern. For example, if an RQ is based on an LC_{50} or EC_{50} value in aquatic species, the LOC is 0.5 for acute risk, 0.1 for acute restricted use, and 0.05 for endangered species.

As discussed in Section 4.3.2, Forest Service risk assessments may use LD_{50}, LC_{50} and other similar values to approximate an NOAEC by multiplying lethality value by the corresponding LOC used by the U.S. EPA/OPP for endangered species. Mathematically, this is equivalent to dividing the lethality value by an uncertainty factor – i.e., an uncertainty factors of 10 for terrestrial species and 20 for aquatic species, both of which are simply the

reciprocals of the LOC values used by the U.S. EPA/OPP for these groups of organisms (Table 19).

As detailed in U.S. EPA/EFED (2004), the U.S. EPA risk assessments conducted by EFED (i.e., within the Office of Pesticide Programs) are part of the pesticide registration process and the specific LOCs detailed in Table 4-2 are considered as screening tools. If a particular risk assessment results in an HQ that exceeds the LOC, additional analyses may be conducted and may involve elaboration or refinement of the dose-response relationships or exposure assessments. This approach reflects the U.S. EPA's interpretation and implementation of its legislative mandates under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA), both amended by the Food Quality Protection Act (FQPA) of 1996.

The risk assessments conducted by or for the USDA Forest Service are in response to the National Environmental Policy Act (NEPA). In implementing the NEPA process, the generic or programmatic risk assessments detailed in this document are only the initial step in the risk assessment process and are analogous (although not identical) to what the U.S. EPA refers to as a screening level risk assessment (U.S. EPA/EFED 2004, p. 31 ff). Some differences between the EPA screening level risk assessments and generic risk assessments conducted for the Forest Service involve the use of extreme values rather than simply the most conservative value. As detailed in Section 4.3.3, the Forest Service risk assessments do not focus solely on the most sensitive species (as in most U.S. EPA risk assessments) but also consider a range of sensitivities within groups of organisms when such information is available. Similar differences exist in the use of extreme values in the exposure assessments (Section 4.2).

As noted above, the generic risk assessments prepared in support of Forest Service programs are only the first step in a much broader process. Typically, before the Forest Service will conduct or support any pesticide application, a programmatic Environmental Impact Statement (EIS) will be prepared that specifically addresses the issues associated with a programmatic goal (e.g., the control of a specific pest or class of pests) in a specific region. Subsequent to the EIS and prior to a pesticide application, an additional site-specific assessment, referred to as an Environmental Assessment (EA) may be conducted. It is during the preparation of an EIS and/or an EA that specific consideration is given to threatened and endangered species, steps that can be taken to mitigate risks, as well as a number of other regional or site-specific conditions that could impact the risk characterization.

Because of the differences in the use of U.S. EPA and Forest Service risk assessments, the system used by U.S. EPA (as summarized in Table 4-2) is not adopted directly. The alternative approach used in Forest Service risk assessments is summarized in Table 4-3. Consistent with the above discussion, the generic Forest Service risk assessments will not typically develop a separate risk characterization for threatened and endangered species. As noted in Section 4.3, the extreme value method will consider variability in all species within a group and will identify information on threatened and endangered species when such information is available. This information may then be used in an EIS or EA.

Notwithstanding the general use of this HQ based approach, some Forest Service risk assessments may use more elaborate dose-response assessments (Sections 4.3). This is similar to methods that are used by U.S. EPA when their screening level risk assessments trigger a more detailed analysis (U.S. EPA/EFED 2004). In some cases, the HQ approach in the risk characterization may be supplemented with discussions concerning the probability, severity, and/or duration of some adverse effect. In other cases, field studies may used to either temper or confirm concerns raised by the HQ approach. In rare cases, the quality of available field studies may be sufficiently high to essentially form the basis of the risk characterization, effectively displacing the use of the HQ approach.

Forest Service risk assessments will often attempt to assess the potential for secondary effects as well as direct toxic effects. For example, the application of any effective herbicide is likely to alter terrestrial vegetation. Similarly, the application of any effective insecticide is likely to alter populations of insects. These alterations could lead to secondary changes that might impact different groups of organisms based on changes in food availability, predator-prey relationships, and/or habitat quality. These types of secondary effects are likely to vary over time and vary among different groups of organisms or species. While the potential for secondary effects is discussed in Forest Service risk assessments, a clear characterization of the potential significance of secondary effects is often limited in the absence of extensive field studies.

While the quantitative risk characterization presented in Forest Service risk assessments is based on the HQ approach, qualitative discussions of risk are also typically presented. These qualitative discussions attempt to reflect limitations of or uncertainties in the available data as well as the ability to interpret the available data. In many respects, a qualitative appreciation of limitations in the information that is available will be as important, if not more important, than the numeric characterization of risk.

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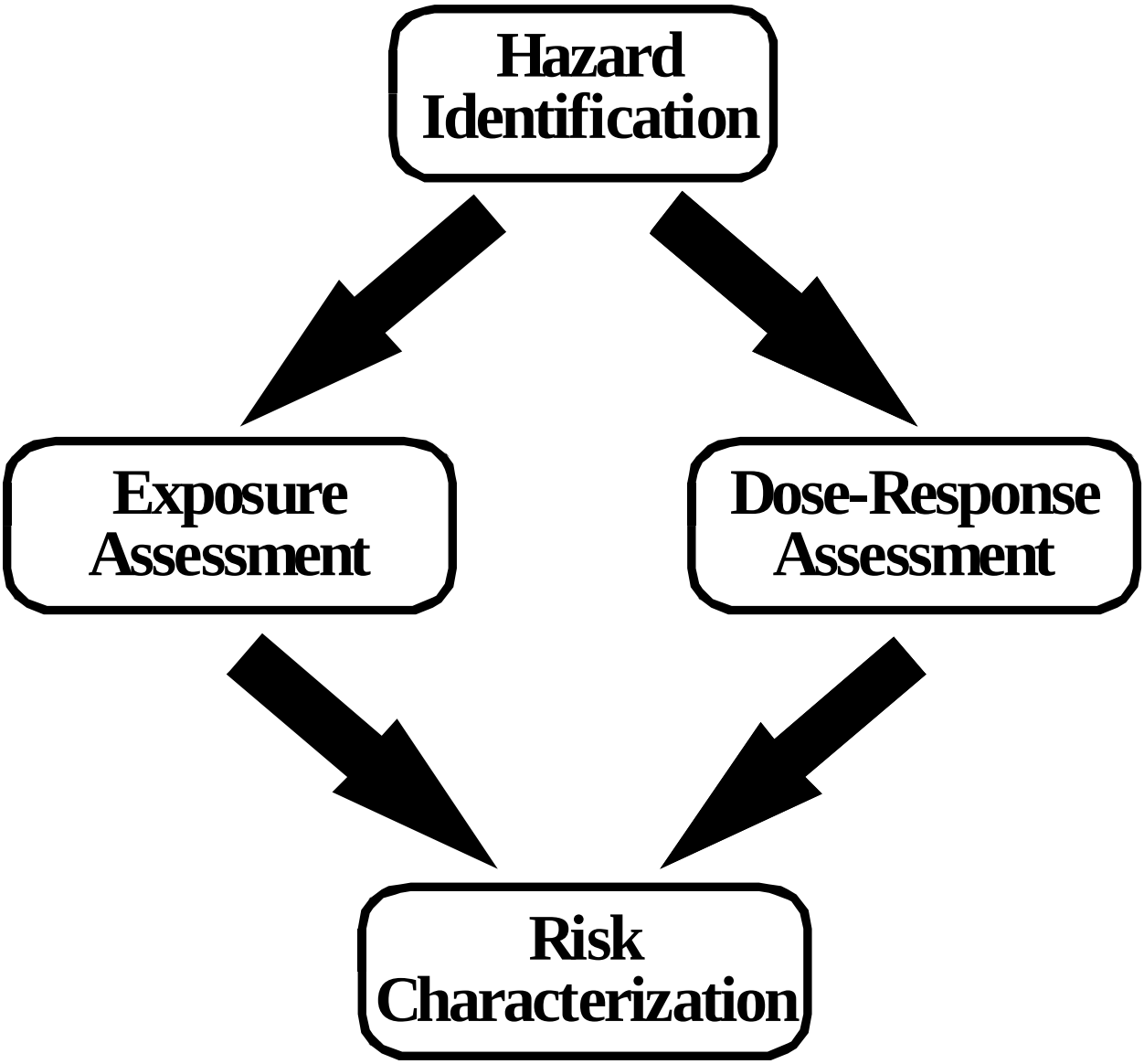

Figure 1: Overview of Risk Assessment Process

See Section 1.2 for discussion.

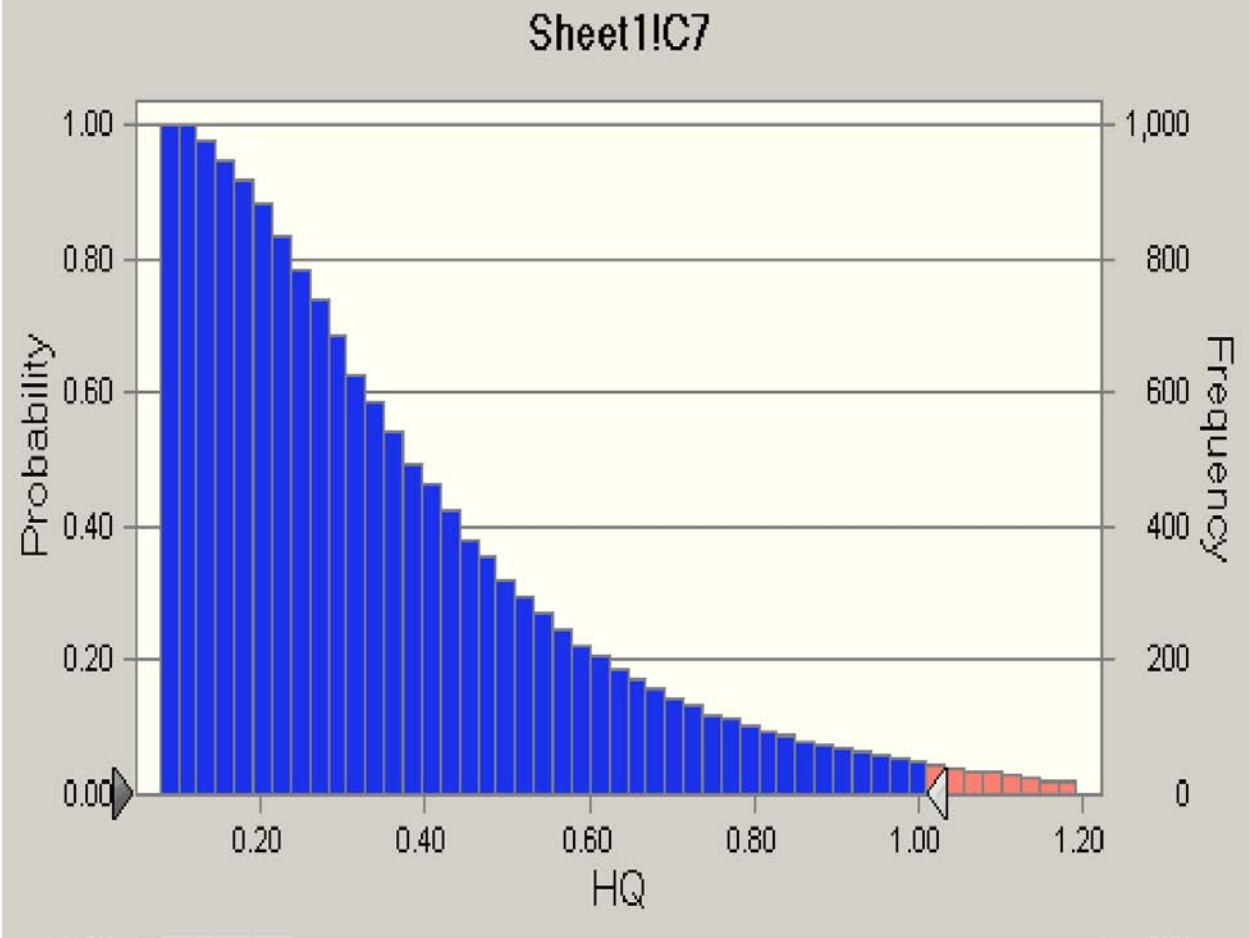

Figure 2: Example of Monte Carlo Analysis

See Section 1.2.2.1 for details and discussion.

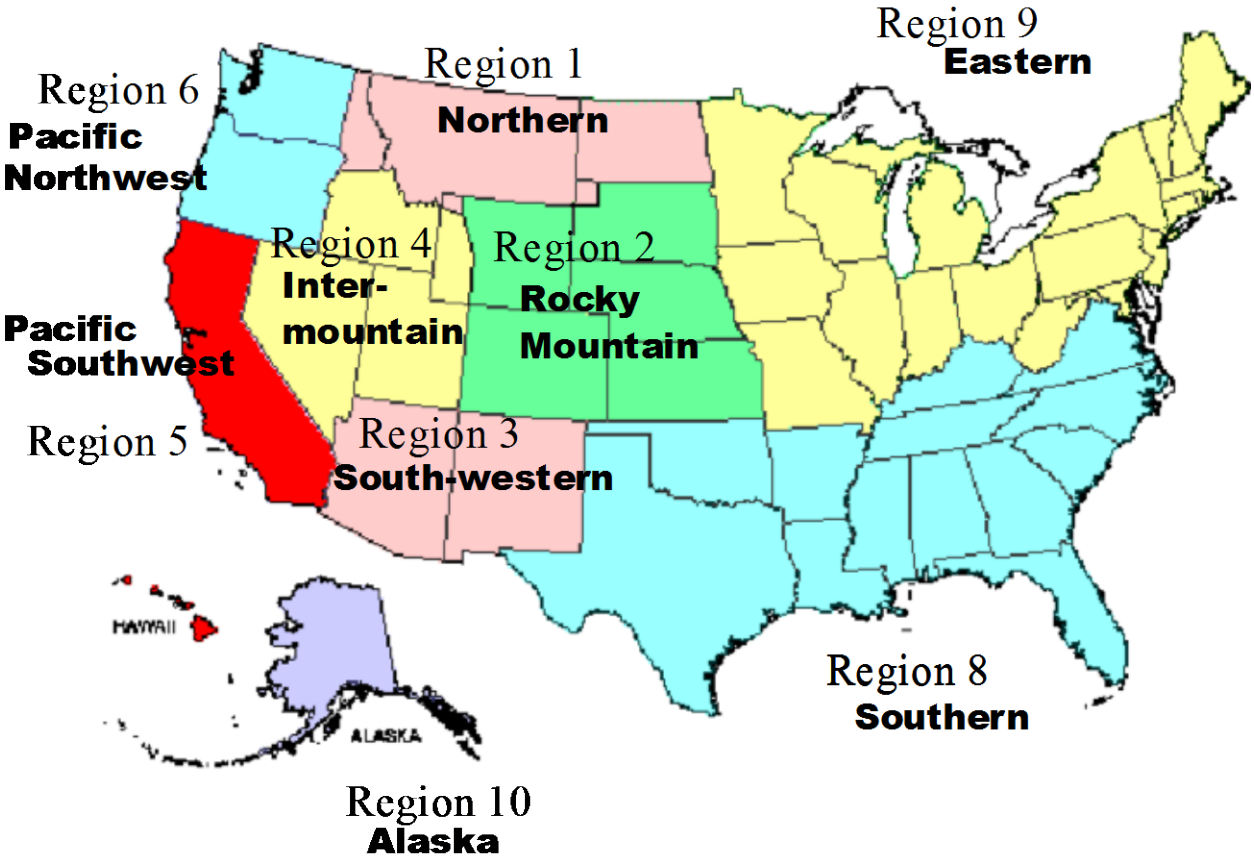

Figure 3: Forest Service Regions

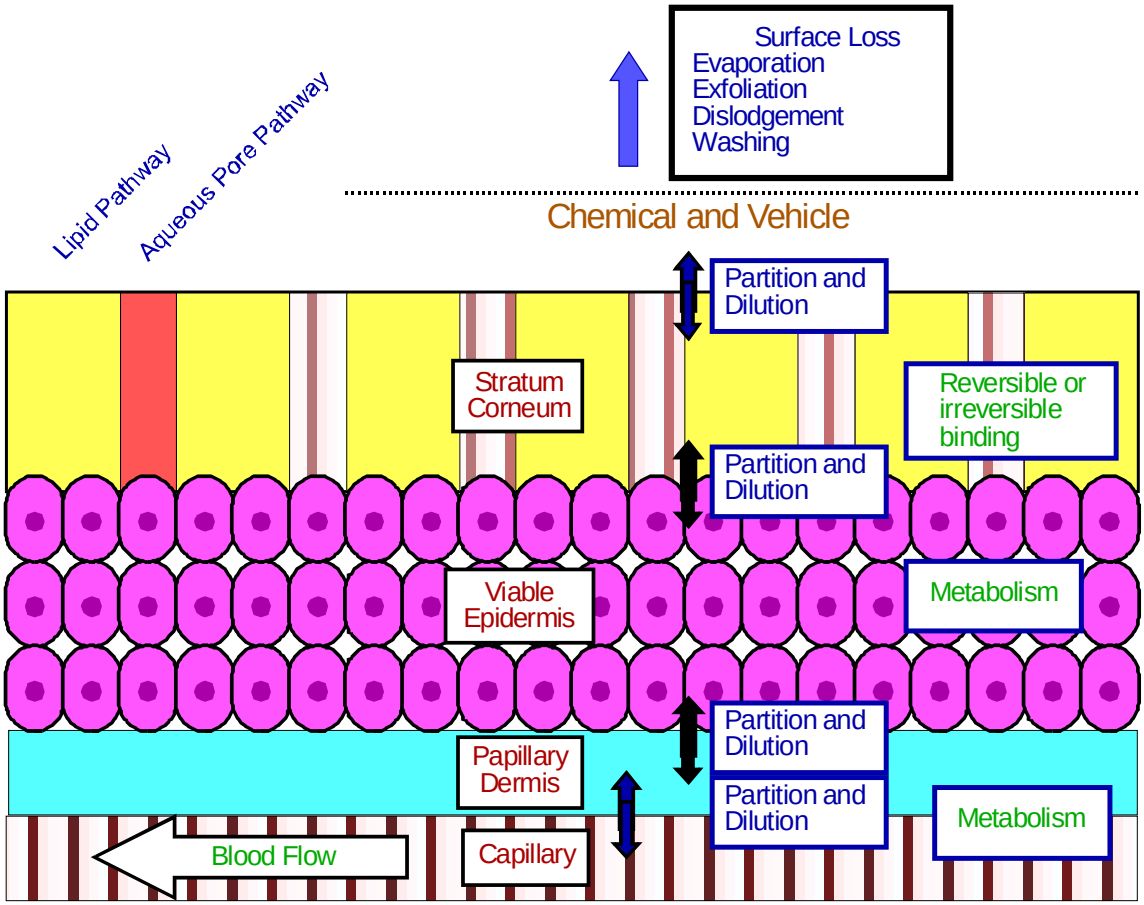

Figure 4: Schematic Overview of Dermal Absorption Processes

Source: Modified from U.S. EPA/ORD (1992, 2007) and Flynn (1990)
See Section 3.1.3.2 for discussion.

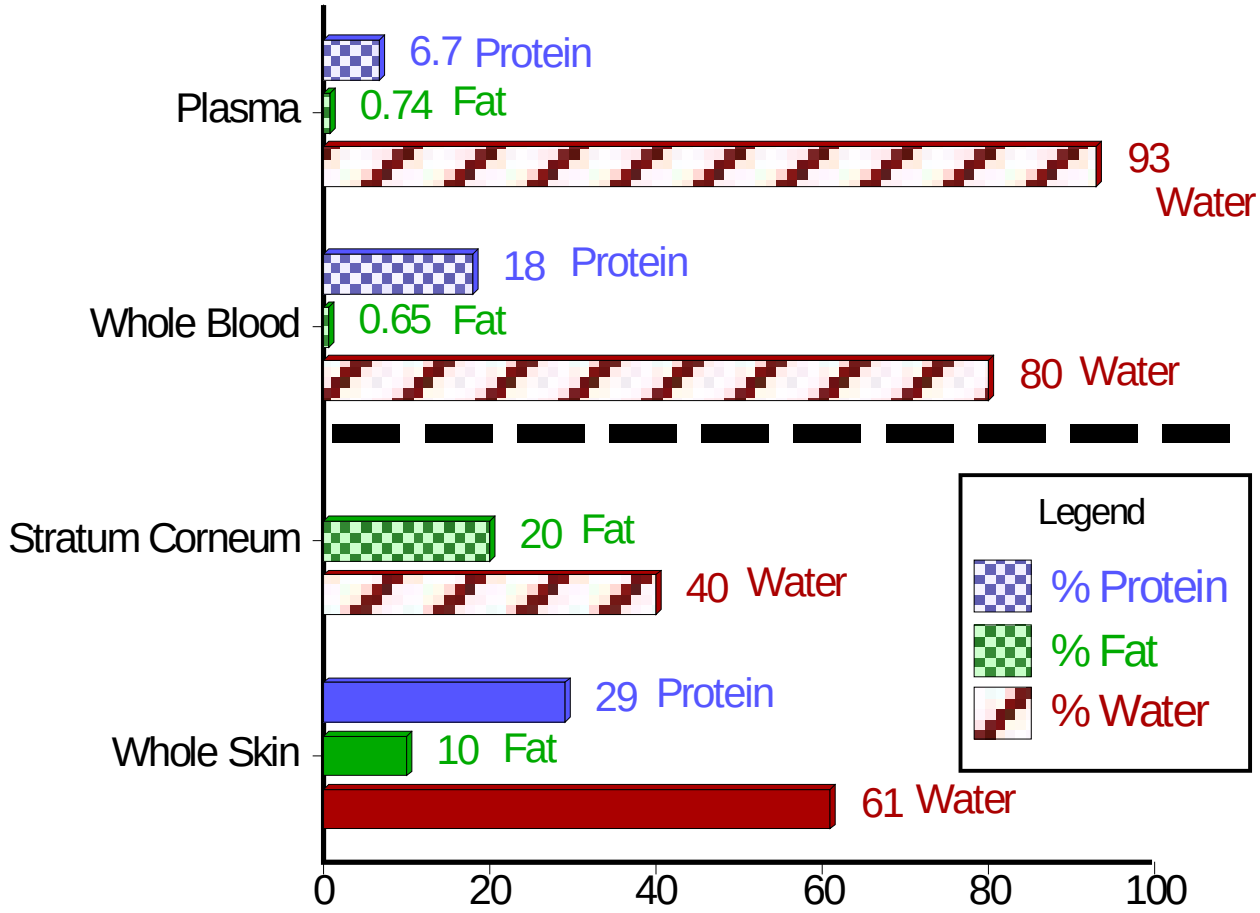

Figure 5: Composition a percentages of the blood and skin

Source: Data from ICRP (1992) and Klein-Szanto et al. (1991).
See Section 3.1.3.2 for discussion.

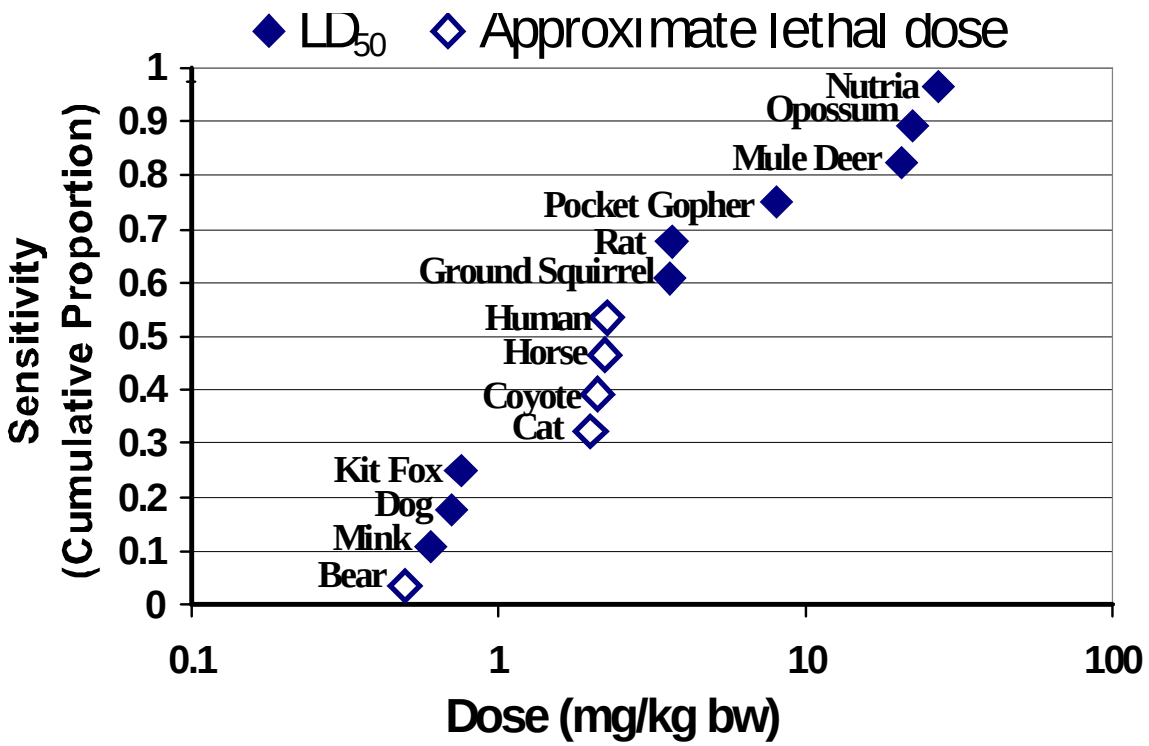

Figure 6: Species sensitivity distribution in mammals for strychnine

Source: SERA (2010b), Figure 10 with data in Table 14 of SERA (2010b)
See Section 4.3.5 for discussion.

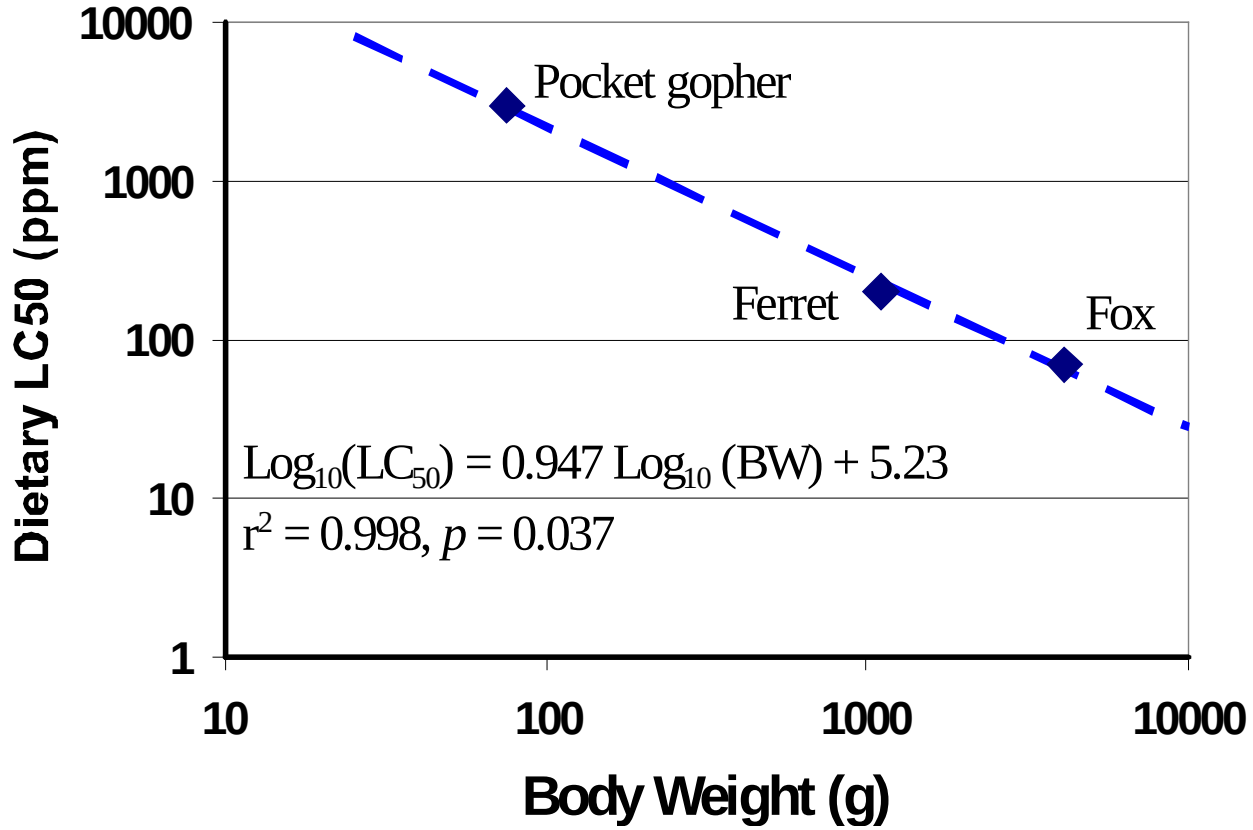

Figure 7: Example of Allometric relationship for dietary exposures in mammals

Source: SERA (2010b), Figure 8
See Section 4.3.5 for discussion.

Table 1: Severity definitions used in human health and ecological risk assessments

HHRA	ERA	Definition
NOEL	NOEC	*No-Observed-Effect Level* (C*oncentration*): No biologically or statistically significant effects attributable to treatment.
NOAEL	NOAEC	*No-Observed-Adverse-Effect Level* (C*oncentration*): No biologically or statistically significant adverse effects attributable to treatment. Effects that are attributable to treatment but do not appear to impair the organism's ability to function and clearly do not lead to such an impairment.
LOAEL	LOAEC	*Lowest-Observed-Adverse-Effect Level* (*Concentration*): The lowest dose or exposure level associated with an adverse effect.
AEL	AEL	*Adverse-Effect Level*: 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.
FEL	FEL	*Frank Effect Level*: Gross and immediately observable signs of toxicity.

Key: HHRA= Human Health Risk Assessment; ERA=Ecological Risk Assessment

See Section 1.2.1.1 for discussion.

Table 2: EPA Guideline Studies for Pesticides

Number	Title	Number	Title
61-1	Chemical Identity	163-1	Soil Leaching/adsorption/desorption
61-2	Description of Beginning Materials and Manufacturing Process	164-1	Terrestrial field dissipation
61-3	Discussion of Formation of Impurities	165-1	Confined rotational crop
62-1	Preliminary Analysis	165-4	Bioaccumulation in fish
62-2	Certification of limits	171-4B	Residue Analytical Methods
62-3	Analytical Method	171-4C	Magnitude of the Residue [by commodity]
63-0	Physical/Chemical Characteristics	171-4A2	Nature of the Residue in Plants
63-17	Storage stability	171-4A3	Nature of the Residue in Livestock
71-1	**Avian Single Dose Oral Toxicity**	830.1550	Product Identity and composition
71-2	**Avian Dietary Toxicity**	830.1600	Description of materials used to produce the product
71-4	**Avian Reproduction**	830.1620	Description of production process
72-1	**Acute Toxicity to Freshwater Fish**	830.1650	Description of formulation process
72-2	**Acute Toxicity to Freshwater Invertebrates**	830.1670	Discussion of formation of impurities
72-3	**Acute Toxicity to Estuarine/Marine Organisms**	830.1750	Certified limits
81-1	**Acute oral toxicity in rats**	830.1800	Enforcement analytical method
81-2	**Acute dermal toxicity in rabbits or rats**	830.6302	Color
81-3	**Acute inhalation toxicity in rats**	830.6303	Physical state
81-4	**Primary eye irritation in rabbits**	830.6304	Odor
81-5	**Primary dermal irritation**	830.6314	Oxidizing or reducing action
81-6	**Dermal sensitization**	830.6315	Flammability
82-1	**Subchronic Oral Toxicity: 90-Day Study**	830.6316	Explodability
82-2	**21-day dermal-rabbit/rat**	830.6317	Storage stability of product
83-1	**Chronic Toxicity**	830.6320	Corrosion characteristics
83-2	**Oncogenicity**	830.7000	pH of water solutions or suspensions
83-3	**Teratogenicity**	830.7100	Viscosity
83-4	**2-Generation Reproduction**	830.7300	Density/relative density
84-2	**Interaction with Gonadal DNA**	835.4100	Aerobic soil metabolism
85-1	**General metabolism**	**870.1100**	**Acute oral toxicity**
122-2	**Aquatic plant growth**	**870.1200**	**Acute dermal toxicity**
123-1	**Seed germination/seedling emergence and vegetative vigor**	**870.1300**	**Acute inhalation toxicity**
141-1	**Honey bee acute contact**	**870.2400**	**Acute eye irritation**
161-1	Hydrolysis	**870.2500**	**Acute dermal irritation**
161-2	Photodegradation-water	**870.2600**	**Skin sensitization**
161-3	Photodegradation-soil	**870.5100**	**Bacterial reverse mutation test**
162-1	Aerobic soil metabolism	**870.5375**	***In vitro* mammalian chromosome aberration test**
162-2	Anaerobic soil metabolism	**870.3050**	**Repeated dose 28-day oral toxicity in rodents**
162-3	Anaerobic aquatic metabolism	N/A	Non-Guideline Study

Guidelines relevant to human health effects and ecological effects are given in bold typeface.
See Section 1.3.2 for discussion.

Table 3: Dermal absorption and estimated permeability of hormones and their esters

Chemical	MW[1]	log K_{ow}[1]	Kp[2]	% Absorption[3]
Hydrocortisone	362.47	1.61	0.00016	1.87
Hydrocortisone acetate	404.51	2.30	0.00028	2.55
Testosterone	288.43	3.32	0.0075	13.24
Testosterone acetate	330.47	4.27	0.020 [0.032]	4.62
Testosterone propionate	344.50	4.77	0.037 [0.032]	3.34

[1] Durkin et al. (1995).

[2] Calculated using Equation 1. Upper limits based on Flynn (1990) in brackets. See text for details.

[3] Feldmann and Maibach (1969). Cumulative percent absorption over a 5-day observation period.

See Section 3.1.3.2 for discussion.

Table 4: Toxicity Categories for Pesticide Labeling

Study Type	Category I	Category II	Category III	Category IV
Acute Oral	≤ 50 mg/kg	>50 to 500 mg/kg	>500 to 5,000 mg/kg	>5,000 mg/kg
Acute Dermal	≤ 200 mg/kg	>200 to 2,000 mg/kg	>2,000 to 5,000 mg/kg	>5,000 mg/kg
Acute Inhalation (4 hour exposure)	≤ 0.05 mg/L	>0.05 to 0.5 mg/L	> 0.5 to 2 mg/L	>2 mg/L
Primary Eye Irritation	Corrosive (irreversible destruction of ocular tissue) or corneal involvement or irritation persisting for more than 21 days	Corneal involvement or other eye irritation clearing in 8-21 days	Corneal involvement or other eye irritation clearing in 7 days or less	Minimal effects clearing in less than 24 hours
Primary Eye Skin Irritation	Corrosive (tissue destruction into the dermis and/or scarring)	Severe irritation at 72 hours (severe erythema or edema)	Moderate irritation at 72 hours (moderate erythema)	Mild or slight irritation at 72 hours (no irritation or slight erythema)

Source: Modified slightly from U.S. EPA/OPP 2010, Label Review Manual, p. 7-2.
See Section 3.1.4 for discussion.

Table 5: Overview of Worker Exposure Methods

Factor	Forest Service	EPA and BLM
General Approach	Absorption based	Deposition based
Database	Worker exposure studies measuring absorbed dose covering all routes of exposure (SERA 2014).	Worker exposure studies on deposition from Pesticide Handlers Exposure Database (PHED Task Force 1995).
Worker groups	Aerial, boom spray, backpack, hack-and-squirt, and basal bark applicators.	37 different groups are defined in database – e.g., mixing, loading, application, flaggers) for different application methods.
Absorption rates	Not explicitly used – i.e., incorporated into studies in database.	Uses daily absorption rates if available, otherwise a default of 10% for dermal and 100% for inhalation.
Personal Protective Equipment	Chemical specific study or taken from PHED	Taken from PHED
Accidental Exposures	Wearing contaminate gloves and spilling pesticide onto skin	None in EPA assessments. One scenario in BLM assessments.

See Section 3.2.2 for discussion.

Table 6: Worker Exposure Rates and Treatment Rates

Worker Exposure Rates (mg/kg bw per lb handled).

Application Method	Reference Chemical	Central Estimate [Confidence Intervals] (Prediction Intervals)[1]	Adjust for Dermal Absorption
Directed Ground			
Backpack Directed Foliar	Glyphosate	0.0003 [0.0002-0.0005] (0.00006-0.002)	Yes
	2,4-D	0.005 [0.003-0.008] (0.001-0.02)	Yes
	Triclopyr BEE	0.01 [0.008-0.01] (0.002-0.06)	Yes
Hack-and-squirt	2,4-D TIPA	0.004 [0.001-0.01] (0.00003-0.5)	Yes
Basal Bark	Triclopyr BEE	0.001 [0.0006-0.003] (0.0001-0.02)	Yes
Broadcast Ground			
Broadcast foliar	2,4-D	0.0001 [0.00004-0.0002] ($2x10^{-6}$-0.005)	Optional
Aerial			
All aerial broadcast	2,4-D	0.00002 [0.000006-0.00007] ($5x10^{-7}$-0.0008)	Optional
Aquatic			
Aquatic broadcast	2,4-D	0.0009 [0.0004-0.002] (0.0002-0.005)	Optional

Acres Treated per Day

Application Method	Central	Lower	Upper
Directed foliar	4.4	1.5	8.0
Broadcast foliar	112	66	168
Aerial	490	240	800

Source: Worker Exposure Rates from SERA 2014.
See Section 3.2.2.1 for discussion.

Table 7: Summary of Exposure Rates from PHED

Exposures in units of mg/lb handled

Scenario	No clothing	Single Layer, No gloves	Single layer, Gloves	Inhalation
1. Dry flowable, open mixing and loading	1.1	0.066	0.066	0.00077
2. Granular, open mixing and loading	0.032	0.0084	0.0069	0.0017
3. All liquids, open mixing and loading	3.1	2.9	0.023	0.0012
4. Wettable powder, open mixing and loading	6.7	3.7	0.17	0.04342
5. Wettable powder, water soluble bags	0.039	0.021	0.0098	0.00024
6. All liquids, closed mixing and loading			0.0086	0.000083
7. Aerial-fixed wing, enclosed cockpit/liquid	0.0050	0.0050	0.0022	0.000068
8. Aerial-fixed wing, enclosed cockpit/granular	0.0044	0.0017	0.0017	0.0013
9. Helicopter application, enclosed cockpit		0.0019	0.0019	0.0000018
10. Aerosol application	480	190	81	1.3
11. Airblast application, open cockpit	2.2	0.36	0.24	0.0045
12. Airblast application, enclosed cockpit			0.019	0.00045
13. Groundboom applications, open cab	0.046	0.014	0.014	0.00074
14. Groundboom applications, enclosed cab	0.010	0.0050	0.0051	0.000043
15. Solid broadcast spreader, open cab, AG	0.039	0.0099		0.0012
16. Solid broadcast spreader, enclosed cab, AG	0.0021	0.0021	0.0020	0.00022
17. Granular bait dispersed by hand			71	0.47
18. Low pressure handwand	25	12	7.1	0.94
19. High pressure handwand	13	1.8	0.64	0.079
20. Backpack applications	680			0.33
21. Hand gun (lawn) sprayer			0.34	0.0014
22. Paintbrush applications	260	180		0.280
23. Airless sprayer (exterior house stain)	110	38		0.830
24. Right-of-way sprayer	1.9	1.3	0.39	0.0039
25. Flagger/Liquid	0.053	0.011	0.012	0.00035
26. Flagger/Granular	0.0050			0.00015
27. WP or liquid/open pour/airblast/open cab	26			0.021
28. WP or liquid/open pour/airblast/closed cab	0.88	0.37	0.057	0.0013
29. Liquid or DF /open pour/ground boom/closed cab	0.22	0.089	0.029	0.00035
30. Granule/open pour/belly grinder	210	10	9.3	0.062
31. Push type granular spreader		2.9		0.0063
32. Liquid/open pour/low pressure handwand	110	100	0.43	0.030
33. WP/open pour/low pressure handwand			8.6	1.1
34. Liquid/open pour/backpack			2.5	0.03
35. Liquid/open pour/high pressure handwand			2.5	0.12
36. Liquid/open pour/garden hose end sprayer	34			0.0095
37. Liquid/open pour/termiticide injection			0.36	0.0022

NB: Note that the above values are in mg/lb handled and **NOT** mg/kg bw per lb handled.

Source: Keigwin (1998)

See Section 3.2.2.1 for discussion.

Table 8: Example of Site Characteristics and Parameters Used in Gleams-Driver Modeling

Field Characteristics	Description	Pond Characteristics	Description
Type of site and surface (FOREST)	Field (0)	Surface area	1 acre
Treated and total field areas	10 acres	Drainage area:	10 acres
Field width	660 feet	Initial Depth	2 meters
Slope	0.1 (loam and clay) 0.05 (sand)	Minimum Depth	1 meter
Depth of root zone	36 inches	Maximum Depth	3 meters
Cover factor	0.15	Relative Sediment Depth	0.02
Type of clay	Mixed		
Surface cover	No surface depressions		

Stream Characteristics	Value
Width	2 meters
Flow Velocity	6900 meters/day
Initial Flow Rate	710,000 liters/day

GLEAMS Crop Cover Parameters[3]	Description	Value
ICROP	Weeds	78
CRPHTX	Maximum height in feet.	3
BEGGRO	Julian day for starting growth	32
ENDGRO	Julian day for ending growth	334

Application, Field, and Soil Specific Factors [1]	Code[3]	Clay	Loam	Sand
Percent clay (w/w/):	CLAY	50%	20%	5%
Percent silt (w/w/):	SILT	30%	35%	5%
Percent sand (w/w/):	N/A	20%	45%	90%
Percent Organic Matter:	OM	3.7%	2.9%	1.2%
Bulk density of soil (g/cc):	BD	1.4	1.6	1.6
Soil porosity (cc/cc):	POR	0.47	0.4	0.4
Soil erodibility factor (tons/acre):	KSOIL	0.24	0.3	0.02
SCS Runoff Curve Number [2]:	CN2	83	70	59
Evaporation constant (mm/d):	CONA	3.5	4.5	3.3
Saturated conductivity below root zone (in/hr):	RC	0.087	0.212	0.387
Saturated conductivity in root zone (in/hr)	SATK	0.087	0.212	0.387
Wilting point (cm/cm):	BR15	0.28	0.11	0.03
Field capacity (cm/cm):	FC	0.39	0.26	0.16

[1] The qualitative descriptors are those used in the QuickRun window of Gleams-Driver. Detailed input values for the soil types are given in the sub-table below which is adapted from SERA (2007b, Tables 2 and 3). All fields are run for about 6 months before the pesticide is applied in early summer.

[2] From Knisel and Davis (Table H-4), *Clay*: Group D, Dirt, upper bound; *Loam*: Group C, woods, fair condition, central estimate; *Sand*: Group A, meadow, good condition, central estimate.

[3]Codes used in documentation for GLEAMS (Knisel and Davis 2000) and Gleams-Driver (SERA 2007a)

See Section 3.2.3.4.3.1 for discussion.

Table 9: Precipitation, Temperature and Classifications for Standard GLEAMS-Driver Sites

Location	Precipitation	Temperature	Average Annual Rainfall (inches)	Average Annual Temperature (◦F)
HI, Hilo	Wet	Warm	126.06	73.68
WA, Quillayute [1]	Wet	Temperate	95.01	49.14
NH, Mt. Washington	Wet	Cool	98.49	27.12
FL, Key West	Average	Warm	37.68	77.81
IL, Springfield	Average	Temperate	34.09	52.79
MI, Sault Ste. Marie	Average	Cool	32.94	40.07
AR, Yuma Test Station	Dry	Warm	3.83	73.58
CA, Bishop	Dry	Temperate	5.34	56.02
AK, Barrow	Dry	Cool	4.49	11.81

[1] Based on composite estimation in WEPP using a latitude of 47.94 N and a longitude of -124.54 W.

See Section 3.2.3.4.3.1 for discussion.

Table 10: Sample Chemical input parameters used in Gleams-Driver modeling

Parameter	Triclopyr BEE	Triclopyr Acid	TCP	Note/Reference
Halftimes (days)				
Aquatic Sediment	1	1300	7.5 (5 to 11.3)	Note 1
Foliar	1.1 - 15	2.6 - 15	2.6 - 15	Note 2
Soil	0.2	14 (8 - 28.4)	69 (40 to 95)	Note 3
Water	0.5	426	6	Note 4
Soil $K_{o/c}$, mL/g	1233 (640 - 1650)	59 (25 to 134)	149 (81 to 242)	Note 5
Sediment K_d, mL/g	12 (0.64-16.5)	0.6 (0.25-1.32)	10.6 (6.5 to 21)	Note 6
Water Solubility, mg/L	7.4	440	100	Note 7
Foliar wash-off fraction	0.7	0.95	N/A (0.5)	Note 8
Fraction applied to foliage	0.5	0.5	N/A (0.5)	Note 9
Coefficient of Transformation	1	1	0.774	Note 10
Coefficient of Uptake	0	0	0	
Number of metabolites	2	1	0	
Depth of Soil Incorporation	1 cm			Surface application

Note 1	BEE: Use twice the half-life in water. Acid: Assume no degradation per U.S. EPA/OPP 2009a (Tables 2-1 and 3-2). Based on field studies, this is probably very conservative. TCP: Sediment half-times in ponds from Petty et al. 2003.
Note 2	For BEE and acid, lower bound is from Thompson and upper bound is from Knisel and Davis (2000). No data for TCP. Value for TCP taken as identical to that for triclopyr acid. Ranges are modeled with a uniform distribution.
Note 3	Acid from U.S. EPA/OPP (2009a). Triclopyr BEE conservatively set to 0.2. Much more rapid dissipation is reported in U.S. EPA/OPP (2009a). Values for TCP from Knuteson 1999. Central values and ranges are modeled with a triangular distribution.
Note 4	BEE: 0.5 days for hydrolysis of triclopyr BEE to acid (U.S. EPA/OPP 2009a, Appendix P, MRID 134174 and McCall et al. 1988); Acid: 426 days (U.S. EPA/OPP 2009a, Table 3-2, PRZM/EXAMS input, this is very conservative.); TCP: lumped aquatic degradation from Knuteson (1999). This appears to be very conservative given the rapid aquatic photolysis of TCP. See Table 1 of this risk assessment.
Note 5	BEE: Cessna et al. 2002. This is consistent with Knisel and Davis (2000) . Acid: Cessna et al. 2002. Central estimate is consistent with U.S. EPA/OPP (2009a) PRZM/EXAMS input. TCP: From Cessna et al. 2002 and consistent with Knuteson 1999. Central values and ranges are modeled with a triangular distribution.
Note 6	BEE and triclopyr: Based on the values for Koc with the assumption of 1% OC in soils. Values for BEE have little impact because of rapid conversion to acid. Estimates for triclopyr are consistent with sediment/water concentrations from Petty et al. (2003). TCP: Based on sediment/water concentrations from Petty et al. (2003). Central values and ranges are modeled with a triangular distribution.
Note 7	BEE: U.S. EPA/OPP (2009a, Appendix P); Acid: U.S. EPA/OPP (2009a, Tables 2-1 and 3-2); TCP: Knuteson (1999).
Note 8	Values for BEE and acid from Knisel and Davis (2000). This parameter is not used by GLEAMS for metabolites.
Note 9	Conservative assumption used in all Forest Service risk assessments for foliar applications. This parameter is not used by GLEAMS for metabolites.
Note 10	For TCP, the value of 0.774 is the ratio of the molecular weight of TCP to triclopyr acid. Because application rates will be expressed in units of a.e., no adjustment is needed for triclopyr BEE to triclopyr acid.

General Note: As indicated above, the Gleams-Driver runs utilize Monte Carlo methods for some input parameters. These are done using the Full Run capabilities in Gleams-Driver. In the database that is released with Gleams-Driver, only central estimates are entered into the chemical data table and only triclopyr TEA and BEE are included. Also note that the modeling of metabolites can only be done using a Full Run. This is true for all pesticides in Gleams-Driver.

Source: SERA (2011c), Forest Service risk assessment on triclopyr, Table 22.
See Section 3.2.3.4.3.1 for discussion.

SPECIAL NOTE: This is an example table taken from SERA (2011c). The references cited in the above table are given in SERA (2011c) but are not included in Section 5 of the current document.

Table 11: Sample Summary of Modeled Concentrations and Monitoring in Surface Water

All concentrations in units of ppb or µg a.e./L for an application rate of 0.32 lb a.e./acre

Scenario	Peak	Long-Term Average
Modeling for This Risk Assessment		
Accidental Spill (Section 3.2.3.4.1)	700 (70-5,800)	
Direct Spray and Spray Drift		
Pond, Direct Spray (Section 3.2.3.4.2)	36	N/A
Pond, drift at 25 feet (Section 3.2.3.4.2)	0.3-8	N/A
Stream, Direct Spray (Section 3.2.3.4.2)	30	N/A
Stream, drift at 25 feet (Section 3.2.3.4.2)	0.2-7	N/A
Gleams-Driver		
Single Application (see Appendix 8 for details)		
Pond, Section 3.2.3.4.4	6.27 (0-73.9)	2.39 (0-26.6)
Stream, Section 3.2.3.4.4	3.42(0-42.9)	0.170 (0-1.57)
Two Applications at 14-day Interval (see Appendix 9 for details)		
Pond, Section 3.2.3.4.4	11.6 (0-131)	4.48 (0-51.2)
Stream, Section 3.2.3.4.4	6.30 (0-86.4)	0.336 (0-3.17)
Three Applications at 14-day Intervals (see Appendix 10 for details)		
Pond, Section 3.2.3.4.4	16.2 (0 to 150)	6.59 (0 to 62.4)
Stream, Section 3.2.3.4.4	8.51 (0-115)	0.490 (0 to 4.32)
EPA Modeling		
U.S. EPA/OPP/EFED 2003a, FIRST, Tier 1. Three applications at 0.375 lb a.i./acre with 14 day interval.	53.327	11.336
U.S. EPA/OPP/EFED 2004a, PRZM/EXAMS, Index Reservoir, 3 applications at 0.375 lb a.i./acre (0.32 lb a.e./acre) with 21-day interval. CA Fruit. PCA 0.87, ranges from Appendix A	5.6 (2.7 to 26)	1.5 (0.74 to 6.84)
U.S. EPA/OPP/EFED 2008, PRZM/EXAMS, **2 applications** at 0.36 kg a.e./ha (0.32 lb a.e./acre)	1.35 to 14.3	N/A
U.S. EPA/OPP/EFED 2010a, PRZM/EXAMS, cites 2008 and 2010 EFED risk assessments.	26.2 to 33.4	N/A
U.S. EPA/OPP/EFED 2010b, Three ground applications, at 0.36 kg a.e./ha (0.32 lb a.e./acre) with 14-day interval. CAGrapes, CAWineGrape, NYGrapesSTD and Citrus using Index Reservoir. Table 3, p. 7.	8.7 to 27.3	2.0 to 4.4

See Section 3.2.3.4.3.1 for discussion.

Table 12: Sample Summary of Concentrations in Surface Water Used in a Risk Assessment

Foliar Broadcast, one application	Peak[1]	Longer-term[1]
Central	0.020	0.0075
Lower	0.002	0.00075
Upper	0.23	0.083
Foliar Broadcast, two applications	**Peak[1]**	**Longer-term[1]**
Central	0.036	0.014
Lower	0.0036	0.0014
Upper	0.41	0.16
Foliar Broadcast, three applications	**Peak[1]**	**Longer-term[1]**
Central	0.05	0.02
Lower	0.005	0.002
Upper	0.47	0.20

[1] All concentrations given as Water Contamination Rates – concentrations in units of mg a.i./L expected at an application rate of 1 lb a.i./acre. Units of mg a.e./L are used in the EXCEL workbook that accompanies this risk assessment.

See Section 3.2.3.4.6 for discussion.

Table 13: Estimated residues in food items in mg/kg wet weight per lb/acre applied

Broadcast Liquid Applications

Food Item	Central [1]	Lower [2]	Upper [1]
Short grass	85	30	240
Tall grass	36	12	110
Broadleaf/forage plants and small insects	45	15	135
Fruits, pods, seeds, and large insects	7	3.2	15

Broadcast Granular Applications [3]

Food Item	Central [1]	Lower [2]	Upper [1]
Short grass	3.4	1.2	9.6
Tall grass	1.44	0.48	4.4
Broadleaf/forage plants and small insects	1.8	0.6	5.4
Fruits, pods, seeds, and large insects	0.28	0.13	0.6

[1] From Fletcher et al. (1997) and U.S. EPA/EFED 2001, p. 44.
[2] Central values × (Central Value ÷ Upper Value).
[3] Based on estimates from granular applications of hexazinone (Michael 1992). See Section 3.2.3.7 for discussion.

Table 14: Dose-response assessments conducted by the federal government

Acronym	Definition	Reference
	Systemic Toxicity (Non-carcinogenic)	
RfD	*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.	U.S. EPA 1987
RfD_s	*Subchronic Reference Dose:* Oral dose (mg/kg/day) not likely to be associated with adverse effects over a less-than-lifetime exposure, in the general population, including sensitive subgroups.	U.S. EPA/ ODW 1990
RfD_{rt}	*Reference Dose for Reproductive Toxicity:* Oral dose (mg/kg/day) not likely to be associated with adverse developmental effects, in the general population, including sensitive subgroups. Used to evaluate effects after single exposure episode.	U.S. EPA/ OERPP 1991a
RfC	*Reference Concentration:* Concentration in air (mg/m^3) not likely to be associated with adverse effects over lifetime exposure, in the general population, including sensitive subgroups.	U.S. EPA/ ORD 1990
MRL	*Minimal Risk Level:* 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.	ATSDR 2004
1-Day HA	*1-Day Health Advisory:* A drinking water concentration (mg/L) not likely to cause adverse effects in the general population, including sensitive subgroups, after 1-day of exposure.	U.S. EPA/ ODW 2005
10-Day HA	*10-Day Health Advisory:* A drinking water concentration (mg/L) not likely to cause adverse effects in the general population, including sensitive subgroups, over a 10-day exposure period.	U.S. EPA/ ODW 2005
TLV	*Threshold Limit Value:* An air concentration (mg/m^3) not likely to cause adverse effects in exposed workers, over a normal period of work.	ACGIH 2011
	Carcinogenicity	
Slope Factor [q_1*]	*Cancer Potency Parameter:* A model-dependent measure of cancer potency $(mg/kg\text{-}day)^{-1}$ 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.]	U.S. EPA/ RAF 2005
Unit $Risk_{air}$	*Unit Risk for Inhalation Exposures:* The risk associated with a continuous lifetime exposure to an air concentration expressed $(mg/m^3)^{-1}$ or $(\mu g/m^3)^{-1}$.	U.S. EPA/ RAF 2005
Unit $Risk_{water}$	*Unit Risk for Water Consumption:* The risk associated with a continuous lifetime exposure to a drinking water concentration expressed $(mg/L)^{-1}$ or $(\mu g/L)^{-1}$.	U.S. EPA/ RAF 2005

See Section 3.3.2 (Chronic) and Section 3.3.3 (Acute) for discussion.

Table 15: Uncertainty factors used to derive reference values

Factor	Basis	ATSDR	U.S. EPA
Inter-human	Use a 10-fold factor when extrapolating from valid experimental results using prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among humans.	yes	yes
Experimental to human	Use a 10-fold factor when extrapolating from valid results of long-term studies on experimental animals when results of studies on human exposure are not available or are inadequate. This factor is intended to account for the uncertainty in extrapolating animal data to humans. If methods are available for a more explicit extrapolation, this uncertainty factor can be reduced or eliminated.	yes	yes
LOAEL to NOAEL	Generally use a 10-fold factor when deriving a reference value, RfD, or MRL from a LOAEL instead of an NOAEL. This factor is intended to account for the uncertainty in extrapolating from LOAELs to NOAELs.	yes - UF always 10	yes -UF varies
Subchronic to chronic	Generally use a 10-fold factor when deriving a reference value or RfD from less than chronic results on experimental animals or humans. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs.	no	yes
Children	An additional uncertainty factor of 10 is required for the protection of children unless the available data indicate that this factor is not required.	no	yes
Incomplete database	Generally use a 10-fold factor when deriving a reference value or RfD from valid results in experimental animals when the data are "incomplete." This factor is intended to account for the inability of any study to address all possible adverse outcomes.	no	yes
Modifying factor	Use professional judgment to determine an additional uncertainty factor that is >1 and ≤10 for deriving a reference value or RfD. The magnitude of the MF depends upon the professional assessment of the scientific uncertainties of the study and database not explicitly treated above. The default for the MF is 1	no	yes

Source: Adapted from ATSDR 2004; U.S. EPA 1987; U.S. EPA/OERD 2000.

See Section 3.3.2 (Chronic) and Section 3.3.3 (Acute) for discussion.

Table 16: Toxicity categories used in ecological risk assessments

Toxicity Category	Avian oral	Avian dietary	Aquatic	Wild mammals oral	Honeybees
Very highly toxic	Less than 10 mg/kg	Less than 50 ppm	Less than 0.1 mg/L	Less than 10 mg/kg	
Highly toxic	10 to 50 mg/kg	50 to 500 ppm	0.1 to 1 mg/L	10 to 50 mg/kg	<2 µg/bee
Moderately toxic	51 to 500 mg/kg	501 to 1000 ppm	Greater than 1 to 10 mg/L	51 to 500 mg/kg	2 to 11 µg/bee
Slightly toxic	501 to 2000 mg/kg	1001 to 5000 ppm	Greater than 10 to 100 mg/L	501 to 2000 mg/kg	
Practically nontoxic	Greater than 2000 mg/kg	Greater than 5000 ppm	Greater than 100 mg/L	Greater than 2000 mg/kg	>11 µg/bee

Source: http://www.epa.gov/oppefed1/ecorisk_ders/toera_analysis_eco.htm.
See Section 4.1.2 for discussion.

Table 17: Terrestrial Nontarget Animals Used in Ecological Risk Assessment

MAMMALS [1]

Animal	Representative Species	W[4]	Food Consumption[5]	Water Consumption
Small mammal	Mice	20	$2.514\ W^{0.507}$ [Eq 3-48]	$0.099\ W^{0.9}$ [Eq 3-17]
Larger mammal	Squirrels	400	$2.514\ W^{0.507}$ [Eq 3-48]	$0.099\ W^{0.9}$ [Eq 3-17]
Canid	Fox	5,000	$0.6167\ W^{0.862}$ [Eq 3-47]	$0.099\ W^{0.9}$ [Eq 3-17]
Large Herbivorous Mammal	Deer	70,000	$1.518\ W^{0.73}$ [Eq 3-46]	$0.099\ W^{0.9}$ [Eq 3-17]
Large Carnivorous Mammal	Bear	70,000	$0.6167\ W^{0.862}$ [Eq 3-47]	$0.099\ W^{0.9}$ [Eq 3-17]

BIRDS [2]

Animal	Representative Species	W[4]	Food Consumption[5]	Water Consumption
Small bird	Passerines	10	$2.123\ W^{0.749}$ [Eq 3-36]	$0.059\ W^{0.67}$ [Eq 3-15]
Predatory bird	Owls	640	$1.146\ W^{0.749}$ [Eq 3-37]	$0.059\ W^{0.67}$ [Eq 3-15]
Piscivorous bird	Herons	2,400	$1.916\ W^{0.704}$ [Eq 3-38]	$0.059\ W^{0.67}$ [Eq 3-15]
Large herbivorous bird	Geese	4,000	$1.146\ W^{0.749}$ [Eq 3-37]	$0.059\ W^{0.67}$ [Eq 3-15]

INVERTEBRATES [3]

Animal	Representative Species	W[4]	Food Consumption[5]
Honey bee [7]	*Apis mellifera*	0.000116	≈2 (1.2 to 4)[6]
Herbivorous Insects	Various	Not used	1.3 (0.6 to 2.2)

[1] Sources: Reid 2006; U.S. EPA/ORD 1993.
[2] Sources: Sibley 2000; Dunning 1993; U.S. EPA/ORD 1993.
[3] Sources: Humphrey and Dykes 2008; Reichle et al. 1973; Winston 1987
[4] Body weight in grams.
[5] For vertebrates, based on allometric relationships estimating field metabolic rates in kcal/day for rodents (omnivores), herbivores, and non-herbivores. For mammals and birds, the estimates are based on Nagy (1987) as adapted by U.S. EPA/ORD (1993). The equation numbers refer to U.S. EPA/ORD (1993). See the following table for estimates of caloric content of food items. For herbivorous insects, consumption estimates are based on fractions of body weight (g food consumed/g bw) from the references in Note 3.
[6] For honeybees, food consumption based on activity and caloric requirements. Used only when estimates of concentrations in nectar and/or pollen can be made, which is not the case in the current risk assessment.
[7] A surface area of 1.42 cm2 is used for the direct spray scenario of the honey bee. This value is based on the algorithms suggested by Humphrey and Dykes (2008) for a bee with a body length of 1.44 cm.

See data on food commodities in following table.
See Sections 4.2.2 and 4.2.3.2 for discussion.

Table 18: Diets: Metabolizable Energy of Various Food Commodities

Food Item	Animal Group	Caloric Value [1] (kcal/g bw)	Water Content [2]	Comment/Source(s)
Fruit	Mammals	1.1	0.77	See Footnote 3
	Birds	1.1	0.77	See Footnote 4
Fish	Mammals	4.47	0.70	Water content from Ali et al. (2005).
	Birds	3.87	0.70	Water content from Ali et al. (2005).
Insects	Mammals	4.47	0.70	Water contents from Chapman 1998 (p. 491). Typical ranges of 60-80%.
	Birds	4.30	0.70	Water contents from Chapman 1998 (p. 491). Typical ranges of 60-80%.
Vegetation (NOS)	Mammals	2.26	0.85	See Footnote 5
	Birds	2.0	0.85	See Footnote 5

[1] Metabolizable energy. Unless otherwise specified, the values are taken from U.S. EPA/ORD (1993), Table 3-1, p. 3-5 as adopted from Nagy 1987.

[2] From U.S. EPA/ORD (1993), Table 4-2, p. 4-14 unless otherwise specified.

[3] Based on a gross caloric value of 2.2 kcal/g bw (U.S. EPA/ORD 1993, Table 4-2). An assimilation factor for mammals eating fruit not identified. Use estimate for birds (see below).

[4] Based on a gross caloric value of 2.2 kcal/g bw (U.S. EPA/ORD 1993, Table 4-2) and an assimilation factor for the consumption of fruit by birds of 51% [2.2 kcal/g bw x 0.51 ≈ 1.1 kcal/g bw]

[5] Based on a gross caloric value of 4.2 kcal/g bw for dicot leaves (U.S. EPA/ORD 1993, Table 4-2). For birds, the value is corrected by an assimilation factor for the consumption leaves by birds of 47% [4.2 kcal/g bw x 0.47 = 1.974 kcal/g bw]

See Sections 4.2.2 for discussion.

Table 19: Overview of EPA/OPP's Risk Presumption System

Risk Presumption	RQ	LOC
	Mammals and Birds	
Acute Risk [a]	EEC[b]/LC_{50} or LD_{50}/sqft[c] or LD_{50}/day[d]	0.5
Acute Restricted Use [e]	EEC/LC_{50} or LD_{50}/sqft or LD_{50}/day (or LD_{50} <50 mg/kg)	0.2
Acute Endangered Species [f]	EEC/LC_{50} or LD_{50}/sqft or LD_{50}/day	0.1
Chronic Risk	EEC/NOEC	1
	Aquatic Animals	
Acute Risk	EEC[g]/LC_{50} or EC_{50}	0.5
Acute Restricted Use	EEC/LC_{50} or EC_{50}	0.1
Acute Endangered Species	EEC/LC_{50} or EC_{50}	0.05
Chronic Risk	EEC/NOEC	1
	Terrestrial and Semi-aquatic Plants	
Acute Risk	EEC/EC_{25}	1
Acute Endangered Species	EEC/EC_{05} or NOEC	1
	Aquatic Plants	
Acute Risk	EEC[h]/EC_{50}	1
Acute Endangered Species	EEC[g]/EC_{05} or NOEC	1

[a] Potential for acute toxicity for receptor species if RQ > LOC (EPA, 2004).
[b] Estimated environmental concentration (ppm) on avian/mammalian food items
[c] mg/ft^2
[d] mg of toxicant consumed per day
[e] Potential for acute toxicity for receptor species, even considering restricted use classification, if RQ > LOC (EPA, 2004).
[f] Potential for acute toxicity for endangered species of receptor species if RQ > LOC (EPA, 2004).
[g] EEC = ppb or ppm in water
[h] EEC = lbs a.i./A

Source: U.S. EPA/OPP 2004

See Section 4.3.2 for discussion.

Appendix 1: Maximum Time-Weighted Averages for Multiple Applications

Simple Case: Single Dose or Application

For a single dose, the calculation of the time-weighted average is relatively simple. First-order kinetics are generally applied where the elimination/dissipation/degradation of a body burden or other concentration is defined by a first-order rate coefficient, ***k***, typically expressed in units of days^{-1}. Under the assumption of first-order elimination, the first-order elimination rate coefficient (***k***) is inversely related to the half-life ($\boldsymbol{T_{50}}$) [$\boldsymbol{k} = \ln(2) \div \boldsymbol{T_{50}}$ and $\boldsymbol{T_{50}} = \ln(2) \div \boldsymbol{k}$]. For the following discussion, the processes of first-order elimination, dissipation, and degradation will be referred to as the general term, *first-order decay*. The discussion will also use the term *dosing* but the term *application* could be used as well.

Under the assumption of first-order decay, the concentration of a compound ($\boldsymbol{X_t}$) at time ***t*** after dosing is:

Equation 1

$$X_t = X_0 e^{-kt} = X_{Dose1} e^{-kt}$$

where $\boldsymbol{X_0}$ (or equivalently $\boldsymbol{X_{Dose\ 1}}$) is the concentration immediately after the first dose. Equation 1 is often referred to as the exponential function or exponential decay.

Risk assessments are always interested in the peak exposure. In the above example, the peak exposure is simply the dose – e.g., the mg/kg bw concentration at time zero. Risk assessments are also interested in longer-term exposures. If the above example is viewed as the "dose" or concentration of the compound on vegetation, scenarios are typically developed 90-day exposures. Over this period, the concentration on vegetation will diminish over time but we need to get a sense of whether or not the longer-term exposure might present a longer-term hazard.

The approach taken to longer-term term exposures to variable concentrations in Forest Service as well as most other risk assessments is based on Haber's Law (Bliss 1940; Gaylor 2000; Witschi 1999). Haber's Law (sometimes referred to a Haber's Principle or Haber's Rule) essentially states that the effect of variable concentrations of a chemical administered over a prolonged period of time is equal to the effect of the time-weighted average concentration over the same period time. Thus, finding the time-weighted average is a common task in risk assessment, including the calculations in WorksheetMaker.

For example, take the very simple case of a compound that does not diminish over time, as illustrated in Figure 1. In the example given in Figure 1, the calculation of the time-weighted average is obvious – the body burden starts at a value of 1 and stays at 1. The units are not critical to the kinetic discussion but the body burden can be thought of in units of mg/kg bw (ppm). The average body burden is thus 1 mg/kg bw over a 90-day period or any other period.

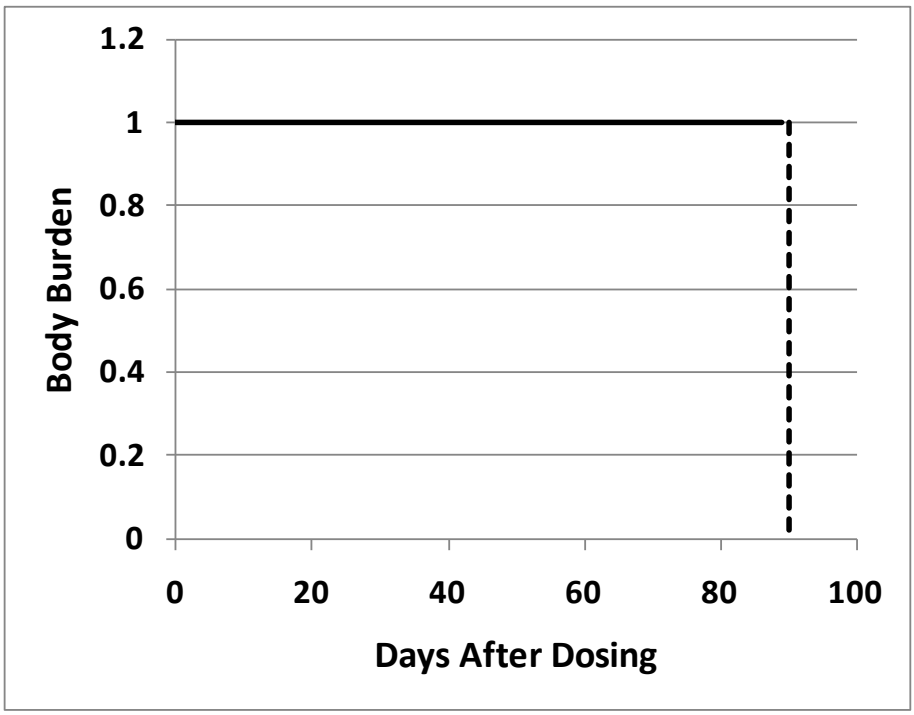

Figure 1: Compound that does not decay

This simple example illustrates a more general rule: the time-weighted average for any function can be calculated as the integral divided by interval. In more formal terms, the example illustrated in Figure 1 is a special case of first-order decay in which the rate coefficient is zero (***k***=0). In this case, Equation 1 reduces to:

Equation 2

$$X_t = X_0 e^{-k\,t} = X_0 e^{-0\,t} = X_0$$

The integral of Equation 2 with respect to ***t*** over the period from time zero to time ***t*** is:

Equation 3

$$\int_0^t X_0 d(t) = X_0\ t$$

and the time-weighted average of $\boldsymbol{X_t}$ is:

Equation 4

$$TWA_{X_t} = \frac{\int_0^t X_0 d(t)}{t} = \frac{X_0\ t}{t} = X_0$$

The explication of the general rule – i.e., the average is the integral divided by interval – for a straight line may seem unnecessarily pedantic but it useful in addressing the general and somewhat more complex conditions of first-order decay, illustrated in Figure 2, in which the values for ***k*** are greater than zero.

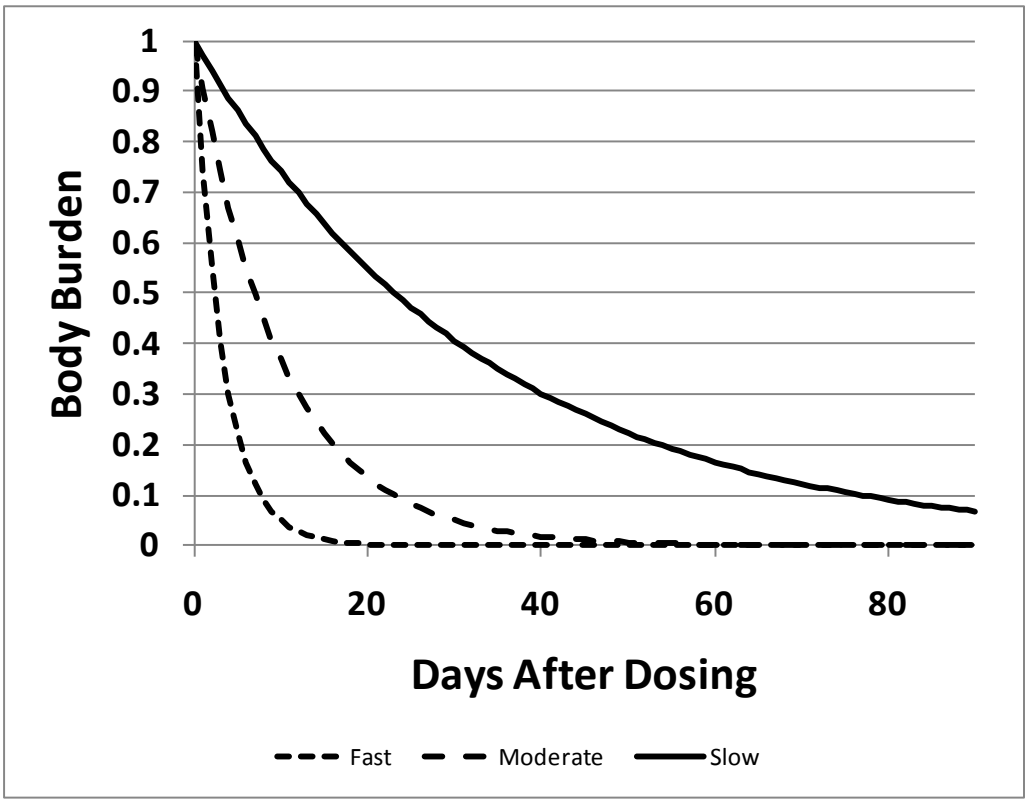

Figure 2: First-order kinetics for slow to fast decay

Specifically, Figure 2 illustrates three first-order decay curves involving relatively fast (***k***=0.3 day^{-1}), moderate (***k***=0.1 day^{-1}), and slow (***k***=0.03 day^{-1}) rate coefficients which correspond to half-lives ($\boldsymbol{T_{50}}$) of about 2.3, 7, and 23 days [$\boldsymbol{T_{50}}$ = ln(2)÷***k***].

Unlike the cased of the "flat curve" in Figure 1, the time-weighted averages for the exponential curves are not readily apparent. The curve for relatively slow decay (***k***=0.03) looks like it might have an average value of about 0.3 but even approximate averages for the other two curves are not obvious. Thus, numerical methods are needed – specifically, we need to integrate with respect to time (i.e., calculate the areas under the curves) and then divide the integral by the interval.

The integral of the exponential function (Equation 1) is very simple:

Equation 5

$$\int_0^t X_0 e^{-kt} = X_0 \int_0^t e^{-kt} = X_0 \frac{1-e^{-kt}}{k}$$

The steps in the above integration notation are included simply to be explicit. $\boldsymbol{X_0}$ is not dependent on time, so it is not actually a part of the integration. $\boldsymbol{X_0}$ does, of course, effect the calculation of and is directly proportional to the value of the integral. The time-weighted

average between dosing at time zero and ***t*** is simply the integral (area under the curve) divided by the interval (or time period):

Equation 6

$$TWA_m = \frac{X_0 \frac{1-e^{-kt}}{k}}{t} = X_0 \frac{1-e^{-kt}}{kt}$$

Note that estimation of the *maximum* time-weighted average in the case of a single dose is obvious in the case of a single dose with first-order decay: The maximum time-weighted average will start at time zero and end at time ***t***, the end of the interval of concern.

The practical need to resort to calculus in calculating time-weighted averages may not be intuitive. EXCEL is very good at taking averages. Equation 1 can be quickly entered into an EXCEL spreadsheet and averages over any period can be calculated. For example, if the value of k is Cell N2 and a series of times (0-90) are in Cells C5:C95, the formula "`=EXP(-N$2*$C5)`" can be entered into Cell D5. This can then be block copied. This is basically how Figure 2 was produced. Once this is done, the EXCEL `Average()` function can be used to calculate the average value from Day 0 to Day 90.

Table A1-1: Estimates of Simple Averages from EXCEL

Item	Low	Medium	High
Halftime:	2.31	6.93	23.10
k:	0.3	0.1	0.03
Empirical Average:	0.04240	0.11546	0.34757
Correct Average:	0.03704	0.11110	0.34548
Relative Error:	14.48%	3.93%	0.61%

The only problem with using EXCEL in this manner is that the answers are not correct, although they are sometimes close. The errors with the simple averaging are summarized in Table A1-1 for the first-order decay curves illustrated in Figure 2.

In all cases, the simple averages over-estimate the correct averages (Equation 6). This bias is due to the fact that the averaging is based on the starting value for each day rather than the average value of the course of each day. As illustrated in Table 1, the extent of the error associated with simple averaging increases as the first-order rate coefficient increases. An error of less than 1% (***k***=0.03) will seldom matter in many applications but an error of about 15% can make a difference.

The more important factor, as a matter of principle, is that there is no reason to tolerate any error because a reasonably simple analytical solution is available – i.e., Equation 6 in the case of a single dose.

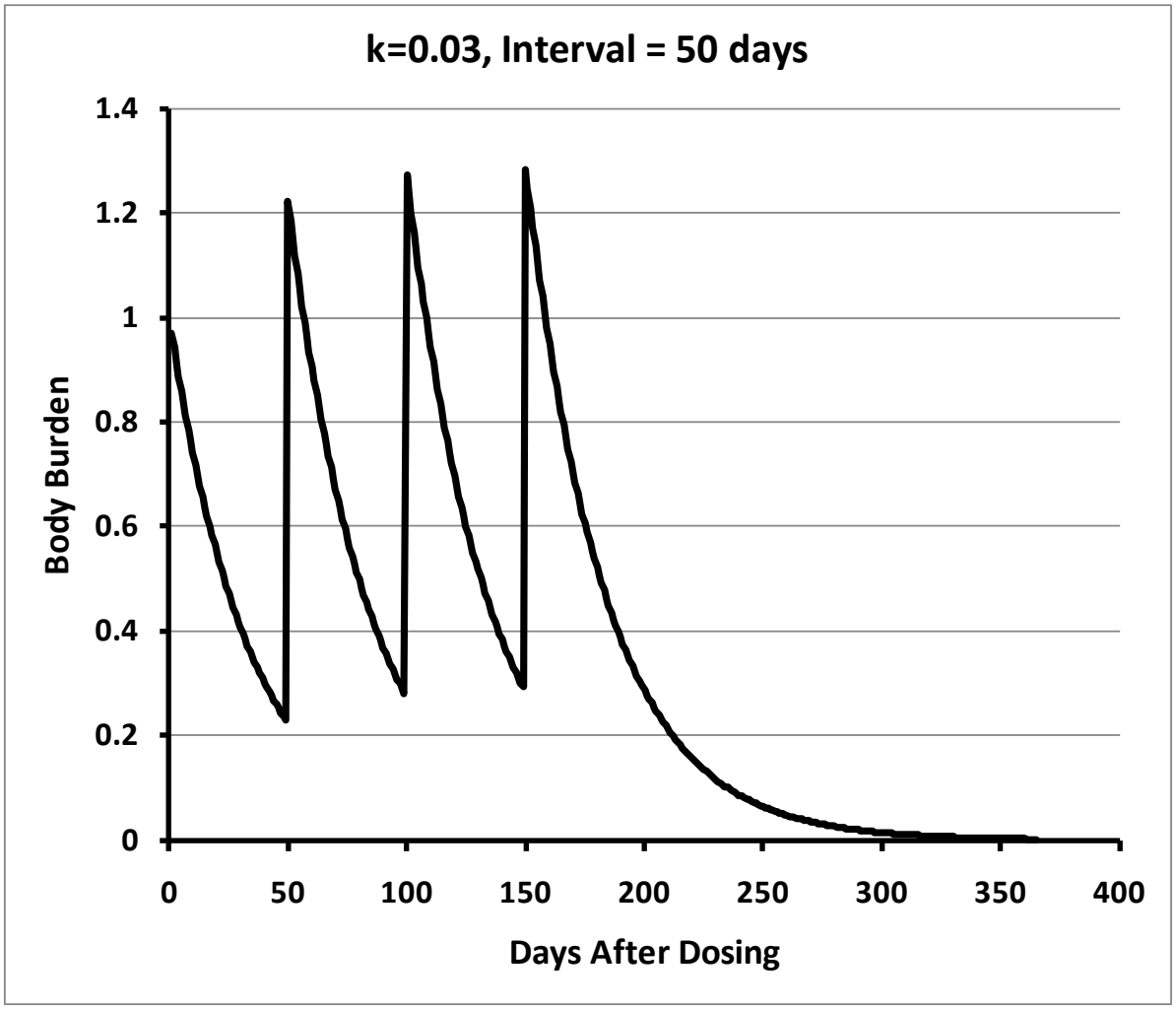

Figure 3: Four Doses at 50 day intervals for k=0.03

Multiple Doses or Applications

Concentration-Time Curves

Figure 3 illustrates the time-course for the body burden following a multiple dosing, specifically 4 unit doses (e.g., 1 mg/kg bw) of a compound with a first-order decay rate (***k***) of 0.03 day^{-1} administered at an interval of 50 days. The basic kinetics of multiple dosing are based on the *plateau principle* (e.g., Goldstein et al. 1974, p. 320 ff). If a chemical with a first-order elimination rate coefficient of ***k*** is administered at fixed time interval (**Δt**) between doses, the body burden after the nth dose ($X_{n\ Dose}$) relative to the body burden immediately following the first dose ($X_{1\ Dose}$) is:

Equation 7

$$X_{n\,Dose} = X_{1\,Dose}\frac{1-e^{-k\,\Delta t\,n}}{1-e^{-k\,\Delta t}}$$

The derivation of the above equation seems to have been proposed initially by (Gaddum 1944). The derivation itself is peripheral to the discussion of time-weighted averaging but

note that the fractional expression is a unitless factor that adjusts the concentration immediately after the first dose ($X_{1\ Dose}$) to the concentration immediately after the nth dose ($X_{n\ Dose}$).

While not explicitly detailed in Gaddum (1944), Equation 7 can be easily converted to estimate the body burden (X_t) at time t after the initial dose:

Equation 8

$$X_t = X_{1Dose} \frac{1 - e^{-k\,\Delta t\, n_t}}{1 - e^{-k\,\Delta t}} e^{-k\,t_e}$$

where n_t is the number of doses administered as of time t and t_e is the time since the last dose – i.e., the effective time period over which elimination without doing has occurred.

The calculation of the number of doses (n_t) up to time t is only modestly complicated by the multiple dose schedule:

Equation 9

$$n_t = \text{Min}[1 + \text{Floor}[t \div \Delta t], N]$$

In Equation 10, *Floor[]* is the integer part of the time since the first dose (which is administered at time zero) divided by the interval between dosing. Taking Figure 3 as an example, if anywhere from 50 to 99 days have elapsed since the first dose ($50 \geq t \leq 89$) and the interval between dosing is between 50 days, Floor[50 to 89/50] is 1. The constant ***1*** is added in Equation 9 because the initial dose is given at time zero. Thus, if a total of 4 doses are to be administered [N=4] at 50 day intervals, only two doses have been administered up to Day 99 – i.e., one on Day 0 and the other on Day 50.

The *Min[]* function in Equation 9 limits the number of doses to be no greater than the number of doses that are administered (N). For example, if 4 doses are administered at 50 day intervals, the number of dose given after any day past Day 150 is still only 4.

The effective time of elimination since the last dose is simply:

Equation 10

$$t_e = t - ((n_t - 1)\,\Delta t)$$

In Equation 10, the constant 1 is subtracted from the number of doses up to time t (n_t) to convert n_t to a zero index origin which is needed for the multiplication.

TWA Concentrations Following the Peak Concentration

Previous versions of WorksheetMaker – i.e., all versions prior to Version 6 – have used Equation 8 for calculating the time-weighted average dose or concentration because $X_{N\ Dose}$ is the maximum dose or concentration that will be attained – i.e., the dose or concentration immediately after administration of the last dose.

Defining m as the averaging period, the time-weighted average concentration (TWA_{Nm}) over the period m following the last (N^{th}) dose can been calculated as in Equation 5:

Equation 11

$$TWA_{Nm} = X_{N\,Dose}\frac{1-e^{-k\,m}}{k\,m}$$

To be explicit, Equation 11is expanded below to more clearly indicate that the estimation of time-weighted average doses for multiple dose regimens involves two different intervals: ***Δt***, the interval between dosing, and ***m***, the interval over which the time-weighted average is to be taken:

Equation 12

$$TWA_{Nm} = X_{1\,Dose}\frac{(1-\left(e^{-k\,\Delta t}\right)^{n})}{1-e^{-k\,\Delta t}}\frac{1-e^{-k\,m}}{k\,m}$$

In the case illustrated in Figure 3 – i.e., 4 doses of 1 unit (e.g. mg/kg bw) with a k of 0.03 day^{-1} – Equation 12 is a very good estimate of the maximum time-weighted average dose so long as we are concerned with an averaging period (***m***) that is less than the dosing interval (***Δt***). However, if we are concerned with a 90 day or some similarly long period relative to the dosing interval (***Δt***), using Equation 12 will calculate the 90-day TWA from Day 150 (the day of the last dose) to Day 240, 90 days after the last dose. Visual inspection of Figure 3 clearly indicates that the maximum TWA will occur over an earlier period that must encompass Day 100 to Day 150.

Time-Weighted Average Over a Specified Interval

Before addressing the issue of calculating the maximum time-weighted average for any averaging period (***m***) and dosing interval (***Δt***), the more basic issue of calculating the time-weighted average for any averaging period (***m***) needs to be addressed.

Because multi-dose regimens involve discontinuous functions – i.e., instantaneous changes in state with each dose – calculating the time-weight average over some averaging period (***m***) involves adding the areas under the time-course curve (AUC) for each dosing period that is partially or completely contained by the averaging period (***m***). This is illustrated in Figure 4 for the 4 dose regime given at 50 day intervals in which the desired averaging period (***m***) covers a 150 day period from Day 20 to Day 170. The general equation for calculating the time-weighted average is:

Equation 13

$$TWA_{Day_x \text{ to } Day_{x+m}} = \frac{\sum_{n=n_F}^{n_L} AUC_n}{Day_{x+m} - Day_x}$$

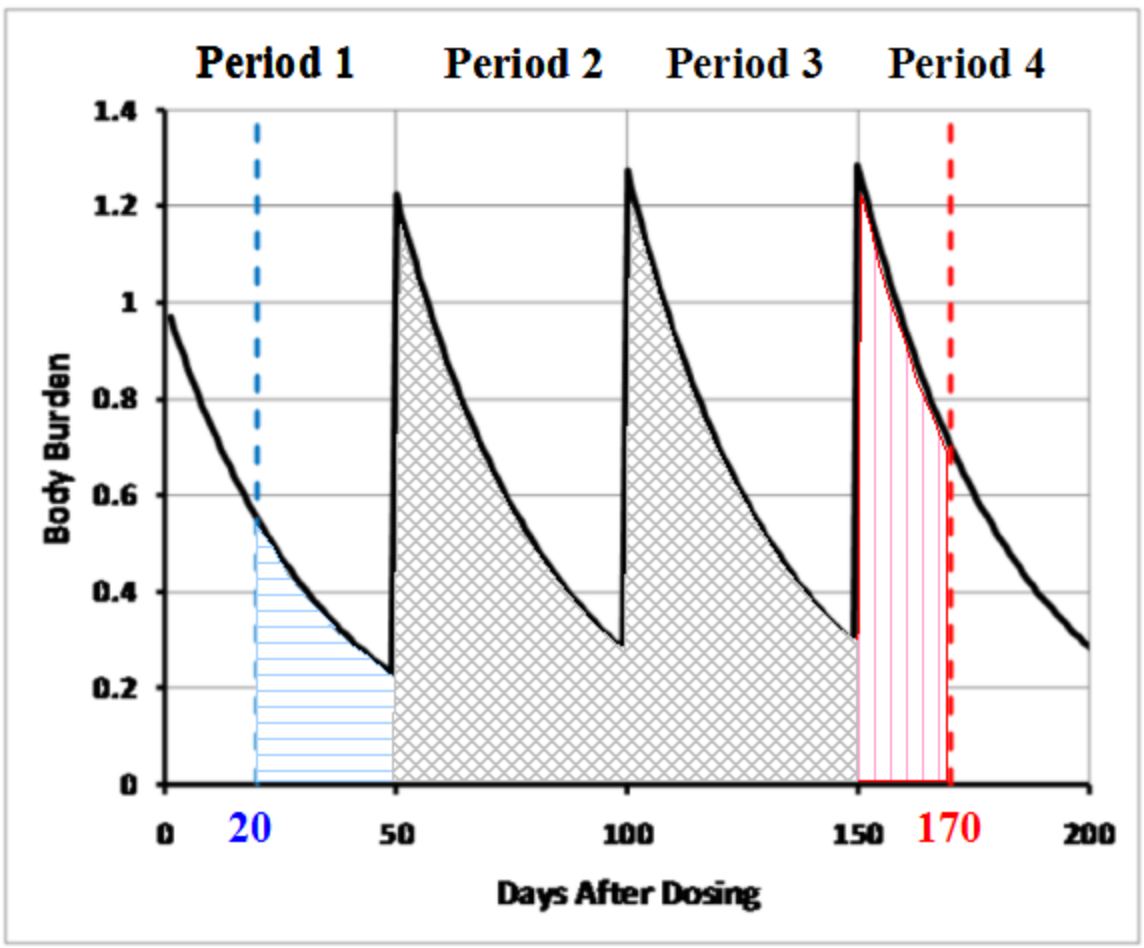

Figure 4: Regions for Estimating the Maximum TWA

Where $\boldsymbol{n_F}$ is the first dosing period encompassed by the interval (***m***) defined by the first day of concern ($\boldsymbol{Day_x}$) to the last day of concern ($\boldsymbol{Day_{x+m}}$), and $\boldsymbol{n_L}$ is the last dosing period covered by this interval. In general, the number of doses ($\boldsymbol{n_t}$) administered up to time ***t*** is given in Equation 9. Substituting ($\boldsymbol{Day_x}$) and $\boldsymbol{Day_{x+m}}$) for ***t*** in Equation 9, the first dosing period ($\boldsymbol{n_F}$) and the last dosing period ($\boldsymbol{n_L}$) can be calculated as:

Equation 14

$$\boldsymbol{n_F} = \text{Min}[1 + \text{Floor}[\boldsymbol{Day_X} \div \boldsymbol{\Delta t}], \boldsymbol{N}]$$

Equation 15

$$\boldsymbol{n_L} = \text{Min}[1 + \text{Floor}[\boldsymbol{Day_{X+m}} \div \boldsymbol{\Delta t}], \boldsymbol{N}]$$

The general equation for calculating the area under the curve for any dose period within $\boldsymbol{n_F}$ and $\boldsymbol{n_L}$ is:

Equation 16

$$AUC_n = X_{n\,Dose}\left(\int_0^{T_{End}} e^{-k\,T_{End}}\,dT_{End} - \int_0^{T_{Init}} e^{-k\,T_{Init}}\,dT_{Init}\right)$$

The first integral defines the area between the start of the nth dose period and the length of the dose period that is encompassed by $\boldsymbol{Day_{x+m}}$, the last day of the interval of concern (***m***). The second integral defines the area from the start of the dose period to the time that is equal to or greater than $\boldsymbol{Day_x}$.

The calculation of the starting point ($\boldsymbol{T_{Init}}$) for the integration in Equation 16 is identical for all dosing periods:

Equation 17

$$\boldsymbol{T_{Init}} = \text{Max}\,[\boldsymbol{Day_F - \Delta t\,(n-1), 0}]$$

where ***n*** is the number of doses that have been given up to the start of the $\boldsymbol{n^{th}}$ dosing interval. Thus, the first day ($\boldsymbol{Day_F}$) of the interval (***m***) may or may not include the first day of the dosing period. The failure to encompass Day 0 of the dosing period can occur only during the first dosing period, $\boldsymbol{n_F}$, as defined in Equation 14. For the example illustrated in Figure 4 in which $\boldsymbol{Day_F}$ is on Day 20, the partial inclusion of the first part of the dosing period occurs during Dosing Period 1 and $\boldsymbol{T_{Init}}$ is equal to Day 20– i.e., Max[20 – 50(1-1), 0] = Max[-30, 0].

The calculation of the ending point ($\boldsymbol{T_{End}}$) for the integration differs between last dosing period and all previous dosing periods. This is because the areas of the first through the penultimate dosing periods are bounded or fixed by the dose interval whereas the last dosing period is unbounded – i.e., the period begins at time the last dose is administered ($\Delta t\ \boldsymbol{n_F}$) but extends to infinity, at least conceptually. For the initial to the penultimate dosing periods, the terminal time of concern within the $\boldsymbol{n^{th}}$ dose period is defined as:

Equation 18

$$\boldsymbol{T_{End}} = \text{Min}[\boldsymbol{Day_L - \Delta t\,(n-1), \Delta t}]$$

Taking the dose schedule illustrated in Figure 4 as an example, $\boldsymbol{T_{End}}$ for the integration is the end of the second dosing period, Dose Period Day 50 – i.e., Min[170 – 50(2-1), 50] = Min[120, 50]. On the other hand, $\boldsymbol{T_{End}}$ for the integration is the end of the fourth dosing period is the Dose Period Day 20 – i.e., Min[170 – 50(4-1), 50] = Min[20, 50]. In other words, the averaging period of concern extends only 20 days into Dosing Period 4. Note that in the context of Equation 15, the only days used in the integrations refer to the days within the dosing period – e.g., Dosing Period Days 0 to 50. This approach is appropriate because the term $X_{n\ Dose}$ accounts for period up to start of the nth dosing period.

For the last dosing period, $\boldsymbol{T_{End}}$ is calculated as above except that the value is not limited by the dosing interval:

Equation 19

$$\boldsymbol{T_{End} = Day_L - \Delta t\,(n - 1)}$$

In other words, Equation 19 simply adjusts the last day of the averaging period, $\boldsymbol{Day_L}$ (which is the number of days relative to Day 0 of dosing) so $\boldsymbol{T_{End}}$ is the number of days since the last dose.

Expanding Equation 15 to more explicitly indicate the calculation,

Equation 20

$$AUC_n = X_{1Dose}\frac{(1-\left(e^{-k\,\Delta t}\right)^n)}{1-e^{-k\,\Delta t}}\left(\frac{1-e^{-k\,T_{Init}}}{k}-\frac{1-e^{-k\,T_{End}}}{k}\right)$$

which simplifies to

Equation 21

$$AUC_n = X_{1Dose}\frac{(1-\left(e^{-k\,\Delta t}\right)^n)}{1-e^{-k\,\Delta t}}\left(\frac{e^{-k\,T_{Init}}-e^{-k\,T_{End}}}{k}\right)$$

Equation 21 can be substituted into Equation 13 to calculate the time-weighted average concentration over any given period.

Equation 22

$$TWA_{Day_x\ to\ Day_{x+m}} = \frac{\sum_{n=n_F}^{n_L} X_{1Dose}\frac{(1-\left(e^{-k\,\Delta t}\right)^n)}{1-e^{-k\,\Delta t}}\left(\frac{e^{-k\,T_{Init}}-e^{-k\,T_{End}}}{k}\right)}{Day_{x+m}-Day_x}$$

Applying Equation 22 to the example in Figure 4 – i.e., averaging from Day 20 to Day 170 with a dose schedule of 4 unit doses at 50 day intervals – the 150 day time-weighted average concentration starting at Day 20 is about 0.632027.

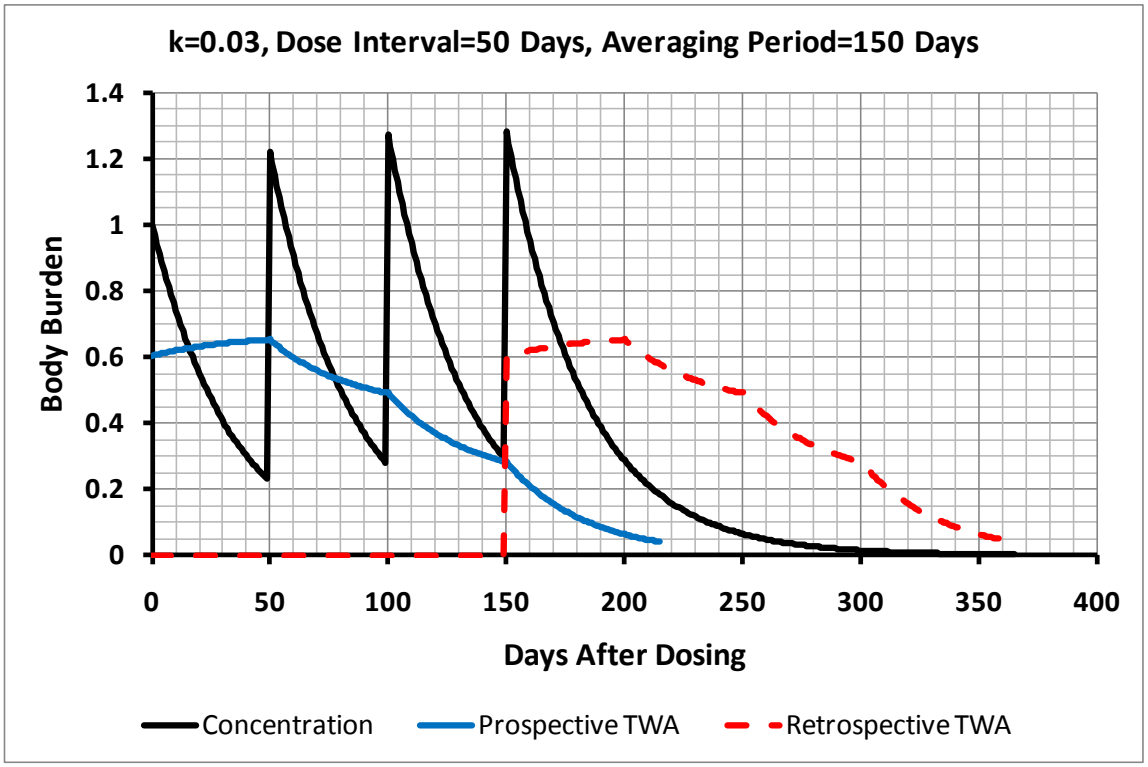

Figure 5: Rolling Time-weighted Averages

Maximum Time-Weighted Average Over a Specified Averaging Time

For the example illustrated in Figure 4, the averaging period from Day 20 to Day 170 is obviously not the maximum 150-day time-weighted average. As the averaging period is shifted to the left (***Day***$_x$ is increased), the contribution from Period 1 (the period with the lowest concentrations) is decreased and the contribution from Period 4 (the period with the greatest concentrations) is increased. This will result in an increase in the 150-day time-weighted average concentration, at least up to a point.

Figure 5 illustrates the rolling 150-day time-weighted averages for the 4-dose example with a k=0.03 and a dosing interval of 50 days. Two sets of time-weighted averages are plotted, one labeled prospective and the other labeled retrospective. This is done simply to illustrate the different ways that time-weighted averages can be viewed. The prospective time-weighted averages plot the time-weighted average that will occur between the specified time and the averaging interval. The retrospective time-weighted averages plot the time at which the average was achieved since the averaging interval. In this example, the maximum time-weighted average occurs between Day 50 and Day 200 – i.e., the period coving the last three doses (Days 50, 100, and 150) plus the dosing interval (to Day 200).

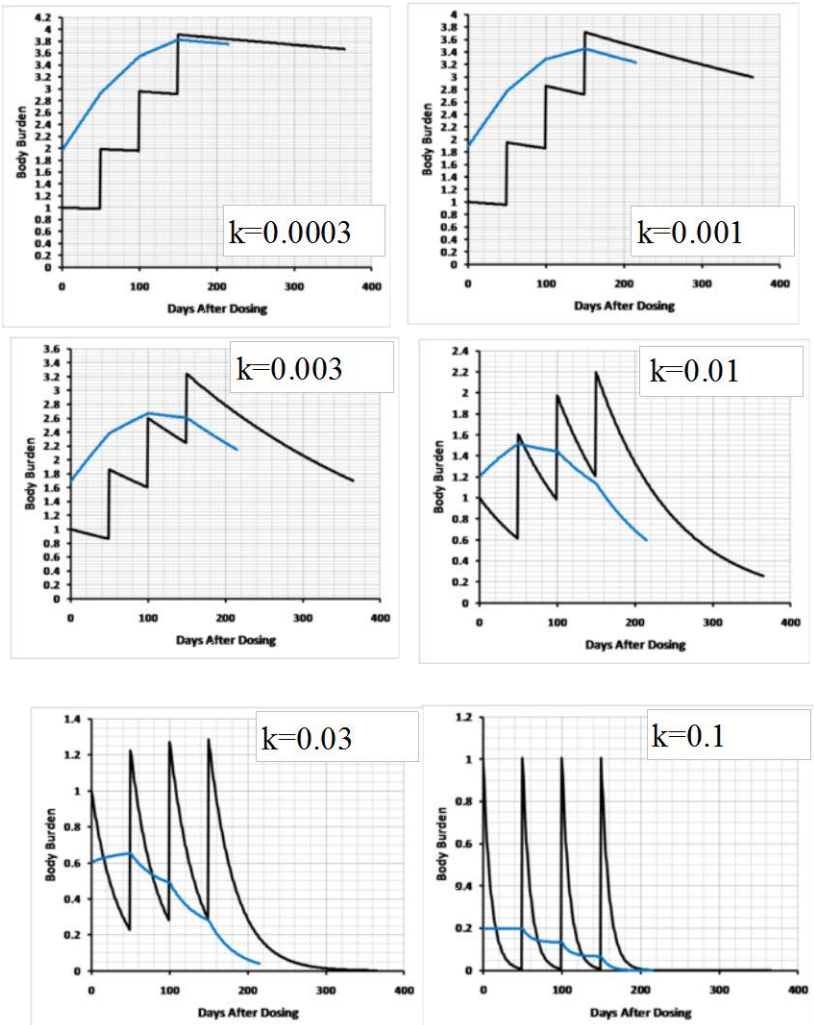

Figure 6: Impact of k on Time to Maximum Time-Weighted

A general method for determining when the maximum time weighted average will occur can be based on either a purely analytical method or a procedural method. The analytical method would involve determining the first-derivative of Equation 22, setting the first-derivative to zero, and then solving for Day_x or Day_{x+m}, to determine the prospective or retrospective time of the maximum time weighted average for the averaging interval of concern (***m***).

While using the analytical method may be possible, implementing this method would be awkward and complex. As indicated in Equation 22, the first-derivative of the equation for the time-weighted average would involve the sum of ***N*** derivatives, where ***N*** is the total number of doses (or applications).

A far simpler and easier method to implement in WorksheetMaker involves a consideration of the concentration at ***m*** (the averaging interval of concern) after the last dose is given relative to the peak concentrations at all but the last dose.

The basis for this procedural method is illustrated in Figure 6 for the 4 doses with a 50 day dosing interval for first-order decay coefficients ranging from 0.0003 day^{-1} to 0.1 day^{-1}. Only the prospective time-weighted average concentrations are illustrated in Figure 6 because only the prospective time-weighted averages are central to the procedural method for calculating the maximum time-weighted average.

The procedural method is based on two factors:

- Factor 1: For any averaging interval, the maximum time-weighted average will encompass at least part of the period following the last dose.
- Factor 2: The prospective day for the maximum time-weighted average (***Day***$_x$) – i.e., the day at which any maximum time-weighted average will start –will always occur on a day of dosing/application.

The first step in the procedure to determine the day on which a maximum time-weighted average will start is to determine the concentration at ***m*** (the averaging interval of concern) days following the last dose. This will be termed the critical concentration (***CC***) for determining whether or not a dosing period can be part of the interval that involves the maximum time-weighted average.

The critical concentration can be determined by substituting ***m*** (the averaging interval of concern) for t_e and ***N*** (the total number of doses to be administered) for n_t in Equation 8:

Equation 23

$$CC = X_{1\,Dose}\frac{1-e^{-k\,\Delta t\,N}}{1-e^{-k\,\Delta t}}e^{-k\,m}$$

As stated above, the critical concentration is simply the concentration at ***m*** days following the last dose. For any dose period (***n***) up to the penultimate (***N***-1) dose, the prospective day for the maximum time-weighted average cannot be in the dose period ***n*** if the peak concentration (Equation 8) is less than the critical concentration.

This rule is illustrated in the top two graphs in Figure 6 (k=0.0003 day^{-1} and k=0.001 day^{-1}). In both of these cases, the critical concentration – i.e., the concentration at Day 300 or 150 days (***m***) after the last dose – is greater than the peak concentration in the penultimate dose (the third dose in this example). Consequently, for these examples, prospective day for the maximum time-weighted average is limited to Dose Period 4. By Factor 2, the maximum 150-day time-weighted average must start on a dosing day. Thus, ***Day***$_x$ is equal to Day 150.

The middle-left graph in Figure 6 (k=0.003 day^{-1}) involves somewhat more rapid decay. In this case, the peak concentration for the third dose – i.e., the penultimate or ***N***-1 dose on Day 100 – is higher than the critical concentration but the peaks of all earlier time periods are lower. Because the dosing interval (***Δt***) is only 50, the maximum 150-day time-weighted average will start on Day 100 (***Day***$_x$) and will extend to Day 250 (***Day***$_{x+m}$).

The middle-right graph in Figure 6 (k=0.01 day^{-1}) involves more rapid decay and the peaks for Dose Period 2 and Dose Period 3 are higher than the critical concentration. Because the twice the dosing interval (***Δt* x 2**) is less than the averaging interval, the maximum 150-day time-weighted average will start on Day 50 (***Day***$_x$) and will extend to Day 200 (***Day***$_{x+m}$).

The bottom two graphs in Figure 6 (k=0.03 day^{-1} and k=0.1 day^{-1}) involve rapid decay and the peaks for all intervals exceed the critical concentration. In both cases, however, maximum 150-day time-weighted average will start on Day 50 (***Day***$_x$) as is the case for the

example in which k=0.01 day^{-1}. This must be the case because of Factor 1 – the period in which the maximum time-weighted average will occur must encompass at least part of the terminal dosing period.

The Algorithm

Based on the above discussion, the algorithm used in Version 6 of WorksheetMaker is relatively simple and the pseudocode the calculating the maximum time-weighted average over any arbitrary averaging interval (***m***) for any number of doses (***N***) administered at any arbitrary dosing interval (***Δt***) is given below.

```
MaxTWA(NDoses, k, Δt, m, Dose)
      'Default assumption of the day of the last dose.
      Dayx = (NDoses-1) * Δt
      DeltaDayx = 0
      CC = Equation 23
      For n = (NDoses-1) to 1 increment -1
            X_nDose = Equation 7(n)
            If X_nDose > CC then
                  'Must have (m – Δt)to ensure that
                  'some of the period past the last
                  'dose in the interval for the TWA.
                  If DeltaDayx < (m - Δt) then
                        DeltaDayx = DeltaDayx + Δt
                  Else
                        Exit For
                  End if
            Else
                  Exit For
            End if
      Next n
      Dayx = Dayx – DeltaDayx
      'Must subtract 1 because we count Dayx as one day
      Dayx+m = Dayx + (m-1)
      MaxTWA = Equation 22(Dayx, Dayx+m)
```

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